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A Comparison of Two Methods for Enhancing the Recovery of Seagrasses into Propellor Scars: Mechanical Injection of a Nutrient and Growth Hormone Solution vs. Defecation by Roosting Seabirds

by

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EXECUTIVE SUMMARY

Based on the recovery rates for *Thalassia testudinum* measured in this study for scars of these excavation depths and assuming a linear recovery horizon, we estimate that it would take ≈ 6.9 years (95% CI. = 5.4 to 9.6 years) for *T. testudinum* to return to the same density as recorded for the adjacent undisturbed population. The application of water soluble fertilizers and plant growth hormones by mechanical injection into the sediments adjacent to ten propellor scars at Lignumvitae State Botanical Site did not significantly increase the recovery rate of *Thalassia testudinum* or *Halodule wrightii*. An alternative method of fertilization and restoration of propellor scars was also tested by a using a method of "compressed succession" where *Halodule wrightii* is substituted for *T. testudinum* in the initial stages of restoration. Bird roosting stakes were placed among *H.wrightii* bare root plantings in prop scars to facilitate the defecation of nitrogen and phosphorus enriched feces. In contrast to the fertilizer injection method, the bird stakes produced extremely high recovery rates of transplanted *H. wrightii*. We conclude that use of a fertilizer/hormone injection machine in the manner described here is not a feasible means of enhancing *T. testudinum* recovery in propellor scars on soft bottom carbonate sediments. Existing techniques such as the bird stake approach provide a reliable, and inexpensive alternative method that should be considered for application to restoration of seagrasses in these environments.

I. INTRODUCTION

A. Background

Seagrass Growth and Reproductive Biology: Seagrasses are clonal plants which propagate both sexually (seeding) and asexually (vegetative extension and new short shoot formation - sometimes called tillering). The contribution of each of these forms of reproduction varies by species and by environmental conditions. For many seagrasses, especially the larger species, their numerical abundance and coverage of the sea floor are maintained almost exclusively by asexual reproduction (Tomlinson 1974). Most asexual reproduction occurs with the division of meristems located on either the vertical or horizontal rhizomes. Rhizomes are also the conduits which physiologically integrate the roots and short-shoots and maintain the physical integrity of the seagrass clones (Tomasko and Dawes 1989, Terrados et al. 1997, Marba and Duarte 1998). In oligotrophic environments roots ensure that a constant supply of nutrients can be derived from the sediment reservoir. Root and rhizome production can be quite large (Duarte et al. 1998, Kaldy and Dunton 2000) and forms a dense, interwove mat of organic matter which stabilize sediments and contributes to building elevated mud banks (Fonseca 1996). While forming mud banks, dead portions of the roots and rhizomes decay very slowly and provide a large and long-lasting supply of organic matter and nutrients which is recycled and utilized by the plants (Kenworthy and Thayer, 1984)

Susceptibility to Mechanical Disturbance: The majority of shallow seagrass banks in south Florida are formed by *Thalassia testudinum* (Zieman 1982). Nearly all of the rhizome system of the *T. testudinum* is buried in the sediment. A mechanical disturbance to sediments, such as a propellor scar or a blowhole excavated by a vessel's propellor or keel, damages the plant's rhizomes and reduces plant abundance and cover, sometimes for many years (*sensu* Zieman 1976, Williams 1988, Durako et al. 1992, Dawes et al. 1997). Once formed, these scars may be vulnerable to further degradation from physical disturbances such as tidal currents, storms, and biological disturbance (e.g., crab and ray burrowing - also termed "bioturbation") (Patriquin 1975,Valentine et al. 1994, Townsend and Fonseca 1998). Furthermore, loss of the buried organic matter results in a direct impact to the biogeochemical cycling of nutrients in the sediments and affects the availability of nutrients for seagrasses attempting to recover in a scar. Since

rhizomes and roots play such a vital role in the spreading, anchoring and nutrition of *T. testudinum*, as well as the physical stabilization of sediments, mechanical damage to these belowground components are some of the most severe injuries that can occur to seagrass meadows. Moreover, once formed these scars have the potential to increase in size far beyond that of the original injury (sensu Patriquin 1975).

Status of Seagrass Disturbance in Monroe County, Florida: It has been estimated that there are 15,490 acres of seagrass moderately or severely damaged by propellor scarring in Monroe County, (see Table 2 in Sargent et al. 1995). For example, in the seagrass meadow in the channel just north of Windley Key leading into Florida Bay, scarring is so severe that the *T. testudinum* bed has physically disintegrated during the past decade (Sargent et al. 1995). The recognition that prop scarring and vessel groundings are a large and chronic problem has lead to widespread interest in curtailing the problem and restoring damaged meadows.

While conscious efforts are underway to minimize damage to seagrasses through public education, channel marking, enforcement, and zoning, there are still many injuries that remain vulnerable to further deterioration. Moreover, injuries continue to occur as increasing numbers of larger power vessels are accessing shallow water. Propellor scars are frequently accompanied by hull groundings and large scour pits (blowholes) forming what we refer to as the typical keyhole feature (Figure 1). Often, the blowholes are formed when the vessels attempt to move under their own power to reach deeper, navigable water and produce more severe injuries than prop scars alone. The most severe injuries are generally deeper holes formed when sediments are excavated from the blowholes and redistributed as raised berms adjacent to the scars, burying the seagrasses and further limiting recovery.

Most of the injured beds are in shallow water and usually dominated by *T. testudinum*, the species with the slowest rate of asexual reproduction of the seagrasses found throughout south Florida (Fonseca et al. 1987, Fonseca et al. 1998). The ability to quickly restore injured *T. testudinum* beds before additional damage is done (e.g., erosion and enlargement of the injury) has not been adequately developed and both scientists and resource managers must recognize the need to develop techniques which can enhance the

recovery of propeller scars, minimize further degradation of *T. testudinum* beds, and calculate the compensation for lost ecological services (Fonseca et al 1998, Fonseca et al. 2000).

Local Factors Limiting Recovery; Nutritional Requirements: Studies on shallow banks in Florida Bay have shown that if light is abundant, seagrasses are nutrient limited and additions of nitrogen and phosphorus stimulate the productivity of *T. testudinum* and *H. wrightii* (Powell et al. 1989, Powell et al. 1991, Fourqurean et al. 1995). Because of their well-developed and deeply- rooted rhizome systems, most of the nutrients used by *T. testudinum* are obtained from pore waters and organic reservoirs in the sediments (Fourqurean et al. 1992a). The specific limiting factor for seagrasses growing on shallow carbonate banks in south Florida is the availability of phosphorus (Powell, et al. 1989, Fourqurean et al. 1992b). Because propellor scarring modifies the physical and chemical properties of the substrate by excavating surface sediments and removing the buried pool of organic matter, the nutrient regime of a propellor scar is different than in sediments of undisturbed beds. Presumably, if nutrient enrichment stimulates leaf productivity in undisturbed seagrass beds where sediment reservoirs are not altered, it should influence other plant growth characteristics, including rhizome growth and vegetative reproduction (Fourqurean et al. 1995). Therefore, we hypothesize that the addition of nutrient fertilizers to propellor scars will increase the recovery rate of seagrasses, assuming that the nutrients are delivered to the plants in the appropriate form and at a rate that is sufficient to stimulate growth and asexual reproduction.

B. Objectives

This project had two objectives: 1) determine if the recovery of propeller scars by asexual reproduction of *T. testudinum* could be significantly increased over that of natural asexual reproduction rates by repeatedly injecting two different formulas of water soluble fertilizers and plant growth hormones into the adjacent *T. testudinum* beds, and 2) to compare the results of the injection method (active fertilization) with a previously developed passive technique where nutrients were added to the prop scars from bird excrement (bird roosting stakes).

The latter objective is based on previous work on nutrient enrichment experiments in Florida Bay (Powell et al. 1989, Powell et al. 1991, Fourqurean et al. 1995) and a modification of the concept of

"compressed succession" (Derrenbacker and Lewis 1983, Durako and Moffler 1984, Lewis, 1987). In a modified compressed succession approach we propose to install bird roosting stakes among faster growing *H. wrightii* planting units in propellor scars inside *T. testudinum* meadows. We hypothesize that fertilization of *H. wrightii* transplants by bird excrement will increase the initial rate of *H. wrightii* growth and establishment in the scars. At the same time this will stimulate the input and accumulation of organic matter into the disturbed sediments, physically stabilize the scar, and eventually contribute to enhancing the recovery of *T. testudinum* in the scar.

II. MATERIALS AND METHODS

A. Experimental Design of the Mechanical Injection Method

Description of the Anderson Fertilizer Injection System: The fertilizer injector delivers a pre-determined volume of liquid fertilizer (plus additives) into the sea floor under mild pressure (20 lbs $in.^{-2}$). The fertilizer was mixed and stored in a 100 gallon tank and delivered to the injectors through a series of tubes. A pair of large (~ 1.5 m diameter), side-by-side wheels with regularly spaced injection pipes (~ 1 cm diameter) protruding from the outer rim of the wheels was mounted on an ~ 7 m long pontoon boat, powered by a small outboard (Figure 2). The wheels are lowered to the sediment surface where they roll over the sea floor as the boat is driven forward. The parallel wheels allow for the injection of fertilizer into each side of a scar. A trip mechanism ensures that an injection pipe is pressurized, delivering the test liquid into the sediment only when that particular pipe is at the bottom dead center of the rotating wheels. This trip mechanism also ensured that the pipe was buried in the sediment and at the point of delivery for the test liquid.

Study sites: The study area was located in Lignumvitae State Management Area (sometimes referred to as LV), Monroe County, FL (Figure 3). The Lignumvitae area is typical of the upper and middle Florida Keys environment, and consists of extensive shallow seagrass flats dominated by *T. testudinum*, interspersed with mangrove islands and deep channels which connect Hawk Channel with Florida Bay. Tides are semi-diurnal with a range of ~0.5 m.

Fifteen propeller scars were identified from recent aerial photography and on-site inspection. The scars were located on two shallow banks (see mechanical fertilization, Figure 3); 1) the south end of Lignumvitae Key Bank (24°53.2780N, 80°41.2960W) , and 2) the southwest corner of Shell Key Bank adjacent to Race Channel (24°54.8210N,80°.3632W). Both of these banks are dominated by *T. testudinum* and patchy, sparsely distributed shoalgrass, *H. wrightii.*

Prop scar selection: To avoid the possible confounding effects from changes in seagrass cover associated with the "bank top die off" in Florida Bay (Hall et al. 1999), only seagrass beds with scars in dense *T. testudinum* cover were chosen for the experimental treatments. Within each of the 15 scars, we arbitrarily delineated a 10 m long interval of the scar and recorded the beginning and ending positions of this interval with a Differential Global Positioning System (DGPS, Trimble ProXL, < 1.0 m accuracy) and permanent PVC stakes. Scars were selected according to the three criteria agreed upon by the project collaborators: 1) scars would be located in seagrass beds dominated primarily by *T. testudinum,* 2) all scars would have similar water depths (0.5-1.0 m), and 3) all scars would have similar excavation depths and widths (see Figure 9).

Fertilizer/Hormone treatments: Because the ages of the scars were not known, varying degrees of natural recovery may have been underway when the experimental treatments were applied. Therefore, each scar was randomly assigned one of three treatments; 1) control with mechanical injection and no fertilizer added , 2) mechanical injection and fertilizer with just nitrogen enrichment and growth hormones, and 3) mechanical injection and fertilizer with nitrogen and phosphorus enrichment plus growth hormones. The nitrogen enriched fertilizer (treatment #2) consisted of a mixture of 100 lb. of prilled nitrogen (44%) plus 2 ounces of synthetic cytokinin and 2 ounces of synthetic gibberellin mixed in 100 gallons of ambient seawater obtained from the study site. The nitrogen and phosphorus enriched fertilizer (treatment #3) consisted of #2 plus an additional 50 lb. of di-ammonium phosphate. This formulation was chosen by the contractor based on their claims of significant effects on regrowth of seagrasses into prop scars in Tampa Bay.

Approximately 10 ml of a fertilizer solution was injected into the sediment every 20 cm along a 10 m length of each treated scar to a depth of ~ 10 cm. The scars were injected with fertilizers on five separate occasions every two weeks beginning on May 15, 1998.

Prop scar monitoring: In April 1998, prior to fertilizer additions, we surveyed each experimental scar by selecting five random paired points along the length of each scar; five points in the scar and five in the adjacent undisturbed bed (Figure 4). At each random point within the scar; 1) a 20 cm by 20 cm PVC quadrat was placed on the sediment in the middle of the scar and the number of seagrass short-shoots in the quadrat were recorded, 2) presence and species composition of macroalgae was noted, 3) the excavation depth was measured at the center of the scar (nearest 0.1 m), and 4) the scar width (nearest 0.1 m) was measured. Finally, the full 10 m test section of each scar was video taped on each visit with a SONY VX-1000 digital video camera mounted in an Amphibico VX-1000 housing. To provide scale in the video, a tape measure was unrolled on the bottom extending up the center of the scar prior to filming.

As a control, seagrass short-shoot densities were counted in a 20 x 20 cm quadrat placed 1 m into the adjacent, undisturbed bed at a ninety degree angle to the alignment of the scar. This created a sample pair (within scar and outside scar) at each randomly selected distance within the 10 m test section. Digital video was also used to record a 10 m segment of the adjacent natural bed, parallel to the scar and encompassing the quadrat count areas to detect if there were any changes in the undisturbed bed during the experimental period. This monitoring was repeated in December 1998 and again on November 1999 (8 months and 19 months following the treatments).

Data Analysis: The five point counts within each scar were averaged and a mean shoot count (n = 5) was used to represent each scar in all analyses. To meet unconfirmed assumptions of heteroscedacity, all *T. testudinum* short-shoot counts were transformed ln(x +1) (Sokal & Rohlf 1969). After transformation, residuals were normal (Shapiro-Wilke statistic W = 0.96, p = 0.1851) and variance among the five replicate scars within a treatment were homogeneous (Cochran's C = 0.28, df = 3, p > 0.05). A two-way repeated measures ANOVA (SAS™ version 6.12) was conducted, with sampling date (elapsed time since

initial survey) and fertilization treatment as factors. A third order polynomial transform was used to specify the spacing of time (0, 224, and 570 days) in the analysis.

B. Experimental Design of Bird Roosting Stakes

Study Site and Prop Scar Selection: Two propellor scars in Lignumvitae State Management area were selected for this experiment; Site1) an 80 m long scar on the southeast corner of Peterson Key Bank adjacent to Lignumvitae Channel and , Site 2) an 80 m segment of another scar approximately 1 km east of Site 1 on Lignumvitae Key Bank (see bird stakes, Figure 3). Both of these scars were located on shallow, nearly monotypic *T. testudinum* banks with minor amounts of *H. wrightii*; environmental conditions similar to those found at the fertilizer injection scars described above in section A. Prior to selection, the seagrass beds and prop scars were visually inspected for the following five criteria: 1) the length was continuous, 2) the scars had well defined edges and were without large-scale regrowth of seagrasses to verified by preliminary sampling, 3) maximum water depth over the scars were $\leq$1.5 m and relief between scar bottom and surrounding sediment no greater than 0.5 m, 4) there was unconsolidated sediment in the scar, and 5) the scars were accessible without damaging adjacent seagrass beds. Prior to initiating the experiment on July 22 and 23, 1994, five positions were randomly selected along each experimental scar to determine the depth of the sediment layer in the scars, the ambient density of *H. wrightii, T. testudinum* and macroalgae within and adjacent to the scars (paired samples), and the average dimensions of each scar.

The bird roosting stakes were constructed of 0.5 in. diameter PVC pipe capped with a 2 in. by 2 in. by 4 in. pressure treated wooden block (Figure 5). Numbers were burned into the faces of the blocks for identification. The wood blocks at the top of the stakes provided a stable surface where comorants and terns roost comfortably (Powell et al. 1989, Fourqurean et al. 1995). While roosting, the birds defecate their nutrient rich excrement into the water and sediments beneath the stakes (Powell et al. 1989); acting as a passive fertilizer delivery system. Control stakes were constructed of 0.5 in. PVC pipe without blocks and were cut diagonally at the top which prevented any birds from roosting on the stakes.

Experimental Design and Fertilizer Treatment: Twenty stakes were placed at 4 m intervals along each of the two experimental scars (Figure 6). Stakes were pushed into the sediment by hand until approximately 0.25 m extended above the surface of the water at mean high tide. Ten stakes were randomly assigned roosting blocks and ten as controls. Five of the ten roosting blocks and five of the ten control stakes were randomly selected for transplanting *H. wrightii*.

Prop Scar Monitoring Prior to Installing the Roosting Stakes: Prior to initiating the experiments, the abundance of seagrass and macroalgae were determined using a non-destructive visual sampling method (Braun-Blanquet 1965). A 0.25 m^2 quadrat was placed on the bottom in the scar 0.5 m from each of the designated stake positions. The same quadrat was also placed 0.5 m outside the scar in the adjacent seagrass bed to complete a paired comparison. The seagrass species and macroalgae occurring within the quadrats were assigned a cover - abundance scale value according to the following categories: 0 = absent, 0.1 - solitary, with small cover; 0.5 - few, with small cover; 1 - numerous, but less than 5% cover; 2 - any number, with 5-25% cover; 3 - any number, with 25 - 50 % cover; 4 - any number, with 50 - 75 % cover; and 5 - any number, with 75 - 100% cover.

Halodule wrightii short-shoots for transplanting were collected from a monotypic bed within 1 km of the experimental scars. Whole shoots with intact roots and rhizomes were collected by hand and planting units (hereafter referred to as PU) were assembled by attaching horizontal rhizomes with their associated short-shoots to a 10 in. U-shaped metal staple using paper-coated wire twist ties (Fonseca et al. 1998). This method is commonly referred to as the “bare root” planting technique. Approximately 17 short-shoots and five rhizome apical meristems were included in each PU. Plants were collected and PU were assembled and planted on July 24, 1994.

The scars were monitored periodically between October 1994 and August 1999. Initially, in October 1994 (71 days after planting) and January 1995 (140 days after planting), survival of PU and the number of short-shoots PU^{-1} were recorded. By January 1995 all of the original transplants were lost. We believe that a combination of factors influenced the original plantings. These factors included grazing by

herbivores following the original planting, and high temperature stress experienced during August and September.

In April 1995 we replanted the two experimental scars using the original design described above with one major modification. For the second planting we used between 30 and 50 short-shoots PU^{-1} as opposed to17. Also, note that by April 1995 the birds roosting on the stakes had been fertilizing the scars for 9 months. After the second planting, PU survival was surveyed in June 1995 (78 days following planting) and again in August 1995 (138 days following planting).

By May 1996 (489 days after planting) many of the PU had coalesced, making it impossible to identify the individual transplant units. So in May 1996 and January 1997 (737days after planting) we mapped the spatial coverage of *H. wrightii* in each scar by measuring the physical dimensions (length and width) of the seagrass cover with a tape measure in order to calculate the percent area of the scar covered by the transplanted *H. wrightii*. We also counted the number of short-shoots per 100 cm^2 using 10 cm by 10 cm quadrats placed around the stakes assigned to each treatment along the length of each scar at the two sites. After transforming the short-shoot counts using the square root of ln + 0.5 we first tested whether transplanting affected shoot density at either site in May 1996 in both the fertilized and not fertilized treatments. Next, we tested whether fertilization had a significant influence on short-shoot density at both sites in May 1996 and January 1997, regardless of transplanting.

In August1999 (1670 days after planting) we mapped the seagrass cover at site 1 only, and since the cover of *H. wrightii* was nearly continuous over the entire length of the scar we counted the number of *H. wrightii* and *T. testudinum* short-shoots in 40 quadrats (100 cm^2 each) located every 2 m along the length of the scar.

Throughout the study period oblique aerial photographs of each of the scars were taken opportunistically by collaborators (Curtis Kruer and Pat Wells). Photos of site 1 one were obtained in December 1996, December 1997, September 1998 and January 2000. Photos of site 2 were obtained in December 1996 and September 1998. The color photos were digitally scanned (300 dpi) and printed in grey scale for presentation in this report.

III. RESULTS

A. Mechanical Fertilizer Injection

Fertilizer/Hormone Effects Within Scars: Time was a significant factor for *T. testudinum* short-shoot counts (Wilkes' Lambda F=71.6, df=2, p<0.0001) but treatment and time * treatment were not (F=0.23, df=2, p=0.79 and Wilkes' Lambda F=1.03, df=4, p=0.42, respectively. (Table 1, Figure 7 top panel). Only the 1st degree polynomial for time was significant (Table 2: F=157, df=1, p<0.0001). *T. testudinum* mean short-shoot counts increased with time in all three treatments (Table 3, Figure 7).

Table 1. Repeated Measures ANOVA Results for Time and Fertilizer Treatment.

Source	Statistic	Value	F Value	Degrees of Freedom	P-value
Time	Wilkes' Lambda	0.0653	71.5972	2, 10	0.0001
Time*Treatment	Wilkes' Lambda	0.6877	1.0294	4, 20	0.4164
Treatment	ANOVA		0.2300	2	0.7948

Table 2. First order polynomial contrast for Time and Treatment.

Source	Degrees of Freedom	Type III SS	Mean Square	F Value	P-value
Mean	1	2.1909	2.9109	157.13	0.0001
Treatment	2	0.0176	0.0088	0.47	0.6344
Error	11	0.2038	0.0185		

Table 3. Mean *Thalassia* shoot counts for each fertilizer treatment.

	Mean *Thalassia* shoot count +/- 1 standard deviation (n)		
Date	Nitrogen Treatment	Nitrogen + Phosphorus Treatment	Control Treatment
April 1998	0.16 +/- 0.17 (5)	0.12 +/- 0.18 (5)	0.24 +/- 0.33 (5)
December 1998	1.32 +/- 0.86 (5)	2.12 +/- 1.74 (5)	1.24 +/- 1.28 (5)
November 1999	5.54 +/- 3.99 (4)	4.68 +/- 2.92 (5)	4.30 +/- 3.10 (5)

Effects on Short-Shoot Density ; Within Scars vs. Undisturbed Controls: *Thalassia testudinum* short-shoot counts inside and outside the scars at the beginning of the monitoring period were 0.17 +/- 0.22 and 22.10 +/- 6.41, respectively (Figure 8). Variances were not homogeneous (F = 0.2, df = 14, p = 0.002), so a two sample t-test was performed on square root transformed short-shoot counts. These were significantly different (t Stat = -22.58, df = 19, p < 0.0001,). In order to assess the recovery of scars 18 months after the beginning of the monitoring period in November 1999, a two sample t-test for equal variances was performed on square root transformed *T. testudinum* short-shoot counts inside the scars and outside in the adjacent undisturbed seagrass beds. Variances were homogenous (F = 0.53, df = 13, and p = 0.13) and the differences between inside and outside shoot counts were normally distributed (Shapiro-Wilke statistic W = 0.90, p = 0.10). A T-test revealed that *T. testudinum* short-shoot counts outside the scars were significantly greater than counts inside the scars (18.51 +/- 3.91 versus 4.79 +/- 2.85; t Stat = -10.6, df = 26, p < 0.0001) (Figure 8).

To avoid concerns that the short-shoot population outside the scars may have declined during the monitoring period and possibly influenced the results of the treatments, we tested whether or not there were changes between the beginning and the end of the experiment. *Thalassia testudinum* short-shoot counts declined slightly from 22.1 ± 6.4 $*$ 400cm^{-2} in April 1998 to 18.51 ± 3.9 $*$ 400cm^{-2} in November 1999. The variances were equal (F = 2.1, df = 14, p = 0.09) and a T-test indicated that there wasn't a significant difference (t Stat = 1.72, df = 27, p = 0.0961)

Scar depths varied considerably both within a time period and over time. Mean scar depth increased between April1998 and December 1998, but decreased slightly in November 1999 (Figure 9 and Table 4).

Table 4. Descriptive Statistics for Scar Depth.

Sampling Date	Sample Size	Mean (cm)	Median (cm)	Range (cm) 25^{th} -75^{th} percentiles	Range (cm) 10^{th} - 90^{th} percentiles	Range (cm) 5^{th} - 95^{th} percentiles
Apr 98	15	1.69	1.6	0 – 3.7	0 – 3.9	0 – 4.8
Dec 98	13	4.78	2.7	2.0 – 7.78	1.8 – 8.9	1.6 – 9.1
Nov 99	14	3.9	3.0	1.6 – 4.8	1.4 – 8.4	1.4 – 10.4

Predicted Recovery Time: Because the fertilizer treatments were not significant, all short-shoot count data were pooled and short-shoot number was regressed on time (linear regression: $r^2 = 0.52$, df = 1, $p < 0.0001$) (Figure 7) to estimate the time to 100% recovery for the propellor scars investigated in this study (Figure 10). The model for the regression, with time in days, was:

$$\text{Shoot Count } (0.04\ m^{-2}) = 0.0082*\text{Days} + 0.0040 \qquad \text{(Eq. 1)}$$

A 95% confidence limit around the slope of the regression line was computed to be: $0.0059 \leq \beta_1 \leq 0.0105$. The time required for *T. testudinum* short-shoot density in these scars to reach the average short-shoot density of the undisturbed, adjacent seagrass bed (20.7 short-shoots * 0.04 m^{-2} or equivalent to 518 short-shoots m^{-2}; within the natural range here of 463 - 552 short-shoots m^{-2}) was predicted to be 6.9 years, with a 95 % confidence interval of 5.4 to 9.6 years (see Figure 10). Because the exact date when these scars were formed is unknown, this is a conservative estimate of recovery.

B. Bird Stake Fertilization

Initial PU Survival After Replanting: At the first monitoring in June 1995 approximately 78 days after replanting, PU survival ranged between 60 and 75% at site 1 and nearly 90% at site 2 (Figure 11). In August, 138 days after planting, survival at site 1 was 68% in the fertilized treatment and only 18% for the unfertilized PU. In August, survival at site 2 remained at about 90%, nearly similar to the 60 day monitoring values.

By May 1996, a little over a year after planting, many of the PU had coalesced and it was impossible to record survival of individual transplants. Therefore, we measured the percentage of the scar within the total staked area covered with seagrass by mapping out the dimensions of the patches with *H. wrightii*,

summing their areas, and dividing this by the total area of the original scar. As part of this mapping effort we also recorded *H. wrightii* short-shoot density (100 cm $^{-2}$) at each of the treatment stakes within each site (scar). Coverage at sites 1 and 2 were 22 and 40%, respectively (Figure 12), with the highest short-shoot densities, 37.6 short-shoots 100 cm^{-2}, at the fertilized and planted treatments at site 2 (Figure 13). We used a T-test to examine if transplanting *H. wrightii* into the treatments at both sites affected short-shoot density. At site 1, planting did not have a significant effect in either the fertilized (t Stat = 0.2706, p = 0.3967, df = 8) or not-fertilized (t Stat = 1.3378, p = 0.1192, df = 5) treatments (Figure 13, top panels). In contrast, there were significant effects due to planting in both the fertilized (t Stat = -3.0475, p = 0.0190) and not fertilized treatments at site 2 (Figure 13, bottom panels). We then disregarded planting and tested whether or not fertilizer made a significant different. Both the fertilized treatments at site 1 (t Stat = 3.1270, p = 0.0029, df = 18) and site 2 (t Stat = 3.5837, p = 0.0024, df = 10) had significantly greater numbers of *H. wrightii* short-shoots than the non-fertilized treatments (Figure 14). Total coverage of *H. wrightii* increased at both sites between May 1996 and January 1997 (Figure 12). Within 20 months of planting, nearly 50% of each scar within the staked area was covered by *H. wrightii* (Figure 12). Likewise, in January 1997 the fertilized treatments at site 1 (t Stat = 2.9570, p = 0.0042, df = 18) and site 2 (t Stat = 4.8589, p = 0.0001, df = 14) had significantly greater numbers of *H. wrightii* short-shoots than the non-fertilized treatments (Figure 14).

Oblique aerial photographs of sites 1 (Figure 15) and 2 (Figure 16) show the progression of coverage by *H. wrightii* in the two scars. The more deeply excavated scar at site 1 was recolonized by *H. wrightii* along the length of the scar while the *H. wrightii* at site 2 spread more horizontally outside the original dimensions of the injury. The growth of *H. wrightii* was so rapid that portions of the scar which originally were not planted and had non-roosting stakes were also colonized by *H. wrightii* growing in from planted and fertilized stakes. By September 1998 more than 80% of the scar within the staked area at site 1 was colonized by *H. wrightii* and sometime between September 1998 and August 1999 the scar became 100% covered as shown in the January 2000 photo. At site 1 in August 1999, *H. wrightii* and *T. testudinum*

short-shoot densities were 3294 ± 281 and 21 ± 6.2 m^{-2}, respectively. Both species were recolonizing the scar but it was clearly dominated by *H. wrightii*.

IV. DISCUSSION

A. How Representative Was the Study Area in General?

Thalassia testudinum short-shoot densities in the Lignumvitae beds (~507 m^{-2}) at the start of the mechanical injection experiment were typical for seagrass banks throughout south Florida (Zieman 1982), therefore we were confident that the fertilizer treatments were applied to seagrass banks with locally representative populations of *T. testudinum.* Eighteen months after the initiation of the experiment, *T. testudinum* short-shoot counts in the undisturbed bank populations adjacent to the experimental scars were nearly identical to counts obtained at the beginning of the study (Figure 8). This reassured us that the experimentally treated *T. testudinum* beds were not experiencing the seagrass "die off" reported for banks nearby in Florida Bay (Hall et al. 1999).

B. Potential Storm Effects

During our study period the Lignumvitae area experienced two Hurricanes; one in late September 1998 (Georges) and another in October 1999 (Irene). As a category 2 storm when it passed through the study area Georges caused extensive damage throughout the middle and lower Florida Keys with winds in excess of 161 kph, whereas Irene's windspeeds in the Lignumvitae area were minimal hurricane strength (~120 kph). Between April and November 1998, the mean depth of the experimental prop scars increased from about 2 to 4.5 cm, suggesting the possibility that the scars were eroded by Hurricane Georges. However, some filling had occurred by November 1999 as scar depth decreased slightly (Figure 9). In absolute terms, these were minor fluctuations and at no time did scar depth ever reach a point where *T. testudinum* regrowth should have been significantly impaired (estimated to be $\geq$20cm, author's unpubl. data). Moreover, based on visual observations, there was no suggestion that erosion had occurred in the adjacent beds where the nutrients had been injected (pers. obs.). Therefore, based on the lack of significant evidence for erosion within and around the scars, we conclude that hurricane Georges did not influence the course of the experiment.

C. Comparison of Fertilization by Mechanical Injection and Bird Stakes

During the study period most of the propellor scars in the mechanical injection experiment surveyed exhibited some degree of asexual recolonization by *T. testudinum* (Figures 7 and 8), however neither of the two mechanical fertilizer/hormone treatments significantly influenced regrowth as measured by short-shoot density of *T. testudinum* within the scar (Figure7, Table 2). This lack of effect by mechanical injection is in distinct contrast with the existing bird stake method (Figures 11, 12, 13, 14 and 15) and previous experimentation on Cross Bank in Florida Bay where phosphorus and nitrogen were supplied to seagrass beds in the form of bird excrement (Powell et al. 1989, Fourqurean et al. 1995). In the Cross Bank studies, *T. testudinum* initially responded to continuous fertilization with bird excrement by increasing leaf size, leaf productivity and standing crop for a 2-3 year period until *H. wrightii* began to replace *T. testudinum* (Fourqurean et al. 1995). After about 2 years *H. wrightii* increased short-shoot density and produced aerial rhizome runners which overgrew and shaded the *T. testudinum* canopy. The authors hypothesized that *H. wrightii* out-competed *T. testudinum* for the available nutrients from the solubilized excrement. Fourqurean et al. (1995) confirmed that *T. testudinum* will respond to fertilizer, particularly phosphorus-rich fertilizer, but it appears that if nutrients are delivered in high concentration for a continuous period of time, *T. testudinum* may be displaced by *H. wrightii*. *Halodule wrightii* is a species with an intrinsically higher population growth rate than *T. testudinum* and appears to thrive better in nutrient enriched environments. Even though there was only a very low background density of *H. wrightii* on Cross Bank it eventually dominated the seagrass cover.

The trends observed on Cross Bank where *H. wrightii* responded opportunistically to nutrient enrichment were not evident at Lignumvitae where scars were mechanically injected with fertilizers and hormone mixtures. Even if *T. testudinum* productivity were enhanced by the soluble fertilizer used in the mechanical injection (productivity was not measured), this did not translate into a corresponding increase in the asexual reproduction rate (recovery) in the scars, nor was there any evidence that growth of the low density ambient populations of *H. wrightii* on the Lignumvitae banks was enhanced by mechanical injection as it had been with bird stakes. As on Cross Bank, patchy and sparse *H. wrightii* were present on

the Lignumvitae banks around the mechanically injected scars as an understory species but it appears that the mechanical injection of soluble fertilizer was unable to stimulate any kind of seagrass response which significantly increased recovery rates in the Lignumvitae sites. Similarly, bird stake fertilization and *H. wrightii* transplanting initiated in July 1994 failed. However, with a modification of our planting technique by using larger PU and planting in April 1995 the transplants rapidly colonized the two experimental scars. Within 1.6 yr the scars were nearly 50% covered (Figure 12) and *H. wrightii* attained short-shoot densities equivalent to between 1000 and 4000 m^{-2} (Figures 13 and 14). These densities are similar to those reached in previous studies of bird stake fertilization on nearby Cross Bank (Fourqurean et al. 1995) and around bird rookery islands in Florida Bay (Powell et al. 1991).

The present study in the two experimental scars differs from previous work on Cross Bank and from the mechanical injection test in the fact that we transplanted *H. wrightii* into the bird stake scars. Our results suggest that transplanting in combination with the roosting stakes initially accelerated *H. wrightii* growth at site 2 only. At site 1 there were no differences between planted and not planted treatments, however, there was evidence that planting treatments may have been obscured by the fact that some of the fertilized treatments grew rapidly down the scar into other not planted and not fertilized locations.

In contrast, mechanical injections alongside *T. testudinum* scars and among understory of *H. wrightii* had no apparent stimulation effect on either species of seagrass, whereas on Cross Bank defecation caused the *H. wrightii* understory to proliferate. By 1.6 years the effect of fertilizer in birds stake experiments obscured any of the possible effects of the initial plantings. Fertilized and planted sites were expanding into non planted and in some cases non fertilized locations. Eventually, the growth of *H. wrightii* was so extensive that all of the initial treatments were obscured.

The failure of our first transplanting effort in the bird stake experiments in July 1994 and the positive results the following April suggests that planting in spring (April - May) is more likely to be successful than summer plantings when water temperatures on the shallow banks are much higher. There are other factors which may have also contributed the success of the second planting. First of all, we used larger planting units with more short-shoots and apical meristems. Second, there was some evidence that the

July plantings were grazed by herbivores. Since we have previously observed severe grazing on *H. wrightii* transplants in nearby back reef environments, we cannot rule out the possibility that herbivores contributed to poor survival. The third factor may be related to a "conditioning process". By April 1995 the sediments in the experimental scars had been fertilized for almost 9 months by the original bird stakes. Bird usage of the stakes was between 75 and 100 % on every site visit, therefore, nutrients were being added to the sediments throughout the period between July 1994 and April 1995. It is possible that by the time we replanted in April the sediments were nutrient enriched and better suited for the survival and growth of the *H. wrightii* than 9 months earlier.

D. Recommendations for Restoration

The bird stake method holds considerable promise as a means of stabilizing and restoring propellor scars and other similar injuries in *T. testudinum* beds by temporarily substituting a faster- growing, opportunistic species for the slower-growing climax plant. This restoration principle, sometimes referred to as "compressed succession" (Derrenbacker and Lewis 1983, Durako and Moffler 1984, Lewis, 1987) should be designed as a stepwise process whereby *H. wrightii* temporarily substitutes for *T. testudinum*. The development of tropical and subtropical seagrass systems of the Atlantic, Caribbean and Gulf of Mexico normally proceeds through a succession of species initiated by the colonization of unconsolidated sediments by small turf-like algal species (e.g., *Chaetomorpha* or *Batophora*), followed by upright calcareous and fleshy macroalgae (e.g., *Caulerpa spp.*) (Zieman 1982, Williams 1990). Depending on the circumstances, faster growing opportunistic seagrasses (either *Halophila spp., H. wrightii or S. filiforme)* will colonize a disturbance along with the macroalgae. If environmental conditions remain suitable and propagules are available, *T. testudinum* will eventually recruit and dominate the community. However, this is typically a slow process and may take more at least three to five years and as long as seventeen years for *T. testudinum* recovery in a disturbance similar to prop scars. A restoration plan using a modification of the concept of "compressed succession" takes advantage of the growth characteristics of the opportunistic species to accelerate colonization and stabilize the sediments. When the colonizing species is established, the treatment responsible for accelerating recovery (fertilization) is removed and,

suggested by several studies (Zieman 1982, Williams 1990), the climax species (*T. testudinum*) will replace the colonizer.

To get a better understanding of how the bird stake method can work, consider the recovery rates of prop scars at Lignumvitae (Figures 6 and 10). The data from this study provided us an opportunity to estimate the recovery time for prop scars of the physical dimensions selected in this study. Assuming that regrowth of *T. testudinum* was linear throughout the recovery period, the regression model predicted it would take 6.9 years (95% CI. = 5.4 to 9.6 years) for the prop scars at Lignumvitae to return to the same *T. testudinum* short-shoot density as the adjacent beds (see Figure 10). This is comparable to the rate of recovery estimated for *T. testudinum* prop scars with similar dimensions in Tampa Bay (3.5-7.6 y; Dawes et al. 1997), and within the range of 3 - 5 years suggested by Zieman (1976) for *T. testudinum* in Florida Bay. In contrast, data for the coverage rates of experimentally fertilized *H. wrightii* transplants growing in *T. testudinum* prop scars at Lignumvitae indicate that *H. wrightii* could reach 50% cover (short-shoot densities $\geq$500 m^{-2}) in 1.5 years (Figures 12, 13, and 14). Consider, however, that the two experimental scars were only partially planted and the PU were randomly distributed over the 80 m long scar. We planted a total of 1200 *H. wrightii* short-shoots and only half of these were assigned to bird roosting stakes. Based on these data, had we planted and staked the entire scar, we estimate that the scar would have been 100% covered in $\leq$ 1.5 years.

Based on these results, we recommend using the bird roosting stakes in conjunction with *H. wrightii* PU for restoration of prop scars and other injuries. Initially, the bird roosting stakes should be placed two meters apart along the length of a scar. After waiting at least 6 months for confirmation of bird use and allowance for the feces to enrich the sediments, three *H. wrightii* PU should be planted 0.5 m apart between each stake. The estimated cost for two stakes with three peat-pots PU of *H. wrightii* is approximately $5.75, including construction and installation, not including logistics and travel costs. In the case of larger injuries, for example, blowholes created by vessel hull groundings (Figure 1), the placement of stakes and PU should follow the same protocols but should be placed in a uniform grid pattern. Place the stakes in an array no more than two meters apart with three PU between stakes.

Ideally after planting, the stakes should remain in place for at least one - two years or until a desired cover of *H. wrightii* is reached; preferably, at least 50% cover. If the stakes remain in the scar for an extended period of time, for example >2-3 years, *H. wrightii* should reach high densities (Figures 12, 13, 14, 15 and 16; also see Fourqurean et al. 1995). The sediments will become enriched with nutrients and organic matter while encouraging the maintenance of *H. wrightii* populations, but excessive fertilization could behave more like a disturbance, reversing the direction of the succession and delaying the recovery of the climax species, *T. testudinum* (Fourqurean et al. 1995). The idea of this restoration method is to reach a balance between gaining seagrass cover (*H. wrightii*) with nutrient fertilization while restoring significant amounts of lost resource services. Once the input of nutrients from the bird excrement ceases, the injury is partially stabilized by the opportunistic species but the sediments have a larger pool of nutrients for *T. testudinum* to draw upon. Since nutrients and organic matter have been incorporated directly into the sediments inside the scar with *H. wrightii* colonization, conditions may be more suitable for vegetative regrowth of *T. testudinum* as well as for the introduction of planted or naturally recruited *T. testudinum* seedlings.

There are fundamental differences in the two methods compared in this report, beginning with the fact that the delivery of nutrients by the bird stakes is relatively continuous and much larger than the five injections of soluble fertilizer. Also, the birds defecate directly into the scar, whereas the injections were directed into the sediments adjacent to the scars. The idea behind the mechanical injections was to stimulate *T. testudinum* at the edge of the scars to grow into the injury. Presumably this could also affect *H. wrightii* growth, however, the mechanical injections never called for stimulation of *H. wrightii* whereas the planted bird stakes were designed to encourage the colonization of *H. wrightii.* Evidently, the amount of soluble fertilizer delivered in the five bi-weekly injection treatments was not sufficient to significantly improve recovery of any species in the scars. Also, even if *H. wrightii* PU were transplanted into the scar, the mechanical injections would not be effective unless they were directed at the PU and not the edge of the scars. Without actually doing the mass balance calculations it is likely that the nutrient requirements for reestablishment of *T. testudinum* biomass into the highly disturbed sediments of a propellor scar, should it

occur at all, would require a much larger and regular fertilization method than could be realistically provided by the mechanical injection in a cost effective manner.

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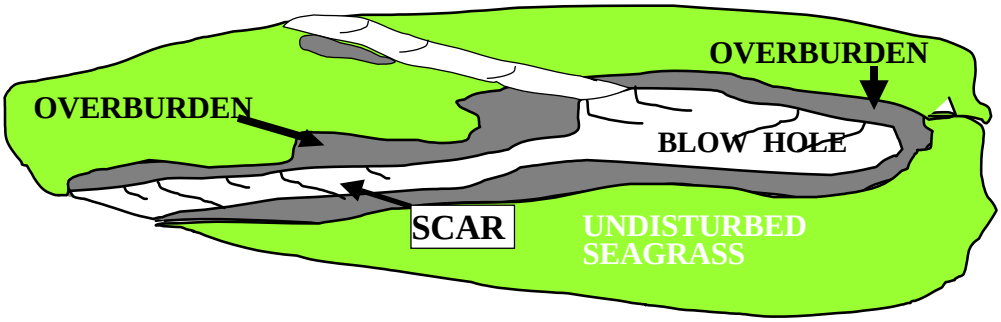
Figure 1: Diagram (top) and photographs (bottom) of typical prop scars and associated blowhole injuries. The diagram on top illustrates the classic keyhole shaped grounding with an inbound propellor scar, a blowhole, sediment overburden, the undisturbed seagrass bed, and an outbound propellor scar. The photo in the lower left is a closeup of a twin propellor (screw) scar and hull grounding from a 42 ft. sport fisherman. The oblique aerial photo on the lower right shows several propellor scars and hull groundings on a portion of Red Bay Bank in the Florida Keys National Marine Sanctuary.

Figure 1



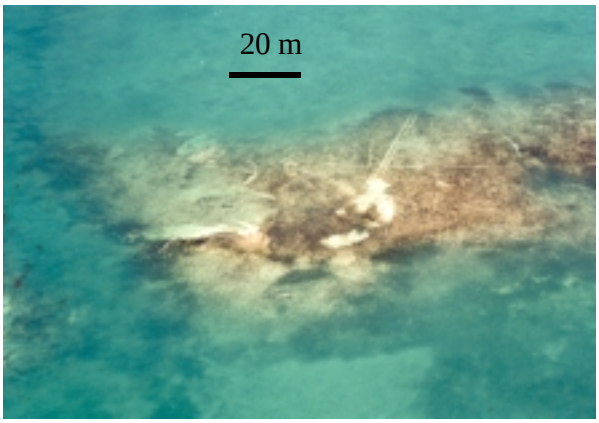


Figure 2: Photograph of the vessel used for mechanical injection of fertilizers (top) and the fertilizer injector wheels (bottom).

Figure 2

Figure 3: Site map of the Lignumvitae Key area and the location of the experimental prop scars for the two studies reported.

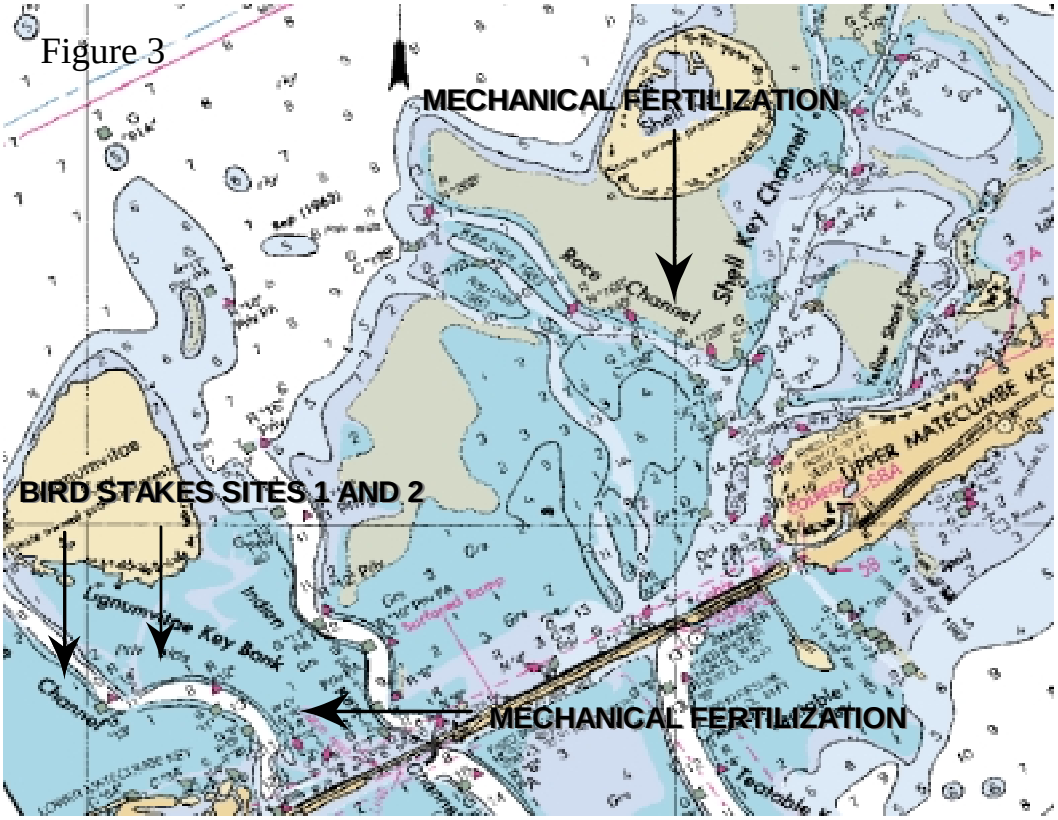


Figure 4: Sampling design for one 10 m long prop scar in the mechanical injection experiments where 15 propellor scars were treated. Shown are the descriptions of the cross sectional dimensions for the width and depth of each scar and the location of paired 20 $*$ 20 quadrats.

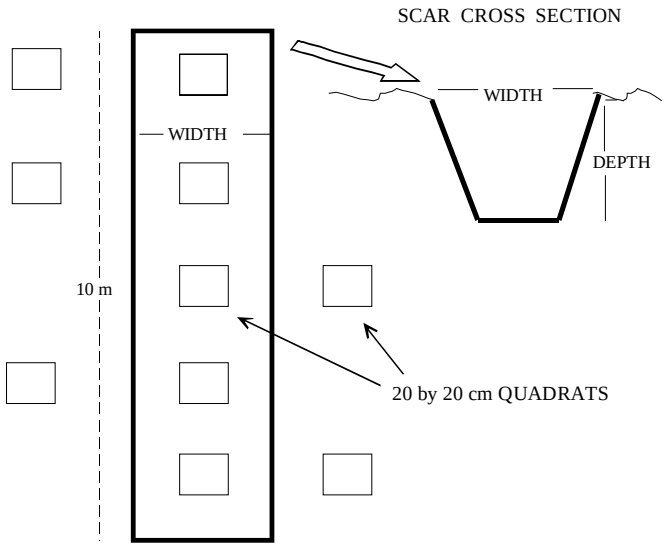


Figure 5: Photograph of comorants roosting on two bird stakes. Also shown are two control stakes without roosting blocks.

Figure 5

Figure 6: Design of the bird roosting stake experiments in two propellor scars at Site 1 (Bird Stake Scar #1) and Site 2 (Bird Stake Scar #2) at Lignumvitae State Management Area. The middle panel shows a roosting stake (1) and the four planting units of *Halodule wrightii* associated with the stake.

Figure 6

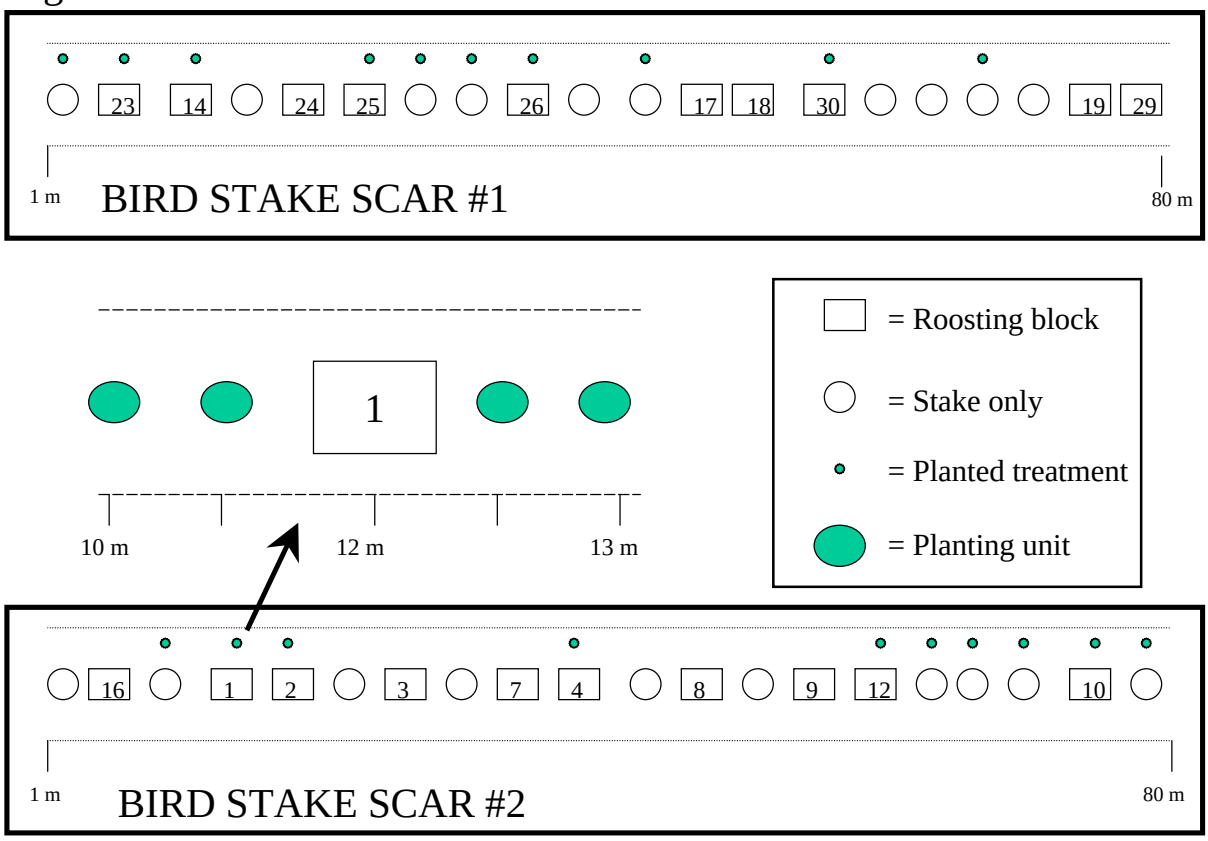


Figure 7: Plots of the mean (± 95% Confidence Intervals) *Thalassia testudinum* short-shoot density per 400 cm^2 versus sampling date for two fertilizer treatments (nitrogen and nitrogen plus phosphorus) and the controls.

Figure 8

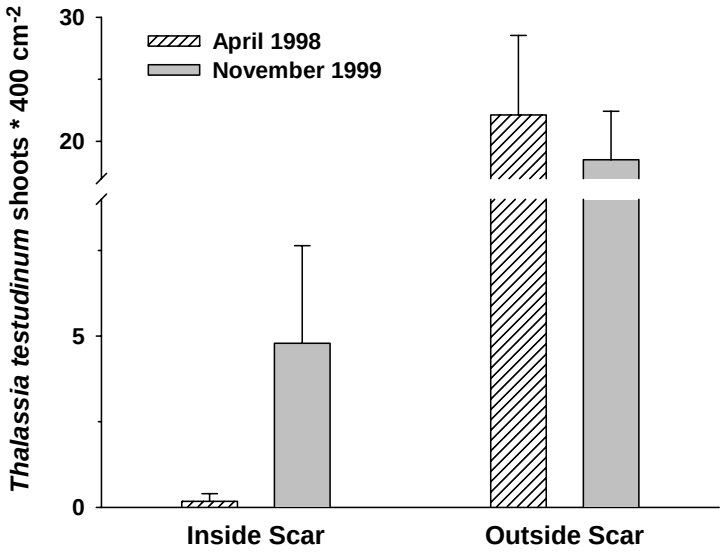


Figure 8: Plot of *Thalassia testudinum* short-shoot density per 400 cm^2 inside and outside the scars at the start of the experiment in April 1998 and at the last sample date in November 1999.

Figure 9: Box plot of the mean, median, range and 95% confidence interval of prop scar depth on three sampling dates for the propellor scars receiving mechanical injection of fertilizer.

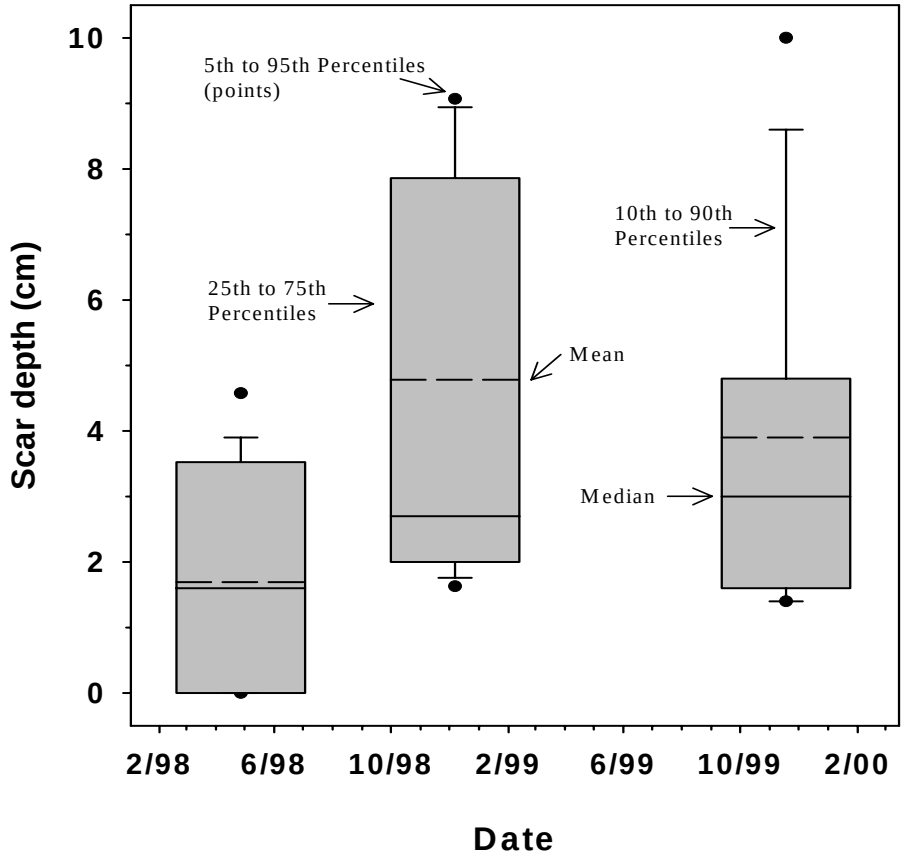


Figure 10: Plot of the linear regression model predicting the time (date) to recovery for *T. testudinum* short-shoot density per 400 cm^2 in propellor scars at Lignumvitae State Management Area. Regression was developed ignoring fertilizer treatments and using all 15 scars.

Figure 10

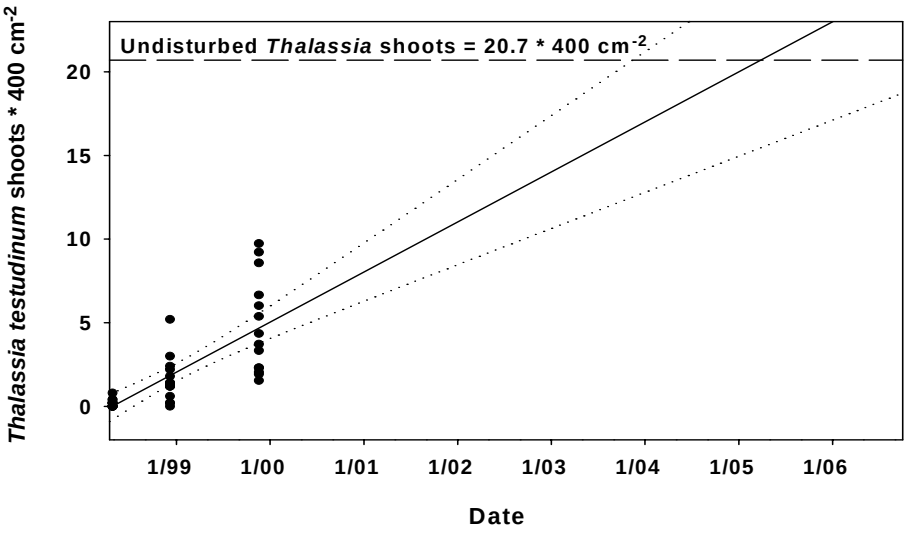


Figure 11: Percent survival of *Halodule wrightii* planting units (PU) on two sampling dates in June 1995 and August 1995 in two propellor scars (Site 1 and Site 2) in treatments that were fertilized by bird stakes (FP) and not fertilized by bird stakes (NFP).

Figure 11

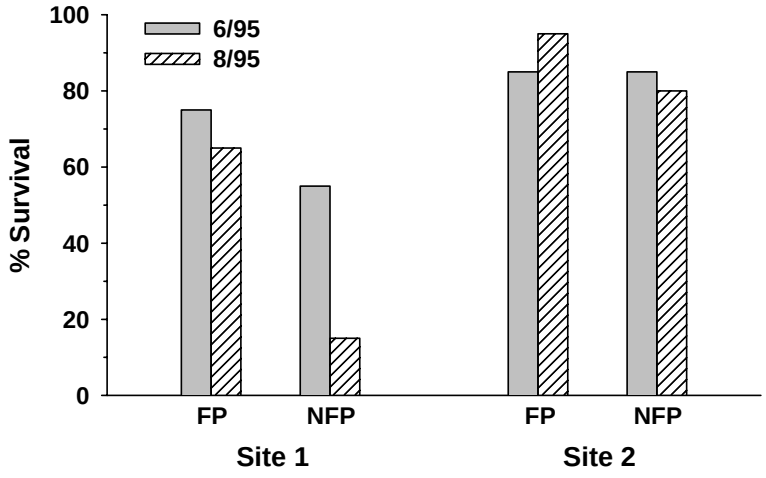


Figure 12: Percent coverage of *Halodule wrightii* in two propellor scars (Site 1 and Site 2) on two sampling dates in May 1996 and January 1997 that were fertilized by bird stakes at Lignumvitae State Management Area.

Figure 12

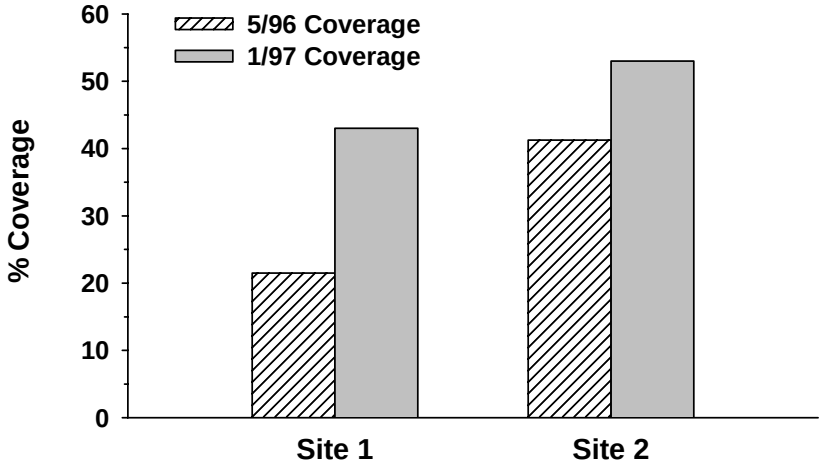


Figure 13: *Halodule wrightii* short-shoots per 100 cm^2 in the four bird stake experimental treatments in May 1996 at Site 1 and Site 2. NFNP = not fertilized and not planted; NFP = not fertilized and planted with *Halodule wrightii*; FNP = fertilized but not planted; FP = fertilized and planted with *Halodule wrightii*. $*$ = significant difference between treatments. Bars indicate one standard error.

Figure 13

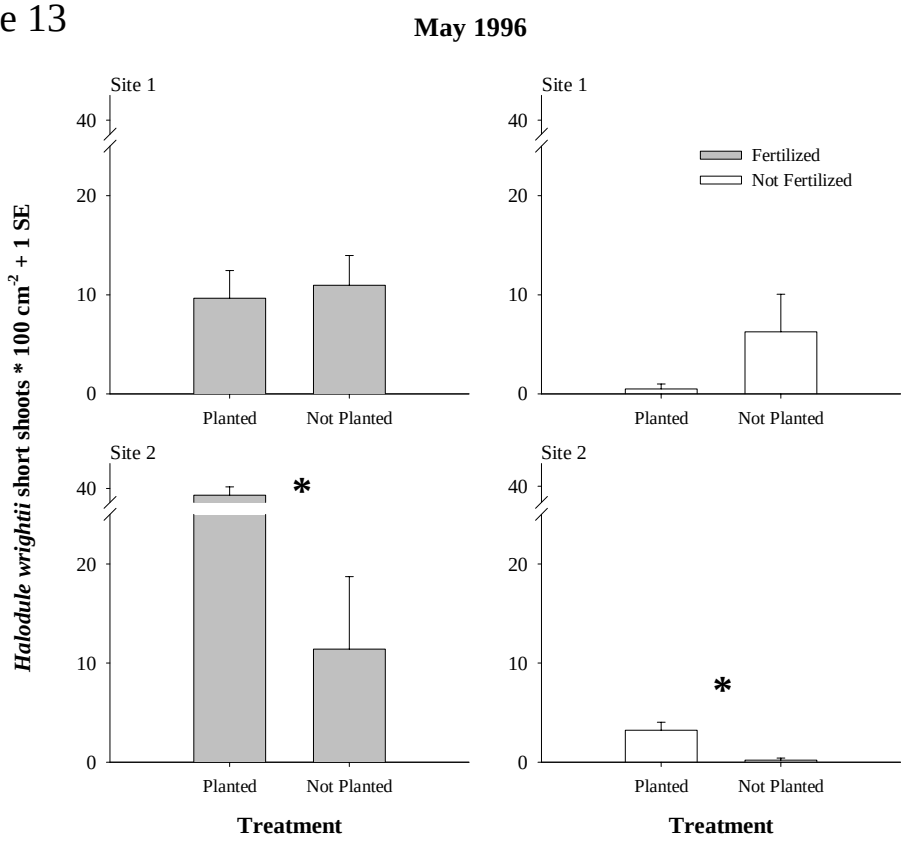


Figure 14: *Halodule wrightii* short-shoots per 100 cm^2 in the fertilized and unfertilized bird stake treatments in May 1996 and January 1997 at Sites 1 and 2. $*$ = significant difference between treatments. Bars indicate one standard error.

Figure 14

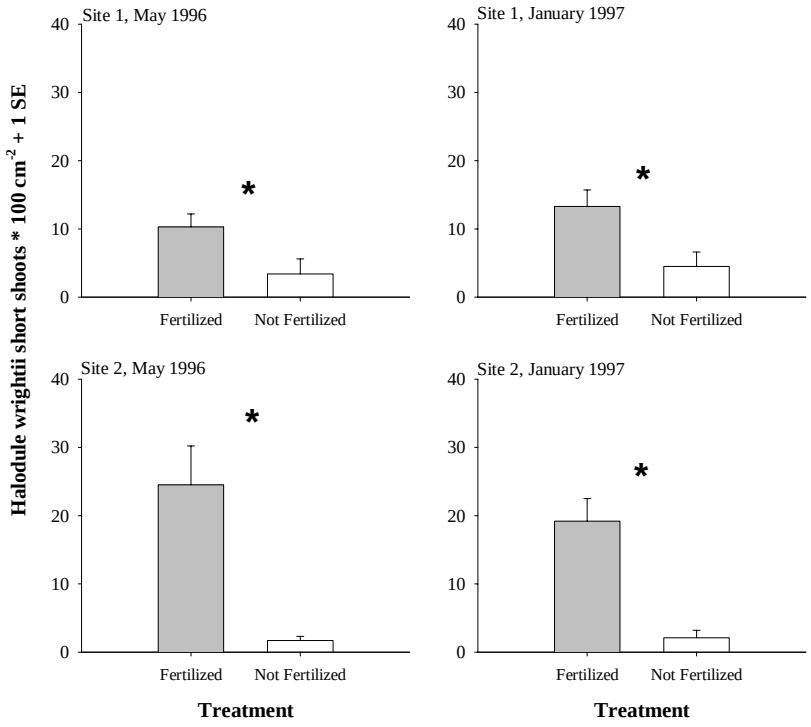


Figure 15: Oblique aerial photographic sequence of the colonization of the bird stake scar at Site 1 by *Halodule wrightii* on four dates: A = December 1996; B = December 1997; C =September 1998 and D = January 2000. The long dark feature indicated by the arrow is the *Halodule wrightii* growing along the length of the scar. Also indicated in Panel D is the recently filled scar adjacent to site 1.

Figure 15

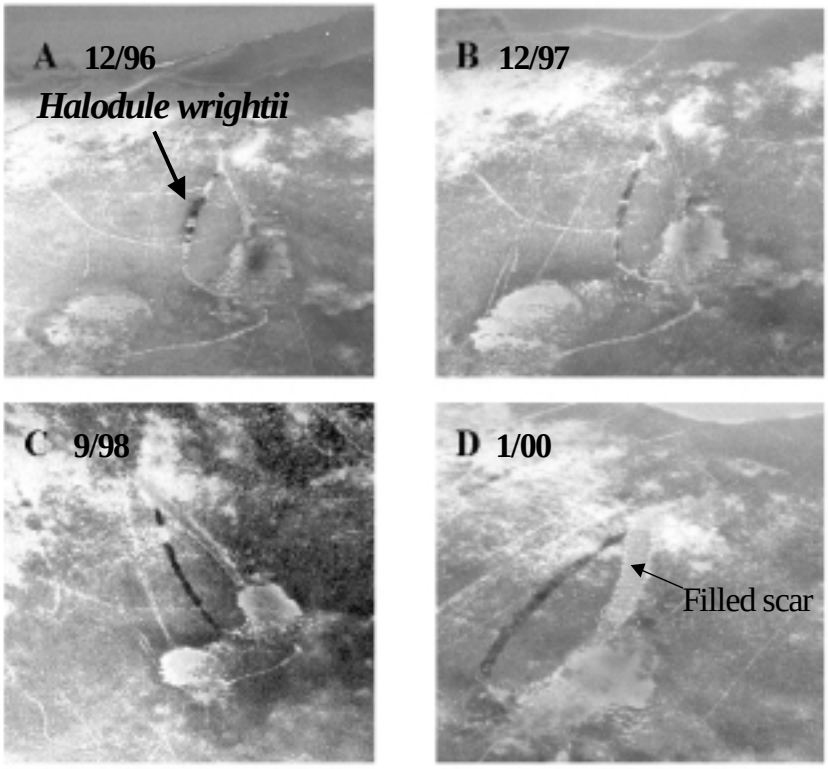


Figure 16: Oblique aerial photographic sequence of the colonization of the bird stake scar at Site 2 by *Halodule wrightii* on two dates 1) December 1996 and; 2) September 1998. The arrow points to the dark oval shaped features where *Halodule wrightii* is colonizing the area inside and outside the scars around the bird stakes.

Figure 16