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An experimental evaluation of methods of surfgrass (*Phyllospadix torreyi*) restoration using early life history stages

Final Technical Summary

Final Study Report

U.S. Department of the Interior
Minerals Management Service
Pacific OCS Region

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FINAL TECHNICAL SUMMARY

STUDY TITLE: An experimental evaluation of methods of surfgrass (*Phyllospadix torreyi*) restoration using early life history stages

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BACKGROUND: Increased urbanization of coastal areas has led to the degradation of many seagrass populations due to increases in nutrient loading, polluted waste from sewage and industrial discharges, coastal development and offshore construction, and recreational and commercial boating and fishing. The high ecological importance of seagrasses, coupled with their susceptibility to damage from a range of human activities and natural disturbances, have sparked much interest in understanding their ecology and developing methods for restoring damaged populations.

Previous attempts to restore seagrass populations often have relied on transplanting older life stages. In many cases, transplanting techniques have been successful in promoting the establishment of new plants. Although the cost-effectiveness of different planting techniques has been examined in some cases, there have been few attempts to measure whether damage to donor populations from which transplants are collected causes long-term losses to otherwise healthy populations. The potential for such damage has prompted studies to explore the use of seeds and cultivated seedlings in seagrass restoration, but relatively little research on this has been done to date.

In the United States, seagrass restoration efforts have focused on species that occupy shallow, soft-bottom habitats such as *Halodule wrightii* and *Zostera marina*. Surfgrasses such as *Phyllospadix torreyi* pose special challenges for restoration because the transplanting techniques that have been developed for other seagrasses are not applicable. Unlike most seagrasses, surfgrasses grow on exposed rocky coasts. Adventitious roots from the rhizome secure the plant to hard substrata. Thus, successful transplantation depends not only on growth following relocation, but also on secure attachment that enables transplants to avoid dislodgement by breaking waves. Populations of *P. torreyi* typically extend from the low intertidal into the subtidal, and because wave forces vary greatly along this depth gradient, different restoration methods might be required at different depths.

Results from our previous MMS-supported research have shown that *P. torreyi* produces copious amounts of seeds, whose germination is easily controlled in laboratory culture. This feature allows it to be propagated from laboratory-raised seedlings, as well as from sprigs or plugs taken from natural populations. Such flexibility could be of significant value in restoration if the relative performance of the different life forms varied along the depth gradient. The inability to restore populations of surfgrass motivated us to develop restoration techniques for this species.

OBJECTIVES: Our research addresses the important issue of mitigation of adverse effects of OCS (Outer Continental Shelf) oil and gas related activities on surfgrass (*Phyllospadix* spp.) communities. *Phyllospadix* is an important structure-forming plant in the intertidal and shallow subtidal zones that is impacted by a number of activities associated with offshore oil and gas production. With funding from Santa Barbara County and the MMS - UC Coastal Marine Institute Program, we have gathered much needed information on the reproductive ecology of *Phyllospadix* and we identified the most appropriate life stages of surfgrass for use in restoration. The primary objectives of this project were to: (1) test the feasibility of various techniques of transplanting laboratory reared seedlings to the field and evaluate their usefulness in restoring damaged surfgrass populations, (2) collect information on the growth and survivorship of naturally recruited surfgrass seedlings for use in estimating the time required for restored populations to fully recover, (3) compare the efficacy of using seedlings, sprigs (short lengths of rhizome containing a few shoots), and plugs (a cohesive clump of shoots and rhizomes) for potential restoration in the intertidal and subtidal, and (4) determine rates and sources of predation on surfgrass seeds.

DESCRIPTION:

Techniques for transplanting laboratory-reared seedlings

Three experiments were conducted to test different outplanting techniques, each lasting approximately three months. The first experiment tested the role of host type on seedling survival, as well as the effects of abrasion from nearby plants. Three types of hosts were tested: two species of red algae (*Bossiella orbigniana* (Dec) Silva and *Corallina officinalis var. chilensis* Dec), and an artificial host consisting of a 20x20cm piece of 1 mm diameter nylon mesh (a fabric diameter similar to that of the algae). Six 30x30cm plots were established in monospecific patches of *Bossiella* and *Corallina*, respectively in the winter of 1998. To test for effects of abrasion on seedling survivorship, the branches of all algae in half of the plots were clipped to within 1cm of the holdfast. The remaining plots were unaltered ('unclipped'). SCUBA divers hooked the bristled arms of seedlings onto branches of clipped and unclipped holdfasts of *Bossiella* and *Corallina* using fine forceps in a manner resembling natural attachment of seeds (40 seedlings per plot, 3 treatment replicates). Artificial hosts were placed near unclipped plots of *Bossiella* and *Corallina* (3 replicates for each species). Divers attached seedlings to the nylon mesh (10 seedlings per replicate), and then used underwater epoxy to fasten the artificial host to the substrate.

In the second and third experiment, only artificial hosts were used. Seedlings were attached in the laboratory and the seeded hosts were then outplanted to the field during the winter of 1999. This method was explored because attaching individual seedlings to natural hosts in the field had proved difficult and time consuming. Two types of artificial hosts were tested: braided nylon string and braided nylon netting. In both cases, an opening was made in the braid by untwisting it one half turn. One of the arms of the germinated seed was inserted into the opening and hooked onto one of the braids. The opening closed upon relaxation, locking the seedling into place. Using this technique, many seedlings could be securely and rapidly fastened to the strings and nets. Seeded strings and nets were kept in seawater tables before transportation to the study site in coolers.

The second experiment used nylon strings as hosts to explore effects of seedling density and abrasion from nearby plants. Strings were stretched across 0.5m square racks made of PVC tubing that were bolted to the ocean bottom using marine epoxy. In high-density and low-density treatments, 10 and 4 equally spaced strings were fastened to a rack, respectively (8 seedlings per string in both treatments). Four racks of each density were placed in dense patches of both clipped and unclipped *Corallina*.

The third experiment used nylon netting as an artificial host to test for effects of seedling density and clipped algae vs. bare rock. Seedlings were outplanted at three densities (5, 20 or 105 seedlings per net of 20 cm by 20 cm) in clipped patches of *Corallina* and in areas scraped to bare rock (4 replicates for each treatment). The netting was glued to the bottom.

To evaluate potential effects of handling on seedling survivorship, we compared the survivorship of seedlings that had and had not been attached to artificial hosts. At the time of the second and third experiment (winter 1999), control groups of 20 seedlings each were handled in the same ways as outplanted seedlings and then returned to laboratory culture. Six groups consisted of seedlings that were attached to strings and six groups consisted of loose seedlings. Each group of 20 seedlings was placed in a separate culture dish. These were maintained in the laboratory and survivorship was measured over 69 days.

Growth and survivorship of naturally recruited seedlings

We measured natural mortality of seedlings to provide a baseline for comparison to outplanting experiments. Mortality of 335 naturally-recruited seedlings was estimated at a site located 20 km north of Santa Barbara, CA USA (34^o 25' N 119^o 57'W). Cohorts of seedlings were followed in nine fixed 0.64 m^2 circular plots (9–241 seedlings present at the first count) for three months. Plots were selected in reef areas covered with *P. torreyi* and a variety of red algae including *Corallina vancouverensis* L. and *Chondracanthus canaliculatus* (Harv.), which have been proven suitable hosts. Each plot was carefully searched on three different occasions and all seedlings were counted.

Efficacy of seedlings, sprigs and plugs for restoration

Three potential restoration methods for *P. torreyi* to intertidal and shallow subtidal reefs near Santa Barbara, CA were compared: (1) seedlings cultivated in the laboratory and transplanted to the field, (2) sprigs (short lengths of rhizome containing a few shoots) collected from and transplanted to the study areas, and (3) plugs (cohesive clumps of rhizomes and shoots) collected from and transplanted to the study areas.

Each method of restoration was evaluated using data collected on survivorship and growth of the transplants after six months, recovery of the donor population from which transplants were collected, and the amount of effort (i.e. time) involved in transplanting. Transplants were considered dead if they had no leaves or were no longer present on the reef. Growth of surviving transplants was estimated as the change in the aerial coverage of rhizome over the six-month experiment as determined by the linear dimensions of the basal area occupied by the rhizomes of each transplant. The percentage change in the number of leaves after six months ([(final number of leaves – initial number of leaves) / initial number of leaves] * 100) was used to assess the condition of surviving transplants.

Seedlings attached to nylon line were transplanted to experimental plots 30 cm x 30 cm in area at the intertidal and subtidal study sites in November 2000 by fastening the ends of each nylon line directly to the reef using Z-Spar A788 marine epoxy putty.

Sprigs were harvested at the intertidal and subtidal sites in November 2000, and each was immediately transplanted just outside (within 2 m) the surfgrass bed to a 15 cm x 15 cm plot that had been cleared of other biota. To harvest sprigs, an unbranched terminal end of an actively growing rhizome was carefully removed from the perimeter of a bed with a knife. The rhizome of each sprig was five cm in length and contained several lateral shoots and a terminal shoot. Sprigs were transplanted to the cleared areas by attaching the cut end of the rhizome to the reef using marine epoxy. The collection of sprigs resulted in a small loss in surfgrass from the donor bed. To measure recovery from this loss, a reference marker was glued to the reef next to the cut end of each donor rhizome (i.e., a rhizome from which a sprig was harvested). Recovery was estimated as the aerial coverage of new rhizome that grew from the cut end of the donor rhizome.

The efficacy of using clumps of mature surfgrass was evaluated at the intertidal and subtidal sites beginning in August 1999. Square plugs of intertwined rhizomes and shoots were harvested from the middle of a bed of *P. torreyi* using a wide bladed putty knife and transplanted outside (within 2 m) of the surfgrass bed to plots that had been cleared of other biota. Clearings were made larger than the plugs in order to provide a 5 cm wide buffer from surrounding biota. Plugs were attached by pulling the leaves through a square piece of 2.5 cm diameter stretch mesh nylon net that was cut to a size that was slightly larger than the square plug. The net was pulled tight over the plug and secured to the reef at the edges with marine epoxy. As done for sprigs, the leaves were trimmed to 20 cm in length to minimize drag. Three different sizes of plugs (small, 5 cm x 5 cm; medium, 10 cm x 10 cm; and large, 20 cm x 20 cm) were transplanted to test whether plug size influenced: (1) survivorship and growth (i.e. increase in aerial coverage) of the transplanted plug, and (2) rate of recovery of the donor area from which the plug was collected. Six plugs of each size were transplanted to separate plots in each of the two sites. The collection of plugs resulted in the immediate loss of surfgrass in the donor beds equal to the aerial coverage of the plugs. To estimate the recovery of bare patches in the donor bed created by harvesting plugs, we marked each donor patch at the time of harvesting and made periodic measurements to assess the extent of in-growth from the edges of the bare patches by neighboring rhizomes. The recovery of donor patches was determined at the end of the six-month experiment by calculating the area of bare space remaining in each donor patch from linear measurements taken with a tape measure.

Rates and sources of predation on surfgrass seeds

Temporal and spatial patterns of predation on seeds of *Phyllospadix torreyi* were quantified at four sites near Santa Barbara, CA. The intensity of predation on fruits prior to maturation and release from the spadix was assessed at each site at the peak of the flowering season (September) in 1997 and in 1998. Seed traps (consisting of two pieces of polyester mesh) were deployed in the surfgrass zone at the four sites from August 1995 until December 1998 to assess patterns of abundance of dehisced fruits (containing seeds) and intensity of predation. Traps were put out at the sites and retrieved a month later, at which time empty traps were re-deployed in the same positions. Upon return to the laboratory, traps were placed in seawater where they were held until seeds were counted (up to 48 hours). The entire trap was searched for fruits which were carefully removed and categorized as whole (intact with a viable seed present), germinated (intact seed with emergent shoot), eaten (seed visibly eaten out of fruit) or partial (only one arm present).

SIGNIFICANT CONCLUSIONS:

Techniques for transplanting laboratory-reared seedlings

Our results suggest that transplanting laboratory –reared seedlings to the field may be a promising approach to enhancing populations of *P. torreyi* on artificial reefs. Artificial hosts may be used, with resulting short-term survival similar to that of naturally recruiting seedlings if appropriate techniques are selected. However, because of the inherently low survivorship of surfgrass seedlings, it may prove more difficult to enhance establishment of surfgrass populations using seedlings compared to other seagrass species. Longer and larger-scale test projects are required to assess whether rhizome attachment and growth rates of outplanted seedlings could match those of natural seedlings.

Growth and survivorship of naturally recruited seedlings

Seedlings of surfgrass naturally suffer high mortality from a variety of physical and biological causes.

Efficacy of seedlings, sprigs and plugs for restoration
Results from our research suggest that efforts to restore damaged populations of *P. torreyi* are highly dependent on the type of enhancement technique used. The evaluation of several techniques revealed that transplanting sprigs was the most effective means of producing a net increase in area of surfgrass habitat per unit effort. This outcome was affected by several factors. While factors affecting favorable growth and survival were significant elements, cost-effectiveness and recovery of donor plots also played important roles in determining the overall success of a technique. Depth also appeared to affect outcome, with subtidal transplants generally outperforming intertidal transplants for the same technique. The development of techniques that allow transplants to better withstand the harsh conditions of the intertidal should benefit restoration efforts in this zone.

Rates and sources of predation on surfgrass seeds
Surfgrass is a long-lived clonal plant that reproduces by vegetative spreading as well as periodic episodes of seedling recruitment. Clearly, the amount of predation loss of seeds of surfgrass is of a magnitude that potentially could contribute to the low numbers of seedlings typically observed in the populations we study. However, even when the amount of seed predation is so great that a majority of seeds that are produced by a population are consumed, there is no consensus regarding the potential for an impact on population dynamics or spatial distribution. Some terrestrial studies have revealed that seed predation can have substantial effects on local populations. Thus, for surfgrass, a high rate of seed predation could potentially influence population abundance if it occurred during times when conditions are favorable for seedling recruitment, and if density-dependent losses from other sources did not offset variation arising from differences in losses to predators. Clearly, resolving the importance of seed losses to population dynamics of surfgrass will require much additional information. This includes a fuller understanding of patterns of spatial and temporal variation in the intensity of seed predation as well as their underlying causes, so that the importance of seed predation relative to other processes that affect distribution and abundance can be assessed.

STUDY RESULTS:

Techniques for transplanting laboratory-reared seedlings
Approximately half of the seedlings survived after seven days in clipped plots of both *Bossiella* and *Corallina* when seedlings were attached by hand onto the host algae. Nonetheless, only a small percentage survived to three months. In contrast, even short-term survival was close to zero in all unclipped plots and none survived 50 days. Careful searches of the host algae revealed that entire seedlings had disappeared, suggesting that the method of attaching seedlings to host algae with forceps resulted in high rates of dislodgement. Survival was higher in seedlings attached to nylon mesh, possibly because they were not as easily dislodged. Survival differed significantly among treatments, but none differed significantly from survival of the naturally recruited cohort. *Post-hoc* comparison of treatment means revealed that seedlings on nylon mesh outplanted to *Corallina* patches experienced significantly lower mortality than those outplanted to *Bossiella* patches or than seedlings attached to clipped algae.

In the second experiment, percentage survival steadily declined over time in all treatments, with less than 10% remaining alive after four months. Seedlings were often abraded and broken, possibly caused by string movements during periods of high turbulence. Survival was similar among all treatments and survival in each treatment was significantly lower than in the natural cohort.

Survival was higher in the third experiment, when seedlings were attached to braided nylon netting. This method apparently minimized movements and prevented dislodgement. Survival in all treatments was similar to survival of naturally recruited seedlings.

Growth and survivorship of naturally recruited seedlings
Naturally recruited seedlings experienced substantial mortality. About half of the 335 seedlings died within three weeks of the initial survey, while 30% survived until the end of the three month study.

Efficacy of seedlings, sprigs and plugs for restoration
Transplanted seedlings survived poorly and had minimal rhizome growth at both the intertidal and subtidal sites, yet the individuals that did survive showed a 275 % increase in leaf number. Survivorship of transplanted plugs was high in both habitats; however, physical disturbances to the donor populations greatly exacerbated damage sustained at the time of collecting, yielding a substantial net loss in surfgrass. Sprigs transplanted to the subtidal had higher survivorship (71% vs. 48%) and a greater increase in the aerial coverage of rhizome (86% vs. 42%) than those transplanted to the intertidal. Of the three techniques, transplanted sprigs had the greatest overall increase in aerial coverage per unit effort, suggesting that this method may be the most effective approach for restoring *P. torreyi.*

Rates and sources of predation on surfgrass seeds
Over a period of four flowering seasons during 1995 - 1998, monthly patterns of seed fall and intensity of seed predation were similar among sites but were temporally quite variable. Abundance of dispersed seeds varied greatly both among seasons and years. Within a year, seeds were present in the environment every month but they peaked in abundance during the fall months following the annual flowering period. Seeds were more abundant during the earlier years of the study. The intensity of predation steadily increased throughout the study period, from a low of $\leq$ 10 percent seeds consumed during 1995 to about 50 percent consumption by 1997, and it was not correlated with abundance of seeds in the environment. Pre-dispersal seed loss also was estimated in two flowering seasons by counting the numbers of seeds consumed prior to release from the plant, and found to be relatively low (< 15 percent). No differences were detected among the four study sites in patterns of pre-dispersal loss. Field surveys were done at two sites to identify potential seed predators. Three of the most abundant species identified in these surveys were tested in the laboratory to determine size-specific patterns of feeding activity and to assess which early life stages of *P. torreyi* (fruits within spadices, dehisced fruits, and seedlings) were most vulnerable to predation. All three of the early life stages were consumed. The crabs *Pugettia producta* and *Pachygrapsis crassipes* were much more voracious predators than the isopod *Idotea resecata.*

STUDY PRODUCTS:

The following publications resulted from this work:

Holbrook, S.J., D.C. Reed and J. S. Bull. 1999. Methods for Enhancing Habitat Value of Artificial Structures by Establishing Surfgrass (*Phyllospadix torreyi*). *In* Proceedings of the Seventh International Conference on Artificial Reefs and Aquatic Habitats, San Remo, Italy. Pp. 582-589.

Holbrook, S.J., D.C. Reed, K. Hansen and C.A. Blanchette. 2000. Spatial and temporal patterns of predation on seeds of surfgrass, *Phyllospadix torreyi.* Marine Biology 136:739-747.

Holbrook, S.J., D.C. Reed and J. S. Bull. 2002. Survival experiments with outplanted seedlings of surfgrass (*Phyllospadix torreyi*) to enhance establishment on artificial structures. ICES Journal of Marine Science 59:S350-S355.

Bull, J.S. 2002. An experimental evaluation of different methods of restoring *Phyllospadix torreyi* (Surfgrass). M.A. Thesis. University of California Santa Barbara. 77 p.

Bull, J.S., D.C.Reed, and S. J.Holbrook. 2003. An experimental evaluation of different methods of restoring *Phyllospadix torreyi* (Surfgrass). In review, Restoration Ecology.

The following presentations were made based on this work:

Melissa Lee and Julia Diener, "Predation on surfgrass, *Phyllospadix torreyii,* spadices." High School Mentorship Participant Presentation, 4th Annual Research Mentorship Symposium, UCSB, 1998.

J. Scott Bull, "Marine Restoration." Habitat Restoration Group, Museum of Systematics and Ecology, UCSB, 1998.

J. Scott Bull, "Evaluation of Tests to Mitigate Losses of Surfgrass (*Phyllospadix torreyi*) from Anthropogenic Impacts." Poster, University of California Toxic Substances Research and Teaching Program 12th Annual Research Symposium, Santa Barbara, CA. 1999.

J. Scott Bull, "Development of Methods for Surfgrass (*Phyllospadix torreyi*) Restoration." Summer Mentorship Program, UCSB, 1999.

Ellen R. Feingold and Katie E. Gibbons, "The Spatial and Temporal Variability in Surfgrass, *Phyllospadix torreyii,* Reproductive Development in Santa Barbara County." High School Mentorship Participant Presentation, 5th Annual Research Mentorship Symposium, UCSB, 1999.

S. Holbrook, D.C. Reed and J. S. Bull. "Methods for Enhancing Habitat Value of Artificial Structures by Establishing Surfgrass (*Phyllospadix torreyi*)." 7th International Conference on Artificial Reefs and Aquatic Habitats, San Remo, Italy, 1999.

D. Reed and S. Holbrook. "Reproductive ecology of surfgrass in the context of restoration." Invited symposium presentation, Western Society of Naturalists, Monterey, CA, 1999.

Holbrook, S. and D. Reed. "Population ecology of surfgrass (*Phyllospadix torreyi*) and its potential application for restoration." 5th International Temperate Reef Symposium, Capetown, South Africa, 2000.

J. S. Bull. "Evaluation of Tests to Mitigate Losses of Surfgrass (*Phyllospadix torreyi*) from Anthropogenic Impacts." Poster, 13th Annual UC Toxic Substances Research and Teaching Program Symposium, San Diego, CA, 2000.

J. S. Bull, "Evaluation of Tests to Mitigate Losses of Surfgrass (*Phyllospadix torreyi*) from Anthropogenic Impacts." Poster, University of California Toxic Substances Research and Teaching Program 14th Annual Research Symposium, Lake Tahoe, CA, 2001.

D. Reed, S.J. Holbrook, C. Blanchette and R.J. Schmitt. "Differential reproductive responses to environmental fluctuations in species with contrasting demographies." Invited workshop talk, 6th International Temperate Reef Symposium, Christchurch, New Zealand, 2003.

FINAL STUDY REPORT

Proceedings Seventh International Conference on Artificial Reefs (7th CARAH): 582 - 589

Methods for Enhancing Habitat Value of Artificial Structures by Establishing Surfgrass (*Phyllospadix torreyi*)

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Abstract

A variety of structures are placed in coastal waters (e.g., artificial reefs, pipelines, jetties and piers) and valuable species often are targeted for establishment on them. One such species along the west coast of North America is the surfgrass *Phyllospadix torreyi,* a seagrass that is an important source of habitat structure. The rate of natural development of populations of surfgrass is very slow, prompting considerable interest in developing techniques to accelerate establishment. Here we present results of studies of techniques that employ seedlings for enhancement. Laboratory-reared seedlings were outplanted using several different techniques for attachment, and survival rates after three months were compared to those of naturally-recruited seedlings. Both natural host plants (algae) and artificial hosts (nylon strings and nets attached to the bottom) were tested. Survival of seedlings that had been attached to host algae by SCUBA divers was poor, probably because they were dislodged by water motion. Seedlings attached to strings and placed on racks on the substrate also survived poorly, apparently due to damage from abrasion. The highest survival rates (about 25 percent) occurred for seedlings outplanted on pieces of nylon net that were secured in patches of coralline algae. Their survival was comparable to that of naturally-recruited cohorts of seedlings, suggesting that outplant of seedlings is a promising approach to enhancement of *P. torreyi*.

Keywords: Seagrass, *Phyllospadix torreyi*, habitat restoration, surfgrass, artificial reefs, enhancement techniques

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Introduction

Certain species of marine plants, including kelps and surfgrasses, are sometimes targeted for establishment on artificial reefs because they are major sources of biogenic habitat structure and provide food, shelter, and nursery grounds for a variety of fish and invertebrates. The rates of natural establishment of these plants can be very slow – years or decades - and this has prompted considerable interest in devising techniques to accelerate development of populations of these species on artificial structures. The same techniques could also be used in restoration efforts involving these species on reefs that are damaged by anthropogenic activities.

Surfgrass (*Phyllospadix torreyi* S. Watson) is an abundant perennial seagrass that occurs in rocky intertidal and shallow subtidal zones along exposed shores of the Pacific coast of North America (den Hartog, 1970, Phillips, 1979, Phillips and Menez, 1988). It frequently grows on artificial structures and reefs placed in shallow nearshore environments. Local populations of *P. torreyi* are maintained both by vegetative propagation (Tomlinson, 1974, Larkum and den Hartog, 1989) and recruitment of seedlings (Turner, 1983, 1985) when patches of bare space are created by small-scale disturbances. However, larger-scale establishment of populations of surfgrass on artificial reefs or in denuded areas following large disturbances can only result from seedling recruitment. Colonization via seedling recruitment entails the dispersal of seeds from mature surfgrass beds, attachment, germination and growth to a large plant which can take years.

Surfgrass plants are dioecious and both sexes produce spikes of flowers called spadices. The nonfleshy fruit of *P. torreyi* contains one seed (Dudley, 1893) whose endocarp forms two arms with stiff bristles. After release from the spadix, the negatively buoyant fruit drifts along the bottom until the arms entangle on a host alga or a conspecific, where the seed germinates. Like other seagrasses, *P. torreyi* can produce large numbers of seeds (up to 10 000 per m^{-1} during its annual flowering season; Williams, 1995, Reed and Holbrook, unpub. data), but typically very few seedlings become established (Stewart, 1989, Williams, 1995, Blanchette *et al.*, 1999). One reason for this is that there can be tremendous losses of seeds to predators (Holbrook *et al.*, 1999). Further, the host plant must survive long enough for the rhizome of the seedling to anchor it to the rocky substrate. Finally, once attached, growth rates of young plants are low. Single rhizomes of established plants elongated a distance of about a meter in two years and small (< 1 m diameter) patches of surfgrass required several years to reach that size (Stewart, 1989).

The process of establishment of *P. torreyi* on artificial reefs potentially could be greatly enhanced by outplanting seedlings. Although most attempts to restore or establish seagrasses have involved the use of older life stages (Fonseca *et al.*, 1982, Williams and Davis, 1996), recent experiments with the seagrass *Posidonia oceanica* (L.) Delile (Balestri *et al.*, 1999) show great promise for the use of seedlings. Techniques for harvesting seeds of *P. torreyi*, storing them in the laboratory, and germinating them in large numbers have been recently developed (Reed *et al.*, 1998), making it feasible to use early life stages in enhancement efforts. Outplanting seedlings rather than seeds or mature plants has several major advantages. First, potential barriers to natural dispersal of seeds from existing beds to the artificial reef and to germination would be overcome. Second, the seed stage, which can experience tremendous losses from predation, would not be subject to predation during cultivation in the laboratory. Third, laboratory germination enables large numbers of seedlings to be outplanted. Lastly, artificial hosts could be used for attachment if suitable natural host algae were not present on a reef. Here we present results of experiments that tested several techniques for outplanting laboratory-reared seedlings of *P. torreyi*, and we discuss strategies for establishment of populations of surfgrass on artificial reefs and other structures in the coastal zone.

Methods

We measured natural mortality of seedlings to provide a baseline for comparison to outplant experiments. Mortality of 335 naturally-recruited seedlings of *P. torreyi* was estimated at a site located 20 km north of Santa Barbara, CA USA (34° 25' N 119° 57'W). Cohorts of seedlings were followed over three months in fixed 0.64 m^2 circular quadrats (N = 9 quadrats with 9 – 241 seedlings present at the first count) for three months. Quadrats were placed on reef areas covered with *P. torreyi* and a variety of species of red algae including *Corallina vancouverensis* L. and *Chondracanthus canaliculatus* (Harv.). The first counts were made in February at the end of the annual winter period of recruitment. Careful searches were made of each plot on three different occasions and all seedlings were counted.

Seedlings for use in outplant experiments were obtained by culturing seeds collected from female *P. torreyi* plants living in the shallow subtidal zone (< 4 m water depth) in the vicinity of the University of California, Santa Barbara, CA. Mature rhipidia were collected and immediately transported to the University's marine biological laboratory where seeds were dehisced by gently running a finger along the length of a female spadix. Batches of up to 150 seeds were placed in culture dishes containing 50 ml of filtered seawater (0.2 µm) and 50 µl of nutrient stock solution (Provosoli, 1968). Petri dishes were maintained at 16° C, with an irradiance of 40-50 $\mu E\ m^{-2}\ s^{-1}$ using a 14:10 h L:D photoperiod under mild agitation. The culture medium was changed each week and germination took place within a month. Seedlings were outplanted approximately one month post germination and at that stage of development contained a single shoot about 5 cm in length, with an intact fruit and no rhizome.

All outplant experiments were conducted on shallow (< 5 m water depth) subtidal reefs near Santa Barbara, CA, where *P. torreyi* is abundant. In these habitats, patches of surfgrass grow interspersed with several species of algae that serve as hosts for seedlings (Blanchette *et al.*, 1999). Experiments were started in the late winter, the time of the year when seedlings of surfgrass have recruited to host algae and thus are most abundant in nature. This enabled us to compare mortality of outplanted seedlings with natural mortality of similarly-aged seedlings.

The first experiment tested whether seedling survivorship varied on algal hosts whose branches had been clipped to reduce abrasion on seedlings compared to those whose branches were left intact. Seedlings were attached by SCUBA divers who hooked the arms of the residual fruits onto branches of the host algae using fine forceps in a manner that attempted to mimic the natural attachment of seeds. Two different host algal species were used – *Bossiella orbigniana* (Dec) Silva and *Corallina officinalis var. chilensis* Dec. For each host species, algae surrounding the groups of outplanted seedlings (N = 40 in each 30 x 60 cm plot) were clipped to the base of the stipe or left standing. Each treatment was replicated three times. In addition, a second attachment method was tested by threading the shoots of seedlings through holes of 20 x 20 cm pieces of nylon mesh (N = 10 seedlings per piece of mesh) then affixing the mesh to bare rocky substrate with underwater epoxy. Three replicates were placed near the *Bossiella* outplant plots and three were placed near the *Corallina* outplant plots.

In subsequent experiments, seedlings were attached to artificial host substrates in the laboratory and the seeded substrates were then outplanted to the field. These methods were explored because the technique of attaching individual seedlings to natural hosts in the subtidal by divers was difficult and very slow, which put a limit on the number of seedlings that could be outplanted. Two different types of artificial substrates were tested – braided nylon string and braided nylon netting. In both cases, an opening was made in the braid by untwisting it one half turn. One of the arms of the fruit was inserted into the opening and hooked onto one of the braids. The opening closed upon relaxation of the braid locking the seedling into place. Using this technique, many seedlings could be securely and rapidly fastened to the strings and nets. Seeded strings and nets were prepared and kept in seawater tables in the laboratory, then later transported to the study site in coolers and fastened to the reef using marine epoxy.

In one experiment, seedlings were outplanted on strings that were stretched between 0.5 m square racks made of PVC tubing that were bolted to the bottom. In high density treatments, 10 strings (each containing 8 seedlings) were fastened to a rack, and in low density treatments 4 strings were attached to the rack. Racks were placed in dense patches of *Corallina officinalis var. chilensis*. To test effects of abrasion by surrounding algal fronds on seedling survival the algae were clipped to the bottom of the stipe in half the plots, and in the other half of the plots algae were left intact. Outplanted seedlings were counted at intervals over a period of four months.

Another experiment explored the effects of seedling density on survival, using patches of nylon netting fastened with epoxy to the bottom to hold the seedlings in place. Seedlings were outplanted at three densities (5, 20 or 105 seedlings per net) in each of two substrate types – outplanted in patches of *Corallina officinalis var. chilensis* that had been clipped to the bottom of the branches and outplanted on bare rock (N = 4 nets for each combination of density and substrate). Following outplanting, seedlings were counted by divers at intervals over a period of about three months.

Results

Naturally-recruited seedlings experienced high mortality. About half of the 335 seedlings at our study site died within three weeks of the initial survey, and twenty-nine percent survived three months (Figure 1). In experiments where seedlings were attached by hand onto natural substrates (the algae *Bossiella* and *Corallina*) short-term (7-day) survival was higher in areas where surrounding algae had been clipped, but after several months few seedlings remained regardless of treatment (Figure 2). The pattern of survivorship was similar for both species of host algae (*Bossiella* and *Corallina*)

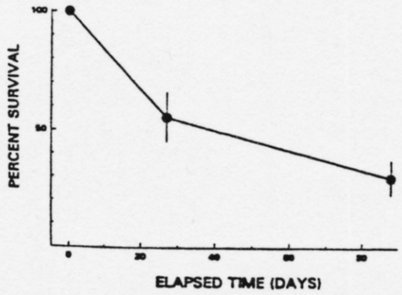

Figure 1. Survival of cohorts of naturally-recruited seedlings of *P. torreyi* near Santa Barbara, CA. Given are mean (± 1 SE, N = 9) percent of seedlings alive at each sampling date.

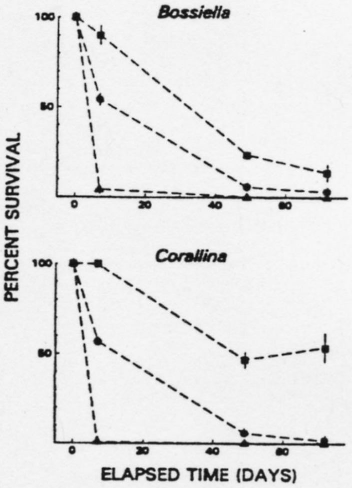

Figure 2. Percent survival (mean ± 1 SE) of seedlings attached to natural host substrates (*Bossiella orbigniana*, top and *Corallina officinalis*, bottom) that were clipped to the base of the stipe (circles) or not clipped (triangles) and to nylon mesh (squares). N = 3 replicates of each type.

examined. Careful searches of the host plants revealed that entire seedlings disappeared, suggesting that the method of attaching seedlings to host algae with forceps was not only time-consuming but did not adequately imitate natural attachment, thus resulting in very high rates of dislodgement. By contrast, survivorship was substantially higher in seedlings attached to artificial hosts (mesh) that were glued to the bottom (Figure 2), possibly because they could not be easily dislodged. The survival rates of these latter groups of seedlings were comparable to survival of the naturally-recruited cohorts (Figure 1).

Seedlings attached to strings in the laboratory that were then fastened to racks on the ocean bottom also survived poorly, with less than 10 percent remaining alive after several months (Figure 3). Seedlings in this experiment were abraded and broken, possibly the result of movement of the strings during periods of high water motion. Survival of seedlings was much higher when they were attached to braided nylon net glued to the bottom (Figure 4). This method prevented dislodgement and minimized movements of the fruits which were held against the bottom. There was no particular pattern to the effect of density on percent of seedlings surviving to three months, but more seedlings survived when the net pieces were affixed in areas of algae than when they were placed on bare rock (Figure 4). Overall, about 25 percent of seedlings outplanted into algae survived three months, which was comparable to survival of naturally-recruited seedlings (29 percent) over a similar time period.

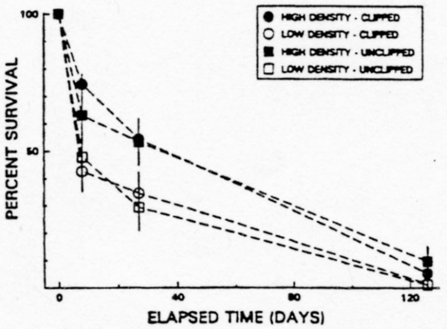

Figure 3. Percent survival (mean ± 1 SE, N = 4 per treatment) of groups of seedlings attached to braided nylon string and then outplanted on racks in patches of the alga *Corallina officinalis*. Algae were clipped to the base of the stipe (circles) or not clipped (squares). Outplanting densities were high (80 seedlings per group, filled symbols) or low (32 seedlings per group, open symbols).

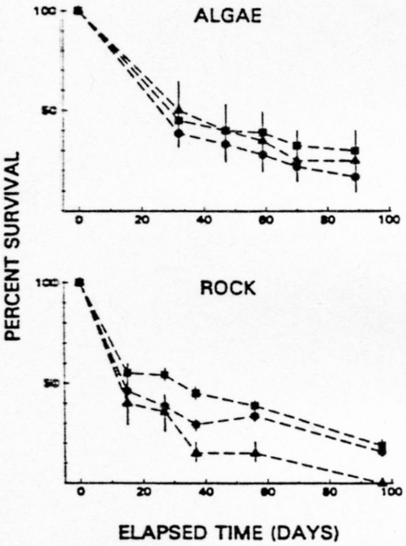

Figure 4. Percent survival (mean ± 1 SE, N = 4 per treatment) of groups of seedlings attached to patches of nylon netting glued on the bottom into patches of the alga *Corallina officinalis* (top) or onto bare substrate (bottom). Outplanting densities were high (105 seedlings per group, circles), medium (20 seedlings per group, squares) or low (5 seedlings per group, triangles).

Discussion

Scientists are often faced with the challenge of enhancing habitat value of artificial reefs and other structures such as jetties, pipelines, or piers that are placed in coastal waters. An ongoing challenge is to develop techniques to foster development of populations of certain species that are of high biological or economic value, such as the surfgrass *P. torreyi*. Previous attempts at establishing populations have relied on collecting adult plants from undisturbed areas and transplanting them to the desired locations, a strategy employed for other seagrass species (Fuss and Kelly, 1969, Kelly *et al.*, 1971, Phillips, 1974, Fonseca *et al.*, 1982, Thom, 1990, Williams and Davis, 1996, Balestri *et al.*, 1999). The collection of established plants not only damages existing populations, but attachment of large numbers of adult plants to rocky substrates in the subtidal zone is logistically difficult. It is thus not surprising that few previous efforts to establish surfgrass in this way have been made and they were not successful. Our results from this study indicate that establishment may be accelerated by outplanting laboratory-reared seedlings to the field. In this way the vagaries of seed dispersal, attachment and germination can be eliminated.

The development of enhancement strategies using early life stages of *P. torreyi* has been hampered by insufficient knowledge about the biology and ecology of these stages as well as a lack of effective outplant techniques. Recently, we developed procedures for collection, storage and germination of large numbers of seeds and for laboratory rearing of seedlings to a stage suitable for use in outplants (Reed *et al.*, 1998). This information made it possible to conduct tests of several different enhancement techniques. We found survival of *P. torreyi* seedlings varied greatly depending on the substrate of attachment. The best results were obtained when artificial hosts (nylon net) were used, possibly because seedlings were attached securely to the net which was fastened firmly against the bottom. The use of artificial hosts such as nylon nets would enable seedlings to be outplanted on reefs that lack the usual algal host plants, a situation that is typical for newly-built artificial reefs.

Seedlings of *P. torreyi* naturally suffer high mortality; we observed only about 30 percent survivorship of naturally-recruited seedlings after 88 days. Our findings indicated that over time periods of several months, survival of outplanted seedlings at best can approximate survival of naturally-recruited seedlings (Figures 1 and 4). Survival of outplanted seedlings of the Mediterranean seagrass *Posidonia oceanica* was much higher than that of *P. torreyi* (about 70 percent surviving after three years, Balestri *et al.*, 1999). However, in both cases rates of survival for outplanted seedlings and naturally-recruited seedlings were about the same (this paper, Balestri *et al.*, 1999). We do not yet know if our outplanted seedlings will perform as well or better than naturally-recruited seedlings over longer time periods. During our experiments rhizomes had not grown enough to attach the plants to the substrate, and it will be important to evaluate this aspect of establishment in future experiments.

The causes of seedling mortality in *P. torreyi* varied among the different enhancement techniques. Dislodgement appeared to be the primary cause of mortality when forceps were used to attach seedlings to natural algal hosts because in this case the entire seedling disappeared. In experiments where seeds were attached to nylon lines or nets the seed generally remained, however, the shoot often was lost. This could have been due to grazing, death of the shoot, or breakage from whiplash or mechanical damage. Despite the high losses of seedlings in our experiments, we predict that mortality rates would be even higher if outplants using (ungerminated) seeds were done. Losses of outplanted *Zostera marina* seeds during 1-week long field experiments ranged up to 96%, and crab predators were the major source of mortality (Fishman and Orth, 1996). Several species of invertebrates, particularly the crabs *Pugettia producta* (Randall) and *Pachygrapsis crassipes* Randall are voracious predators on seeds of surfgrass, and at some of our study sites nearly all released ungerminated seeds are damaged or consumed by predators (Holbrook *et al.*, 1999). There is relatively little predation on seeds while they are developing in the spadix prior to release into the environment (Holbrook *et al.*, 1999). Thus, collecting spadices to obtain seeds for laboratory culture bypasses a life stage where mortality risk is extreme.

The results reported here suggest that outplanting seedlings is a promising approach to enhancing populations of *P. torreyi* on artificial reefs. It appears that artificial hosts can be used and that short-term survival can approximate natural survival rates. However, because of the inherent low survivorship of surfgrass seedlings, it may be more difficult to enhance the establishment of *P. torreyi* compared to other species of seagrass. Longer tests that assess whether rhizome attachment and growth rates of outplanted seedlings could match or even exceed those of natural seedlings are needed.

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Spatial and temporal patterns of predation on seeds of the surfgrass *Phyllospadix torreyi*

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Abstract Spatial and temporal patterns of predation on seeds of the seagrass *Phyllospadix torreyi* S. Watson were quantified at four sites near Santa Barbara, California, USA. Over a period of four flowering seasons during 1995 to 1998, monthly patterns of seed fall and intensity of seed predation were similar among sites, but were temporally quite variable. Abundance of dispersed seeds varied greatly both among seasons and years. Within any one year, seeds were present in the environment every month, but they peaked in abundance during the fall months following the annual flowering period. Seeds were more abundant during the earlier years of the study. The intensity of predation steadily increased throughout the study period, from a low of ≤10% seeds consumed during 1995 to ~50% consumption by 1997, and it was not correlated with abundance of seeds in the environment. Pre-dispersal seed loss also was estimated in two flowering seasons by counting the numbers of seeds consumed prior to release from the plant, and was relatively low (<15%). No differences were detected among the four study sites in patterns of pre-dispersal loss. Field surveys were done at two sites to identify potential seed predators. Three of the most abundant species identified in these surveys were tested in the laboratory to determine size-specific patterns of feeding activity and to assess which early life stages of *P. torreyi* (fruits within spadices, dehisced fruits, seedlings) were most vulnerable to predation. All three of the early life stages were consumed. The crabs *Pugettia producta* (Randall) and *Pachygrapsus crassipes* Randall were much more voracious predators than the isopod *Idotea resecata* Stimpson.

Introduction

Seagrasses are the primary group of seed-producing plants in marine environments. They can produce copious amounts of seeds, but similar to terrestrial plants, seedling abundance is usually low relative to seed production (Turner 1985; Fishman and Orth 1996; authors' unpublished data). Reasons for the relatively low rates of seedling establishment are poorly understood, as the processes that affect seeds during maturation, dehiscence, and germination have been little investigated. The few studies conducted to date have identified two factors that contribute to loss of mature seeds within seagrass beds: rafting of seeds still attached to reproductive shoots was estimated to account for loss of over one-third of the seeds from a bed of *Zostera marina* L. (Gates 1984), whereas predation accounted for up to 65% of the losses of seeds of *Z. marina* observed during 2wk-long experiments (Fishman and Orth 1996). The relative importance of these and other mechanisms that contribute to seed loss in seagrasses undoubtedly will vary both spatially and temporally as well as among species. However, so little is known about the patterns and causes of seed loss that it is not yet possible to make interspecific comparisons or predictions about the consequences to population dynamics, particularly regarding the potential for population recovery via seedling recruitment following large disturbances.

A variety of birds, fishes and invertebrates (including crustaceans and molluscs) has been identified as predators of seagrass seeds (Adams 1976; O'Brien 1984; Agami and Waisel 1986; Wassenberg and Hill 1987; Wigand and Churchill 1988; Wassenberg 1990; Williams 1995; Fishman and Orth 1996). However, relatively few

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attempts have been made to quantify and compare losses of seeds among different sites, relate losses to the action of specific predators, or explore whether variation in predation intensity is related to variation in abundance of seeds. Here, we explore effects of predation on seed loss in the surfgrass *Phyllospadix torreyi* S. Watson. This species occurs in rocky intertidal and shallow subtidal zones and, unlike most other seagrasses, its distribution is limited to rocky reefs in areas of high wave-action (Phillips 1979; Phillips and Menez 1988). Plants are dioecious, and both sexes produce spikes of flowers called spadices. The non-fleshy fruit of *P. torreyi* contains a single seed (Dudley 1893) whose endocarp forms two arms with stiff bristles that attach it to a host plant (Gibbs 1902; Turner 1983; Blanchette et al. 1999). After release from the spadix, the negatively buoyant fruit (commonly referred to as a seed) drifts along the bottom until it entangles on a host alga (or sometimes a conspecific plant), where the seed germinates. After many months, development of the rhizome of the seedling is sufficient to anchor it firmly to the rocky substrate, and the host plant is no longer needed. Like other seagrasses, *P. torreyi* can produce large numbers of seeds (up to 10 000 per m^2 during its annual flowering season: Williams 1995; Reed and Holbrook unpublished data), but typically very few seedlings become established (Stewart 1989; Williams 1995; authors' unpublished data).

The goals of this study were to examine spatial and temporal patterns of predation in natural populations of *Phyllospadix torreyi* as well as conduct feeding trails in the laboratory to establish which early life stages of surfgrass were consumed by several species of predators. Over a period of four flowering seasons from 1995 to 1998, we quantified patterns of seed fall and estimated the strength of predation on post-dispersal seeds at four sites. Pre-dispersal loss also was estimated during two flowering seasons by counting the number of fruits consumed prior to release from the spadix. These measurements enabled us to establish spatial and temporal patterns of seed predation and to explore the relationship between abundance of seeds and intensity of predation. We did field surveys and laboratory experiments to ascertain which species of invertebrates were potential predators. We conducted tests on three species (the crabs *Pugettia producta* (Randall) and *Pachygrapsus crassipes* Randall and the isopod *Idotea resecata* Stimpson) to determine size-specific patterns of feeding activity and to assess which early life stages of *Phyllospadix torreyi* (ripe seeds within spadices, dehisced fruits, seedlings) were vulnerable to predation.

Materials and methods

Study sites

Field work was conducted in *Phyllospadix torreyi* S. Watson beds at four moderately wave-exposed intertidal sites (Alegria, Shoreline, Devereux, Hendry's) located in Santa Barbara County, California, USA. In these areas, *P. torreyi* forms extensive beds on rocky benches and boulders from the low intertidal zone into the subtidal to depths of ~4 m. An ecologically-similar congener, *P. scouleri* W. Hooker, which can co-occur with *P. torreyi*, was not present at these sites. The four study sites were located along a 58 km stretch of shoreline. Shoreline and Hendry's were separated by a distance of ~3 km, and were the southernmost of the sites. Alegria and Devereux were ≃55 and 15 km to the north, respectively. There were no obvious differences among the sites as to wave exposure, seawater temperature or other environmental conditions.

Patterns of pre-dispersal seed predation

Flowering in *Phyllospadix torreyi* is seasonal (Williams 1995). At our study sites, flowers first appear in early summer (June July), reaching peak densities in late summer (usually September: Reed and Holbrook unpublished data). Fruits ripen and are released from the spadix in the late summer and fall. On average, there are 10 to 13 flowers per female spadix on plants growing in the intertidal zone.

The intensity of predation on fruits prior to maturation and release from the spadix was assessed at each site at the peak of the flowering season (September) in 1997 and in 1998. At each site, 10 to 30 rhipidia (reproductive shoots containing one or more spadices) containing a total of 36 to 162 spadices were haphazardly collected on each sampling date and returned to the laboratory for examination. Each spadix was categorized as immature (spadix not open and no flowers are exposed) or opened (at least one flower exposed). Flowers and fruits in opened spadices were classified into the following groups: (1) receptive (flowers open and stigmas exerted); (2) immature (green ripening fruits with developing seeds); (3) mature (dark-brown seed coat visible); (4) dehisced (fruit missing, no sign of damage to spadix); (5) aborted (small abnormal fruit showing signs of atrophy); (6) eaten (seed absent, fruit or spadix cleanly ripped or torn). We explored patterns of pre-dispersal predation in two ways. First, the proportion of spadices from a site that was damaged by predators during each sampling period was calculated by dividing the number of spadices with at least one eaten fruit by the total number of spadices examined. Differences among sites and between years in the proportion of spadices damaged by predators were evaluated with a two-factor ANOVA (without replication) with site and year as fixed factors. Although this analysis does not allow tests of interactions between factors, it does provide a test of main effects. Second, the proportion of fruits eaten per spadix was determined by dividing the number of eaten fruits in a spadix by the total number of fruits in that spadix. The effects of site and year on the mean proportion of eaten fruits per spadix were evaluated by two-way ANOVA, with site and year as fixed factors. Data were transformed to arcsine square-root prior to analysis to meet the assumptions of normality and homoscedasticity.

Patterns of post-dispersal seed predation

Seed traps were deployed in the surfgrass zone at the four sites from August 1995 until December 1998 to assess patterns of abundance of dehisced fruits (containing seeds) and intensity of predation. Each trap consisted of two pieces of 30 × 30 cm polyester mesh tied together at their centers, where they were bolted to the substrate along permanent transects within the surfgrass zone (N = 10 traps per site). The two pieces of fabric had different mesh sizes to accommodate the slight variability in fruit size: 1.7 mm-diam polyester stretch mesh with round openings (polyester netting #9622, Research Nets, Inc., Redmond, Washington) and 1.0 mm-diam mesh with square openings (nylon netting Delta 1004A, Memphis Net and Twine). The fabric diameter of the mesh was similar to that of branched algae known to facilitate fruit attachment, and laboratory tests in an oscillating flow tank revealed that fruits attached to the fabric of the collectors at rates similar to that on many common host algae (Blanchette et al. 1999). Traps were put out at the sites and retrieved a month later, at which time empty traps were re-deployed in the same positions. Upon return to the laboratory, traps were placed in seawater where they were held until seeds were counted (up to 48 h). The entire trap was searched for fruits, which were carefully removed and categorized as whole (intact with a viable seed present), germinated (intact seed with emergent shoot), eaten (seed visibly eaten out of fruit) or partial (only one arm present) (Fig. 1). The condition of the exocarp of eaten fruit was variable. In some cases the exocarp was intact, and in others it was partially missing. It was unlikely that fruits found in the traps and categorized as "eaten" were actually damaged by predators prior to their release from the spadix (and thus actually represented pre-dispersal seed predation). The reason for this is that predators that consumed fruits from spadices damaged them extensively, leaving little or no intact exocarp and greatly damaging the bristled arms that are necessary for attachment to a host alga or the mesh of the traps.

The effects of site on the abundance of fruits caught in traps was evaluated by an analysis of covariance with site as a fixed factor and sample date (month and year) as a covariate. Spatial (among site) and temporal patterns of seed predation were investigated by calculating the proportion of total fruits whose seeds had been eaten (E) for each site during each monthly period as

$$E = \frac{e}{w + g + e + 0.5p}, \qquad (1)$$

where e = number of eaten fruit, w = number of whole fruit, g = number of germinated fruit and p = number of partial fruit. The value of p was divided by two to insure that the number of total fruit was not overestimated by counting two detached arms from the same fruit as two different fruits. The effect of site on the proportion of total fruits with eaten seeds was evaluated by analysis of covariance, whereby site was considered a fixed factor and sample date the covariate. Data were transformed to arcsine square-root prior to analysis. We also examined the relationship between intensity of predation (mean proportion of eaten seeds in each monthly sample) and abundance of seeds (mean number of seeds caught per trap) in a linear regression analysis.

Patterns of abundance of predators in the field

We conducted surveys in the surfgrass zones at two of the study sites, Devereux and Hendry's during fall 1997, spring 1998 and fall 1998 to estimate abundance of potential macro-invertebrate seed predators. Sites were visited a total of 5 (Devereux) or 6 (Hendry's) times in order to obtain time-averaged estimates of abundance. During each visit, 23 to 50 1 × 1 m^2 quadrats were randomly placed in the low intertidal zone and searched for potential predators of surfgrass seeds. Only four species [the hermit crab *Pagurus hirsutiusculus* (Dana), the cancer crab *Cancer productus* Randall, the kelp crab *Pugettia producta* (Randall) and the shore crab *Pachygrapsus crassipes* Randall] of potential seed predators were sufficiently abundant. They were counted and measured (carapace width). The isopod *Idotea resecata* (Stimpson) was commonly observed on the leaves of surfgrass, but was not counted. Half the quadrats were placed within the surfgrass bed, the other half in adjacent rocky areas lacking surfgrass. We sampled both areas because the potential predators are mobile and can forage in both areas, and because surfgrass seeds disperse into both the surfgrass bed and surrounding habitat. The density of each species (number m^{-2}) at each site was calculated using quadrats as replicates.

Laboratory tests of predation on early life stages of *Phyllospadix torreyi*

Laboratory experiments were conducted to assess level of predatory activity of four species of invertebrates on three early life stages of *Phyllospadix torreyi* – seeds within spadices, dehisced fruits, and 2 to 3 wk-old seedlings with fruits still attached. The species screened for evidence of predatory activity were *Pagurus hirsutiusculus*, *Pugettia producta*, *Pachygrapsus crassipes*, and *Idotea resecata*. In these tests individuals of each species were placed in running seawater in separate flow-through containers with spadices, fruits or seedlings, and evidence of consumption was assessed after 48 h. Based on their predatory activity, *Pugettia producta*, *Pachygrapsus crassipes* and *I. resecata* were chosen for more detailed study. Although *Pagurus hirsutiusculus* was very abundant in our field surveys, it did not consume early life stages of surfgrass in the preliminary trials, so it was not considered further.

In the laboratory trials, individual predators were offered one of three early life stages of *Phyllospadix torreyi* – spadices containing fruits, dehisced fruits, or seedlings (< 5 cm shoot length) for a period of 4 d. Fruits were obtained from female plants of *P. torreyi* growing in the shallow subtidal zone (< 4 m water depth) adjacent to the University of California, Santa Barbara (34°25′N; 119°57′W). Mature reproductive shoots (rhipidia) were collected and immediately transported to the laboratory, where they were maintained in seawater for use in experiments. Fruits were dehisced by gently running a finger along the length of a female spadix. Mature fruits dislodged easily with one pass of the finger. Seedlings were obtained by culturing seeds in the laboratory (for details see Reed et al. 1998).

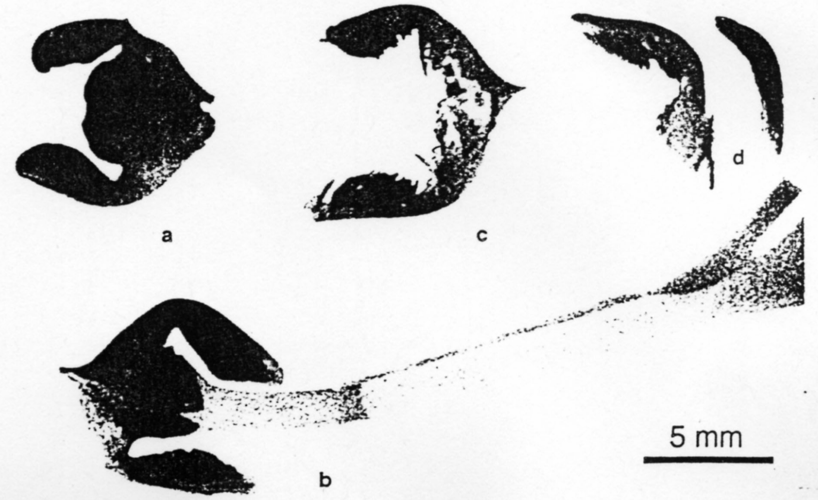

Fig. 1 *Phyllospadix torreyi.* Photograph of fruits collected in seed traps placed in surfgrass beds. Fruits were categorized for analysis as (**a**) whole, (**b**) germinated, (**c**) eaten and (**d**) partial (only one arm present)

Once experiments had been initiated, daily observations were made to assess consumption of the food items, but no additional food was added. In addition, the type of damage inflicted was noted for comparison with fruits caught in the seed traps. Controls in each experiment consisted of containers (N = 2 to 4) with food items but without predators. Predators of a range of body sizes were collected from the field just prior to initiation of the experiments, and housed individually in the laboratory in running seawater. *Idotea resecata* used ranged from 2.5 to 12.8 mm in total length (excluding antennae). *Pugettia producta* were 7.0 to 76.0 mm in carapace length, and *Pachygrapsus crassipes* were 8.0 to 31.7 mm in carapace length. All predators were maintained in the laboratory on *Macrocystis pyrifera* (L.) (giant kelp). Prior to each experiment, predators were starved for 24 h to standardize their hunger level. Experiments began when predators were placed in running seawater with spadices, fruits, or seedlings in separate flow-through containers (N = 24 to 28 individuals for each species of predator). Two spadices were placed with each predator, and their numbers of fruits and total weight were determined at the beginning and end of the 4 d experiment. A total of eight fruits or eight seedlings were given to each predator. The body size of each predator was determined at the beginning of each experiment by measuring the width and length of the carapace for crabs and total length (excluding antennae) for *I. resecata*. The relationship between body size and degree of consumption of seeds, seedlings and spadices was explored for each species of predator.

Results

Patterns of pre-dispersal seed predation

Surveys revealed that predation damage to *Phyllospadix torreyi* seeds prior to their release from the spadix was relatively low. The proportion of spadices at each site that was damaged by predators ranged from 7 to 24% overall (Fig. 2A). These values did not differ among sites ($F_{3,3}$ = 4.52, P = 0.12); in contrast, damage to spadices did differ between years (two-factor ANOVA without replication, $F_{1,3}$ = 12.0, P = 0.04), with a greater proportion of spadices having eaten fruits in 1997. Even though predators attacked a relatively low proportion of spadices overall, they frequently did extensive damage. Considering all damaged spadices from both years combined, in more than half the cases every one of the fruits in the spadix was consumed (54 out of 105 spadices).

Overall, the proportion of fruits in a spadix that was consumed prior to dehiscence was ~15% in 1997 and less than half that amount in 1998 (Fig. 2B). Two-way ANOVA revealed no significant differences among the four sites ($F_{3,592}$ = 0.59, P = 0.61), but a significant difference between years ($F_{1,592}$ = 5.25, P < 0.025). This difference between years was observed at all four sites, and there was no significant site × year interaction ($F_{3,592}$ = 0.03, P = 0.99 for site × year interaction).

Patterns of post-dispersal seed predation

Availability of dehisced seeds in the environment was temporally highly variable. The number of fruits caught in traps peaked during the fall months (September to November), following the annual fruiting period (Fig. 3). Inter-annual variation in abundance of fruits was also marked, with very low numbers observed during 1998 compared to the other three years (Fig. 3). Results of an analysis of covariance revealed that the effects of site on mean number of fruits caught in traps did not vary over time ($F_{3,149}$ = 2.43, P = 0.07 for site × date interaction). The mean number of fruit caught per trap was similar among sites but varied greatly over time [$F_{3,152}$ = 0.76, P = 0.52 for site and $F_{1,152}$ = 10.17, P = 0.002 for date in the reduced ANCOVA model, where the interaction term was dropped (as per Neter et al. 1996)]. Similarly, the proportion of fruits caught in traps that were missing seeds due to predation did not differ among the sites, but differed significantly over time [$F_{3,131}$ = 0.6, P = 0.62 for site and $F_{1,131}$ = 86.06, P < 0.0001 for date in the reduced ANCOVA model in which the non-significant inter-

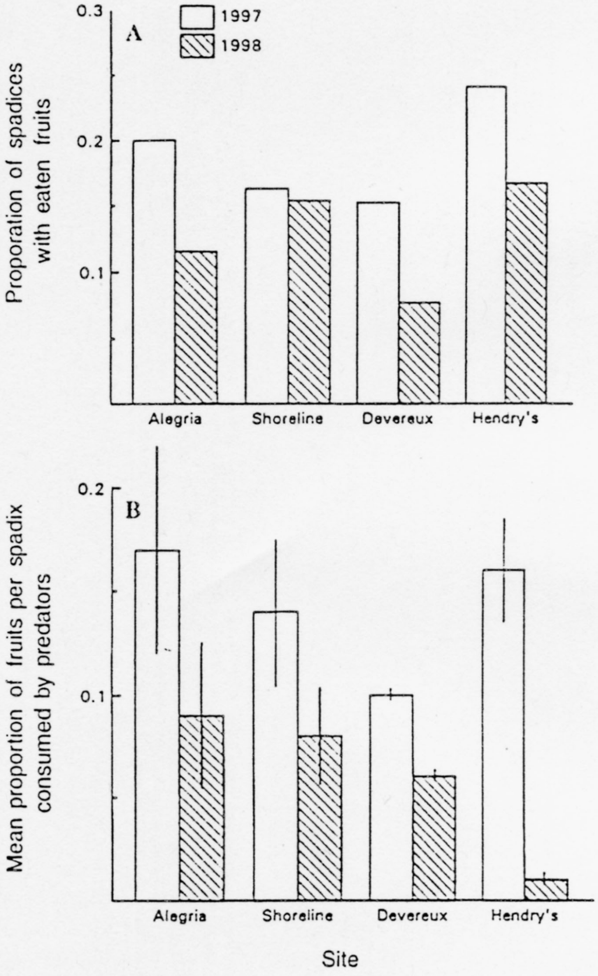

Fig. 2 *Phyllospadix torreyi*. Proportion of spadices with at least one eaten fruit (A) and mean proportion of fruits eaten per spadix (± 1 SE) (B) at four intertidal sites during peak flowering season (September) in 1997 and 1998. (N = 39 to 162 spadices per site per year)

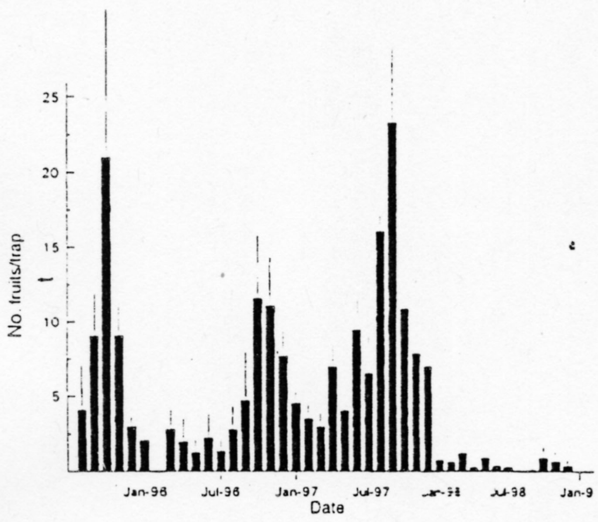

Fig. 3 *Phyllospadix torreyi*. Temporal pattern of abundance of dehisced fruits in the environment. Data are mean (+1 SE) number of fruits caught per trap per site per month. (*N* = 4 sites)

action term site × date ($F_{3,128} = 1.84$, $P = 0.14$) was dropped]. We observed a steady increase over time in the proportion of fruit caught each month whose seeds had been eaten by predators during the first three years of the study (Fig. 4). At the beginning of the study in 1995, fewer than 10% of fruits caught had lost their seeds to predation, but this amount had risen to ~50% by the end of 1997, at which time predation leveled off. High variability in the proportion of seeds eaten in 1998 reflected the low numbers of seeds caught during that time. Linear regression revealed no significant relationship between the intensity of seed predation (proportion of fruit with eaten seeds) and seed abundance (mean number of fruit in the trap: $F_{1,134} = 0.06$, $P = 0.80$).

Patterns of abundance of potential seed predators

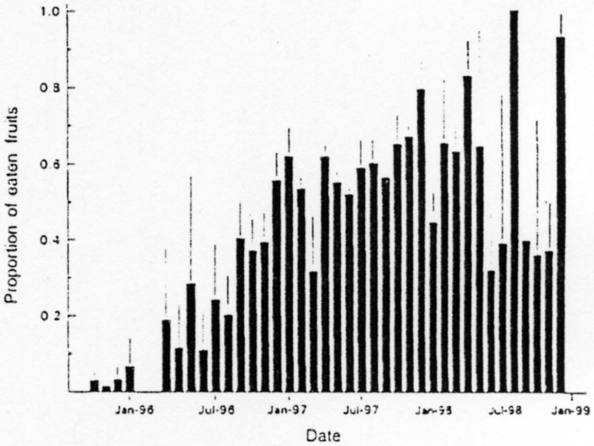

Fig. 4 *Phyllospadix torreyi*. Temporal pattern of predation on surfgrass seeds. Data are mean (+1 SE) proportion of fruits caught in seed traps each month whose seeds had been eaten by predators [E: Eq. (1) in "Materials and methods"] (*N* = 4 sites)

Several species of crabs occurred in and adjacent to *Phyllospadix torreyi* beds at the study sites. *Pagurus hirsutiusculus* was by far the most numerous, occurring in almost every quadrat sampled at the sites, and *Cancer productus* Randall was the rarest (Table 1).

Laboratory tests of predation on early life stages of *Phyllospadix torreyi*

In laboratory feeding trials, the species of predators varied greatly in the life stages of *Phyllospadix torreyi* that they consumed, as well as in the intensity of consumption. The degree to which each predator consumed life stages of *P. torreyi* depended on both the proportion of individual animals that fed during a feeding trial as well as on how much was consumed by each individual that did feed. The proportions of predators that fed actively, and the proportion of food items that only these active feeders consumed during experiments are presented in Table 2. Figure 5 shows the overall proportion of food items consumed by all individuals (those that fed and those that did not feed) of each species tested during a feeding trial.

All three species of predators consumed seeds from the spadix, but *Pugettia producta* was by far the most

Table 1 *Phyllospadix torreyi*. Patterns of abundance of potential seed predators of this plant at two intertidal sites, Devereux and Hendry's. Data are means (SE) of individuals counted per 1 m² quadrat during three surveys. For *Pagurus hirsutiusculus*, *N* = 59 quadrats for Devereux and *N* = 40 quadrats for Hendry's; for all other species, *N* = 204 quadrats for Devereux and *N* = 163 quadrats for Hendry's

Site	*Pagurus hirsutiusculus*	*Cancer productus*	*Pugettia producta*	*Pachygrapsus crassipes*
Devereux				
Mean	5.7	0.06	0.89	1.53
(SE)	(0.73)	(0.20)	(0.18)	(0.22)
Hendry's				
Mean	16.21	0.55	0.29	0.15
(SE)	(2.77)	(0.03)	(0.06)	(0.04)

Table 2 *Phyllospadix torreyi*. Summary of feeding activity on three early life stages of this plant by three species of predators (*Idotea resecata*, *Pugettia producta*, and *Pachygrapsus crassipes*; arranged in increasing order of importance) during 4 d-long laboratory experiments. Data are proportion of individuals of each predator that consumed a life stage, total number of predators tested, and mean (± 1 SD) proportion of each life stage eaten by those predator individuals that actively fed. Life stages offered to each predator were spadices (*N* = 2) containing mature fruits, dehisced fruits (*N* = 8) and seedlings (*N* = 8)

Life stage	Proportion of predators	No. of predators	Mean proportion consumed
Spadices			
Idotea resecata	0.21	28	0.06 (0.05)
Pugettia producta	0.94	18	0.90 (0.23)
Pachygrapsus crassipes	0.38	24	0.10 (0.05)
Dehisced Fruits			
Idotea resecata	0	28	0
Pugettia producta	0.50	26	0.96 (0.24)
Pachygrapsus crassipes	0.36	25	0.69 (0.40)
Seedlings			
Idotea resecata	0	25	0
Pugettia producta	0.83	24	0.96 (0.16)
Pachygrapsus crassipes	0.89	27	0.96 (0.18)

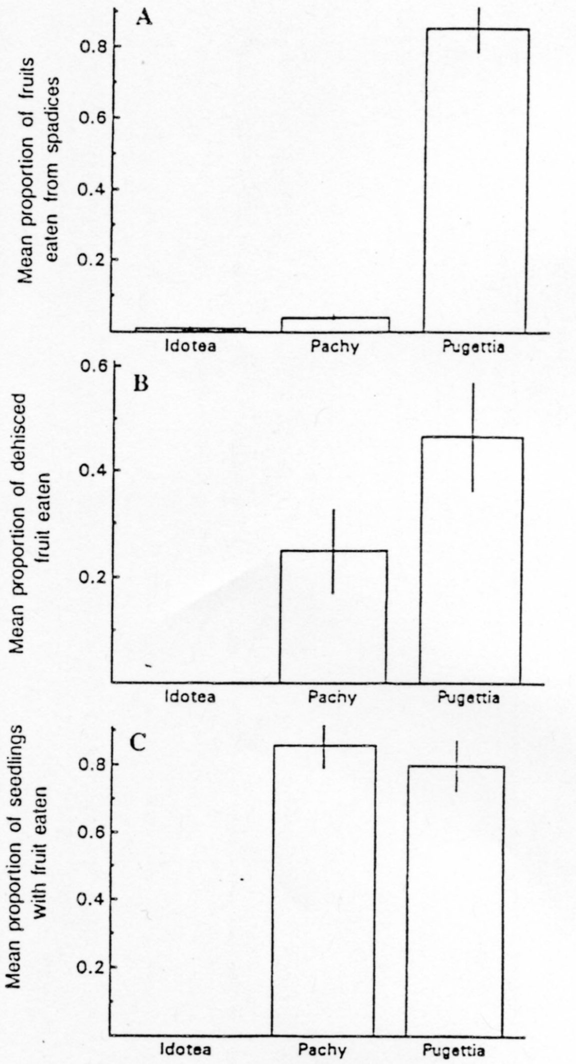

Fig. 5 *Phyllospadix torreyi*. Damage to spadices, dehisced fruits and seedlings by three predators during 4 d-long laboratory feeding experiments. Data are mean proportions (± 1 SE) of eaten fruits from spadices (A), groups of dehisced fruits (B), and seedlings (C). Number of replicate predators in each trial was 18 to 28; predators arranged in increasing order of importance (*Idotea* = *Idotea resecata*; *Pachy* = *Pachygrapsus crassipes*; *Pugettia* = *Pugettia producta*)

voracious. All but one *P. producta* individual ate seeds from spadices during the experiment, whereas fewer than half the individuals of the other two species consumed seeds from spadices (Table 2). Overall, *P. producta* consumed 85% of the seeds offered to them during the spadix experiment while *Pachygrapsus crassipes* and *Idotea resecata* consumed <5% (Fig. 5A). When a particular spadix was attacked by *P. producta* during an experiment, its seeds tended to be almost entirely consumed (Table 2). In contrast, *P. crassipes* and *I. resecata* attacked relatively few of the spadices offered during the experiment and, when an individual predator attacked a spadix, it consumed <10% of its seeds (Table 2).

Idotea resecata did not consume dehisced seeds, regardless of whether they were germinated or not. In contrast, both species of crabs readily consumed dehisced seeds. Overall, during the 4 d experiment, about half the seeds were eaten by *Pugettia producta* and about one-fourth by *Pachygrapsus crassipes* (Fig. 5B). The type of damage done by crabs during the laboratory experiment was similar to that sustained by fruits caught in the seed traps (Figs. 1c and 6). Feeding behavior varied substantially among individual crabs of both species. A large fraction (up to 64%) of the individuals tested in each experiment consumed no seeds at all, but the individuals that did eat during the trial consumed on average most of the seeds offered them (Table 2).

None of the *Idotea resecata* ate seeds attached to seedlings (Fig. 5C, Table 2); they did graze a small amount of leaf tissue from the seedlings (authors' personal observations). In contrast, both species of crabs consumed the seeds from ~80% of the total seedlings offered them during the course of the experiment (Fig. 5C). The majority of individual crabs fed, and those that did consumed virtually all the seeds from the available seedlings (Table 2).

Predator size had only minor effects on foraging efficiency in our experiments. *Pugettia producta* and *Pachygrapsus crassipes* < 10 mm carapace width did not consume any fruits or seeds. Other than this threshold in body size, there was no relationship between predator size and amount of spadices, seeds, or seedlings consumed by these two species. Very few *Idotea resecata* ate fruits or seeds in our experiments, despite testing individuals that spanned the natural range of sizes for this species.

Fig. 6 *Phyllospadix torreyi*. Photograph of fruits whose seeds were consumed by *Pugettia producta* during laboratory trials. All seeds have been removed from the fruits, and extent of damage is variable

Discussion

Several previous studies have indicated that in short-term experiments, predation rates on seagrass seeds can be extremely high. For example, losses of outplanted *Zostera marina* seeds during brief (1 wk) field experiments ranged up to 96%, and predator enclosure-experiments identified blue crabs (*Callinectes sapidus* Rathbun) as the major predator (Fishman and Orth 1996). In their experiments, predators were argued to account for up to 65% of the overall loss of seeds (Fishman and Orth 1996). Our studies on the surfgrass *Phyllospadix torreyi* at four intertidal sites over several years suggest that the high rates of predation observed in short-term experiments can occur on a sustained basis in the field. After an initial period in 1995 of relatively low seed predation, losses of seeds at our study sites rose steadily and have remained high. During the last 2 yr of our study, 7 to 15% of seeds were eaten prior to dehiscence, and half or more of the dehisced seeds caught in traps each month were consumed by predators. All four sites examined showed similar temporal patterns in the intensity of seed predation.

Of the species tested in our laboratory experiments, the crabs *Pugettia producta* and *Pachygrapsus crassipes* were the most active predators on early life stages of *Phyllospadix torreyi*. *Pugettia producta* was particularly voracious, consuming most of the seeds from spadices, as well as virtually all seeds and seedlings offered to it in laboratory experiments. Comparisons of seeds and seedlings exposed to crab predators in the laboratory to those obtained in seed traps showed similar patterns of damage, in that the seed and much of the exocarp were removed, leaving one or both of the bristled arms behind. These patterns are not consistent with mechanical damage that could arise in the high-energy surfgrass habitat. Early life stages of *Phyllospadix torreyi* can also be vulnerable to additional predators that we did not consider in our feeding trials. For example, Williams (1995) found the herbivorous fish *Girella nigricans* (Ayres) caused characteristic damage in a small proportion of male, but not female, spadices of *P. torreyi* at Santa Catalina Island, California. *G. nigricans* is not common at our study sites, and we did not observe its characteristic pattern of damage to spadices during our study; thus we attribute most of the predation observed in our study to crabs.

The results of our laboratory experiments are largely consistent with previous laboratory investigations of the behavior of predators on seeds of other seagrasses, particularly *Zostera marina*. All but the smallest individuals (< 10 mm carapace length) of both crabs (*Pugettia producta* and *Pachygrapsus crassipes*) used in the experiments could easily consume seeds and seedlings, and once this threshold size was reached there was no relationship between predator size and amounts of spadices, seeds, or seedlings consumed. Similarly, Wigand and Churchill (1988) argued for a threshold body size (of between 7 and 9 mm carapace length) in the ability of hermit crabs (*Pagurus longicarpus* Say) to feed on eelgrass (*Z. marina*) seeds in laboratory experiments. We found that all sizes of *Idotea resecata* tested were capable of some degree of consumption of seeds from the spadix, but they did not attack dehisced seeds or seedlings. This may result from a need for *I. resecata* to grasp the spadix in order to break open the seed. The behavior of *I. resecata* appears similar to that observed for juvenile prawns (*Penaeus esculentus* Haswell), who search for and eat mature seeds of *Z. capricorni* Aschers when they are still attached to the plant (Wassenberg 1990).

Our field studies revealed strong seasonal and interannual variation in the abundance of seeds as estimated by captures in seed traps. However, we did not detect a relationship between intensity of predation and abundance of seeds, which might be expected if predators became satiated when seeds were abundant. Reasons for the steady increase in the proportion of seeds eaten during our 4 yr study are unknown. There was no indication that weather conditions were the underlying cause. The observed pattern could result from a temporal decline in the availability of more preferred, alternate food, which increased the tendency of crab predators to feed on surfgrass seeds. This phenomenon has been observed in a laboratory feeding study: Wigand and Churchill (1988) found that several species of crustaceans and molluscs displayed a reduced tendency to consume seagrass seeds when alternate foods (particularly animal tissue such as pieces of clam or scallop) were present. Similarly, McGuinness (1997) argued that spatial variation in consumption rates of seeds of various species of mangroves by red mangrove crabs [*Sesarma* (*Neosarmatium*) *meinerti* de Man] resulted from variation in availability of more highly preferred foods. Thus, it is possible that the increasing rates of seed predation during the course of this study resulted from a per capita decrease in availability of more preferred alternate foods for surfgrass seed predators. This lowered supply could be the result of higher populations of predators, lower amounts of the alternate foods, or both.

Surfgrass is a long-lived clonal plant that reproduces by vegetative spreading as well as through periodic episodes of seedling recruitment. Clearly, the amount of predation loss of seeds of surfgrass is of a magnitude that potentially could contribute to the low numbers of seedlings typically observed in the populations we study. However, even when the amount of seed predation is so great that a majority of seeds that are produced by a population are consumed (Price and Jenkins 1986; McGuinness 1997; Wurm 1998), there is no consensus regarding the potential for an impact on population dynamics or spatial distribution. Some studies have revealed that seed predation can have substantial effects on local populations. For instance, removal of rodent seed-predators greatly affected both the distribution and abundance of grassland species in the Chihuahua Desert (Brown and Heske 1990). In contrast, in some cases even very high rates of losses of seeds to predators did not affect population abundance due to density-dependence in processes operating at other life stages (Borchert and Jain 1978). Thus, for surfgrass, a high rate of seed predation could potentially influence population abundance if it occurred during times when conditions are favorable for seedling recruitment, and if density-dependent losses from other sources did not offset variation arising from differences in losses to predators. Clearly, resolving the importance of seed losses to population dynamics of surfgrass will require much additional information. This includes a fuller understanding of patterns of spatial and temporal variation in the intensity of seed predation as well as their underlying causes, so that the importance of seed predation relative to other processes that affect distribution and abundance can be assessed.

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Survival experiments with outplanted seedlings of surfgrass (*Phyllospadix torreyi*) to enhance establishment on artificial structures

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Holbrook, S. J., Reed, D. C., and Bull, J. S. 2002. Survival experiments with outplanted seedlings of surfgrass (*Phyllospadix torreyi*) to enhance establishment on artificial structures. – ICES Journal of Marine Science, 59: S350–S355.

Surfgrass (*Phyllospadix torreyi*) is considered a valuable plant for enhancing biogenic habitat on artificial structures on the west coast of North America. Its populations establish only slowly, prompting efforts to develop techniques to accelerate the process. We present results from outplanting tests of laboratory-reared seedlings in their natural environment using various techniques. Survival rates after 3 months were compared with those of naturally recruited seedlings. Seedlings require a host plant during their early stages, and both natural hosts (algae) and artificial hosts (nylon strings and nets attached to the bottom) were tested. Survival of naturally recruited seedlings was only 30%, and only in the case where seedlings were hooked into braided nylon netting glued to the substrate were comparable survival rates observed. The results suggest that using this technique may be a promising approach to establishing populations of *P. torreyi* on artificial structures.

Keywords: artificial reefs, enhancement techniques, habitat restoration, *Phyllospadix torreyi*, seagrass, surfgrass.

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Introduction

Certain marine plant species, including kelp and seagrass, are important building blocks of biogenic habitat, and their communities provide food, shelter, and nursery grounds for fish and invertebrates (Phillips, 1979; Dayton, 1985; Turner and Lucas, 1985; Holbrook *et al.*, 1990). Therefore, artificial reef management is often targeted at their establishment. However, the low rates of natural development of these plant populations (often measured in years or decades) has stimulated interest in devising techniques to accelerate the colonization process. The development of strategies for enhancing long-lived marine plant populations is challenging because a good understanding of the life history is required to identify the demographic bottlenecks. Only then can techniques be devised that enable enhanced establishment of populations in desired locations.

Surfgrass (*Phyllospadix torreyi* S. Watson) is an abundant perennial seagrass of rocky intertidal and shallow subtidal zones along exposed shores of the Pacific coast of North America (den Hartog, 1970; Phillips, 1979; Phillips and Menez, 1988). It frequently grows on artificial structures deployed in shallow nearshore environments. Local surfgrass populations are maintained by both vegetative propagation (Tomlinson, 1974; Larkum and den Hartog, 1989) and recruitment of seedlings (Turner, 1983, 1985) into patches of bare space created by small-scale disturbances. Establishment of surfgrass on artificial reefs or in large denuded areas always requires seedling recruitment. This type of colonization entails dispersal of seeds from mature beds, attachment, germination and growth to maturity, a process that may take many years.

Surfgrass is dioecious and both sexes produce spikes of flowers called spadices. The non-fleshy fruit contains one seed (Dudley, 1893) and has two arms with stiff

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bristles. After release from the spadix, the negatively buoyant fruit drifts along the bottom until the arms entangle on a host alga or a conspecific, where the seed germinates. Like other seagrass species, *P. torreyi* can produce large numbers of seeds (up to 10 000 m^{-1} of surfgrass during the annual flowering season; Williams, 1995), but typically very few develop into seedlings (Stewart, 1989; Williams, 1995; Blanchette *et al.*, 1999). One reason is that many seeds succumb during dispersal and never contact a suitable host to which they can attach. Holbrook *et al.* (2000) observed that predators consumed up to 15% of seeds prior to release from the spadix (Holbrook *et al.*, 2000). Seeds fortunate enough to find suitable attachment sites may be subjected to predation rates that exceed 50% (Holbrook *et al.*, 2000). Furthermore, the turbulent physical conditions in these coastal habitats result in losses of seedlings through dislodgement from the host plants or death caused by entanglement with, or abrasion from, whipping branches of the host alga or its neighbours. If host plant and seedling survive, after a period of months the rhizome of the seedling has grown enough to anchor it onto the rocky substrate. Once attached to the substrate, growth of young plants remains slow. Single rhizomes of established plants grew about 1 m in 2 years and development to a small (<1 m diameter) patch required several years (Stewart, 1989).

The low rate of recruitment in natural populations, even when there is adequate seed production, suggests that establishment of *P. torreyi* on artificial reefs might be greatly accelerated by outplanting seedlings. Although most attempts to restore or establish seagrasses have involved the use of older life stages (Fonseca *et al.*, 1982; Williams and Davis, 1996), recent experiments with *Posidonia oceanica* (L.) Delile (Balestri *et al.*, 1999) reveal great promise for the use of seedlings. Recently developed techniques for harvesting seeds of *P. torreyi*, storage in the laboratory, and germination in large numbers (Reed *et al.*, 1998) make it feasible to use seedlings in enhancement efforts. Outplanting seedlings rather than seeds or mature plants has major advantages. First, potential barriers to natural dispersal of seeds from existing beds to a reef (including predation) and to germination would be overcome. Secondly, transplantation of mature plants from natural beds may have unacceptable side effects. However, if suitable natural host algae are not present for seedling attachment on a reef, artificial hosts will have to be developed.

We present results of three experiments in which several techniques for outplanting laboratory-reared seedlings of *P. torreyi* have been tested. Specifically, each experiment tested whether: (1) survival of outplanted and naturally recruited seedlings differed, and (2) survival of outplanted seedlings varied with technique applied. We also discuss strategies for establishing surfgrass populations on artificial reefs and other areas in the coastal zone.

Methods

We measured natural mortality of seedlings to provide a baseline for comparison to outplanting experiments. Mortality of 335 naturally recruited seedlings was estimated at a site located 20 km north of Santa Barbara, California, USA (34°25′N 119°57′W). Cohorts of seedlings were followed in nine fixed 0.64 m^2 circular plots (9–241 seedlings present at the first count) for 3 months. Plots were selected in reef areas covered with *P. torreyi* and a variety of red algae, including *Corallina vancouverensis* L. and *Chondracanthus canaliculatus* (Harv.), which have proved suitable hosts (Blanchette *et al.*, 1999). The first counts were made at the end of the winter of 1996. Each plot was carefully searched on three different occasions and all seedlings were counted.

Experimental seedlings were obtained by culturing seeds collected in the shallow subtidal zone (<4 m depth) near Santa Barbara. Mature spadices were collected and immediately transported to the laboratory where fruits were dehisced by gently running a finger along the length of the spadix. Batches of up to 150 fruits were placed in culture dishes containing 50 ml of filtered seawater (0.2 µm) and 50 µl of nutrient stock solution (Provosoli, 1968). Petri dishes were maintained at 16°C, with an irradiance of 40–50 $\mu E\ m^{-2}\ s^{-1}$ using a 14:10 h L:D photoperiod under mild agitation. The culture medium was changed each week and germination took place within a month. Seedlings were outplanted approximately 1 month after germination when they consisted of a single 5-cm-long shoot attached to the armed endocarp and no rhizome.

All experiments were conducted on shallow (<5 m depth) subtidal reefs. In these habitats, patches of surfgrass grow interspersed with several species of algae that serve as hosts for seedlings (Blanchette *et al.*, 1999). Experiments were started in late winter, the time of year when natural seedlings of surfgrass have just recruited to host algae. This enabled us to compare survival of outplanted and naturally recruited seedlings of a similar age simultaneously.

Three experiments were conducted to test different outplanting techniques (Table 1), each lasting for approximately 3 months. The first experiment tested the role of host type on seedling survival, as well as the effects of abrasion from nearby plants. Three types of hosts were tested: two species of red algae (*Bossiella orbigniana* (Dec) Silva and *Corallina officinalis* var. *chilensis* Dec), and an artificial host consisting of a 20 × 20 cm piece of 1-mm diameter nylon mesh (a fabric diameter similar to that of the algae). Six 30 × 30 cm plots were established in monospecific patches of

Table 1. Mean survival of outplanted seedlings (S, %), difference from naturally recruited seedlings (D, %) and test results (REGWQ: treatments with the same letter are not significantly different from each other at $p=0.05$; Dunnett's t: *significant difference between survival between outplanted and naturally recruited seedlings at $p<0.05$, n.s., not significant, —, not tested because mean and variance 0 were zero) by experiment (ah: artificial host; for treatments and tests, see Methods section).

Treatment	S	D	REGWQ	t-test
Experiment 1 (ah: 1-mm netting compared to natural substrate)				
Bossiella, unclipped	0	− 30	—	—
Bossiella, clipped	3.3	− 26	B	n.s.
Bossiella, ah	13.3	− 16	B	n.s.
Corallina, unclipped	0	− 30	—	—
Corallina, clipped	1.7	− 28	B	n.s.
Corallina, ah	53.3	24	A	n.s.
Experiment 2 (ah: braided strings)				
H-density, unclipped	9.6	− 20	A	*
H-density, clipped	5.3	− 24	A	*
L-density, unclipped	2.0	− 28	A	*
L-density, clipped	1.5	− 28	A	*
Experiment 3 (ah: braided netting)				
H-density, *Corallina*	17.6	− 12	A	n.s.
M-density, *Corallina*	30.0	1	A	n.s.
L-density, *Corallina*	25.0	− 5	A	n.s.
H-density, rock	15.7	− 14	A	n.s.
M-density, rock	18.8	− 11	A	n.s.
L-density, rock	0	− 30	—	—

Bossiella and *Corallina*, respectively, in the winter of 1998. To test for effects of abrasion on seedling survivorship, the branches of all algae in half of the plots were clipped to within 1 cm of the holdfast. The remaining plots were unaltered ("unclipped"). SCUBA divers hooked the bristled arms of seedlings onto branches of clipped and unclipped holdfasts of *Bossiella* and *Corallina* using fine forceps in a manner resembling natural attachment of seeds (40 seedlings per plot, three treatment replicates). Artificial hosts were placed near unclipped plots of *Bossiella* and *Corallina* (three replicates for each species). Divers attached seedlings to the nylon mesh (ten seedlings per replicate), and then used underwater epoxy to fasten the artificial host to the substrate.

In the second and third experiments, only artificial hosts were used. Seedlings were attached in the laboratory and the seeded hosts were then outplanted to the field during the winter of 1999. This method was explored because attaching individual seedlings to natural hosts had proved difficult and time-consuming. Two types of artificial hosts were tested: braided nylon string and braided nylon netting. In both cases, an opening was made in the braid by untwisting it one half turn. One of the arms of the germinated seed was inserted into the opening and hooked onto one of the braids. The opening closed upon relaxation locking the seedling into place. Using this technique, many seedlings could be securely and rapidly fastened to the strings and nets. Seeded strings and nets were kept in seawater tables before transportation to the study site in coolers.

The second experiment used nylon strings as hosts to explore effects of seedling density and abrasion from nearby plants. Strings were stretched across 0.5-m square racks made of PVC tubing that were bolted to the ocean bottom using marine epoxy. In high-density and low-density treatments, ten and four equally spaced strings were fastened to a rack, respectively (eight seedlings per string in both treatments). Four racks of each density were placed in dense patches of both clipped and unclipped *Corallina*.

In the third experiment, nylon netting as artificial host was used to test for effects of seedling density and clipped algae versus bare rock. Seedlings were outplanted at three densities (5, 20, or 105 seedlings per net of 20 × 20 cm) in clipped patches of *Corallina* and in areas scraped to bare rock (four replicates for each treatment). The netting was glued to the bottom.

To evaluate potential effects of handling on seedling survivorship, we compared the survivorship of seedlings that had and had not been attached to artificial hosts. At the time of the second and third experiments (winter 1999), control groups of 20 seedlings each were handled in the same ways as outplanted seedlings and then returned to laboratory culture. Six groups consisted of seedlings that were attached to strings and six groups consisted of loose seedlings. Each group of 20 seedlings was placed in a separate culture dish. These were

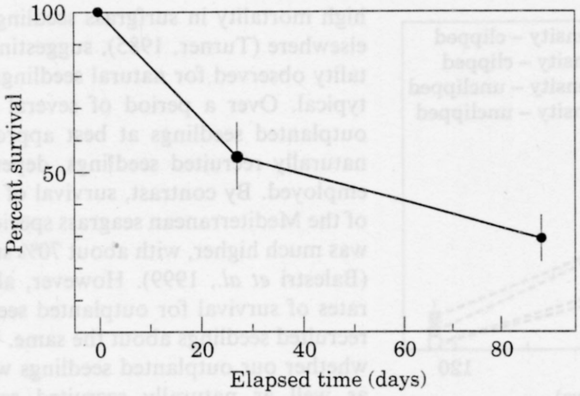

Figure 1. Mean survival (% ± s.e.; n=9) of cohorts of naturally recruited surfgrass seedlings at consecutive sampling dates.

maintained in the laboratory and survivorship was measured over 69 days.

For each experiment, a one-way ANOVA followed by a Dunnett's t-test was used to evaluate whether seedling survival in each treatment differed significantly from survival of naturally recruited seedlings. Differences in survivorship among treatments within an experiment were evaluated using a one-way ANOVA with Ryan–Einot–Gabriel–Welsch Multiple Range Test (REGWQ) for *post-hoc* comparisons of means. The statistical assumption of equal variances was met by transforming all data to arcsin square root. Treatments having a mean survivorship (and variance) equal to zero were excluded from statistical analyses because their inclusion led to violations in this assumption.

Results

Naturally recruited seedlings experienced substantial mortality. About half of the 335 seedlings died within 3 weeks of the initial survey, while 30% survived until the end of the 3-month study (Figure 1).

Seedlings reared in the laboratory for comparison to outplanted seedlings survived very well. Mean survival of six groups of seedlings attached to strings was virtually the same as survival of loose seedlings (in both cases, mean=90%; s.e.=5) after 69 days. These results indicate that outplanted seedlings were viable, and that handling mortality must have been low.

Approximately half of the seedlings survived after 7 days in clipped plots of both *Bossiella* and *Corallina* when seedlings were attached by hand onto the host algae (Figure 2). Nonetheless, only a small percentage survived to 3 months. In contrast, even short-term survival was close to zero in all unclipped plots and none survived 50 days. Careful searches of the host algae revealed that entire seedlings had disappeared, suggesting that the method of attaching seedlings to host algae with forceps resulted in high rates of dislodgement. Survival was higher in seedlings attached to nylon mesh, possibly because they were not as easily dislodged. Survival differed significantly among treatments ($F_{4,16}=4.0$, $p<0.02$), but none differed significantly from survival of the naturally recruited cohort (Table 1). *Post-hoc* comparison of treatment means revealed that seedlings on nylon mesh outplanted to *Corallina* patches experienced significantly lower mortality than those outplanted to *Bossiella* patches or than seedlings attached to clipped algae.

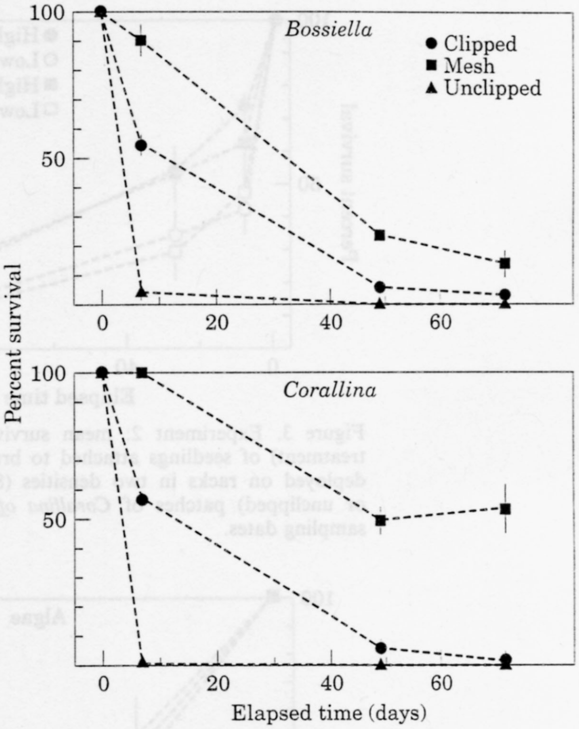

Figure 2. Experiment 1: mean survival (% ± s.e.; n=3 per treatment) of seedlings attached to holdfasts of two natural host substrates (clipped or unclipped) or to a 1-mm nylon netting deployed in these communities (top: *Bossiella orbigniana*; bottom *Corallina officinalis*) at consecutive sampling dates.

In the second experiment, percentage survival steadily declined over time in all treatments, with less than 10% remaining alive after 4 months (Figure 3). Seedlings were often abraded and broken, possibly caused by string movements during periods of high turbulence. Survival was similar among all treatments ($F_{3,20}=1.8$, $p<0.18$) and survival in each treatment was significantly lower than in the natural cohort (Table 1; $F_{4,28}=6.0$, $p<0.001$).

Survival was higher in the third experiment, when seedlings were attached to braided nylon netting (Figure 4). This method apparently minimized movements and prevented dislodgement. Survival in all treatments was similar to survival of naturally recruited seedlings

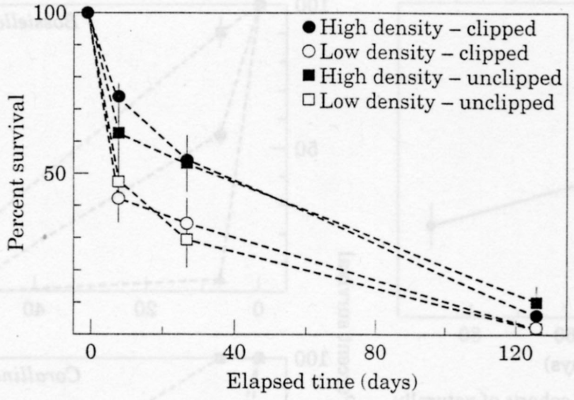

Figure 3. Experiment 2: mean survival (% ± s.e.; n=6 per treatment) of seedlings attached to braided nylon string and deployed on racks in two densities (80 and 32) in (clipped or unclipped) patches of *Corallina officinalis* at consecutive sampling dates.

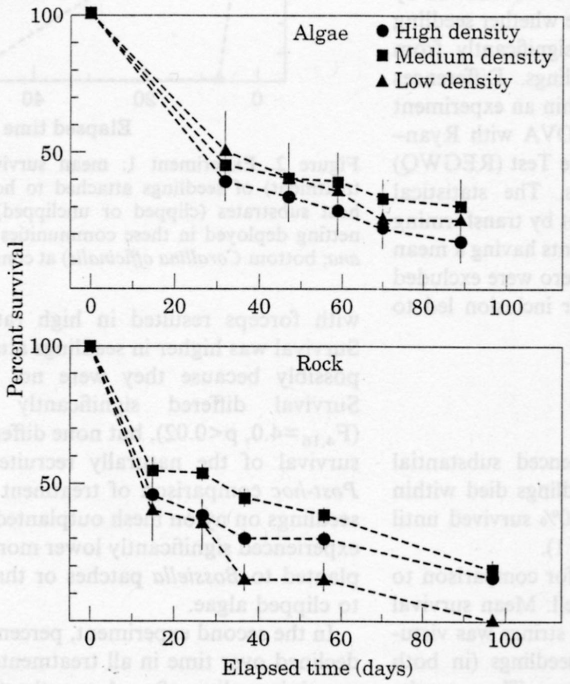

Figure 4. Experiment 3: mean survival (% ± s.e.; n=4 per treatment) of seedlings attached in three densities (105, 20, and 5) to braided nylon netting glued on the bottom into patches of clipped *Corallina officinalis* (top) or onto bare rock (bottom) at consecutive sampling dates.

(Table 1; $F_{5,23}=0.7$, $p>0.6$), and to each other ($F_{4,15}=0.8$, $p>0.5$).

Discussion

Seedlings of surfgrass naturally suffer high mortality and only 30% survival was observed after 88 days. Similar high mortality in surfgrass seedlings has been observed elsewhere (Turner, 1985), suggesting that the high mortality observed for natural seedlings during our study is typical. Over a period of several months, survival of outplanted seedlings at best approximated survival of naturally recruited seedlings, depending on techniques employed. By contrast, survival of outplanted seedlings of the Mediterranean seagrass species *Posidonia oceanica* was much higher, with about 70% surviving after 3 years (Balestri *et al.*, 1999). However, also in this case were rates of survival for outplanted seedlings and naturally recruited seedlings about the same. We still do not know. whether our outplanted seedlings will grow and survive as well as naturally recruited seedlings over longer periods. During the relatively brief experiments, rhizomes had not yet grown enough to attach the plants to the substrate, and it will be important to evaluate this aspect of establishment in future experiments.

The causes of seedling mortality seemed to vary among the different techniques applied. Dislodgement appeared to be the primary cause of mortality when forceps were used to attach seedlings to natural algal hosts because entire seedlings disappeared. In experiments where seedlings were attached to nylon lines or nets the seed generally remained, while the shoot often was lost. This could have been due to grazing, death of the shoot, or mechanical damage caused by moving branches of algae. Despite the high losses of seedlings, we predict that mortality rates would be even higher if ungerminated seeds were outplanted, because seed predation may result in even higher mortality. Losses of outplanted *Zostera marina* seeds during 1-week field experiments ranged up to 96%, crab predators being the major source of mortality (Fishman and Orth, 1996). Several species of invertebrates, particularly the crabs *Pugettia producta* and *Pachygrapsis crassipes*, are voracious predators on surfgrass seeds, and at some sites nearly all ungerminated seeds released were damaged or consumed by predators (Holbrook *et al.*, 2000). In comparison, predation on seeds while they are developing in the spadix is relatively low (Holbrook *et al.*, 2000). Thus, collecting spadices to obtain seeds for laboratory culture bypasses a life stage where mortality risk can be large.

Our results suggest that outplanting seedlings may be a promising approach to enhancing populations of *P. torreyi* on artificial reefs. Artificial hosts may be used to approximate short-term survival of naturally recruiting seedlings if appropriate techniques are selected. However, because of the inherently low survivorship of surfgrass seedlings, it may prove more difficult to enhance establishment of surfgrass populations compared to other seagrass species. Longer test projects are required to assess whether rhizome attachment and growth rates of outplanted seedlings could match those of natural seedlings.

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An Experimental Evaluation of Different Methods of Restoring *Phyllospadix torreyi* (Surfgrass)

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ABSTRACT

The surfgrass *Phyllospadix torreyi* is an abundant seagrass found on rocky exposed shores of the Pacific coast of North America. In southern California, surfgrass populations are adversely affected by a range of natural events and anthropogenic activities. Few attempts have been made to develop restoration methods for surfgrass, and none have investigated the efficacy of using different life stages. We evaluated several techniques for restoration in intertidal and subtidal habitats using: 1) laboratory-reared seedlings transplanted to the field, 2) sprigs (short lengths of rhizome containing a few shoots) transplanted from undisturbed populations, and 3) plugs (a cohesive clump of shoots and rhizomes) transplanted from undisturbed populations. We calculated net change in the aerial coverage of surfgrass after six months, taking into account the recovery or additional losses from the donor population, and amount of effort involved in transplanting. Transplanted seedlings survived poorly and had minimal rhizome growth at both the intertidal and subtidal sites, yet the individuals that did survive showed a 275 % increase in leaf number. Survivorship of transplanted plugs was high in both habitats; however, physical disturbances to the donor populations exacerbated damage sustained at the time of collecting, yielding a substantial net loss in surfgrass. Sprigs transplanted to the subtidal had higher survivorship (71 % vs. 48 %) and a greater increase in the aerial coverage of rhizome (86 % vs. 42 %) than those transplanted to the intertidal. Of the three techniques, transplanted sprigs had the greatest overall increase in aerial coverage per unit effort, suggesting that this method may be the most effective approach for restoring *P. torreyi.*

Key Words: cost-effectiveness, *Phyllospadix torreyi*, plugs, restoration, rhizome growth, seagrass, seedlings, sprigs, surfgrass, survivorship, transplant

INTRODUCTION

Increased urbanization of coastal areas has led to the degradation of many seagrass populations due to increases in nutrient loading (Short & Burdick 1996; Short & Wyllie-Echeverria 1996), polluted waste from sewage and industrial discharges (Santour & Castel 1995; Morand & Briand 1996; Clark 1997; Schiewer 1998), coastal development and offshore construction (Cambridge & McComb 1984; McLusky et al. 1992; Sheridan et al. 1998), and recreational and commercial boating and fishing (Zieman 1976; Walker et al. 1989; Dawes et al. 1997). The high ecological importance of seagrasses (Harlin 1975; Stewart & Myers 1980; Fonseca et al. 1990), coupled with their susceptibility to damage from a range of human activities and natural disturbances, have sparked much interest in understanding their ecology (Duarte 1999) and developing methods for restoring damaged populations (Thorhaug 1986; Larkum et al. 1989; Edgar 1990; Williams & Davis 1996; Orth et al. 1999; Paling et al. 2001a, 2001b; Short et al. 2002).

Previous attempts to restore seagrass populations often have relied on transplanting older life stages (Thorhaug 1986; Thom 1990; Davis & Short 1997; Orth et al. 1999; Paling et al. 2001a, b). In many cases, transplanting techniques have been successful in promoting the establishment of new plants (Fuss & Kelly 1969; Kelly et al. 1971; Phillips 1974; Fonseca et al. 1994; Balestri et al. 1998). Although the cost-effectiveness of different planting techniques has been examined in some cases (e.g., Thorhaug 1986), there have been few attempts to measure whether damage to donor populations from which transplants are collected causes long-term losses to otherwise healthy populations. The potential for such damage has prompted studies to explore the use of seeds and cultivated seedlings in seagrass restoration (Balestri et al. 1998; Harwell & Orth 1999; Holbrook et al. 2002), but relatively little research on this has been done to date (Orth et al. 2000).

In the United States, seagrass restoration efforts have focused on species that occupy shallow, soft-bottom habitats such as *Halodule wrightii* and *Zostera marina* (Thom 1990). Surfgrasses such as *Phyllospadix torreyi* pose special challenges for restoration because the transplanting techniques that have been developed for other seagrasses are not applicable. Unlike most seagrasses, surfgrasses grow on exposed rocky coasts. Adventitious roots from the rhizome secure the plant to hard substrata (den Hartog 1970; Phillips 1979; Cooper & McRoy 1988). Thus, successful transplantation depends not only on growth following relocation, but also on secure attachment that enables transplants to avoid dislodgement by breaking waves. Populations of *P. torreyi* typically extend from the low intertidal into the subtidal, and because wave forces vary greatly along this depth gradient, different restoration methods might be required at different depths.

P. torreyi produces copious amounts of seeds, whose germination is easily controlled in laboratory culture (Williams 1995; Reed et al. 1998). This feature allows it to be propagated from laboratory-raised seedlings, as well as from sprigs or plugs taken from natural populations. Such flexibility could be of significant value in restoration if the relative performance of the different life forms varied along the depth gradient. The inability to restore populations of surfgrass motivated us to develop restoration techniques for this species. Here we investigated the efficacy of using seedlings, sprigs and plugs for restoration in the intertidal and subtidal. Specifically, we evaluated the three life forms with respect to: 1) increase in aerial coverage and survivorship of transplants, (2) effort involved in collecting, preparing and transplanting, and (3) residual damage to donor populations.

METHODS

Three potential restoration methods for *P. torreyi* to intertidal and shallow subtidal reefs near Santa Barbara, CA were compared: (1) seedlings cultivated in the laboratory and transplanted to the field, (2) sprigs (short lengths of rhizome containing a few shoots) collected from and transplanted to the study areas, and (3) plugs (cohesive clumps of rhizomes and shoots) collected from and transplanted to the study areas. Intertidal studies were conducted during low tide at More Mesa Reef ($34^0$25’N, $119^0$57’W), a broad, gently sloping reef fractionated by sand channels. Patches of the red algae *Corallina vancouveriensis, Gelidium purpurascens*, and *Chondracanthus canaliculatus* [which serve as attachment sites for surfgrass seeds (Blanchette et al. 1999)] were common in the study area. *P. torreyi* formed dense beds on most of the emergent reef in the low intertidal zone to the shallow subtidal zone at More Mesa Reef. Seedlings, sprigs and plugs were transplanted into cleared areas on the reef adjacent to *P. torreyi*. The sand channels at More Mesa broaden with depth, resulting in little rocky habitat for surfgrass in the subtidal zone. Consequently, subtidal studies were done using SCUBA at nearby Mohawk Reef (34^0 24’ N, $119^0$44’ W), where *P. torreyi* forms large, patchy beds on a broad, gently sloping reef interspersed with stands of coralline algae consisting primarily of *Bossiella orbigiana* and *Corallina officinalis*. Seedlings, sprigs and plugs were transplanted to depths of 2.5 to 4 m below MLLW in plots cleared of coralline algae and other biota that were located next to established surfgrass beds.

Transplant experiments were initiated in the late summer and fall, the time of year when most surfgrass seeds are released and germinate in southern California (Williams 1995, Holbrook & Reed unpublished data). Each method of restoration was evaluated using data collected on survivorship and growth of the transplants after six months, recovery of the donor population from which transplants were collected, and the amount of effort (i.e. time) involved in transplanting. Transplants were considered dead if they had no leaves or were no longer present on the reef. Growth of surviving transplants was estimated as the change in the aerial coverage of rhizome over the six-month experiment as determined by the linear dimensions of the basal area occupied by the rhizomes of each transplant. The percentage change in the number of leaves after six months ([(final number of leaves – initial number of leaves) / initial number of leaves] * 100) was used to assess the condition of surviving transplants. We measured leaf number as opposed to the more traditional metric of shoot number because it was difficult to accurately count shoots in the wave swept surf zone where shifting sand frequently buried shoots above the sheath. However, the number of leaves per shoot at our sites was very consistent (mean 2.99 $\pm$ 0.05 SE, N = 210 shoots), and thus it can be used to estimate the number of shoots.

Seedlings

Seedlings were germinated from seeds obtained from reproductive shoots of plants collected in September 2000 at the two study sites and other nearby intertidal and subtidal locations. Reproductive shoots were cut several centimeters above the rhizome, transported to the laboratory, and placed in a flow through seawater tank until fruits dehisced (about 1 month). Dehisced fruits were placed in culture as per the methods of Reed et al. (1998), and the single seed within the fruits germinated within one month of fruit dehiscence. At the time of transplanting (approximately one month post-germination) seedlings lacked rhizomes and consisted of a single leaf approximately two cm in length that was attached to the endocarp of the fruit.

Each seedling was attached to a 7 cm long piece of braided nylon line (1 mm diameter) by inserting one of the arms of the fruit into an opening in the line made by untwisting the braids one-half turn (Figure 1a). Tiny bristles along the inside arm of the fruit hooked onto one of the braids, locking the seedling in place. Attached, seedlings were placed in plastic bags with seawater and transported to the field in insulated containers.

Seedlings attached to nylon line were transplanted to experimental plots 30 cm x 30 cm in area at the intertidal and subtidal study sites in November 2000 by fastening the ends of each nylon line directly to the reef using Z-Spar A788 marine epoxy putty. Eighteen seedlings were transplanted in a uniform grid to each of 12 plots at each of the two sites and their survivorship was monitored periodically during the six month experiment. All experimental plots were located adjacent to healthy stands of surfgrass and were cleared of algae and sand prior to transplanting to facilitate attachment with epoxy. Branches of coralline algae growing adjacent to the plots were trimmed to reduce abrasion and dislodgement of the newly transplanted seedlings.

Sprigs

Sprigs were harvested at the intertidal and subtidal sites in November 2000, and each was immediately transplanted just outside (within 2 m) the surfgrass bed to a 15 cm x 15 cm plot that had been cleared of other biota. To harvest sprigs, an unbranched terminal end of an actively growing rhizome was carefully removed from the perimeter of a bed with a knife. The rhizome of each sprig was five cm in length and contained several lateral shoots and a terminal shoot. The mean number of leaves per sprig was 11.9 (± 0.9 SE) for subtidal transplants and 26.6 (± 1.5 SE) for intertidal transplants. Sprigs were transplanted to the cleared areas by attaching the cut end of the rhizome to the reef using marine epoxy (Figure 1b). At least 1.5 cm of the actively growing terminal end of the rhizome of each sprig was exposed and not in contact with the epoxy. Leaves of transplanted sprigs were trimmed to 20 cm in length prior to attachment to minimize drag and reduce the chance of dislodgement while the epoxy hardened. Pilot studies determined that reducing drag by trimming leaves was a necessary condition for effective attachment of transplants. Forty-two sprigs were transplanted to an equal number of plots at each of the two sites.

The collection of sprigs resulted in a small loss in surfgrass from the donor bed. To measure recovery from this loss, a reference marker was glued to the reef next to the cut end of each donor rhizome (i.e., a rhizome from which a sprig was harvested). Recovery was estimated as the aerial coverage of new rhizome that grew from the cut end of the donor rhizome. We were not able to relocate all of the donor rhizomes at the end of the study; six and nine donor rhizomes were used to calculate recovery from harvesting sprigs from the subtidal and intertidal sites, respectively.

Plugs

The efficacy of using clumps of mature surfgrass was evaluated at the intertidal and subtidal sites beginning in August 1999. Square plugs of intertwined rhizomes and shoots were harvested from the middle of a bed of *P. torreyi* using a wide bladed putty knife and transplanted outside (within 2 m) of the surfgrass bed to plots that had been cleared of other biota. Clearings were made larger than the plugs in order to provide a 5 cm wide buffer from surrounding biota. Plugs were attached by pulling the leaves through a square piece of 2.5 cm diameter stretch mesh nylon net that was cut to a size that was slightly larger than the square plug (Figure 1c). The net was pulled tight over the plug and secured to the reef at the edges with marine epoxy. As done for sprigs, the leaves were trimmed to 20 cm in length to minimize drag. Three different sizes of plugs (small, 5 cm x 5 cm; medium, 10 cm x 10 cm; and large, 20 cm x 20 cm) were transplanted to test whether plug size influenced: (1) survivorship and growth (i.e. increase in aerial coverage) of the transplanted plug, and (2) rate of recovery of the donor area from which the plug was collected. Six plugs of each size were transplanted to separate plots in each of the two sites.

The collection of plugs resulted in the immediate loss of surfgrass in the donor beds equal to the aerial coverage of the plugs. To estimate the recovery of bare patches in the donor bed created by harvesting plugs, we marked each donor patch at the time of harvesting and made periodic measurements to assess the extent of in-growth from the edges of the bare patches by neighboring rhizomes. The recovery of donor patches was determined at the end of the six-month experiment by calculating the area of bare space remaining in each donor patch from linear measurements taken with a tape measure.

Efficacy of different methods of restoration

We estimated the amount of effort (E) per unit transplant spent collecting, preparing and transplanting seedlings, sprigs and plugs for both intertidal and subtidal habitats. The effort (i.e., person hours) involved in the collection of seedlings was based on the time spent collecting reproductive shoots in the field, gathering and cleaning fruits dehisced in seawater aquaria, germinating seeds, and cultivating seedlings in laboratory cultures. Effort in preparing seedlings for transplanting consisted of the time spent in the laboratory attaching seedlings to nylon lines, while the effort in transplanting seedlings was based on the time spent attaching them to the reef (this included clearing the transplant plots on the reef of algae and debris). Estimates of effort for sprigs and plugs were based on the time spent collecting in the field, trimming leaves in preparation for transplantation, and transplanting units to adjacent areas on the reef. In the case of plugs, additional preparation time was spent cutting the netting used to secure the plugs and pulling the leaves of the plugs through it. The transport of transplant units, the set-up of laboratory cultures, and the mobilization and demobilization of gear and assistants are factors that are likely to vary greatly among different restoration projects depending on the proximity of the laboratory to the restoration site, and the size of the area to be restored. Consequently we excluded these factors in our estimates of effort.

The efficacy of the three different methods was evaluated by calculating the mean net change in the aerial coverage of rhizome of a transplant unit per unit effort measured in person hours. The mean net change in aerial coverage of rhizome (A) was calculated as:

$$A = TS - (D_{initial} - D_{final}),$$

where T is the mean aerial coverage of rhizome (cm^2) of the transplant unit six months after transplantation, S is the proportion of transplant units surviving six months, $D_{initial}$ is the aerial coverage of rhizome (cm^2) collected from the donor patch, and D_{final} is the mean net change in aerial coverage of rhizome (cm^2) in the donor patch six months later.

The mean net change in the aerial coverage of rhizome of a transplant unit per work hour (CPWH) was calculated as:

$$CPWH = A / E.$$

Analyses

A t-test using Satterthwaite's approximation for the degrees of freedom (SAS 2001) was used to test differences in survivorship between seedlings transplanted to the intertidal vs. the subtidal. Survivorship was estimated from twelve plots each of which contained eighteen seedlings. Two by two contingency tables were used to evaluate habitat-specific differences in survivorship for sprigs and plugs. A two-way fixed factor ANOVA was used to test for the effects of site (intertidal vs. subtidal) and restoration method (seedling, sprig, and small, medium and large plugs) on the growth of surviving transplants. Differences in percent change in leaf number among treatment means were evaluated using Ryan-Einot-Gabriel-Welch multiple range test (SAS 2001).

The hypothesis that the aerial coverage of rhizome of transplanted sprigs and plugs changed significantly during the six months was tested using one-sample, two-tailed, t-tests, where the population mean μ was the aerial coverage of rhizome at the beginning of the experiment. Similar analyses were done on the donor plots from which sprigs and plugs were harvested to assess whether any significant recovery of the donor plots had occurred. Analyses testing the significance of changes in the aerial coverage of rhizomes in transplanted seedlings were not done because all seedlings lacked rhizomes at the start of the experiment and had zero variance in aerial coverage of rhizome at the end of the experiment (the final aerial coverage of rhizome of seedlings was uniformly small and at the lower limit of our measurement capabilities).

RESULTS

Survivorship and change in leaf number

Substantial mortality of seedlings occurred within the first few days of transplanting at both the intertidal and subtidal sites, and few of the 432 seedlings survived six months (Figure 2). Survivorship was equally poor at both the intertidal and subtidal sites ($t_{16} = 0.98$, $P = 0.341$). Survivorship of sprigs and plugs was substantially greater than that of seedlings (Table 1), with sprigs surviving significantly better in the subtidal than in the intertidal (71.4 % vs. 47.6 %; $\chi^2_{1\ DF} = 11.81$, $P = <0.001$). No sprigs died as a result of becoming dislodged. Instead, evidence of death was based on necrosis and loss of leaves. Plugs had the greatest survivorship, as nearly all were alive after six months, except large plugs transplanted to the intertidal where only one of six survived. The five large plugs that died became dislodged and disappeared within days after transplanting.

Production of new leaves by transplant units that survived for six-months was affected by both site and the method of restoration. Seedlings, sprigs, and plugs transplanted to the subtidal produced more than three times as many new leaves as those transplanted to the intertidal (Figure 3a, $F_{1,79} = 12.93$, $P <0.001$). Differences in leaf production among methods were equally large (Figure 3b, $F_{4,79} = 12.93$, $P <0.001$). The few seedlings that survived grew well and showed nearly a 300 % increase in the number of leaves. Substantial leaf production also occurred in transplanted sprigs and small plugs, but was somewhat less than that of seedlings. In contrast, medium and large sized plugs grew poorly and actually lost leaves over the course of the experiment. Differences in leaf production among the different restoration methods were generally similar at the intertidal and subtidal sites ($F_{4,79} = 1.47$. $P = 0.221$ for the site x method interaction).

Rhizome aerial coverage and donor recovery

Changes in the aerial coverage of rhizome material varied substantially among transplanted seedlings, sprigs and plugs (Table 2). Rhizomes of surviving sprigs consistently grew the most. The mean aerial coverage of the rhizomes of sprigs transplanted to the intertidal increased by 42% while that transplanted to the subtidal increased by 86%. Rhizome growth of the different-sized plugs varied inconsistently between the intertidal and subtidal sites. For example, significant increases in rhizome coverage were observed in small plugs transplanted to the intertidal, but not in small plugs transplanted to the subtidal (Table 2). Conversely, rhizome coverage increased significantly in medium plugs transplanted to the subtidal, but not in medium plugs transplanted to the intertidal. There was no significant change in the mean rhizome coverage of large plugs transplanted to the subtidal, whereas the area covered by rhizomes of the lone surviving large plug in the intertidal increased slightly (i.e. 9%). Rhizomes were typically poorly developed in six-month old seedlings. Nonetheless, the few seedlings that survived in the intertidal and subtidal had developed small rhizomes by the end of the experiment.

Striking differences were observed in the recovery of donor plots from which sprigs and plugs were harvested (Table 3). In the case of sprigs, full recovery was observed at both the intertidal and subtidal sites as the re-growth of cut rhizomes more than compensated for the loss incurred from harvesting. In contrast, harvesting plugs led to significant additional losses of surfgrass in areas adjacent to donor plots. These losses were observed within a few weeks of harvesting, and appeared to result from water motion that undermined the edges of cut rhizomous mats that were exposed when the plugs were removed. These subsequent losses were substantial, ranging from 67% to 533% of the initial donor area, and they were generally larger in the intertidal than in the subtidal. Large plugs taken from the intertidal had the largest mean net loss from harvesting. However, because of high variability among replicate donor plots, these were the only type of plug whose subsequent losses of surfgrass were not statistically significant (Table 3).

Efficacy of different methods of restoration

Overall, restoration using seedlings and sprigs required less effort than restoration with plugs (Figure 4). Seedlings, which were reared in the laboratory and attached to strings prior to transplanting, required more effort for transplant preparation than for collecting or transplanting. In contrast, relatively little effort was allocated to transplant preparation for sprigs and plugs; most of the effort for sprigs and plugs involved collecting and transplanting. In general, less effort was needed to collect and transplant sprigs and plugs in the subtidal than in the intertidal. This occurred because material for transplanting was more readily available in the subtidal resulting in shorter collection times. Moreover, transplanting sprigs and plugs required more effort in the intertidal because of the increased difficulty of clearing sand and debris there.

The change in area of surfgrass habitat per unit effort varied tremendously among the methods tested (Table 4). The use of sprigs proved to be the most effective method for restoring surfgrass to both the intertidal and subtidal sites. The mean change in aerial coverage of rhizome per work hour (CPWH) for sprigs in the subtidal was more than five times that in the intertidal. This was a result of greater increases in net rhizome coverage and smaller effort involved in transplanting at the subtidal site. The small values of CPWH observed for seedlings reflected the high mortality and small gains in rhizome coverage observed over a six-month period. Interestingly, CPWH for all three sizes of plugs was negative for both the intertidal and subtidal sites. The negative values for plugs resulted from the subsequent losses of surfgrass to donor populations as a result of harvesting. Even if these subsequent losses were discounted, CPWH for medium and large plugs would be low due to the limited (and in some cases negative) growth of transplanted plugs, coupled with the relatively large amount of effort required for transplant.

DISCUSSION

Techniques permitting large-scale restoration efforts have been developed and tested for a few seagrass species that grow rapidly and occupy sheltered habitats, (e.g., Fonseca et al. 1998; Orth et al. 1999; Short et al. 2002). For example, restoration programs for *Zostera marina* now can include such elements as economic and ecological analyses to determine the scale of the restoration (Fonseca et al. 2000), use of site selection models to identify optimal areas for transplant (Short et al. 2002), and employment of a range of transplant techniques involving various life stages (adult plants, seeds, or seedlings; Fonseca et al. 1998, Orth et al. 1999). However, for many seagrasses, much less progress has been made. For example, in Western Australia, seagrasses (*Amphibolis* sp. and *Posidonia* sp.) normally live in areas of high water motion, and restoration attempts in these habitats consistently failed until recently, when underwater harvesting and planting machines that permit extraction and planting of seagrass plugs that are sufficiently large to withstand storm damage were developed (Paling et al. 2001a, 2001b). Surfgrass (*P. torreyi*), which occupies exposed rocky coasts of western North America, is another example of a species for which restoration strategies have been lacking. Due to the difficulty of anchoring transplant units to the rocky substrata in turbulent water, few attempts have been made to explore transplanting techniques, and the lack of developed techniques has prevented efforts of large-scale restoration.

Although the majority of attempts to restore seagrasses have involved the use of older life stages (reviewed in Thorhaug 1986; Thom 1990), concerns about donor bed recovery, coupled with the fact that harvesting large numbers of early life stages (such as seeds) would have relatively little impact on donor populations, have led to increased interest in the use of early life stages for restoration (Balestri et al. 1998; Orth et al. 2000; Holbrook et al. 2002). Like many other seagrass species, *P. torreyi* tends to produce large numbers of seeds (up to 10,000 per square meter of surfgrass bed during the annual flowering season; Williams 1995). Typically, few seeds develop into seedlings (Stewart 1989; Williams 1995; Blanchette et al. 1999, Holbrook et al. 2000), and early survival of seedlings is poor (Blanchette et al. 1999; Holbrook et al. 2000). Many seeds are consumed by predators, either prior to their release from the spadix or afterwards (Holbrook et al. 2000). Turbulent conditions in the surf zone frequently cause surfgrass seedlings to become dislodged from their host plants or suffer abrasion from nearby vegetation (Blanchette et al. 1999). Burial by sand and desiccation in the intertidal zone are additional causes of mortality of young seedlings (Bull 2002). Recently-developed techniques for harvesting seeds of *P. torreyi*, and for laboratory storage, germination and propagation (Reed et al. 1998), make it possible to consider using seedlings of this species for restoration.

Results from this study raise some concern as to whether the survival bottlenecks in the early life history stages can be overcome as part of the restoration strategy. Our use of nylon line as an attachment host attempted to minimize dislodgement of seedlings. Dislodgement of the strings during the first few days after transplanting accounted for approximately 20% and 14% of the total mortality of seedlings transplanted to the intertidal and subtidal sites, respectively (Bull 2002). Strings that remained glued to the reef appeared to be quite adept at retaining seedlings as most remained attached to the strings even after their shoots were gone. The vast majority of mortality resulted from factors that caused seedlings to die in place. The specific causes of seedling mortality during our study remain unknown. Afternoon low tides undoubtedly create harsh conditions for seedlings in the intertidal zone and desiccation and high temperatures likely contribute to the mortality of seedlings in this habitat. Such factors are not important in the subtidal, yet survivorship was equally poor in this habitat. Small herbivorous crustaceans, mollusks and fish are commonly abundant in intertidal and subtidal habitats where surfgrass is found and these grazers are very capable of consuming the small delicate leaves of surfgrass seedlings (Holbrook et al. 2000). Despite the poor survivorship of seedlings, those that did survive grew extremely well, increasing their leaf numbers by nearly four fold in six months. This suggests that there is promise in using early life stages of surfgrass for restoration, especially when the small impacts on the beds caused by collecting seeds are taken into account. Additional research is warranted to develop methods to reduce losses of transplanted seedlings.

Harvesting plant material from natural, healthy beds can be a large concern for resource managers charged with restoring damaged seagrass populations, especially in the case of slow-growing species such as *Thalassia testudinum* and *P. torreyi.* Our results indicate that such concerns are very well justified for *P. torreyi.* Although survival of plugs was generally high, they grew poorly, and more importantly, harvesting plugs resulted in additional subsequent losses to the donor population. The cut rhizomes at the edges of the holes became eroded by waves and were easily removed from the rocky substrate. Consequently, the holes created by collecting plugs became significantly larger during the six months following harvesting. This was most pronounced at the intertidal site where wave forces are expected to be greater. Recovery via in-growth from neighboring rhizomes was slow in donor plots and after 18 months, surfgrass had regrown to occupy only about 55 % of area of the harvested plots (Bull 2002). For these reasons, it may be unwise to collect plugs of material from surfgrass beds for use in restoration. By contrast, sprigs survived and grew reasonably well, and re-growth of rhizomes that were cut to obtain sprigs for transplanting was rapid. The patterns of growth and survival of transplants and of recovery of donor plots, combined with the amount of effort involved, revealed that the largest gain in rhizome coverage per unit of effort occurred when sprigs were used. Moreover, sprigs suitable for transplanting required relatively little effort to prepare and were abundant at the study sites (Bull 2002), suggesting that collection of sprigs for transplanting would not have a large impact to existing *P. torreyi* beds. Collectively these features rendered sprigs the most acceptable form for use in restoration.

P. torreyi occurs along a depth gradient, from the intertidal to the shallow subtidal zone. As such, it encounters a wide range of physical conditions, and knowledge of the tolerance of the species to factors such as light, sedimentation, temperature, and wave generated water motion provides insight into possible restoration strategies. Not surprisingly, the efficacy of the three restoration methods that we tried varied considerably between the intertidal and subtidal sites. In general, survivorship and growth of transplants and the recovery of donor plots all tended to be higher in the subtidal than in the intertidal, and the effort expended in transplanting was also less in the subtidal. That all three methods of restoration varied consistently in this regard suggests that while restoration success in surfgrass is likely to vary along a depth gradient, it may not require different techniques be used in different depths.

CONCLUSION

Results from our research suggest that efforts to restore damaged populations of *P. torreyi* are highly dependent on the type of enhancement technique used. The evaluation of several techniques revealed that transplanting sprigs was the most effective means of producing a net increase in area of surfgrass habitat per unit effort. This outcome was affected by several factors. While factors affecting favorable growth and survival were significant elements, cost-effectiveness and recovery of donor plots also played important roles in determining the overall success of a technique. Depth also appeared to affect outcome, with subtidal transplants generally outperforming intertidal transplants for the same technique. The development of techniques that allow transplants to better withstand the harsh conditions of the intertidal should benefit restoration efforts in this zone.

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Table 1. Survivorship of transplanted surfgrass after six months at an intertidal and subtidal site. Data for seedlings are means ± 1 SE.

Method	Number transplanted	Survivorship (%)
Intertidal		
Seedling	12 plots of 18 seedlings	2.3 ± 1.3
Sprig	42	47.6
Plug (small)	6	100
(medium)	6	100
(large)	6	16.7
Subtidal		
Seedling	12 plots of 18 seedlings	0.9 ± 0.6
Sprig	42	71.4
Plug (small)	6	100
(medium)	6	100
(large)	6	100

Table 2. Mean aerial coverage of rhizome material (cm^2) of transplant units at the end of six months. µ represents the rhizome aerial coverage (cm^2) of the transplant unit at the beginning the experiment. P is the probability that the mean is ≠ µ. Statistically significant P-values (i.e., <0.05) are in bold. NA indicates that data did not meet the assumptions for a t-test or that a t-test was not applicable.

Method	Mean	SE	µ	df	t	P
Intertidal						
Seedling	0.5	0	0	NA	NA	NA
Sprig	7.1	0.9	5	18	2.452	**0.021**
Plug (small)	56.5	11.4	25	5	2.771	**0.042**
(medium)	86.8	18.4	100	5	-0.717	0.401
(large)	437	NA	400	0	NA	NA
Subtidal						
Seedling	0.5	0	0	NA	NA	NA
Sprig	9.3	0.9	5	29	4.845	**<0.001**
Plug (small)	33.7	4.4	25	5	1.962	**<0.001**
(medium)	146.2	12.1	100	5	3.807	**0.013**
(large)	388.8	57.6	400	5	-0.194	0.858

Table 3. Mean net change in the aerial coverage of rhizomes (cm^2) of the donor plots from which the different transplant units were harvested six months after donor collection. μ represents the rhizome coverage (cm^2) of the donor plot at the beginning the experiment. P is the probability that the mean is $\neq \mu$. Statistically significant P-values (i.e., <0.05) are in bold. NA indicates that a t-test was not applicable. Negative values indicate the area of bare space in the donor plot increased after donor collection.

Method	Mean	SE	μ	df	t	P
Intertidal						
Seedling	0	0	0	NA	NA	NA
Sprig	2.67	0.61	5	5	3.846	**0.021**
Plug (small)	-70.1	33.23	25	5	-2.861	**0.031**
(medium)	-532.7	214.16	100	5	-2.954	**0.036**
(large)	-1065.8	620.47	400	4	-2.362	0.080
Subtidal						
Seedling	0	0	0	NA	NA	NA
Sprig	2.92	1.0 4	5	6	2.011	0.101
Plug (small)	-16.64	9.74	25	3	-4.274	**0.031**
(medium)	-252.19	107.41	100	3	-3.279	**0.049**
(large)	-670.00	205.82	400	2	-5.199	**0.015**

Table 4. Mean net change in aerial coverage of rhizome material (A), effort involved in transplanting per unit transplant (E), and net change in the coverage of rhizome area per work hour (CPWH) for the different methods of restoration at an intertidal and subtidal site. See text for details of how A, E and CPWH were calculated.

Method	**A (cm^2)**	**E (h)**	**CPWH (cm^2 rhizome·work h^{-1})**
Intertidal			
Seedling	1.0×10^{-2}	0.27	0.04
Sprig	1.05	0.25	4.2
Plug (small)	-38.60	0.57	-67.72
(medium)	-545.90	0.73	-747.81
(large)	-1392.82	1.00	-1392.82
Subtidal			
Seedling	4.5×10^{-3}	0.25	0.02
Sprig	4.56	0.18	25.33
Plug (small)	-7.94	0.47	-16.89
(medium)	-205.99	0.58	-355.16
(large)	-681.20	0.73	-933.15

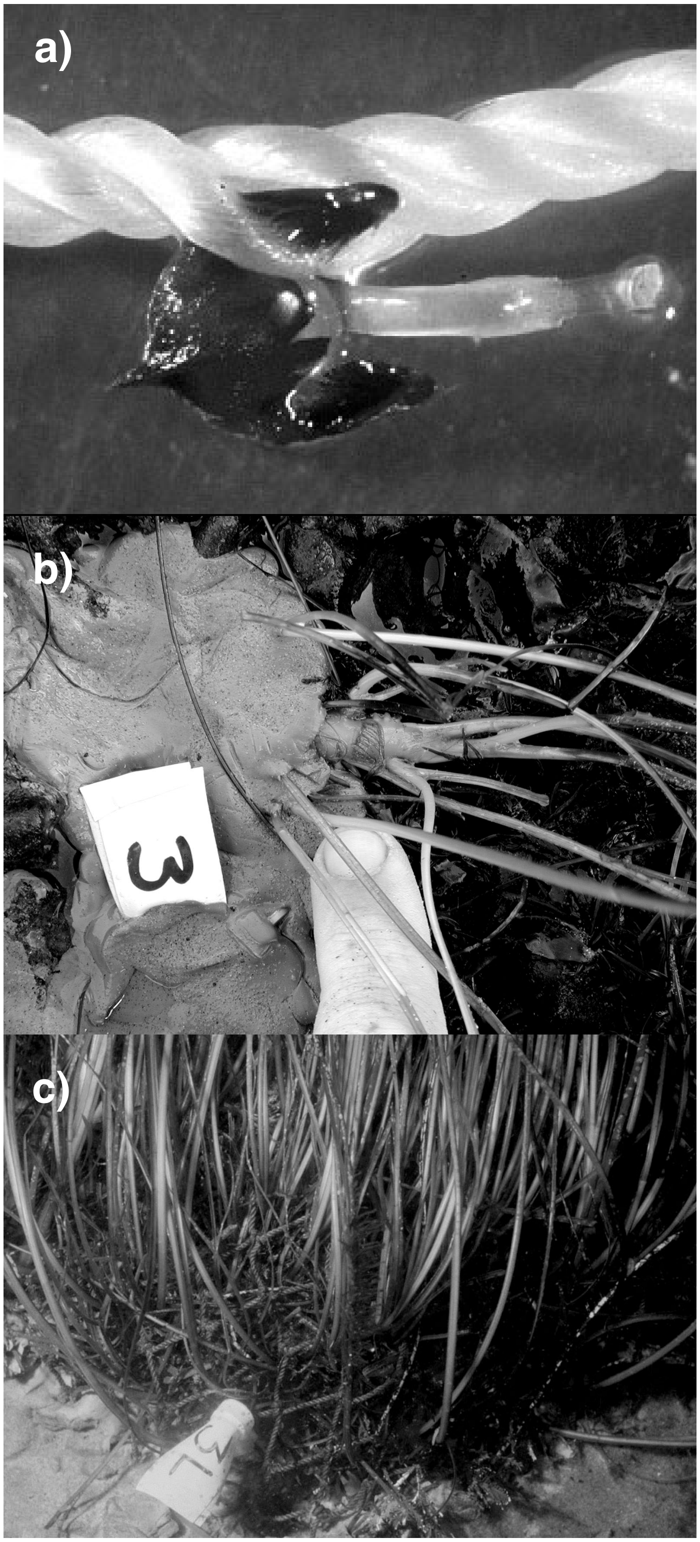

Figure 1. Three methods of restoring *P. torreyi* tested in this study. a) seedling attached to braided nylon line, b) sprig glued to the reef with marine epoxy, and c) plug woven through nylon net fastened to the reef by marine epoxy.

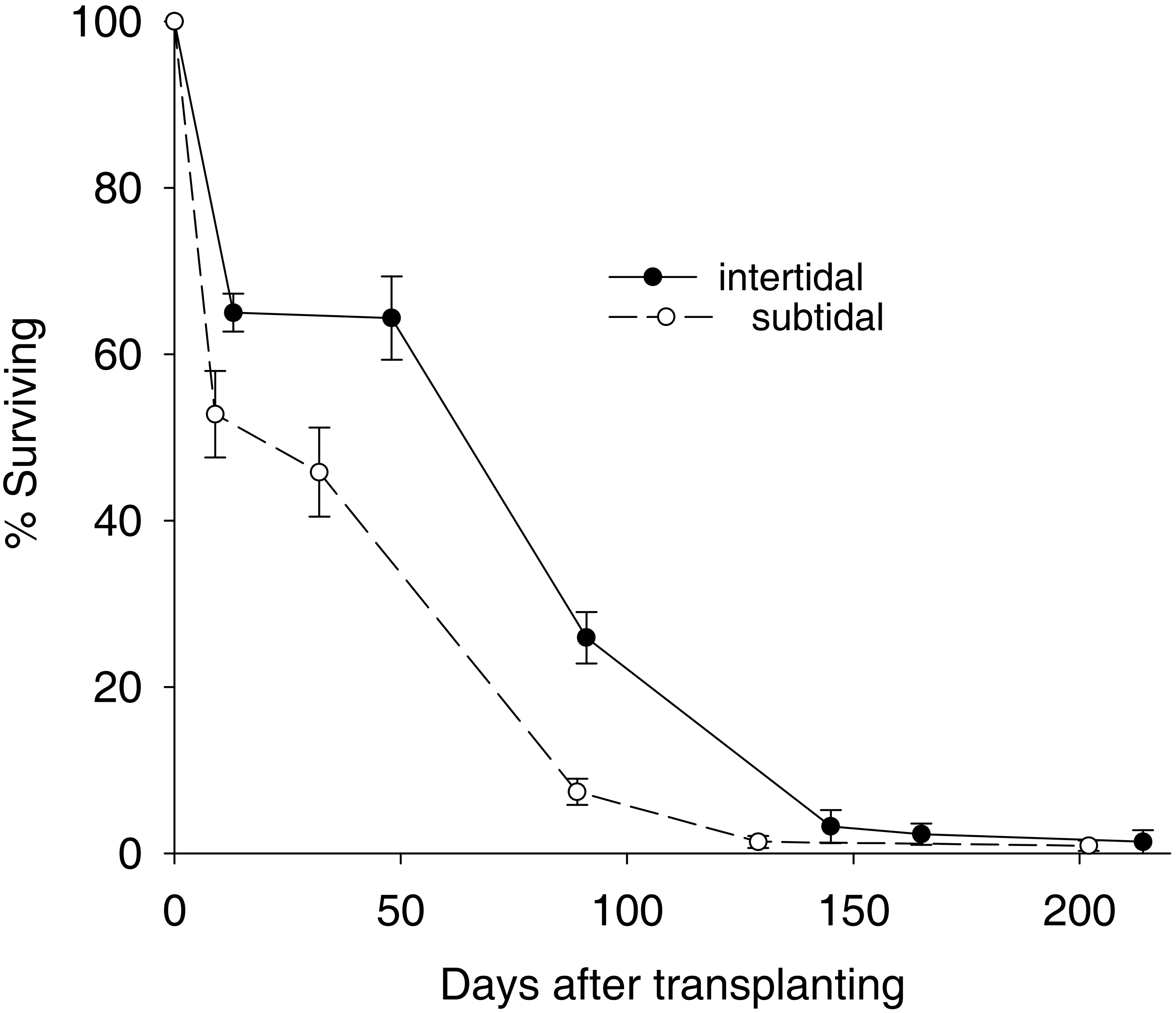

Figure 2. Survivorship of seedlings transplanted to the intertidal and subtidal sites.

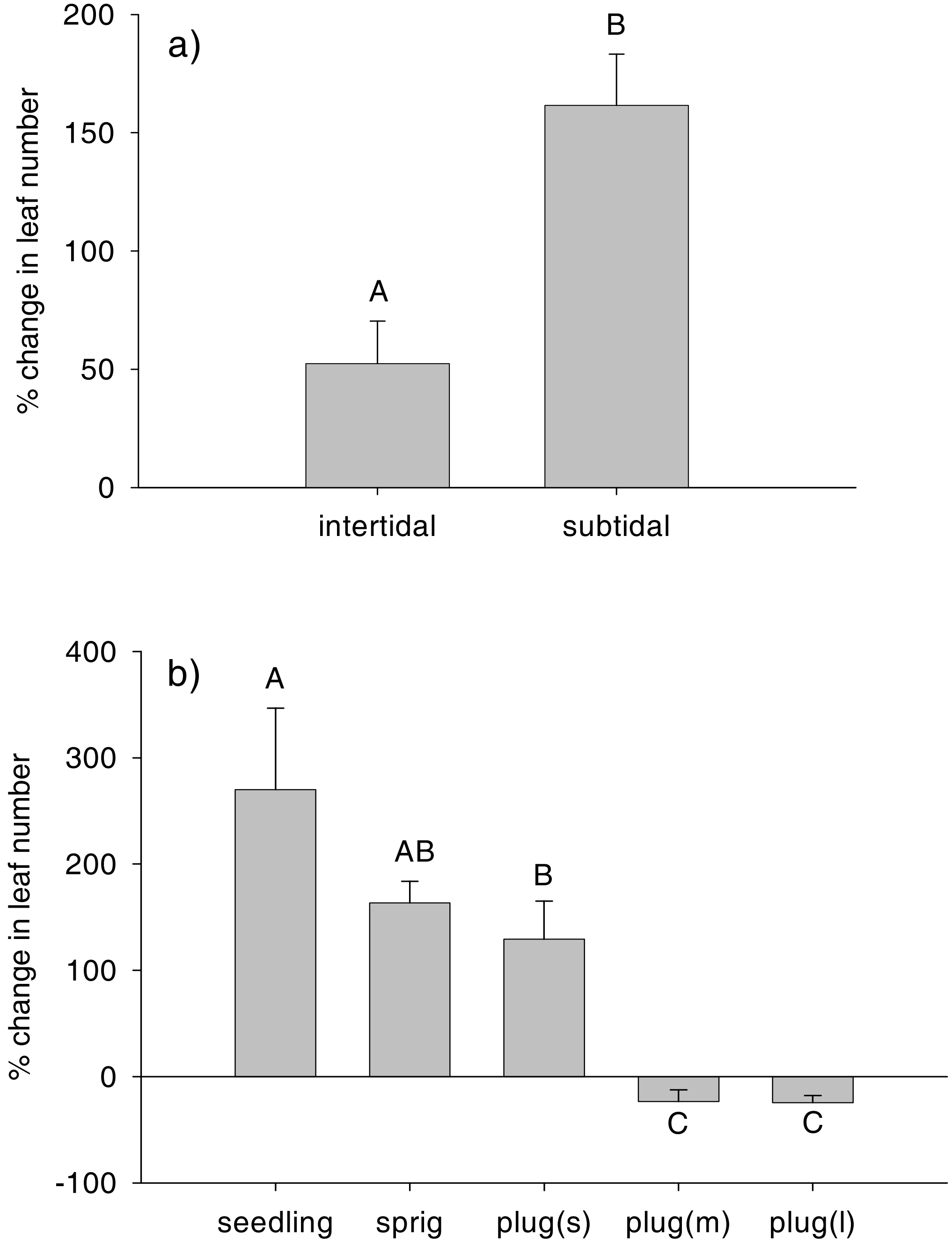

Figure 3. The effects of a) site and b) restoration method on the percentage change in the number of leaves of surviving transplants for the different methods of restoration [(s), (m) and (l) refer to small, medium and large plugs Data are means ± 1 SE. Means sharing the same letter are not significantly different from each other based on Ryan-Einot-Gabriel-Welch multiple range test.

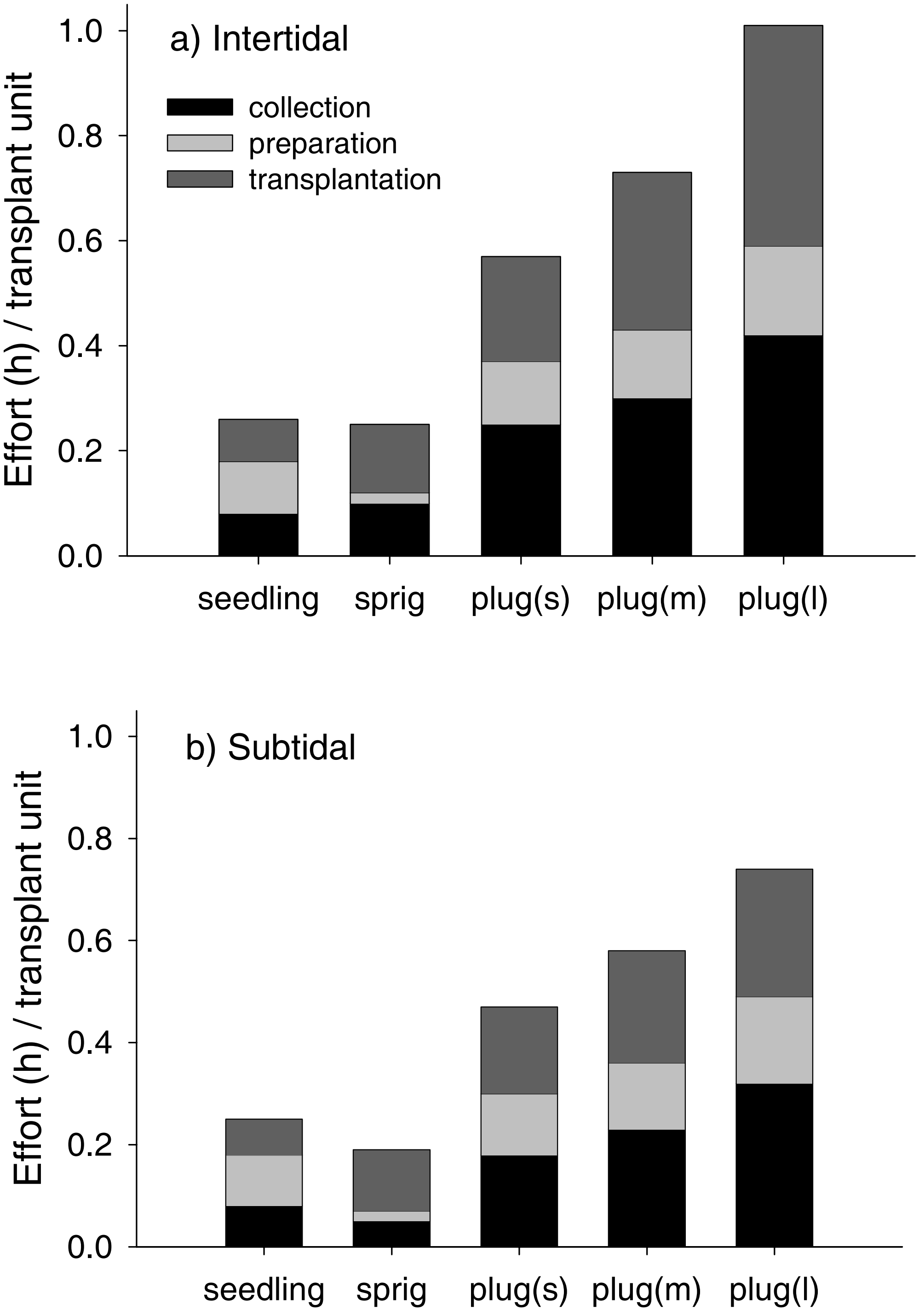

Figure 4. The amount of effort allocated to collection, preparation and transplantation for the different methods of restoration for a) intertidal and b) subtidal sites [(s), (m) and (l) refer to small, medium and large plugs]. See text for details on the work entailed in each category of effort for the different restoration methods.

UNIVERSITY OF CALIFORNIA
Santa Barbara

An Experimental Evaluation of Different Methods of Restoring Surfgrass (*Phyllospadix torreyi*)

A thesis submitted in partial satisfaction
of the requirements for the degree of Master of Arts
in Ecology, Evolution, and Marine Biology

by

J. Scott Bull

Committee in charge:
Professor Sally J. Holbrook, Co-chair
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ABSTRACT

AN EXPERIMENTAL EVALUATION OF DIFFERENT METHODS OF RESTORING SURFGRASS (*PHYLLOSPADIX TORREYI*)

By
J. Scott Bull

Surfgrass, *Phyllospadix spp*., is an ecologically important species that provides habitat for a diverse array of organisms in the intertidal and sublittoral zones of rocky shores. In southern California, *Phyllospadix torreyi* can be adversely affected by a range of natural and anthropogenic activities. Developing effective methods for restoring damaged surfgrass habitat is of great importance, yet few attempts have been made and none have investigated the efficacy of using different life stages. Here I evaluated several techniques for restoring populations of surfgrass in intertidal and subtidal habitats using 1) outplanted laboratory-reared seedlings, 2) transplanted rhizome sprigs (collected *in situ* from within the existing bed) and 3) transplanted rhizome plugs. I calculated net change in surfgrass habitat for each method after six months taking into account the recovery or additional losses from the donor population, and amount of effort required. Outplanted seedlings survived poorly and had minimal rhizome growth over the six-month study period for both intertidal and subtidal sites yet on average showed a 275% increase in leaf number. The survivorship of transplanted plugs was high in both intertidal and subtidal habitats; however, additional disturbances to the donor population caused a substantial net loss in surfgrass habitat using this technique. Sprigs transplanted in the subtidal had higher survivorship than those transplanted to the intertidal with intertidal (71.4% vs. 47.6%), and showed the highest increase in area of surfgrass habitat (42% and 86% for intertidal and subtidal transplants, respectively). Of the three techniques, transplanted sprigs showed the greatest overall increase in surfgrass habitat per unit effort for both subtidal and intertidal habitats. This research suggests that the use of sprig transplants may be the most effective approach for restoration of *P. torreyi*.

CHAPTER 1

AN EXPERIMENTAL EVALUATION OF DIFFERENT METHODS OF RESTORING SURFGRASS *(PHYLLOSPADIX TORREYI)*

INTRODUCTION

A wide variety of biological, environmental and logistical issues must be considered during restoration of long-lived species of plants that have been damaged or destroyed. A number of factors could affect the choice of methods for restoring such species including: (1) ease of propagation, (2) constraints on collection, cultivation and transplantation, (3) fitness of transplanted individuals, (4) desired genetic diversity of the restored population, and (5) the amount of damage suffered by donor populations and the likelihood of recovery. Furthermore, the potential restoration methods for a given species may vary depending on the environmental conditions experienced at the restoration site. Many restoration projects often must proceed without detailed consideration of all the pertinent issues, which can make it difficult to evaluate their long-term success and cost-effectiveness.

One case in point involves the restoration of seagrasses, whose populations have been degraded by nutrient loading (Short & Burdick 1996; Short & Wyllie-Echeverria 1996), polluted waste from sewage and industrial discharges (Sautour & Castel 1995; Morand & Briand 1996; Clark 1997; Schiewer 1998; Castel *et al*. 1996), coastal development and offshore construction (Cambridge & McComb 1984; Short *et al*. 1991; McLusky *et al*. 1991; Sheridan *et al*. 1998), and recreational and commercial boating and fishing (Zieman 1976; Walker *et al*. 1989; Dawes *et a*l. 1997). Seagrasses typically are a dominant organism in the areas in which they occur, providing an important source of food and shelter for a variety of nearshore organisms (Harlin 1975; Stewart & Myers 1980; Fonseca *et al*. 1990; WyIlie-Echeverria & Phillips 1994). Their high ecological importance, coupled with their susceptibility to damage from a range of human activities and natural disturbances, have sparked much interest in developing effective methods for restoring damaged populations (Thorhaug 1986; Larkum *et al*. 1989; Edgar 1990).

Previous attempts to restore seagrass populations often have relied on transplanting older life stages that were collected from healthy stands (Thorhaug 1986; Thom 1990; Orth *et al*. 1999, 2000; Williams & Davis 1996; Paling *et al*. 2001a, b). In many cases, transplanting techniques have been successful in promoting the establishment of new plants (Fuss and Kelly 1969; Kelly *et al*. 1971; Phillips 1974; Fonseca *et al*. 1994; Thorhaug 1986; Thom 1990; Williams & Davis 1996; Balestri *et al*. 1998). Although cost-effectiveness of different planting techniques has been examined (e.g., Thorhaug 1986), there have been few attempts to measure the damage to donor populations that results from collecting materials for use in transplanting. Hence, there is growing concern among resource managers that for some species, restoration projects have the potential to cause long-term losses to otherwise healthy populations. Such concern has prompted studies to explore the use of seeds and cultivated seedlings in restoration (Balestri *et al*. 1998; Harwell & Orth 1999; Holbrook *et al*. 1999); however, relatively little research on this topic has been done to date (Orth *et al*. 2000).

Restoration of sea grasses has been carried out in many areas of the world on a variety of species, but in the United States efforts have focused on species such as *Halodule wrightii* and *Zostera marina* that occupy shallow, soft-bottom habitats (Thom 1990). Surfgrasses (i.e., seagrasses in the genus *Phyllospadix*) pose special challenges for restoration because the transplanting techniques that have been developed for other seagrasses are not appropriate for them. Unlike most seagrasses that live in soft sediments of protected bays or estuaries, surfgrasses grow on rocky shores of exposed coasts. Adventitious roots from the rhizome secure the plant to the rocky substratum (Chrysler 1907; den Hartog 1970; Phillips 1979; Tomlinson 1980; Cooper & McRoy 1988). Their penchant for growing on rocks in wave swept areas greatly complicates efforts to restore them because successful transplantation depends not only on the ability to grow following relocation, but also on the ability of transplanted individuals to avoid becoming dislodged by the forces of breaking waves. Because surfgrass rhizomes are not inherently "sticky", this latter requirement invariably necessitates the use of a glue or epoxy to secure a transplanted individual to rock. Another factor that complicates the restoration of surfgrass is that populations typically extend from the low intertidal into the shallow subtidal and thus are subjected to a wide range of biological (e.g. competition, grazing) and physical (e.g., temperature, desiccation, waves, light) conditions (Turner 1983, 1985; Stewart 1989; Blanchette *et al*. 1999; Holbrook *et al*. 2000) that influence their growth and survivorship. This may dictate the use of different methods for restoration of plants at different depths. To date, there has been little progress in the development of efficient strategies that enable restoration of surfgrass populations (Holbrook *et al*. 2002).

Surfgrass is a clonal plant that produces copious amounts of seeds whose germination is easily controlled in laboratory culture (Williams 1995; Reed et a!. 1998). As such, it has the potential to be propagated readily from seeds, sprigs, or plugs. Such flexibility could be of significant value in restoration if the relative performance of the different forms varies along the depth gradient. Here I experimentally investigated the cost effectiveness of restoring surfgrass from seedlings, sprigs and plugs at two different depths with the goal of determining the optimal method(s) for restoring damaged populations. Specifically, I evaluated the efficacy of the three methods of restoration based on: 1) the growth and survivorship of transplanted individuals, (2) the effort involved in collecting, preparing and transplanting, and (3) the residual damage to donor populations.

METHODS

This study compared the efficacy of three methods of restoring populations of surfgrass (*Phyllospadix torreyi*) to intertidal and shallow subtidal reefs near Santa Barbara, CA. The three methods were: (1) seedlings cultivated in the laboratory and outplanted to the field, (2) sprigs of actively growing rhizome tips collected in the field and transplanted to the study areas, and (3) plugs of rhizomous mats collected in the field and transplanted to the study areas. Intertidal studies were conducted at More Mesa Reef (34°25'N, 119°57'W), a broad, gently sloping, wave-cut bench fractionated by sand channels. Patches of the red algae *Corallina vancouveriensis, Gelidium purpurascens,* and *Chondracanthus canaliculatus* [which serve as attachment sites for surfgrass seeds (Blanchette *et al*. 1999)] were common in the study area. *P. torreyi* formed dense beds on most of the emergent reef in the low-intertidal zone to the shallow subtidal zone at More Mesa Reef. Transplanted seedlings, sprigs and plugs were placed in bare spaces on the reef adjacent to *P. torreyi.* Subtidal studies were done at Mohawk Reef (34°24' N, 119°44' W) where

P. torreyi forms large, patchy beds on broad, gently sloping benches that are interspersed with stands of coralline algae consisting primarily of *Bossiella orbigiana* and *Corallina officinalis.* Seedlings, sprigs and plugs at this site were transplanted to depths of 2.5 to 4 m below MMLW in areas cleared of coralline algae that were adjacent to established surfgrass beds.

Transplant experiments using all three methods were initiated in the late summer and fall months, the time of year when most surfgrass seeds are released and germinate in Santa Barbara County (Holbrook and Reed unpublished data). All experimental transplants were followed for six months. The efficacy of each method of restoration was evaluated using data collected on survivorship and growth of the transplants after six months, recovery of the donor population from which transplants were collected, and the amount of effort (i.e. time) involved in transplanting. Transplants were considered dead if after six months they had no leaves or were removed from the substrate. The growth of surviving transplants was estimated as the change in rhizome area over the course of the six-month experiment. The percentage change in the number of leaves after six months ([(final number of leaves — initial number of leaves) / initial number of leaves] * 100) was used to assess the condition of surviving transplants.

Seedlings

Seedlings used in this study were germinated from seeds obtained from reproductive shoots of mature female *P. torreyi* plants collected in September 2000 at the two study sites and other nearby intertidal and subtidal locations within 15 Km of the sites. Reproductive shoots were cut several centimeters above the rhizome and their collection did not result in the loss of surfgrass habitat. Upon collection, reproductive shoots were transported to the marine biological laboratory at University of California, Santa Barbara where they were placed in a flow through seawater tank until fruits (hereafter referred to as seeds) dehisced (about 1 month). Dehisced seeds were placed in clear aquaria containing filtered seawater (0.2μm) enriched with nutrient stock solution (Provosoli; 1968). Aquaria were maintained at 15°C at an irradiance of 40-50 $\mu E\ m^{-2}\ s^{-1}$ using a 14:10 h L:D photoperiod under mild agitation. Culture media were changed weekly and seeds were cleaned and rinsed with dionized water to inhibit fouling. Under these conditions, seeds germinated within one month of dehiscence (Reed *et al*. 1998). At the time of outplanting (approximately one month post-germination) seedlings lacked rhizomes and consisted of a single leaf approximately two cm in length that was firmly attached to the endocarp of the fruit.

I attached each seedling to a 7 cm long piece of braided nylon line (1 mm diameter) in the laboratory to facilitate outplanting to the field. Seedlings were attached to the line by inserting one of the arms of the fruit into an opening in the line made by untwisting the braids one-half turn (Figure la). Tiny bristles along the inside arm of the fruit hooked onto one of the braids, locking the seedling in place once the line was relaxed and the opening closed. Once attached to the nylon line, seedlings were put into re-sealable plastic bags containing seawater and transported to the field in coolers. Seedlings attached to nylon line were outplanted to experimental plots 30 cm x 30 cm in area at the intertidal and subtidal study sites in November 2000 by fastening the ends of each nylon line directly to the reef using Z-Spar A788 marine epoxy putty. Eighteen seedlings were outplanted in a uniform grid to each of 12 plots at each of the two sites. All experimental plots were located adjacent to healthy stands of surfgrass and were cleared of algae and sand prior to outplanting to facilitate attachment with epoxy. Branches of coralline algae growing adjacent to the plots were trimmed to reduce abrasion and dislodgement of seedlings.

Sprigs

I transplanted apical tips of actively growing rhizomes of *P. torreyi* from established surfgrass beds to evaluate the feasibility and effectiveness of using sprigs to restore damaged populations of surfgrass. Sprigs used in this study were harvested from established beds at the intertidal and subtidal sites in November 2000, and immediately transplanted to adjacent areas lacking surfgrass. To harvest sprigs, an unbranched tip was carefully cut from an actively growing rhizome with a sharp knife. Care was taken to minimize damage to the rhizome roots. Harvested sprigs were five cm in length. Leaf numbers varied from 5 to 36 (mean 11.9 ± 0.9 SE) for subtidal transplants and 12 to 51 leaves (mean 26.6 ± 1.5 SE) for intertidal transplants. Sprigs were transplanted to experimentally cleared areas by attaching the cut end of the sprig to the reef using marine epoxy (Figure 1b). At least 1.5 cm of the actively growing apical end of each sprig was exposed and not in contact with the epoxy. The leaves of transplanted sprigs were trimmed to 20 cm in length prior to attachment to minimize drag and reduce the chance of dislodgement while the epoxy hardened. Forty-two sprigs were transplanted to each of the two sites.

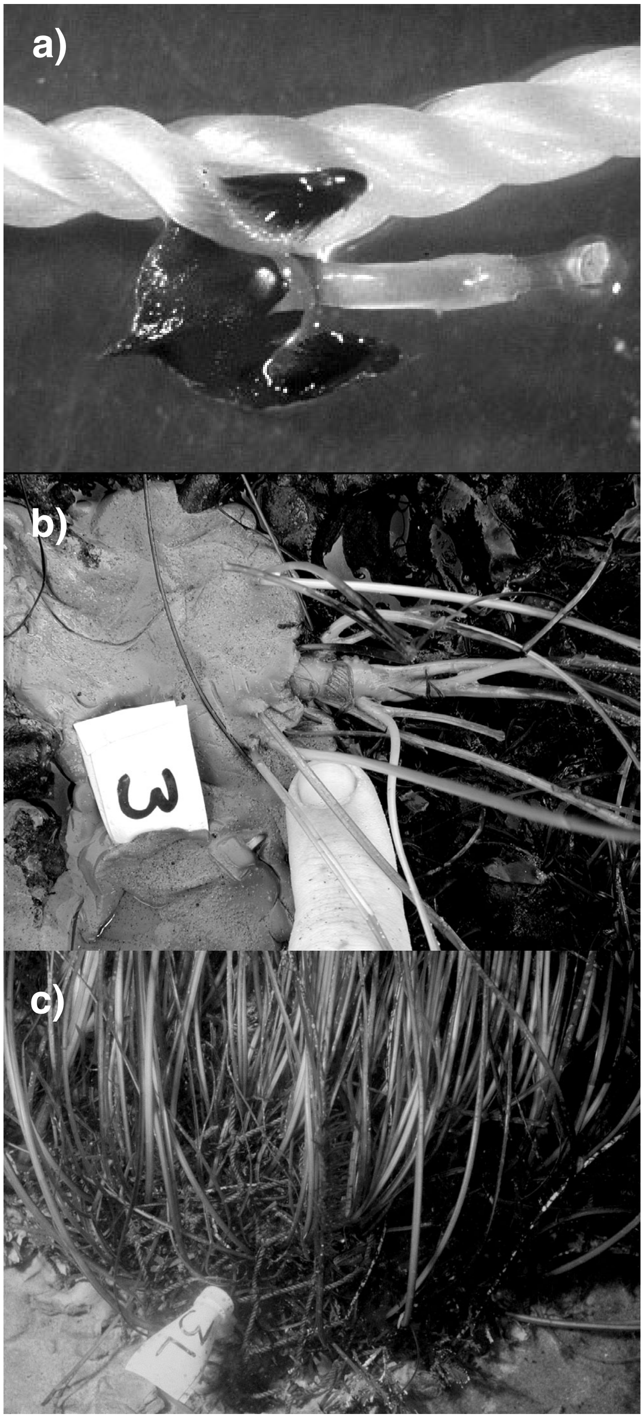
The collection of sprigs resulted in a small loss of surfgrass from the donor site. To measure recovery from this loss, a reference marker was glued to the reef next to the cut end of each donor rhizome (i.e., a rhizome from which a sprig was harvested). Recovery was estimated as the area of new rhizome that grew from the cut end of the donor rhizome. Because I was not able to relocate many of the donor rhizomes at the end of the study, only six and nine donor rhizomes were used to calculate recovery from harvesting sprigs from the subtidal intertidal and sites, respectively.

Plugs

The feasibility and effectiveness of using clumps of mature surfgrass to restore damaged populations was evaluated at the intertidal and subtidal sites beginning in August 1999. Square plugs of intertwined rhizomes and leaves were harvested from the middle of an established bed of P. *rorreyi* using a wide bladed putty knife and transplanted to adjacent areas lacking surfgrass. Plugs were attached to the reef by pulling the leaves through a square piece of 2.5 cm diameter stretch mesh nylon net that was cut to a size that was slightly larger than the square plug (Figure 1c). The net was pulled tight over the plug and secured to the reef at the edges with marine epoxy. Care was taken to insure that the plugs did not come into contact with the epoxy. The leaves of all plugs were trimmed to 20 cm in length prior to attachment to minimize drag and reduce the chance of dislodgement while the epoxy hardened. Three different sizes of plugs (small, 5 cm x 5 cm medium, 10 cm x 10 cm and large, 20 cm x 20 cm) were transplanted to test whether plug size influenced (1) the survivorship and growth of the transplanted plug, and (2) the rate of recovery of the donor area from which the plug was collected. Six plugs of each size were transplanted to each of the two sites.

The collection of plugs resulted in the immediate loss of surfgrass habitat in the donor beds equal to the rhizome area of the plugs (i.e., 25 cm^2, 100 cm^2 and 400 cm^2 for small, medium and large plugs, respectively). To facilitate estimates of the recovery of the bare patches in the donor bed created by harvesting plugs, I marked each donor patch at the time of harvesting and made periodic observations during the experiment to assess the extent of in-growth from the edges of the bare patches by neighboring rhizomes. The recovery of donor patches was determined at the end of the six-month experiment by measuring the area of bare space remaining in each donor patch.

Figure 1. Three methods of restoring *Phyllospadix* tested in this study. a) seedling attached to braided nylon line, b) sprig glued to the reef with marine epoxy, and c) plug woven through nylon net fastened to the reef by marine epoxy.



Effort and efficacy of different methods of restoration

I estimated the amount of effort (E) per unit transplant spent collecting, preparing and transplanting seedlings, sprigs and plugs for both intertidal and subtidal habitats. The effort (i.e., person hours) involved in the collection of seedlings was based on the time spent collecting reproductive shoots in the field, gathering and cleaning seeds dehisced in seawater aquaria, germinating seeds, and cultivating seedlings in laboratory cultures. Effort in preparing seedlings for outplanting consisted of the time spent in the laboratory attaching seedlings to nylon lines, while the effort in transplanting seedlings was based on the time spent attaching them to the reef (this included clearing the transplant plots on the reef of algae and debris). Estimates of effort for sprigs and plugs were based on the time spent collecting in the field, trimming leaves in preparation for transplantation, and transplanting units to adjacent areas on the reef. In the case of plugs, additional preparation time was spent cutting the netting used to secure the plugs and pulling the leaves of the plugs through it. The transport of transplant units, the set-up of laboratory cultures, and the mobilization and demobilization of gear and assistants were not included in any estimates of effort.

The efficacy of the three different methods of restoration was evaluated by calculating the mean net change in the rhizome area of a transplant unit per unit effort measured in person hours. The mean net change in rhizome area (A) was calculated as:

$$A = TS - (D_{\text{initial}} — D_{\text{final}}),$$

where T is the mean rhizome area (cm^2) of the transplant unit six months after transplantation, S is the proportion of transplant units surviving six months, D_{initial} is the mean area (cm^2) of rhizome collected from the donor patch, and D_{final} is the mean net change in rhizome area (cm^2) in the donor patch six months after donor collection.

The mean net change in the rhizome area of a transplant unit per unit effort (CPUE) was calculated as:

$$\text{CPUE} = A / E.$$

Analyses

Differences in survivorship between intertidal and subtidal habitats were tested individually for the different methods of restoration. A t-test using Satterthwaite's approximation for the degrees of freedom (SAS v. 8.02) was used to test differences in survivorship between seedlings outplanted to the intertidal vs. the subtidal. Survivorship was estimated from twelve plots of eighteen seedlings. Two by two contingency tables were used to evaluate habitat specific differences in survivorship for sprigs and plugs.

I tested the hypothesis that the rhizome area of transplanted sprigs and plugs changed significantly during the six month experiments using one-sample, two-tailed, t-tests, where the population mean μ was the area of rhizome at the beginning of the experiment. Similar analyses were done on the donor plots from which sprigs and plugs were harvested to assess whether any significant recovery of the donor plots bad occurred. Analyses testing the significance of rhizome growth in transplanted seedlings were not done because all seedlings lacked rhizomes at the start of the experiment and bad zero variance in rhizome area at the end of the experiment (the final rhizome area of seedlings was uniformly small and at the lower limit of my measurement capabilities).

RESULTS

Survivorship and leaf development

Extreme differences in survivorship were observed among the different methods of restoration (Table 1). Very few of the 432 seedlings outplanted on nylon lines were alive after six months. Survivorship was equally poor at both the intertidal and subtidal sites ($t_{16} = 0.98$, $P = 0.34\ 1$). The use of epoxy and nylon line and the transport of seedlings to the field did not contribute substantially to low survivorship in the field; seedlings treated the same way but maintained in the laboratory showed 95% survivorship after two months (Appendix IV). The causes of high mortality in seedlings appeared to differ between the two habitats. The majority of seedlings at the intertidal site appeared to die in place. These seedlings lacked leaves after six months, but remained attached to the nylon line by the exocarp of the fruit. In contrast, poor survivorship at the subtidal site reflected high rates of dislodgement as most seedlings were completely missing from the outplanted nylon line at the end of the six-month study.

Table 1. Survivorship of transplanted surfgrass after six months for the three methods of restoration in intertidal and subtidal habitats. Data for seedlings are means ± 1 SE.

Method	Number transplanted	Survivorship (%)
Intertidal		
Seedling	12 plots of 18 seedlings	2.3 ± 1.3
Sprig	42	47.6
Plug (small)	6	100
(medium)	6	100
(large)	6	16.7
Subtidal		
Seedling	12 plots of 18 seedlings	0.9 ± 0.6
Sprig	42	71.4
Plug (small)	6	100
(medium)	6	100
(large)	6	100

Survivorship of sprigs and plugs was substantially greater than that of seedlings (Table 1). Sprigs fared significantly better in the subtidal than the intertidal (71.4 % vs. 47.6 %; $\chi^2_{1DF} = 11.81$, P = <0.001), where desiccation appeared to be a major cause of death. No sprigs transplanted to either site died as a result of becoming dislodged. Instead, evidence of death in sprigs was based on necrosis and loss of leaves. Plugs had the greatest survivorship as nearly all were alive after six months, with the exception of large plugs transplanted to the intertidal (where only one of six transplants survived). The five large plugs that died became dislodged within days after transplanting and quickly disappeared.

The production of new leaves by transplant units that survived the six-month experiments was affected by both the site to which they were transplanted and the method of restoration. On average, seedlings, sprigs, and plugs transplanted to the subtidal produced more than three times as many new leaves as those transplanted to the intertidal (Figure 2a, $F_{1,79} = 12.93$, P <0.00 1). Differences in leaf production among restoration methods were equally large (Figure 2b, $F_{4,79} =$ 12.93, P <0.001). The few seedlings that survived grew remarkably well and showed nearly a 300 % increase in the number of leaves after six months. Substantial leaf production also occurred in transplanted sprigs and small plugs, but was somewhat less than that of seedlings. In contrast, medium and large sized plugs grew poorly and actually lost leaves over the course of the experiment. Differences in leaf production among the different restoration methods were generally similar at the intertidal and subtidal sites ($F_{4,79}$ 1.47, P = 0.221 for the site x method interaction).

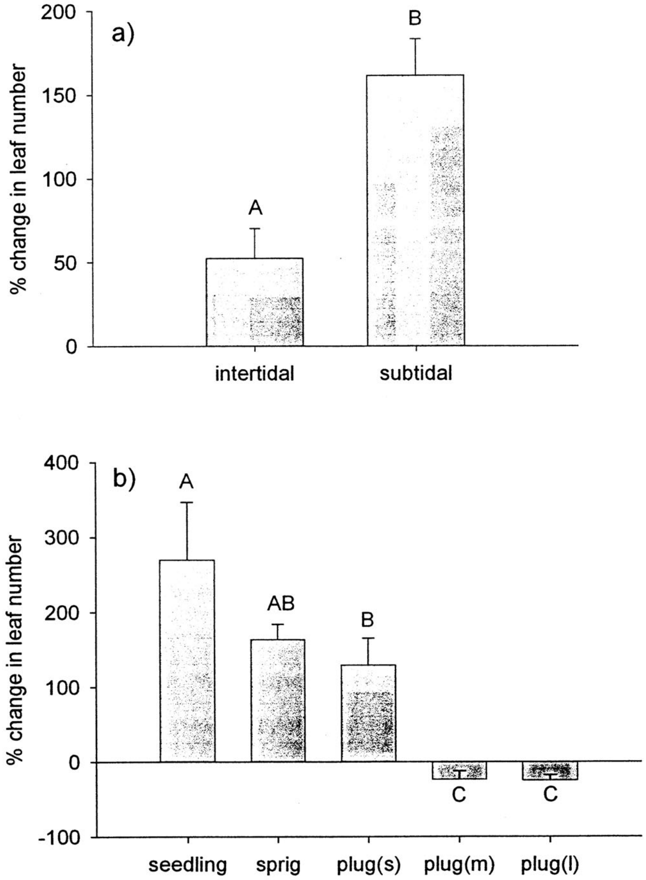


Figure 2. The effects of a) site and b) restoration method on the percentage change in the number of leaves of surviving transplants. Percentage change was calculated as [(final number of leaves - initial number of leaves) / initial number of leaves) * 100]. Data are means ± 1 SE. Means sharing the same letter are not significantly different from each other based on Ryan-Einot-Gabriel-Welch multiple range test (SAS v. 8.02).

Rhizome growth and donor recovery

Rhizome growth, which was used to estimate changes in the amount of surfgrass habitat, varied substantially among transplanted seedlings, sprigs and plugs that survived the six-month experiments (Table 2). Rhizomes of surviving sprigs consistently grew the most. The average rhizome area of sprigs transplanted to the intertidal increased by 42% while those transplanted to the subtidal increased by 86% Rhizome growth of the different-sized plugs varied inconsistently between the intertidal and subtidal sites. For example, significant rhizome growth was observed in small plugs transplanted to the intertidal, but not in small plugs transplanted to the subtidal (Table 2). Conversely, rhizome area increased significantly in medium plugs transplanted to the subtidal, but not in medium plugs transplanted to the intertidal. There was no significant change in the mean rhizome area of large plugs transplanted to the subtidal, whereas the rhizome area of the lone surviving large plug in the intertidal showed a slight increase (i.e. 9%). Rhizomes were typically poorly developed in six-month old seedlings. Nonetheless, the few outplanted seedlings that survived in the intertidal and subtidal had developed small rhizomes by the end of the experiment.

Table 2. Mean rhizome area (cm^2) of the different transplant units at the end of the six month experiments. μ represents the rhizome area (cm^2) of the transplant unit at the beginning the experiment. P is the probability that the mean is $\neq \mu$. * <0.05, ** <0.02, *** <0.001. NA indicates that data did not meet the assumptions for a t-test.

Method	Mean	SE	µ	df	t	P
Intertidal						
Seedling	0.5	0	0	NA	NA	NA
Sprig	7.1	0.9	5	18	2.452	*
Plug (small)	56.5	11.4	25	5	2.771	*
(medium)	86.8	18.4	100	5	-0.717	
(large)	437	NA	400	0	NA	NA
Subtidal						
Seedling	0.5	0	0	NA	NA	NA
Sprig	9.3	0.9	5	29	4.845	***
Plug (small)	33.7	4.4	25	5	1.962	
(medium)	146.2	12.1	100	5	3.807	**
(large)	388.8	57.6	400	5	-0.194	

Striking differences were observed in the recovery of donor plots from which sprigs and plugs were harvested (Table 3). In the case of sprigs, full recovery was observed at both the intertidal and subtidal sites as the re-growth of cut rhizomes more than compensated for the loss of surfgrass incurred from harvesting. In sharp contrast, harvesting plugs led to significant losses of additional surfgrass because areas adjacent to donor plots sustained losses of surfgrass as well. These additional losses were observed within a few weeks of harvesting, and appeared to result from water motion that undermined the edges of cut rhizomous mats that were exposed when the plugs were removed. These subsequent losses of additional surfgrass were substantial, ranging from 67% to 533% of the initial donor area, and they were generally larger in the intertidal than in the subtidal. Large plugs taken from the intertidal had the largest mean net loss from harvesting. However, because of high variability among replicate donor plots, large plugs were the only type of donor plug whose subsequent losses of surfgrass were not significant (Table 3).

Table 3. Mean net change in rhizome area (cm^2) of the donor plots from which the different transplant units were harvested six months after donor collection. μ represents the rhizome area (cm^2) of the donor plot at the beginning the experiment. P is the probability that the mean is $\neq \mu$. * <0.05, ** <0.02. NA indicates that a t-test was not applicable. Negative values indicate the area of bare space in the donor plot increased after donor collection.

Method	Mean	SE	μ	df	t	P
Intertidal						
Seedling	0	0	0	NA	NA	NA
Sprig	2.67	0.61	5	5	3.846	*
Plug (small)	-70.1	33.23	25	5	-2.861	*
(medium)	-532.7	214.16	100	5	-2.954	*
(large)	-1065.8	620.47	400	4	-2.362	0.08
Subtidal						
Seedling	0	0	0	NA	NA	NA
Sprig	2.92	1.04	5	6	2.011	0.1
Plug (small)	-16.64	9.74	25	3	-4.274	*
(medium)	-252.19	107.41	100	3	-3.279	*
(large)	-670	205.82	400	2	-5.199	*

Effort and efficacy of different methods of restoration

Overall, restoration using seedlings and sprigs required less effort than restoration with plugs (Figure 3). Seedlings, which were reared in the laboratory and attached to strings prior to outplanting, required more effort for transplant preparation than for collecting or transplanting. In contrast, relatively little effort was allocated to transplant preparation for sprigs and plugs; most of the effort for sprigs and plugs involved collecting and transplanting. In general, less effort was needed to collect and transplant sprigs and plugs in the subtidal than in the intertidal. This occurred because material for transplanting was more readily available in the subtidal resulting in shorter collection times. Moreover, transplanting sprigs and plugs required more effort in the intertidal because of the increased difficulty of clearing sand and debris there.

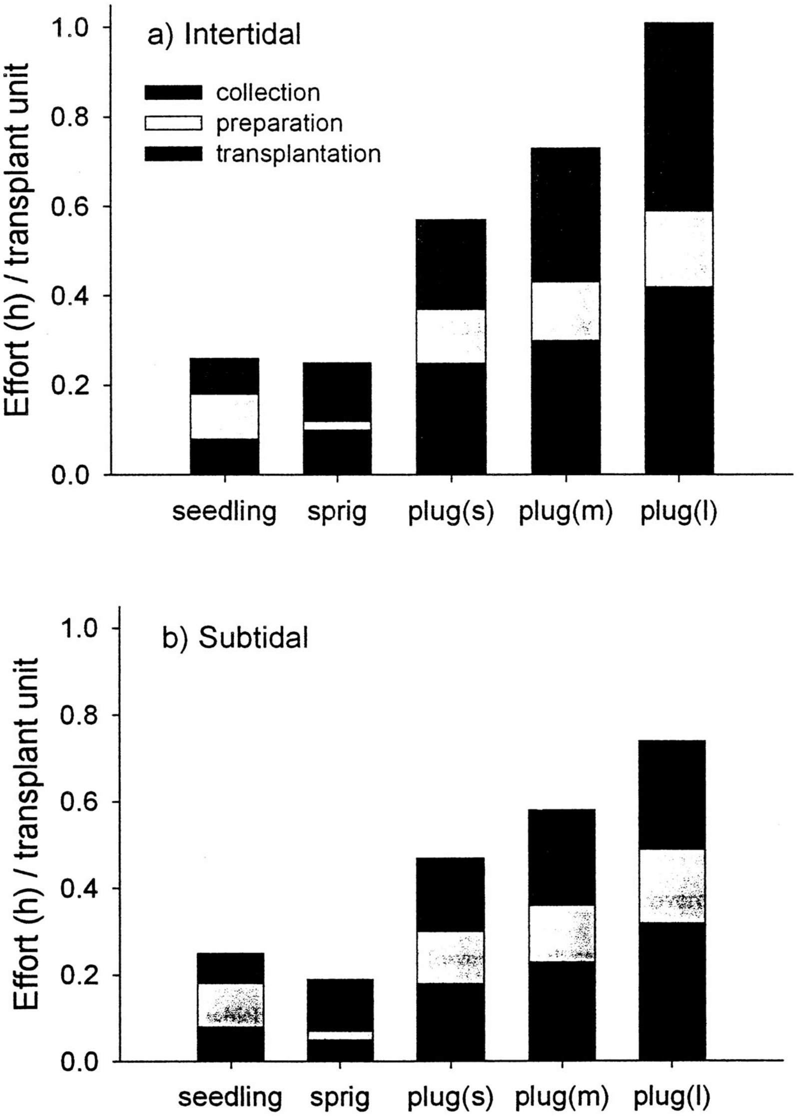


Figure 3. The amount of effort allocated to collection, preparation and transplantation for the different methods of restoration for a) intertidal and b) subtidal sites. See text for details on the work entailed in each category of effort for the different restoration methods.

The change in area of surfgrass habitat per unit effort varied tremendously among the methods of restoration tested (Table 4). The use of sprigs proved to be the most effective method for restoring surfgrass to both the intertidal and subtidal sites (as determined by changes in rhizome area after six months per work hour). Change in rhizome area per unit of effort (CPUE) for sprigs in the subtidal was more than five times that in the intertidal (25.33 cm^2 rhizome • work h^{-1} compared to 4.2 cm^2 rhizome • work h^{-1}). This was a result of greater increases in net rhizome area and smaller effort involved in transplanting at the subtidal site. Small changes in rhizome area per unit effort for seedlings with < 0.04 cm^2 rhizome • work h^{-1} reflected the high mortality and small gains in rhizome area observed over a six-month period. Interestingly, change in rhizome area per unit effort of plugs was negative for all three sizes at both the intertidal and subtidal sites (Range= -16.89 cm^2 rhizome • work h^{-1} to -1392.82 cm^2 rhizome • work h^{-1}), suggesting that this method of restoring surfgrass should be avoided. The negative values for plugs resulted from the additional losses of surfgrass over time incurred from harvesting. Even if these losses were discounted, change in rhizome area per unit effort for medium and large plugs would be exceedingly low due to the limited (and in some cases negative) growth observed in transplanted plugs, coupled with the relatively large amount of effort required to transplant them.

Table 4. Mean net change in rhizome area of surfgrass (A), effort involved in transplanting per unit transplant (E), and net change in rhizome area per unit effort (CPUE) for the different methods of restoration in intertidal and subtidal habitats. See text for details of how A, E, and CPUE were calculated.

Method	A (cm^2)	E (h)	CPUE (cm^2 rhizome • work h^{-1})
Intertidal			
Seedling	$1.0 * 10^{-2}$	0.27	0.04
Sprig	1.05	0.25	4.2
Plug (small)	-38.60	0.57	-67.72
(medium)	-545.90	0.73	-747.81
(large)	-1392.82	1.00	-1392.82
Subtidal			
Seedling	$4.5 * 10^{-3}$	0.25	0.02
Sprig	4.56	0.18	25.33
Plug (small)	-7.94	0.47	-16.89
(medium)	-205.99	0.58	-355.16
(large)	-681.20	0.73	-933.15

DISCUSSION

In the past several decades numerous attempts to restore a variety of seagrass species have been made. Spatial scales have ranged from small, experimental plots to large areas that are hectares in extent, and a variety of planting techniques using different life stages (plugs, seeds, seedlings, and sprigs) have been utilized (Thorhaug 1986; Thom 1990). The success of these efforts has been highly variable. For example, Thorhaug (1986) reported that 75 of 165 attempts resulted in some degree of re-establishment of seagrass populations. Similarly, 11 of the 17 projects reviewed by Thom (1990) were considered successful, so far as during the period of study seagrass survived and grew in at least a portion of the restoration site. Not surprisingly, both the life stage and the local environmental conditions (including the presence of species that consume seagrasses) at the restoration site affected outcome. In my study, both of these aspects (life stage and location) were explored with respect to developing restoration strategies for surfgrass, *Phyllospadix torreyi.* Unlike other seagrasses, surfgrass lives attached to hard substrates, so restoration strategies previously developed for seagrasses that inhabit soft bottom areas are not applicable. Few attempts have been made to restore surfgrass, and there have been no prior attempts to evaluate the efficacy of using different life stages (Holbrook *et al*. 2002). This motivated me to test the performance of several life stages along the habitat gradient (rocky intertidal into shallow subtidal) normally occupied by the species.

Growth and survivorship differed greatly among transplanted seeds, sprigs and plugs of surfgrass. Interestingly, growth was often inversely related to survivorship. Plugs, which as a group survived the best, grew the least, with medium and large-sized plugs actually losing leaves and showing little change in rhizome area over the duration of the study. In contrast, seedlings survived poorly, but those that did survive grew extremely well, increasing their leaf number by nearly four fold in six months. Survivorship and leaf growth for transplanted sprigs were intermediate between seedlings and plugs, while rhizome growth in transplanted sprigs was quite substantial. Similarly, there were great differences in recovery of donor plots from which sprigs and plugs were harvested for use in transplants. Re-growth of rhizomes that were cut to obtain sprigs was rapid, and at the end of six months had more than compensated for the harvesting loss, while harvesting of plugs led to substantial additional loss of surfgrass from the donor site as the holes experienced additional disturbance. The patterns of growth and survival of outplants and of recovery of donor plots, combined with the amount of effort involved, suggested that the largest gain in cover of rhizomes per unit of effort occurred when sprigs were used. Moreover, sprigs suitable for transplanting required relatively little effort to prepare and were relatively abundant at the study sites (Appendix V) suggesting that a minimal collection of sprigs for transplanting will not have a large impact to the existing *P. torreyi* beds. Collectively these features rendered sprigs the most acceptable form for use in restoration. It should be noted that sprig transplants compared to natural undisturbed sprigs over the same time period had very similar rhizome growth in the intertidal, yet sprig transplant only grew 1/3 as much as undisturbed sprigs in the subtidal (Appendix III).

Although the majority of attempts to restore seagrasses have involved the use of older life stages (Williams & Davis 1996), concerns about donor bed recovery and the fact that harvesting large numbers of early life stages (such as seeds) would have relatively little impact on donor populations have led to increased interest in the use of these stages for restoration (Balestri *et al*. 1998; Holbrook *et al*. 1999). Like many other seagrass species, *P. torreyi* can produce large numbers of seeds (up to 10,000 per square meter of surfgrass bed during the annual flowering season (Williams 1995). Typically, few seeds develop into seedlings (Stewart 1989; Williams 1995; Blanchette *et al*. 1999; Holbrook *et al*. 2000), and early survival of seedlings is poor (Blanchette *et al*. 1999; Holbrook *et al*. 2000). Studies of three seedling cohorts that recruited naturally to the intertidal at More Mesa Reef showed an exponential decline in survivorship similar to that which was observed in laboratory reared seedlings outplanted to the field (Appendix I). Several factors account for the low numbers of seedlings that become established in surfgrass populations. Many seeds are consumed by predators, either prior to their release from the spadix or afterwards (Holbrook *et al*. 2000). The turbulent physical conditions in the coastal habitats occupied by surfgrass result in losses of seedlings via dislodgment from their host plants or from abrasion by nearby plants (Blanchette *et al*. 1999). Burial by sand in the subtidal zone and desiccation in the intertidal zone are additional causes of mortality of young seedlings (Appendix I).

Recently-developed techniques for harvesting seeds of *P. torreyi,* and for laboratory storage, germination and propagation (Reed *et al*. 1998), make it possible to consider use of seedlings of this species for restoration, but concern now is whether the survival bottlenecks in the early life history stages can be overcome as part of the restoration strategy. The use of nylon line as an attachment host attempted to minimize dislodgement, a key element in recruitment (Blanchette *et al*. 1999). In my technique nearly 70% of the initial mortality in the intertidal after the first few days was due to the inability to secure the nylon line to the reef (Appendix II). However, of the nylon lines still secured to the reef after six months, I found that many seedlings were still attached to the nylon line even after the shoots were gone. This suggests that the primary factor contributing to the low survivorship in the intertidal (if the nylon line remained attached to the reef) appeared to be desiccation. Placement of the seedlings in areas cleared of algae protected them from abrasion from neighboring plants, but probably increased the desiccating effects of exposure during low tides. Only 12% of the seedlings survived after exposure during just two tidal cycles. Compared to natural recruits in the intertidal (which attach directly to algae that can be used as cover from effects of exposure) outplanted seedling survivorship was approximately three times lower over the same time period (Appendix I). Desiccation was not a factor for subtidal seedlings, but these also experienced rapid mortality, with fewer than 50% surviving after just 10 days. Unlike seedlings outplanted intertidal, only 30% of the initial mortality observed in the subtidal was due to removal of the nylon line (Appendix II). This suggests that I had better success in securing the nylon line to the reef in the subtidal compared to the intertidal. Dislodgement of seedlings from the nylon line appeared to be the major cause of loss of subtidal seedlings. It is possible that drift algae could have hooked the unattached arm of the fruit. Additional research is needed to develop methods to prevent losses of outplanted seedlings from desiccation and dislodgement for this technique to be effective in restoration.

The apparent sources of mortality of seedlings in my experiments contrast with results of several other studies that reported poor survival of transplanted seeds and seedlings of seagrasses due to consumption by crabs (Davis *et al*. 1998) and other consumers (Harwell & Orth 1999). An additional problem that I observed in my experiments was that after six months, outplanted seedlings had not formed adventitious roots [compared to naturally recruited seedlings which had rhizome growth over 4 cm (Appendix I)]. All surviving outplanted seedlings were still attached to the line at the end of the experiment. It may well be the case that the seedlings will have difficulty attaching to the rocky substrate and growing rhizomes while on the nylon line, but longer-term experiments would be required to determine this.

Harvesting of plant material from natural, healthy beds can be a large concern for restoration managers. While this is less of a concern for faster-growing species such as *Halodule wrightii* and *Syringodium filiforme,* for slower-growing species such as *Thalassia testudinum* and *P. torreyi* it would be unwise to collect large plugs of material for restoration. My results indicate that the concerns are well justified, because not only did plugs grow poorly, collecting from surfgrass beds resulted in additional damage to the existing population. The cut material on the outer edges of the holes became eroded and was easily removed from the rocky substrate when water motion was high. This had the result that the holes grew even larger after the plugs were removed. Even if the transplanted plugs had survived and grown well, the additional loss of surfgrass from the donor plot and the failure of any re-growth to occur in the donor areas after six months suggest that use of this life stage for restoration should be avoided. In addition, observations after eighteen months found that approximately only 55% of the area removed from donor plugs had recovered overall.

P. torreyi occurs along a depth gradient, from the intertidal to the shallow subtidal zone. As such, it encounters a wide range of physical conditions, and knowledge of the tolerance of the species to factors such as light, sedimentation, temperature, and susceptibility to dislodgement from water motion provides insight into possible restoration strategies. Not surprisingly, the efficacy of the three restoration methods that I tried varied considerably between the intertidal and subtidal. In general, survivorship and growth of transplants and the recovery of donor plots all tended to be higher in the subtidal than in the intertidal, and the effort expended in transplanting was also less in the subtidal. That all three methods of restoration varied consistently in this regard suggests that while restoration success in surfgrass is likely to vary along a depth gradient, it probably will not require that different techniques be used in different depths.

CONCLUSION

This research suggests successful restoration to increase vegetative growth of *P. torreyi* habitat is highly dependent on type of technique. Through evaluation of several potential restoration techniques, I found that transplanting of sprigs provided the highest net change in surfgrass habitat per unit effort. The outcome was affected by several factors that contributed to the overall change in habitat. Favorable growth and survival were significant elements, however, further investigation on cost-effectiveness and recovery of donor plots from which the sprigs and plugs were collected played important roles in evaluation of overall success. Furthermore, site selection along the gradient of surfgrass habitat also affected outcome with subtidal transplants outperforming intertidal transplants for the same technique. Additional tests need to be conducted to identify ways to increase survivorship of transplants due to physically harsh intertidal habitats and to assess long-term costs to host beds from intensive collections. Although I found results to be pertinent to my study sites, further experimentation is needed to evaluate performance under variable geographical locations.

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APPENDIX I

GROWTH AND SURVIVORSHIP OF NATURALLY RECRUITED SURFGRASS (*PHYLLOSPADIX TORREYI*) SEEDLINGS AT A ROCKY INTERTIDAL REEF

METHODS

I followed the survivorship and growth of naturally occurring surfgrass seedlings *(Phyllospadix torreyi)* to obtain a baseline for comparison to experimentally outplanted seedlings and investigate recovery following natural disturbance. The study was conducted at More Mesa Reef (see Chapter 1 for site description). Field surveys of the *P. torreyi* population at this site were conducted from 1994 to 2001 as part of a larger study to gather baseline data on the natural distribution of *P. torreyi* and algae. One 25 m long transect was established running parallel to the coastline at about -0.07 feet mean low water level. The area was sampled using a point contact method each summer and winter to assess *P. torreyi* percent cover. Surveys in winter 1994 indicated that the area within the study area was densely covered in *P. torreyi* with up to 71.4% covered by leaves (Reed and Holbrook, unpublished data). However, high sand flux from December 1995 to June 1999, which entirely buried *P. torreyi* and algae in sand, potentially led to the mortality of all existing *P. torreyi* in the study area. This was the basis for my census to investigate natural recovery of *P. torreyi* in the absence of sand in the following years.

A 25 m long x 12 m wide area was selected within the survey area and stainless steel eyebolts (5/16" X 3") were screwed into the substrate at each of the four corners. Naturally recruited *P. torreyi* seedlings were mapped within the designated area. Distances were then taken from each seedling to at least two permanent bolts in order to relocate the seedling at later (census) dates. Survivorship and growth of three cohorts of seedlings recruited to the study area were followed from December 1998 to June 2001. The first census in December 1998 found 30 naturally recruited seedlings in the vicinity of the experimental area (98-99 cohort). The young seedlings at this time had between 11 to 58 leaves (mean 29.5 +/- 4.1) and were assumed to be of the same cohort, recruited within that year. Unfortunately, seedlings within this cohort became entirely buried in sand soon after census. Mean depth of sand covering the seedlings was measured and persisted for 162 days (Figure 1). Subsequently the sand never returned. The second census mapped 148 total new naturally recruited seedlings one year later in January 2000 (99-00 cohort) with <5 leaves per seedling. The most recent census found a total of 194 new *P. torreyi* seedling recruits to the study area in December 2000 (00-01 cohort) and had on average 3.06 +/- 0.2 leaves per seedling. Based on my laboratory observations of seedling growth I assumed seedlings for each of the last two censuses were all from the same cohort and were no more than one-month post germination.

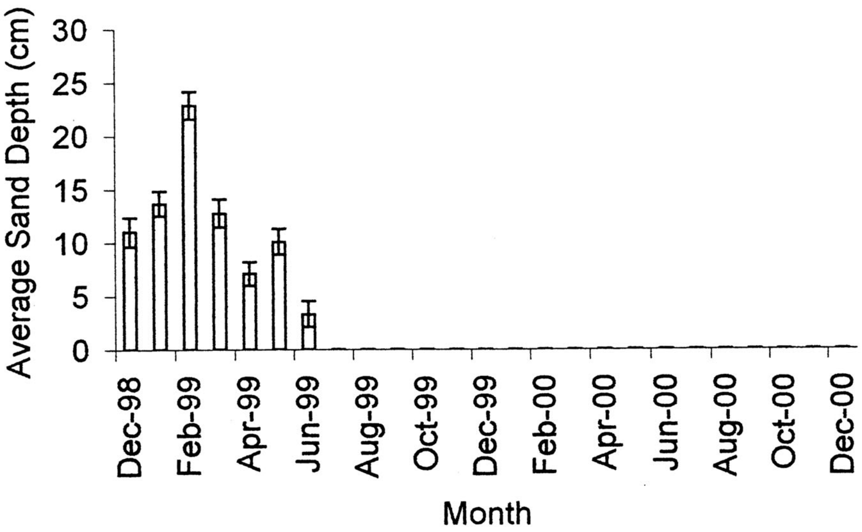

Figure 1. Average depth of sand covering 1998-1999 seedling cohort at More Mesa from December 1998 to December 2000.

Data on individual seedling survivorship and growth for each of the three cohorts were collected periodically through June 2001. Survivorship was recorded by presence or absence of each seedling at each census date. Seedlings were considered dead if no leaves were present over two consecutive sample periods or if the seedling was missing. Growth of surviving *P. torreyi* seedlings was measured by mean leaf length, longest leaf length, the number of leaves per seedling, and mean rhizome length.

RESULTS

Survivorship for naturally occurring seedlings in the 98-99 cohort showed dramatic reduction in survivorship with only 40% remaining after the first 70 days (Figure 2a). However, after nearly 220 days, survivorship leveled off at 8% at the end of the study. A similar initial reduction in survivorship was observed for the 99-00 and 00-01 cohorts with approximately 40% survivorship after 100 and 75 days of the initial survey, respectively (Figure 2b, 2c). Survivorship also tapered off with 18% remaining at the end of the study after 550 days for the 99-00 cohort and 25% after nearly 200 days for the 00-01 cohort. Mortality of seedlings is potentially due to dislodgement or desiccation. Seedlings observed to have the majority of their leaves brown and dry did not survive the subsequent census. Naturally occurring seedling recruits that survived typically were firmly attached to the base of the stipe of the host algae. This allowed the fruit to stay moist and covered from harmful sunlight during extreme low tides.

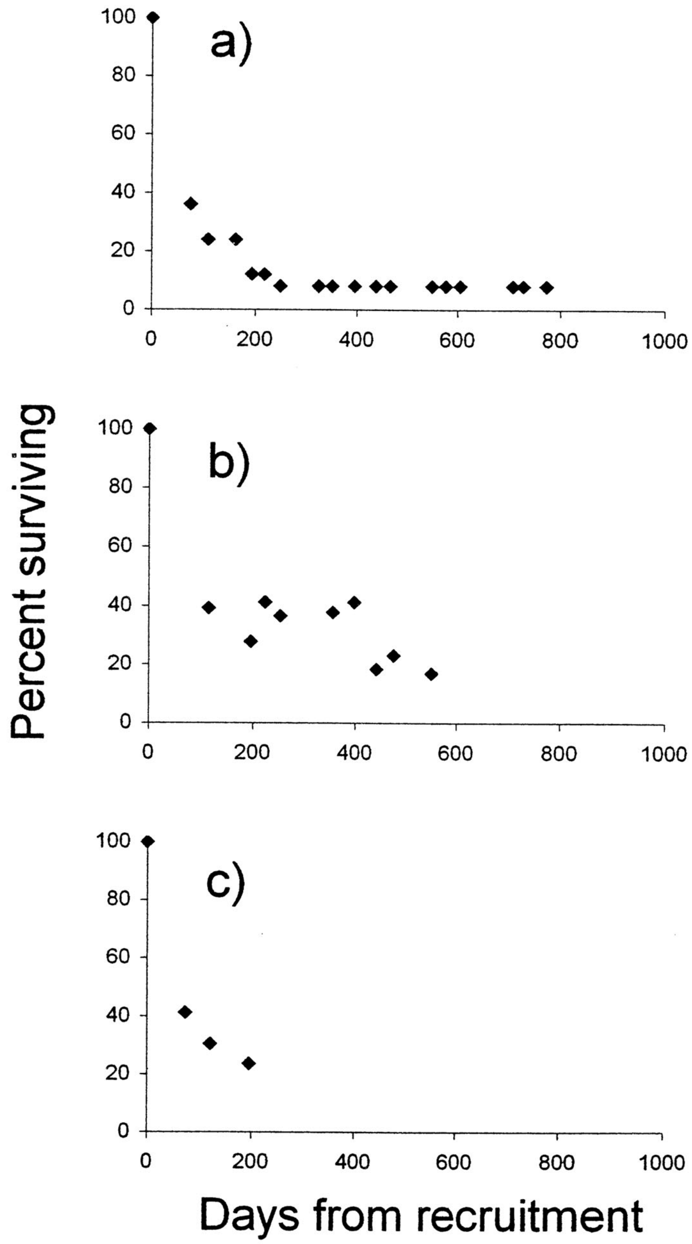

Figure 2. Percent survivorship of natural *P. torreyi* seedling recruits on host algae for a) 98-99 cohort, b) 99-00 cohort, and b) 00-01 cohort at More Mesa Reef study site.

Surviving seedlings grew rhizomes at an exponential rate. The mean rhizome length of all cohorts at first census was <0.5 cm yet after 400 days, mean rhizome length of the 98-99 cohort was approximately 12 cm and grew to over 40 centimeters after nearly 800 days. The mean rhizome length of the 99-00 cohort quadruped after 400 days (Figure 3b). Likewise, rhizome length doubled after 200 days for the 00-01 cohort (Figure 3c). Although only rhizome length was measured, width also increased dramatically as the rhizome bifurcated rapidly. Mean number of leaves per seedling also grew substantially over time at an exponential rate (Figure 4). Surprisingly, the one surviving seedling had over 1000 leaves after 800 days (Figure 4a). Mean leaf length and longest leaf length were similar between cohorts (Figure 5 and Figure 6). Growth was linear over time yet varied through censuses with seasonal declines during the fall and winter when low tides occur in the afternoon. Rhizome length had significant linear correlation with number of leaves ($p < 0.0001$) for all cohorts (Figure 7).

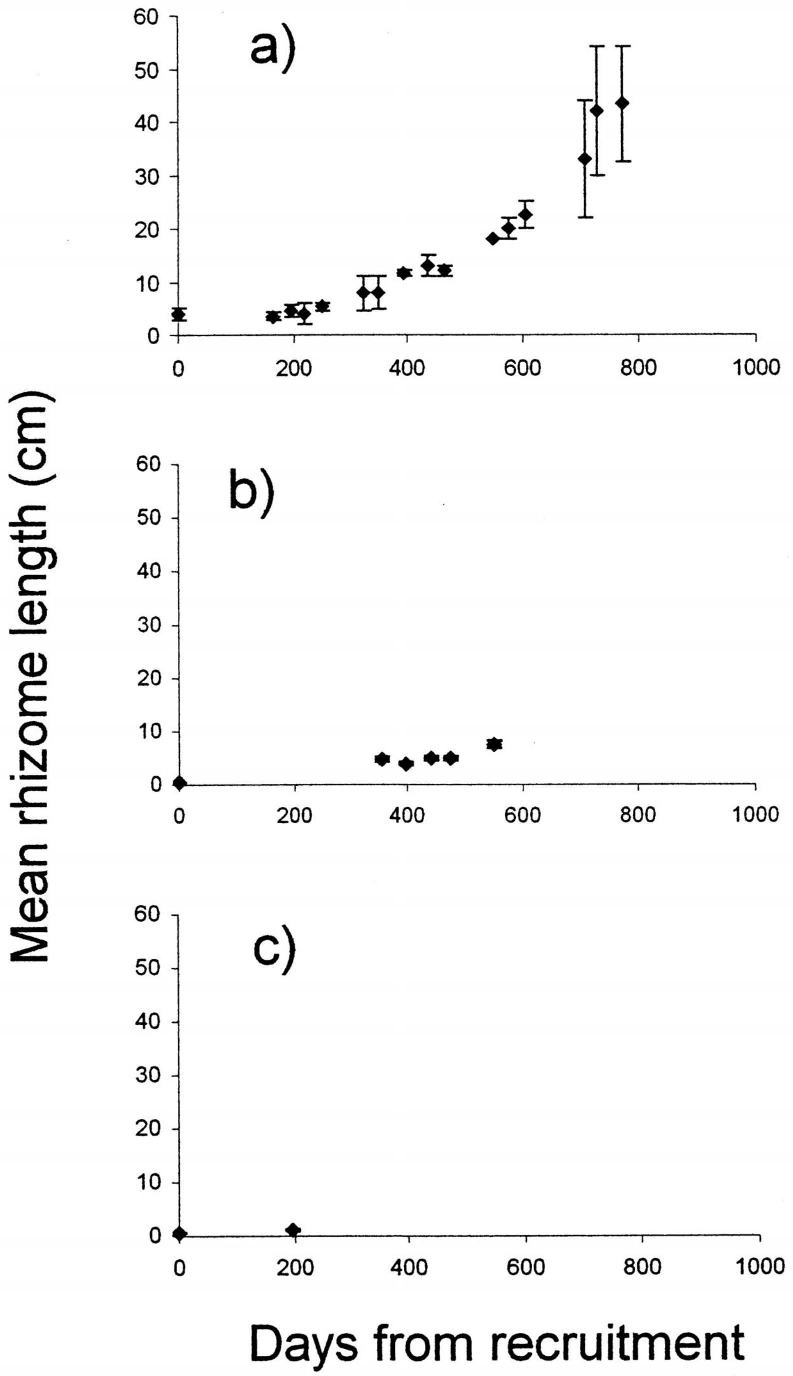

Figure 3. Mean rhizome length of natural *P. torreyi* seedling recruits (+/- SE) on host algae for a) 98-99 cohort, b) 99-00 cohort, and b) 00-01 cohort at More Mesa Reef study site.

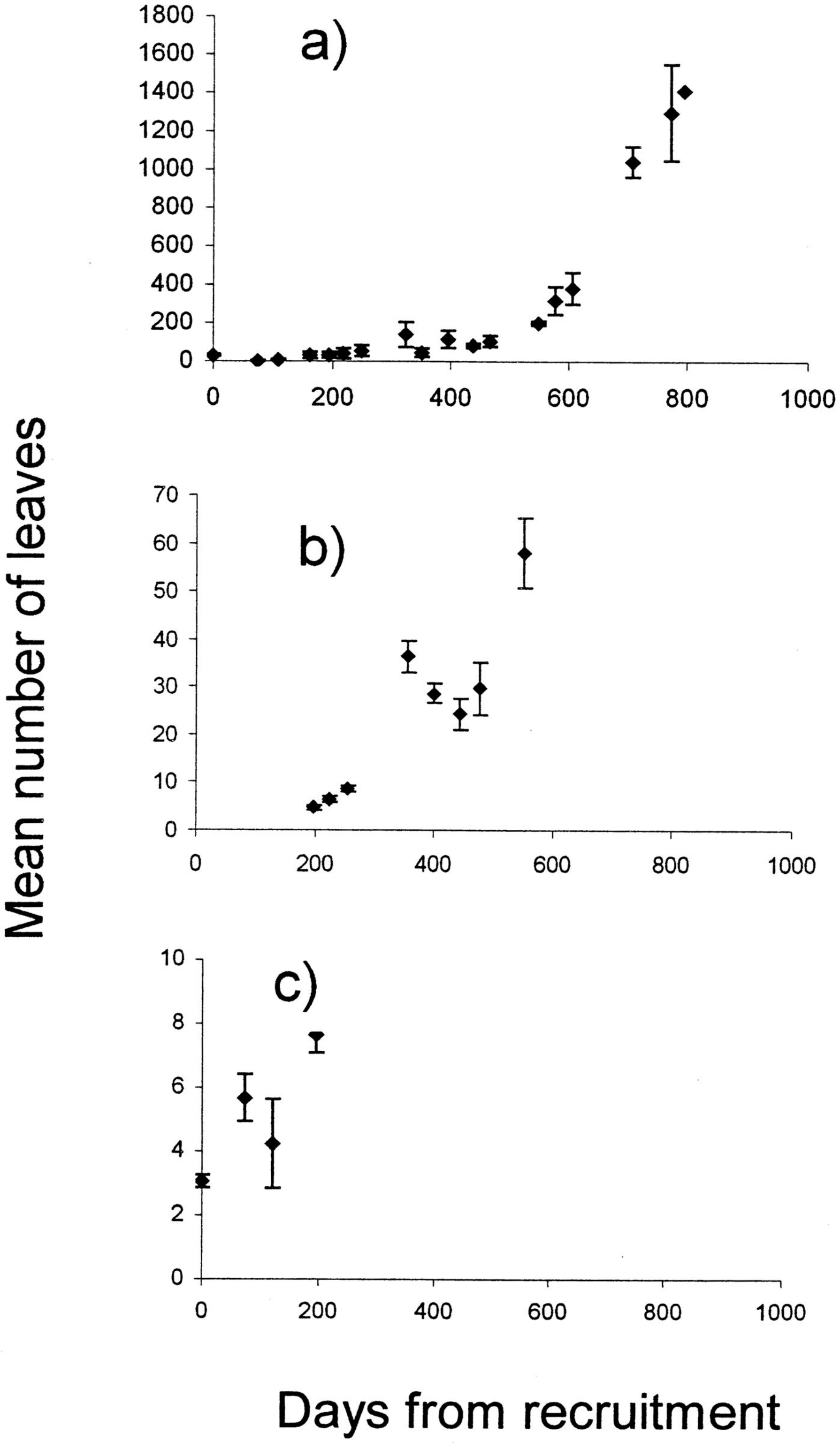

Figure 4. Mean number of leaves (+/- SE) on natural *P. torreyi* seedling recruits on host algae for a) 98-99 cohort, b) 99-00 cohort, and b) 00-01 cohort at More Mesa Reef study site.

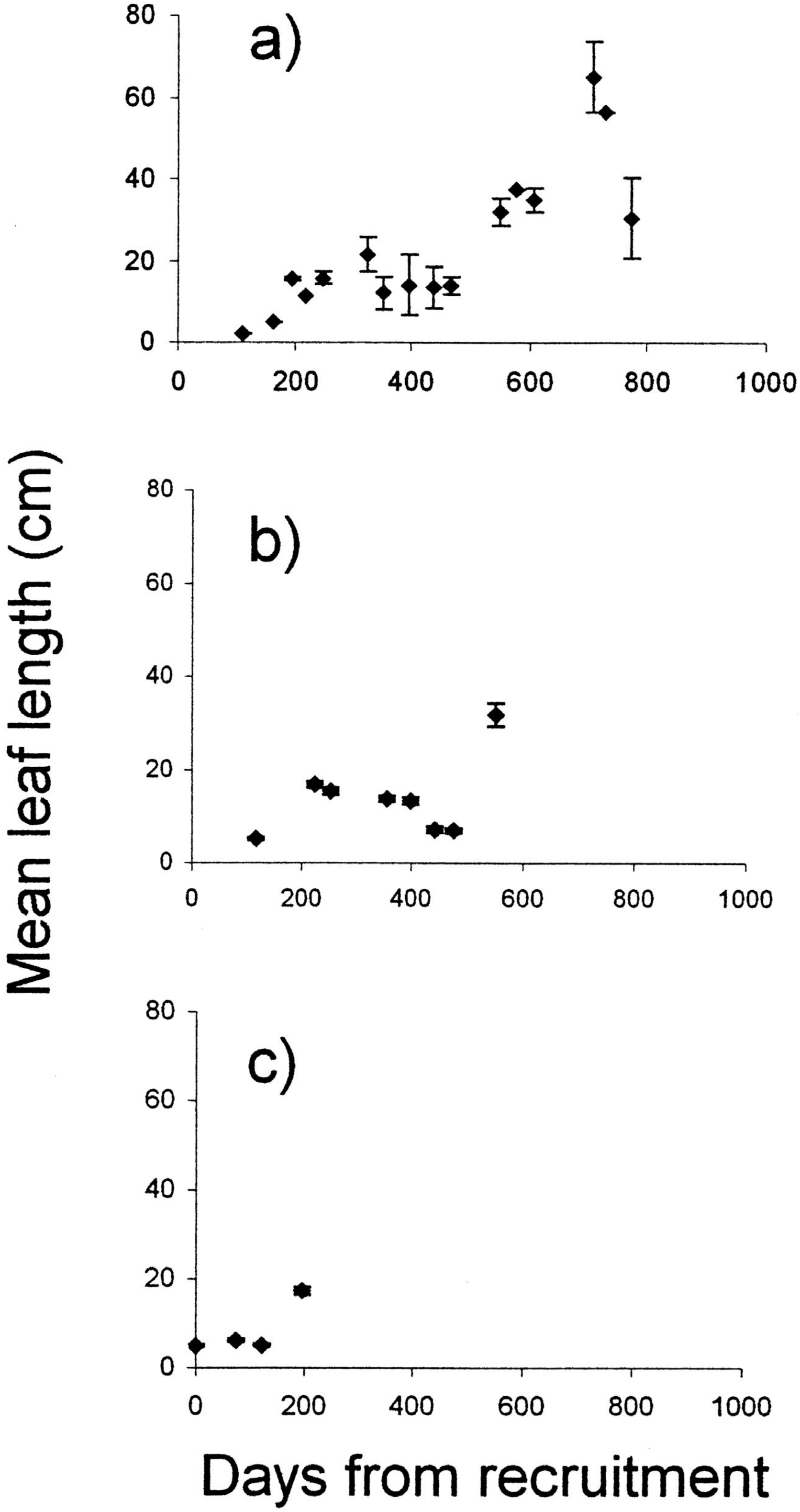

Figure 5. Mean length of leaves on natural *P. torreyi* seedling recruits (+/- SE) on host algae for a) 98-99 cohort, b) 99-00 cohort, and b) 00-01 cohort at More Mesa Reef study site.

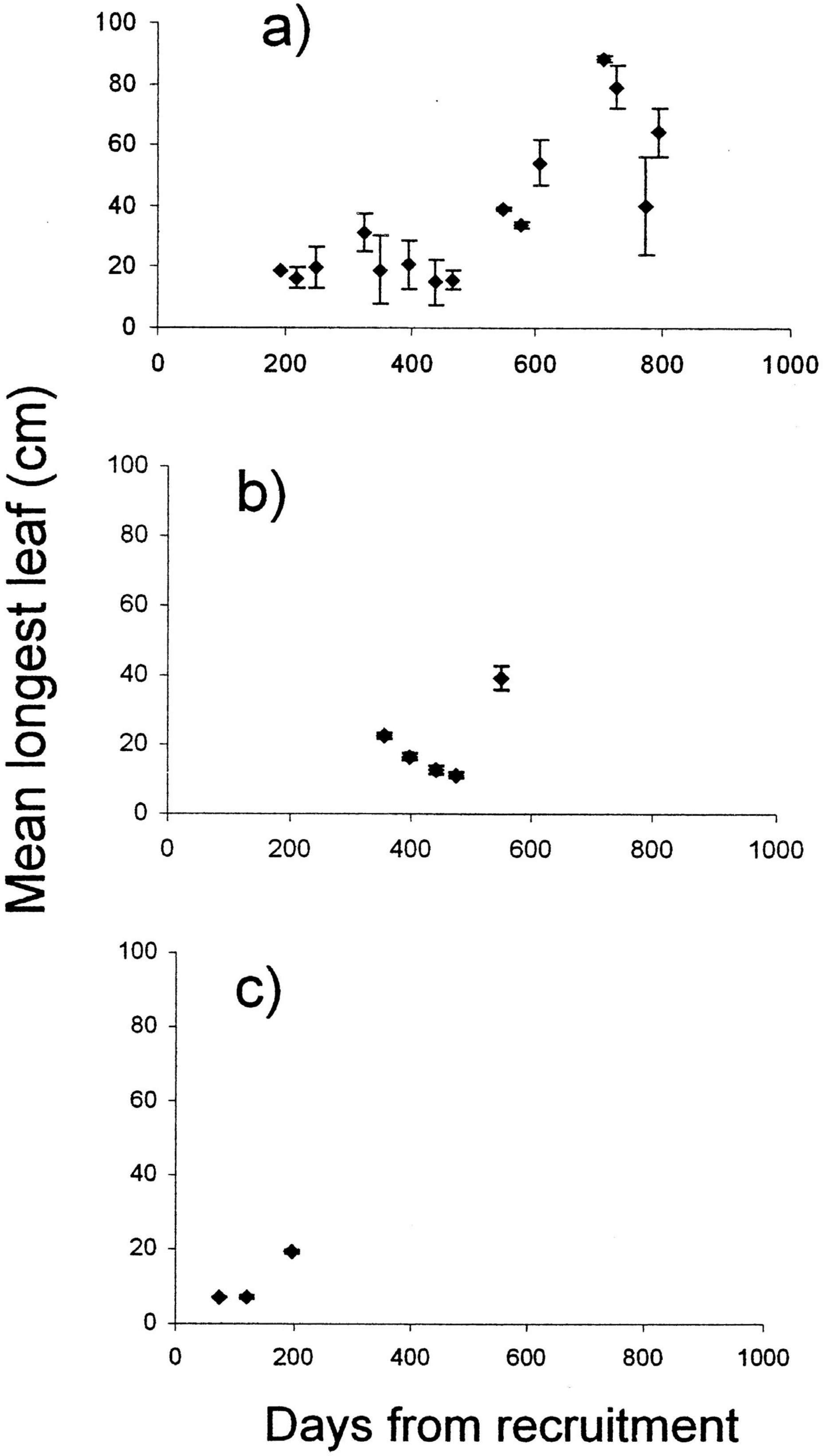

Figure 6. Mean longest leaf length on natural *P. torreyi* seedling recruits (+/- SE) on host algae for a) 98-99 cohort, b) 99-00 cohort, and b) 00-01 cohort at More Mesa Reef study site.

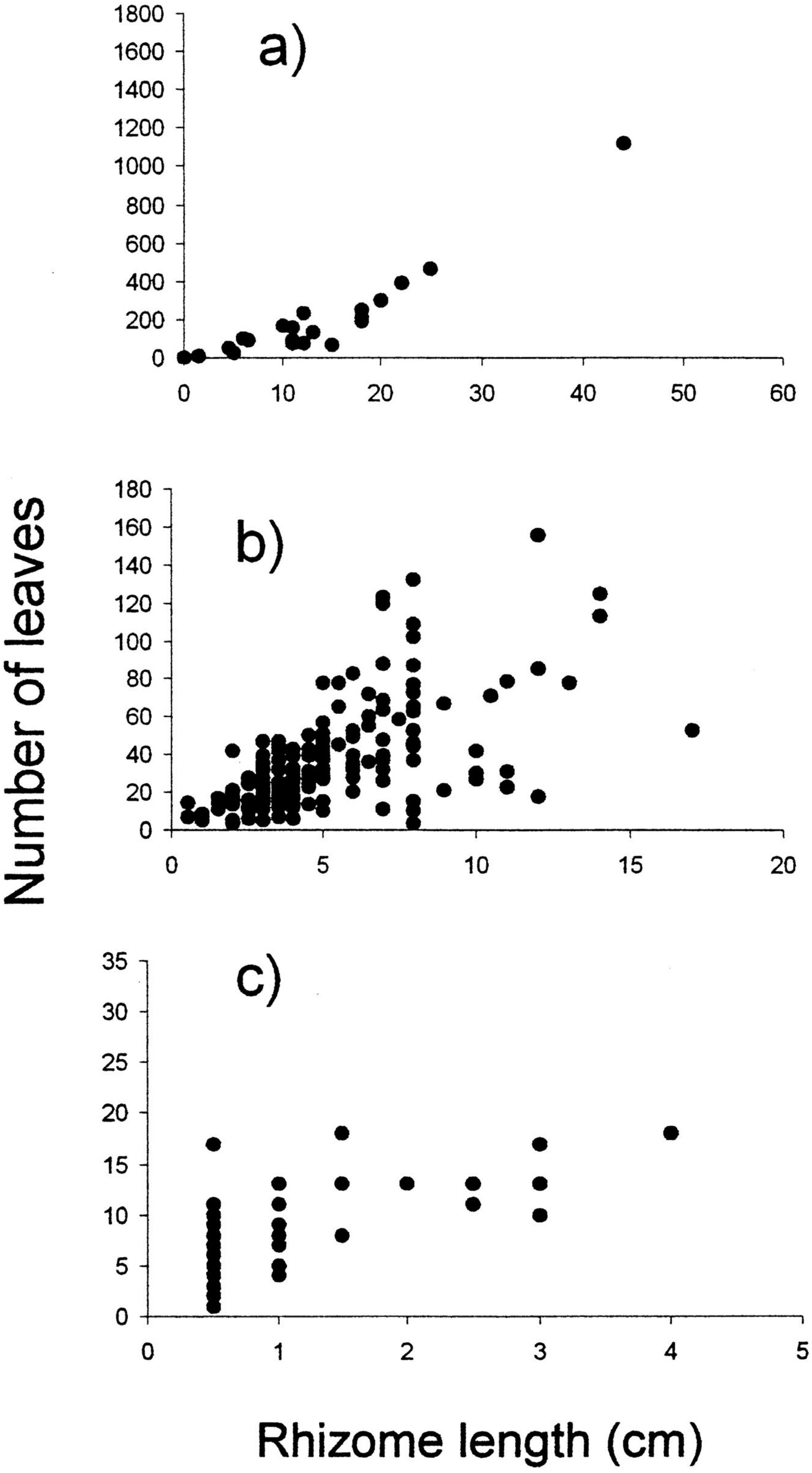

Figure 7. Rhizome length vs. number of leaves for natural *P. torreyi* seedling recruits on host algae for a) 98-99 cohort, b) 99-00 cohort, and b) 00-01 cohort at More Mesa Reef study site.

APPENDIX II

GROWTH AND SURVIVORSHIP OF SURFGRASS (*PHYLLOSPADIX TORREYI*) SEEDLINGS OUTPLANTED ON EPOXY BEADS

METHODS

I tested direct attachment of surfgrass seedlings *(Phyllospadix torreyi)* set in epoxy in the laboratory and transplanted to the field to evaluate this method as a viable technique for restoration. This experiment coincided with that involving seedlings outplanted on nylon lines (see Chapter 1). Here I attempted to minimize seedling dislodgement in the field by embedding seedlings in marine epoxy prior to outplant. Seedlings were collected in the field and reared in the laboratory as described in Chapter 1. Viable seedlings were selected and embedded in a small "bead" of marine epoxy (1 cm in diameter) in the laboratory using fine forceps such that the emergent leaves from the seedling were not covered by the epoxy. A small "bead" was formed around the exocarp of the fruit which held it to the epoxy (Figure 1). The epoxy and seedling unit is referred to as a seedling bead. Seedling beads were then placed in a flow through seawater tank to keep the water clean while the epoxy hardened. Once dry, the seedling beads were stored in the laboratory under the same conditions as the seedlings on nylon line described in Chapter 1 before outplant. In November 2000, seedling beads were outplanted to 30 cm x 30 cm experimental plots at intertidal and subtidal study sites (More Mesa Reef and Mohawk Reef, respectively; see Chapter 1 for site descriptions). Seedling beads were fastened to the reef using small amounts of Z-Spar A788 marine epoxy putty. Efforts were taken to ensure that the shoots of seedlings were upright. Eighteen seedlings were outplanted in a uniform grid to each of 12 experimental plots at each of the two sites adjacent to healthy stands of surfgrass. Branches from surrounding algae were clipped to inhibit dislodgement of seedlings by whipping.

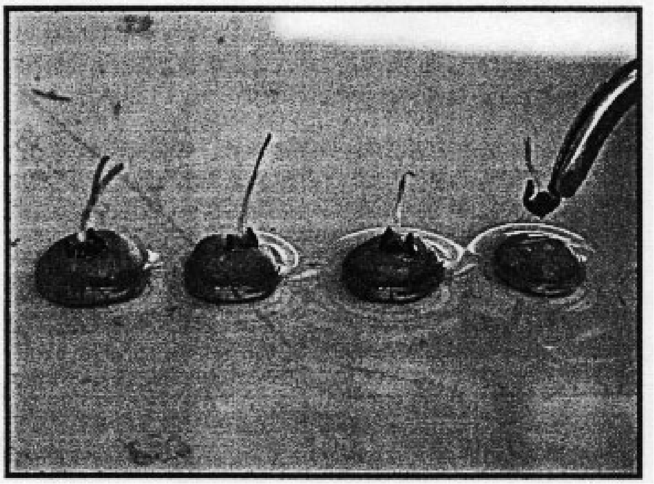

Figure 1. Seedlings are firmly attached in the laboratory to the artificial substrate host "beads" made of marine epoxy prior to outplant.

The efficacy of this method of restoration was evaluated using data collected on survivorship and growth of the seedling outplants after six months. Mortality of seedlings occurred either by 1) the inability to secure the seedling bead to the reef, 2) seedling death in place (loss of leaves with fruit still attached) or 3) dislodgment of the seedling directly from the bead. Survivorship of seedling beads at More Mesa and Mohawk was followed for over 200 days. To assess early sources of mortality, outplanted seedlings were surveyed after 13 days for subtidal outplants and 8 days for intertidal outplants. Data from the seedlings outplanted on nylon lines are included for comparison (see Chapter 1 for methods). Data collected, however, did not distinguish between seedlings that had died in place or were removed directly from the bead. The growth of surviving outplanted seedlings was evaluated by leaf counts per surviving seedling and calculating mean leaf length from measurements. Potential causes for mortality were evaluated after the first census date. I noted if the seedling was either dislodged (from the bead or dead in place) or if the z-spar failed to secure the seedling bead to the reef.

RESULTS

For both the More Mesa and Mohawk seedling bead outplants, a sharp decline in survivorship was seen after the first 90 days (Figure 2). After two hundred days very few seedlings remained regardless of the site. As time progressed, mortality decreased suggesting that the seedlings' survivorship would eventually level off at some point yet overall survivorship for this technique was poor.

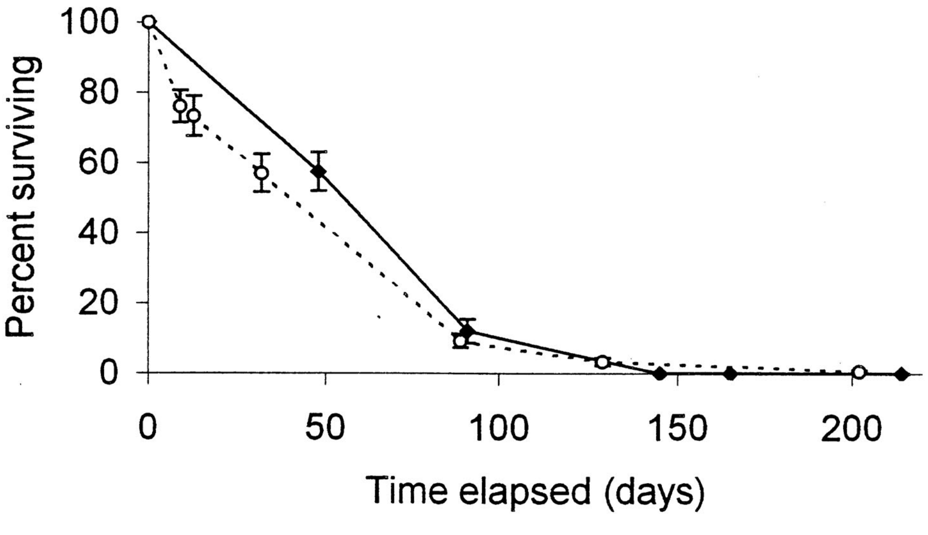

Figure 2. Percent surviving (+/- SE) of outplanted seedlings on epoxy beads to More Mesa Reef (hatched) and Mohawk Reef (solid line) study sites. Data are shown for 12 groups of 18 seedlings.

The primary cause of mortality of seedlings was different depending on type of technique and location outplanted (Figure 3). A greater percentage of seedlings died in place or were removed directly from the bead outplants at More Mesa Reef. However, seedlings outplanted on nylon line had higher mortality at More Mesa due to the inability to secure the line to the reef. Seedlings outplanted on beads were more susceptible to desiccation which dried seedlings in place at More

Mesa and resulted in a higher mortality. Nylon lines had more drag and potentially were more easily removed during periods of high water flow. In addition, individual seedlings on nylon lines outplanted to Mohawk Reef could be more susceptible to removal by drift algae or whipping of unclipped adjacent algae that hooked the second barb of the fruit and broke the seed from the bead or nylon line.

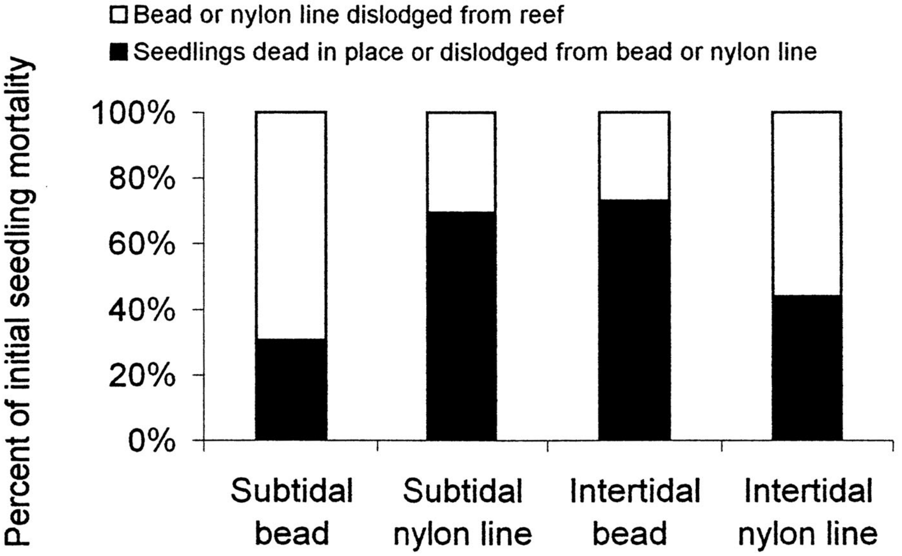

Figure 3. Initial sources of mortality in seedling outplant experiments. Sources of mortality of outplanted seedlings were either by the inability of the seedling bead or nylon line to secure to the reef or the seedling dying in place or being dislodged from the bead or nylon line.

Growth of seedlings based on new leaves and mean leaf length was dependent on site outplanted. After 200 days, seedlings outplanted to the subtidal site at Mohawk Reef on average developed slightly more leaves and were approximately 10 cm longer than seedlings outplanted to the intertidal site at More Mesa (Figure 4 and Figure 5). It should be noted that mean values for seedlings in the later portion of the experiment were calculated from very low numbers (1 seedling and 3 seedlings for Mohawk and More Mesa, respectively).

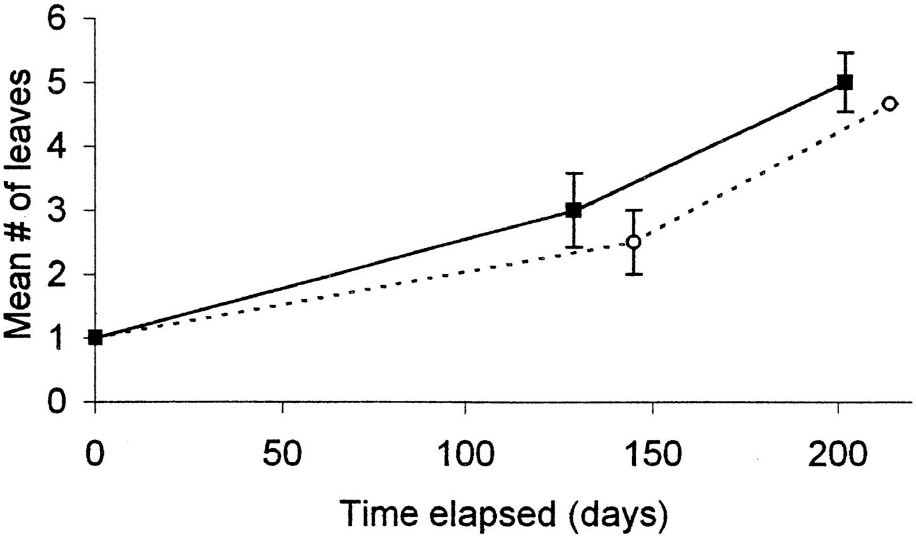

Figure 4. Mean development of leaves (+/- SE) of outplanted seedlings on epoxy beads to More Mesa Reef (hatched) and Mohawk Reef (solid line) study sites.

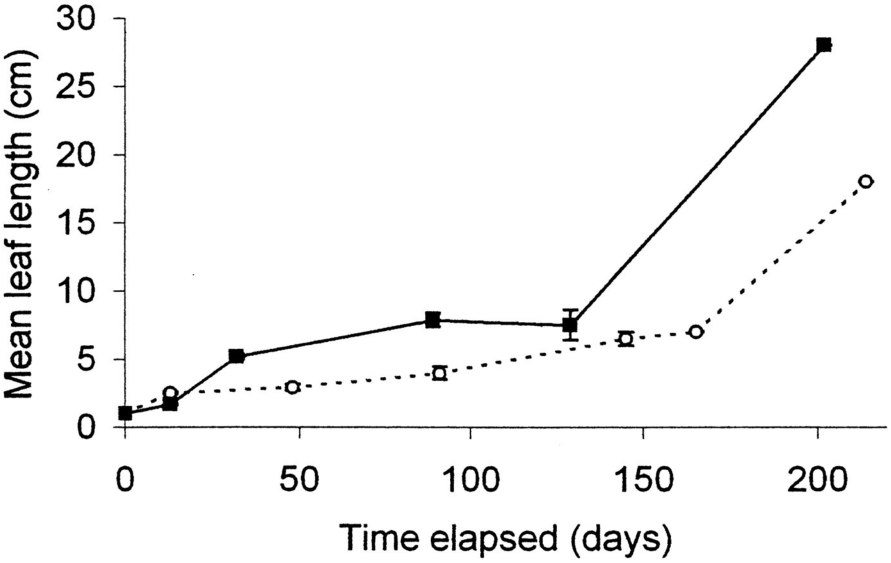

Figure 5. Mean leaf length (+/- SE) of outplanted seedlings on epoxy beads to More Mesa Reef (hatched) and Mohawk Reef (solid line) study sites.

APPENDIX III

GROWTH COMPARISON OF TRANSPLANTED AND UNDISTURBED SURFGRASS (*PHYLLOSPADIX TORREYI*) RHIZOME SPRIGS

METHODS

To estimate natural growth of undisturbed *Phyllospadix torreyi* rhizomes and associated leaves and compare growth to transplanted sprigs in Chapter 1, I marked undisturbed rhizomes and monitored growth for approximately six months. Experiments were initiated in both summer and winter for seasonal comparisons. For summer experiments, undisturbed rhizome sprigs (N=11), were mapped in May 2000 at More Mesa Reef (Chapter 1 for site description) which coincided with the harvest and transplantation of sprigs (N=20) from established beds to adjacent areas lacking surfgrass. The experiment was also replicated in the subtidal study site at Mohawk Reef (see Chapter 1 for site description) in June 2000 with 15 and 30 replicates for undisturbed and transplanted sprigs, respectively. Transplanted sprigs were harvested and secured to the reef as described in Chapter 1. A reference marker was glued to the reef next to each undisturbed and transplanted sprig to aid in relocating them for purposes of measuring growth. Mean increase in rhizome area was estimated as the length of new rhizome (measured to nearest ½ cm) that had grown from the end of the undisturbed rhizome sprig or transplant, multiplied by the rhizome width (typically 1 cm). Only leaves associated with new growth of the rhizome were counted. The experiments were followed for approximately six months.

To evaluate variation in rhizome and leaf growth between seasons and compare rhizome growth of transplanted rhizome sprigs conducted in Chapter 1, the experiment was repeated in November 2000. Undisturbed rhizome sprigs were marked at More Mesa Reef and Mohawk Reef (N=21 and 10, respectively) and sprigs were transplanted to adjacent areas (N=42 for both sites). Increase in rhizome area and leaves associated with rhizome growth were followed for approximately six months.

RESULTS

Differences in rhizome growth of undisturbed and transplanted sprigs varied inconsistently between sites and seasons. Summer More Mesa sprigs (Figure lb) and winter Mohawk sprigs (Figure 1c) had virtually no difference in rhizome growth. However, winter More Mesa (Figure la) and summer Mohawk (Figure 1d) undisturbed rhizome sprigs had approximately 3 cm^2 greater area after approximately 175 days. The highest growth occurred for both undisturbed and transplanted sprigs at Mohawk Reef during the summer season study.

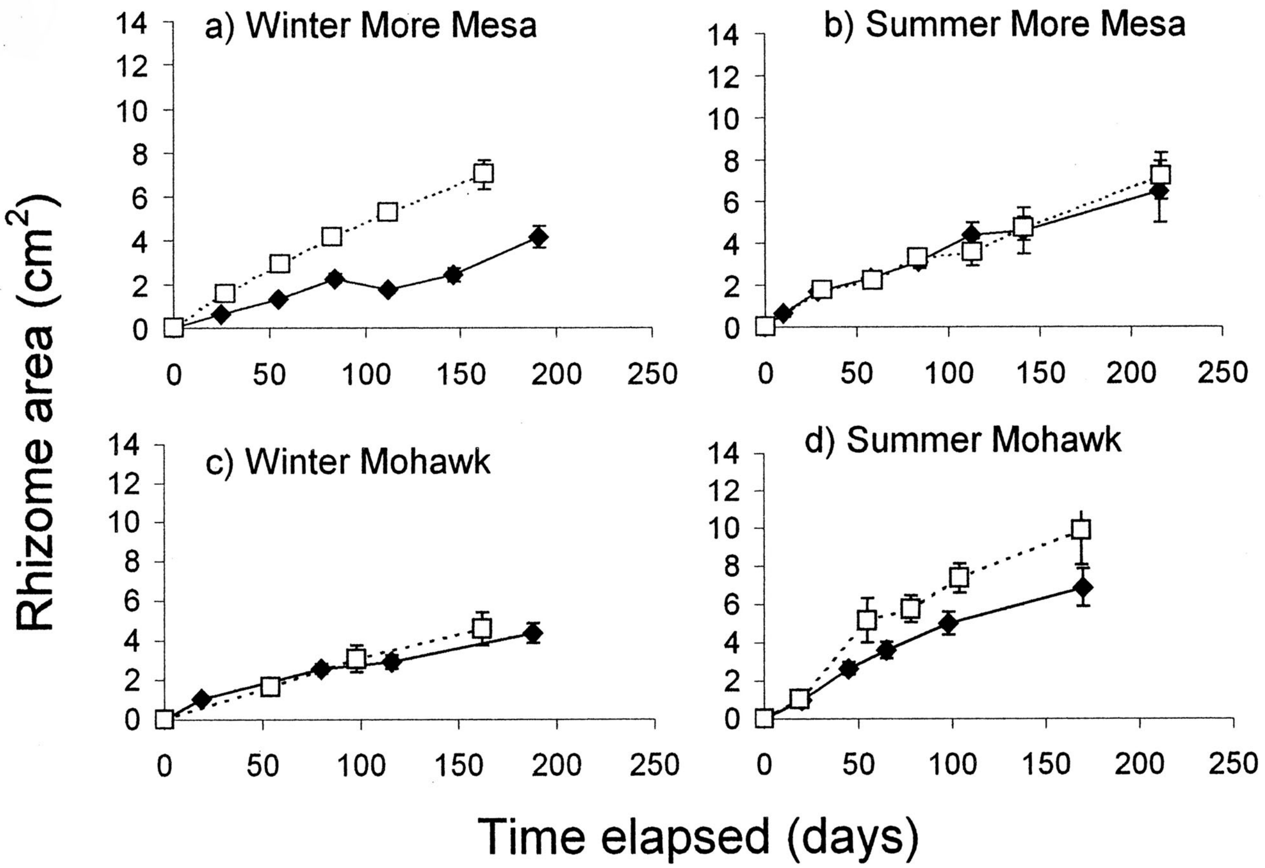

Figure 1. New rhizome area for transplant and undisturbed rhizome sprigs during a) winter More Mesa study sprigs (mean +/- 1 SE, N = 42 and 21 for each treatment, respectively), b) summer More Mesa study sprigs (mean +/- 1 SE, N = 20 and 11 for each treatment, respectively), c) winter Mohawk study sprigs (mean +/- 1 SE, N = 42 and 10 for each treatment, respectively) d) summer Mohawk study sprigs (mean +/- 1 SE, N = 30 and 15 for each treatment, respectively). Hatched line is data for undisturbed rhizome sprigs, and solid line is data for transplanted rhizome sprigs.

Mean new number of leaves associated with new rhizome growth was similar across transplants and undisturbed sprigs regardless of season (Figure 2). The highest growth of new leaves occurred for summer More Mesa sprigs with approximately 50 new leaves for both transplant and undisturbed rhizomes after 200 days (Figure 2b). Winter Mohawk sprigs developed fewest leaves with only approximately 20 leaves after 150 days (Figure 2c).

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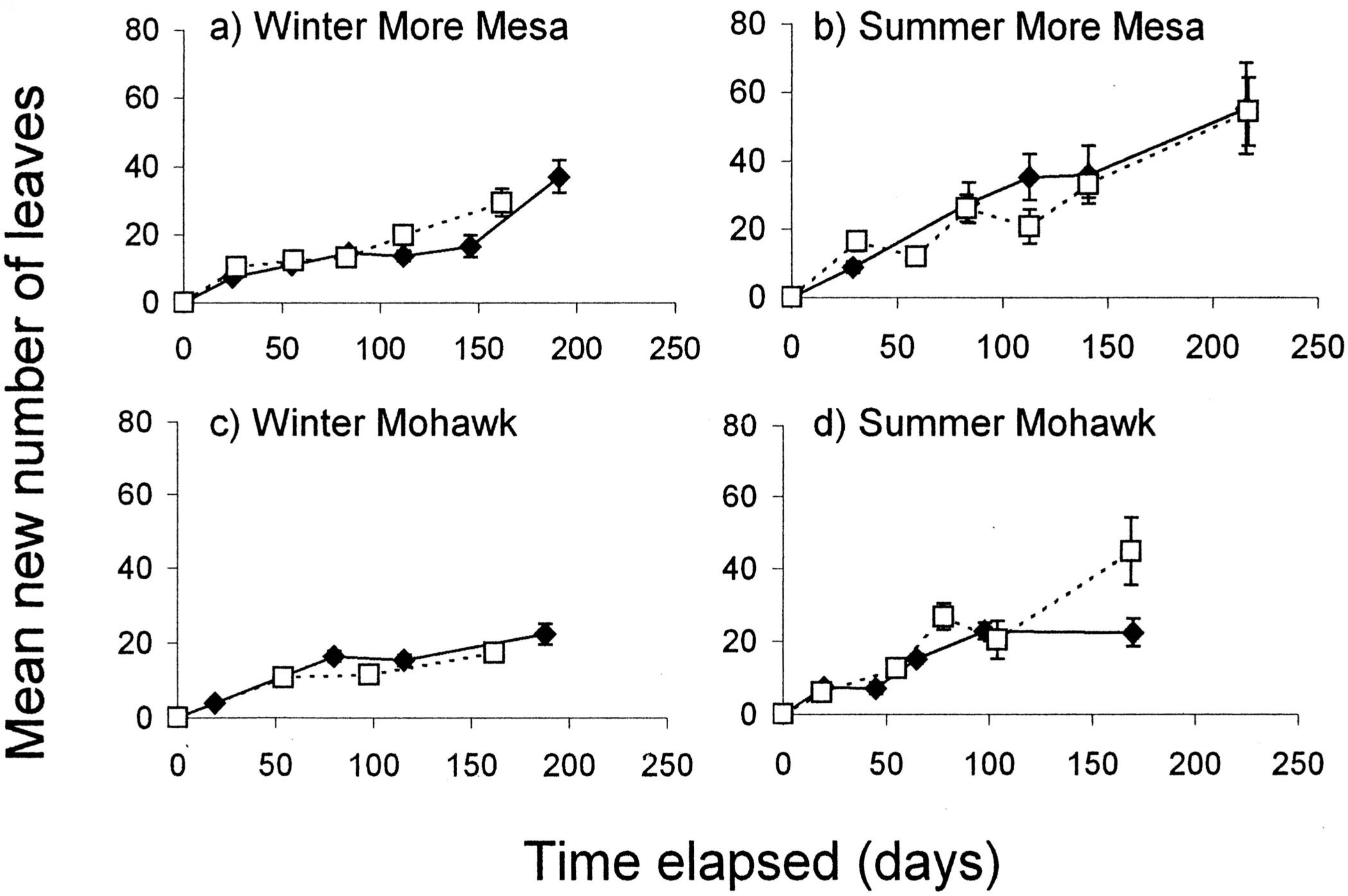

Figure 2. Mean increase in number of leaves for transplant and undisturbed rhizomes for a) winter More Mesa study sprigs (mean +/- 1 SE, b) summer More Mesa study sprigs (mean +/- 1 SE), c) winter Mohawk study sprigs (mean +/- 1 SE), d) summer Mohawk study sprigs (mean +/- 1 SE). Hatched line is data for undisturbed rhizome sprigs, and solid line is data for transplanted rhizome sprigs.

APPENDIX IV

SURVIVORSHIP OF SURFGRASS (*PHYLLOSPADIX TORREYI*) SEEDLING LABORATORY CONTROLS

METHODS

To evaluate the effect of nylon line or epoxy on the early survivorship of *Phyllospadix torreyi* seedlings, seedling controls were grown in the laboratory. The experiment investigated the consequences of using material to attach seedlings to the reef and stresses from transport of outplant units in the field, Treatment controls consisted of (l) loose seedlings with no material, (2) loose seedlings with an equal number of loose epoxy beads, approximately 1 cm in diameter (3) loose seedlings with an equal number of 5 cm lengths of loose nylon line, (4) seedlings attached to 5 cm lengths of nylon line (see Chapter 1 for description), (5) seedlings embedded in epoxy beads (see Appendix III for description), (6) seedlings attached to nylon line brought to Mohawk Reef under similar transport stress as outplants and returned to laboratory culture, (7) seedlings attached to epoxy beads brought to Mohawk Reef under similar transport stress as outplants and returned to laboratory culture, (8) seedlings attached to epoxy beads brought to More Mesa Reef under similar transport stress as outplants and returned to laboratory culture, and (9) seedlings on nylon line brought to More Mesa Reef under similar transport stress as outplants and returned to laboratory culture. Seedlings were collected in the field and reared in the laboratory as described in Chapter 1. Six replicate groups of 20 seedlings per treatment were placed in culture dishes with 50 ml of sterile filtered seawater (0.2µm) enriched with 50ul nutrient stock solution (Provosoli 1968) for each of the nine controls. The culture dishes were maintained at 15°C at an irradiance of 40-50 µE m^{-2} s^{-1} using a 14:10 h L:D photoperiod under mild agitation. All seedlings were rinsed with dionized water and the culture media were changed on a weekly basis. Seedling survivorship was assessed after two months.

RESULTS

Seedling survivorship was relatively high for all laboratory control treatments after a two-month period (Table 1). Most treatments had over 95% survivorship with no direct correlation to a particular material. In addition, a certain percentage can be expected to not survive even under optimal growing conditions. These data demonstrate that the use of nylon line or epoxy used to attach the seedlings to the reef as a potential restoration technique has no negative effects on early seedling mortality.

Table 1. Two-month percent survivorship of laboratory controls. Data are 20 seedlings per treatment (n=6 replicates per treatment).

Treatment	Survivorship
Loose seedlings	93.33%
Loose seedlings with epoxy bead	99.17%
Loose seedlings with nylon line	98.33%
Seedlings attached to nylon line	95.83%
Seedlings embedded in epoxy	95.83%
Transported seedlings attached to nylon line to subtidal	95.00%
Transported seedlings embedded in epoxy to subtidal	93.33%
Transported seedlings embedded in epoxy to intertidal	90.83%
Transported seedlings attached to nylon line to intertidal	98.33%

APPENDIX V

THE AVAILABILITY OF SURFGRASS (*PHYLLOSPADIX TORREYI*) SPRIGS FOR TRANSPLANTING IN AN INTERTIDAL AND SUBTIDAL ROCKY REEF

METHODS

To assess the availability *of Phyllospadix torreyi* rhizome tips for use as sprigs for restoration, I conducted surveys of the density of rhizome sprigs in intertidal and subtidal habitats in March 2001. At Mohawk Reef and More Mesa Reef (subtidal and intertidal habitats, respectively, see Chapter 1 for site descriptions), I placed a meter ruler haphazardly next to the edge of the existing *P. torreyi* bed and counted the number of sprigs within 10 cm of the meter rule. Forty-six 1 m x 10 cm areas were sampled along approximately two hundred meters parallel to the shore at Mohawk Reef and eighteen 1 m x 10 cm areas were sampled along approximately one hundred meters at More Mesa Reef. The sample areas at each site were not contiguous and most were greater than 10 meters apart.

RESULTS

I found that Mohawk Reef had slightly more sprigs than More Mesa, with an average of 10.1 +/- 0.5 sprigs per linear meter compared to 8.1 +/- 1.2, respectively.

APPENDIX VI

LEAF AND RHIZOME BIOMASS OF SURFGRASS (*PHYLLOSPADIX TORREYI*) RHIZOME SPRIGS USED IN RESTORATION EXPERIMENTS

METHODS

To provide a means to assess biomass production of *P. torreyi* sprigs transplants, I harvested sprig rhizomes (>5cm) and the attached leaf material for analysis in April 2001. This study interprets restoration growth measured by rhizome primary cover and new number of leaf counts in Chapter 1, in terms of biomass production per 1 cm^2 rhizome and biomass production for 1 cm leaf lengths. Sprigs were harvested from established subtidal beds at Mohawk Reef (see Chapter 1 for site description) by removing an unbranched tip from an actively growing rhizome with a sharp knife without damaging the root hairs. Nineteen samples were collected in the field and brought to the laboratory. Each harvested sprig was cut in the laboratory to exactly 5 cm measured from the cut end to the base of the emerging shoots at the apical end of the rhizome. The width of each rhizome sprig was 1 cm for all harvested sprigs. Data recorded for each 5 cm sprig included number of leaves, longest leaf length (in centimeters), and estimated average leaf length (in centimeters). Leaves were then cut from the base of the rhizome and wet weights (in grams) were recorded for the rhizome sprigs and the leaves. Mean biomass of a 1 cm^2 rhizome sprig was calculated by:

Wet weight of rhizome/ (total rhizome length x width).

The mean biomass of a 1 cm leaf length was calculated by:

Wet weight of leaves/ (total # leaves x average leaf length).

RESULTS

I found that a 5 cm long unbranched sprig in the field had on average 16.95 +/- 0.86 leaves with an average leaf length of 29.42 +/- 1.50 cm with the longest being 47.90 +/- 2.79 cm. Mean wet weights of leaves were found to be 3.48 +/- 0.24 grams, and wet weights of rhizomes were slightly greater at 3.87 +/- 0.13 grams. Biomass of a 1 cm^2 rhizome sprig and a 1 cm long leaf was calculated to be 0.774 +/- .025 and 0.0072 +/- .0004 grams, respectively.

ACKNOWLEDGEMENTS

I would like to dedicate this thesis to Mike Anghera, who as a mentor provided the guidance and encouragement to get where I am today. For me and for many others, you are most deserving of recognition for your capability and solid work ethic in everything you do. Mike, I will never forget our long days on the boat, nights in the intertidal, and all the hard work and good times side by side in the Reed Lab. Your friendship will always be cherished.

A sincere thank you to Dan and Sally who gave me the opportunity and freedom to pursue this exciting research. Their tireless hard work and thorough, intellectual direction throughout the long process of drafts led to the completion of this thesis. To Scott H, who provided enthusiastic comments, I hope to meet up again out in the surf.

To Shane, Terry, and Dave, you guys were always there to lend a helping hand or offer much needed advice, and to Bonnie for always coming through in the end.

To all the field, lab and diving volunteers and general support team, Mike Almer, Carrie Baer, Ian Blake, Bryn Evans, Maria Fontes, Chris Faist, Rachel Fogelman, Jennifer Heizer, Rochelle King, Patric Larush, Stu Levenbach, Jessica VanLeuven, Julie Love, Mike Mazur, Mike Muzzio, Brent Maridian, Steph Mutz, Wendy Manard, Seth Reder, Ben Reder, Reed Sante, Garrett Snyder, Reed Sante, Allan Willis and Brian Zamacona, I hope you all learned something from this project while having fun at the same time. And very special thanks to my dedicated crew, Jobnathan Blythe, Charlie Boch, Jake Bunch, Lauren Deeb, Maria Fuchs, Carey Gaist, Andy Quinn, Holly Rodrigues, Ashleigh Rossman, and Mark Brinkman, for going way beyond. This research would not have been possible without all of your support, hard work and dedication. Many of you started as field assistants and became close friends. You guys are the best and have made this experience the greatest it could be.

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.