Evaluating the Feasibility of Transplanting to Promote Seagrass Recovery in the Indian River Lagoon

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Executive Summary

Catastrophic loss of seagrass occurred in the Indian River Lagoon, Florida, due to two consecutive years (2011-2012) of unprecedented phytoplankton “super blooms”. Shading resulted in widespread loss of seagrass, up to 100% at many sites in the northern IRL; lagoon-wide approximately 60% (47,000 acres) was lost. Two years later, some sites had begun to recover, but large areas of the Lagoon showed no recovery, despite seemingly adequate water quality for growth. This project evaluated whether the lack of seagrass recovery in IRL is due to the lack of available recruits, vegetative fragments (shoots, roots, and/or rhizomes), or if other environmental factors are limiting recovery. The evaluation method consisted of transplanting seagrass (shoal grass, *Halodule wrightii*) using tested techniques to determine whether seagrass can grow in areas previously covered by seagrass and deemed suitable, based primarily on recent water clarity, for seagrass survival and expansion.

In July 2013, we established experimental plots at three sites to determine if seagrass recruitment might be limiting recovery. At each recipient site, transplants were made in unprotected plots (without enclosure) and protected plots (within enclosures) due to potentials impacts of grazers. In 2014, an additional site was added and modifications of enclosures were tested at one of the initial sites. Key metrics to assess transplant success were: initial survival of planting units, and subsequent monitoring of cells occupied per m^2 plot, visual cover, and canopy height. These proposed metrics readily demonstrated survival, growth, and expansion rates, and are recommended for use in future studies.

Results of these test transplants suggested that, in the absence of grazing pressure, the environmental conditions present at three of the four sites are favorable for seagrass recovery. These observations support the hypothesis that the lack of natural recruitment and recovery in areas of the IRL system nearly devoid of seagrass is due to the lack of seagrass vegetative stock and vegetative reproduction. The use of enclosures in these experiments demonstrated that grazing may be a major impediment to seagrass recolonization at the three northern sites. Grazing varied tremendously at our sites and is higher in the summer than the rest of the year.

Whether or not restoration in large, denuded areas of the Lagoon, can be accelerated by vegetative planting is still an open question. Clearly, grazing pressure may limit large-scale restoration. In areas with little or no grazing pressure on colonizing seagrass, fairly small efforts strategically located might be able to jump start seagrass restoration. But given the encouraging signs of initial seagrass recovery at a number of locations with severe seagrass losses in 2011-2012, it may be that significant natural/unassisted recovery will occur in a fairly short time frame, possibly 5 years or less. Such recovery would be more desirable than investing significant amounts of money into large-scale restoration efforts.

Given the uncertainty of that natural recovery, the next step in considering seagrass restoration might be consider doing a pilot-scale test, drawing on what has been learned in this project. Recommendations for future seagrass transplanting in the IRL include: conduct monitoring of natural recruitment patches, conduct a pilot-scale seagrass restoration test, develop a land-based experimental seagrass nursery facility, and engage citizen scientists in seagrass restoration.

Introduction

Catastrophic loss of seagrass occurred in the Indian River Lagoon (IRL), due to two consecutive years (2011-2012) of unprecedented phytoplankton "super blooms". Shading resulted in widespread loss of seagrass, with up to 100% loss at many sites in the northern IRL; lagoon-wide approximately 60% (47,000 acres) was lost. Two years later, some sites had begun to recover, but large areas of the Lagoon showed no recovery, despite seemingly adequate water quality for growth.

The goal of this project was to evaluate whether the lack of seagrass recovery in the IRL is due to the lack of available recruits, vegetative fragments (shoots, roots, and/or rhizomes), or if other environmental factors are limiting recovery. The evaluation method consisted of transplanting seagrass (shoal grass, *Halodule wrightii*) using tested techniques to determine whether seagrass can grow in areas previously covered by seagrass and deemed suitable, based primarily on recent water clarity, for seagrass survival and expansion. In our previous Year 1 and Year 2 reports we provided progress reports for those two periods. Key sections of those reports have been included below in this final report, while others have been updated with Year 3 as appropriate

Task 1 (from Year 1 Report, September 2013)

Task 1a. Kick-off Meeting

A Kick-off Meeting was held on July 10, 2013, in the District's Palm Bay Office to ensure all parties understood the objectives and tasks to be completed. A schedule was finalized that specified the timing of the tasks in Phase 1 (1b-1d below), the associated deliverable schedule, and a tentative task schedule for the remaining contract (assuming transplant success). The work load for Phase 1 was identified and discussed, including location of transplant sites, along with available budget, volunteers and other outside commitments to the District that would help in completing Task 1b and 1c. This meeting was summarized in Deliverable 1a, previously submitted and accepted by the District, and included here as Appendix 1.

Task 1b. Development of Work Plan and Experimental Design

The participants at the Kick-Off Meeting examined a series of GIS seagrass maps compiled from biennial aerial surveys by the District, as well as the results of the semiannual transect monitoring sites within the region, to determine where seagrass had been consistently abundant in the past and where it has been absent since 2011. Transplanting methods, an initial protocol, and plans to start the field work were discussed. Transplant sites selected were located where: current water quality is supportive for seagrass recovery; no seagrass currently exists, but did prior to 2011; and there are existing seagrass transect monitoring sites. In addition, the Sebastian Inlet District identified three transplant sites, two on the flood tidal shoal at Sebastian Inlet and one south of the inlet.

Subsequent to the Kick-off Meeting, a study plan was developed that includes the experimental design, such as transplanting protocol, plot size, sampling units, performance measures, and a schedule for transplanting and follow-up monitoring. This plan was Deliverable 1b, previously submitted and accepted by the District, and included here as Appendix 2.

Task 1c. Transplanting and Monitoring

The HBOI transplanting of *Halodule wrightii* was conducted during the week of July 22, 2013, at 3 sites, near the District's existing transects #17 ("Southwest Pineda" Causeway in Banana River), 34 ("Exxon," south of Turkey Creek), and 48 ("ELC," south of Wabasso bridge; Fig. 1). For the first two sites, the donor site for PUs (Planting Units) was near Transect #20 on the north side of Titusville. For the ELC site, the donor site was located on the southeast side of the spoil island just north of the 17th Street Bridge at Vero Beach.

Additionally, the Sebastian Inlet District established three sites around Sebastian Inlet, two on the flood tidal shoals (North Shoal and South Shoal) and one at Transect #44 (T44) south of the inlet along the eastern shoreline of the IRL (Figs. 1, 2). All of the Sebastian Inlet sites used the Vero Beach donor site (Fig. 1).

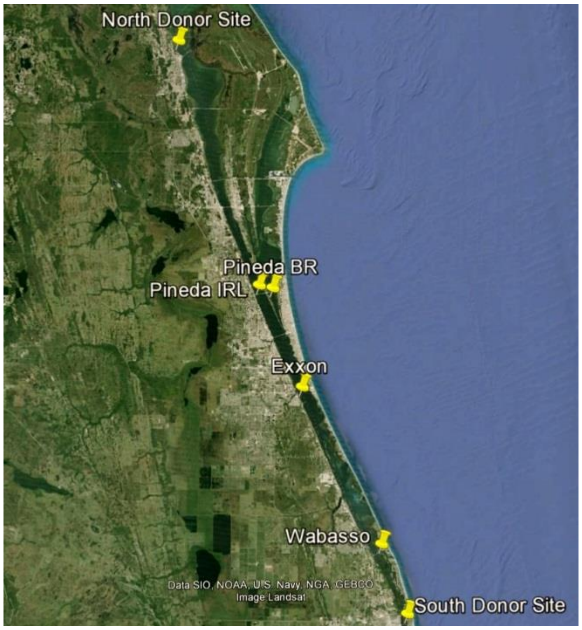


Figure 1. Map of donor and recipient sites in this study.

At the donor sites, a control (3 unprotected plots, ca. 2 m part) was established to test if our transplanting methods do work and that PUs survive the stress of the handling process. At each recipient site, there were 3 blocks (= replicates) of each set of 4 treatments (Fig. 3). The position of each of the 4 treatments was randomized within a block. The treatments per block were:

1) An unprotected plot (without enclosure), with 5 PUs in an X array, with each PU 28 cm apart on centers, to allow for vegetative spread
2) A protected plot (within enclosures), also with 5 PUs in an X array and 28 cm apart on centers
3) An unprotected plot (without enclosure), with an X array, 28 cm apart, of 40-cm long 2-cm by 2-cm wooden stakes driven into the sediment and extending 10 cm above the sediment, to test whether there are any available *Halodule wrightii* vegetative fragments which would be captured by the stakes and also to measure sediment loss and/or gain
4) An unprotected 1-m^2 bare plot, to test for unassisted natural recruitment.

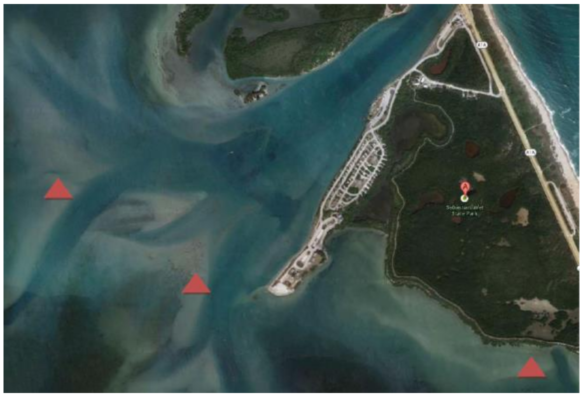


Figure 2. Approximate location of the three Sebastian Inlet sites.

Figure 3. PVC poles mark all plots to facilitate their location (Exxon site, September 19, 2013).

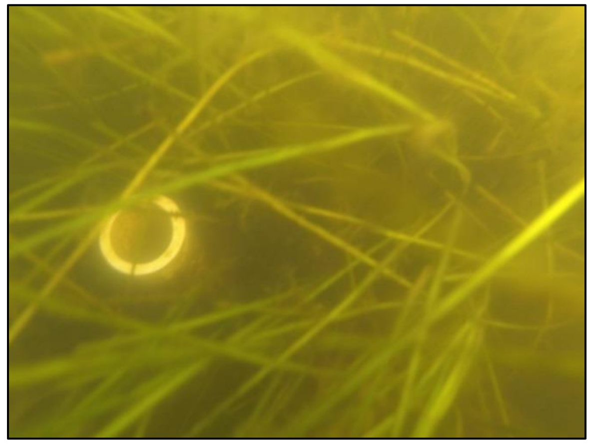


Figure 4. Transplanted seagrass plugs (to the right of PVC marker) completely blended into the surrounding bed at the donor sites (Titusville site, September 19, 2013).

Initial monitoring of the three HBOI transplant sites and northern donor site was conducted at approximate 2-week intervals, on August 9, August 22, September 4, and September 19, 2013. Monitoring of the 3 Sebastian Inlet sites was conducted separately by Atkins North America (Atkins) on August 4, August 11, August 14, August 28, and September 14, 2013. At the Titusville donor site, all transplanted plugs survived, with no evidence of impact (Fig. 4). Monitoring of the Vero Beach donor site was conducted by Atkins with similar results.

In our initial monitoring of the transplants we observed considerable variation among the three HBOI recipient sites (Tables 1, 2), which will be reviewed site-by-site below. Observations consistent at all three recipient sites were:

1) In protected treatments (cages), there was 100% survival of PUs.
2) No stakes trapped any seagrass fragments.
3) There was no recruitment of *Halodule* into any control (bare) plots.

Table 1. Survival of PUs (%, n = 5 per treatment) at the three HBOI recipient sites during four monitoring visits.

Date (2013)	Site	Unprotected Treatment				Protected Treatment			
		Block 1	Block 2	Block 3	Mean	Block 1	Block 2	Block 3	Mean
August 9	Pineda BR	0%	0%	0%	0%	100%	100%	100%	100%
	Exxon	0%	20%	40%	20%	100%	100%	100%	100%
	Wabasso	100%	100%	100%	100%	100%	100%	100%	100%
August 22	Pineda BR	0%	0%	0%	0%	100%	100%	100%	100%
	Exxon	0%	0%	0%	0%	100%	100%	100%	100%
	Wabasso	100%	100%	100%	100%	100%	100%	100%	100%
September 4	Pineda BR	0%	0%	0%	0%	100%	100%	100%	100%
	Exxon	0%	0%	0%	0%	100%	100%	100%	100%
	Wabasso	100%	100%	100%	100%	100%	100%	100%	100%
September 19	Pineda BR	0%	0%	0%	0%	100%	100%	100%	100%
	Exxon	0%	0%	0%	0%	100%	100%	100%	100%
	Wabasso	100%	100%	100%	100%	100%	100%	100%	100%
October 4	Pineda BR	0%	0%	0%	0%	100%	100%	100%	100%
	Exxon	0%	0%	0%	0%	100%	100%	100%	100%
	Wabasso	100%	100%	100%	100%	100%	100%	100%	100%

Table 2. Monitoring status of key metrics at the HBOI sites during the last monitoring visit (September 19, 2013).

Metric	Site	Unprotected Treatment				Protected Treatment			
		Block 1	Block 2	Block 3	Mean	Block 1	Block 2	Block 3	Mean
PUs Live (Number)	Pineda BR	0	0	0	0	5	5	5	5
	Exxon	0	0	0	0	5	5	5	5
	Wabasso	5	5	5	5	5	5	5	5
Cells Occupied (Number)	Pineda BR	—	—	—	—	17	14	20	17
	Exxon	—	—	—	—	16	16	24	19
	Wabasso	30	34	28	31	19	18	28	22
Halodule (% Cover, Visual)	Pineda BR	—	—	—	—	10%	3%	5%	6%
	Exxon	—	—	—	—	3%	1%	4%	3%
	Wabasso	10%	16%	12%	13%	4%	6%	12%	7%
Canopy Height (cm)	Pineda BR	—	—	—	—	22	13	15	17
	Exxon	—	—	—	—	1.5	1.5	2	1.7
	Wabasso	11	12	8	10	15	7	19	14
Drift Algae (% Cover, Visual)	Pineda BR	20%	0%	5%	8%	60%	45%	5%	37%
	Exxon	0%	0%	0%	0%	0%	0%	0%	0%
	Wabasso	70%	30%	0%	33%	5%	10%	5%	7%

At the Pineda BR site, on August 9, 2013, it was observed that all of the PUs in the unprotected treatments were completely lost, likely due to grazing, given that the protected ("caged") treatments all had five healthy PUs. While both manatees and turtles are possible suspects, we could not determine which were responsible. We suspected manatees as we believe that turtles likely would not have dug up the entire plants. In contrast, there were no losses of PUs in the protected treatments; plants looked healthy and some of the PUs have begun to spread. There were large quantities of drift algae (primarily *Acanthophora spicifera*, which was very abundant offshore of our plots) and the bryozoan *Zoobotrryon*. The cages trapped the drift more than the other treatments. The drift algae did not seem to have a negative impact on *Halodule* – even with the thick algae, all 5 PUs could be easily seen through the top of the cage, with blades sticking above the algae canopy (Fig. 5). The drift was removed from the cages on each visit, but it likely returns quickly, given its nearby abundance. The drift algae on the last Year 1monitoring visit (September 19, 2013) had declined from the previous visit.

Figure 5. Despite heavy coverage of drift algae and *Zoobotryon* at the Pineda site, *Halodule* PUs are doing well (September 19, 2013).

At the Exxon site, on August 9, 2013, all of the unprotected treatments showed signs of being grazed, with surviving PUs (only 20% of what was transplanted) only about 5 cm tall. Unlike at Pineda, there also was clear evidence of grazing in the protected treatment with blades of about 15 cm. By the second monitoring (August 22, 2013), all of the unprotected PUs were gone, and the PUs in the protected treatments appeared to be further grazed (<5 cm, with two of the plots uniformly grazed to within 1 cm of the sediment surface). Based on the way the seagrass blades are being sheared off, the working hypothesis is that the unknown grazer may be juvenile green turtles. These unexpected observations stimulated discussion with Bob Chamberlain and Karen

Holloway-Adkins on consideration of adding some finer mesh to the cages at the Exxon site, so that the experiment could continue there, rather than losing all the transplants to grazers. During the third monitoring on September 4, green plastic mesh, 40 inches x 40 inches, with openings of 1 inch x 1 inch, were secured with tie-wraps on the tops of cages in two of the three protected treatments to determine if additional protection will stop the grazing (Fig. 6). However, we also noted that *Halodule* in the protected treatments had possible signs of recovery/less grazing; a few shoots were longer than in the second monitoring, and new shoots had formed. Unfortunately, the additional mesh did not result in less grazing; on September 19, 2013, the shots were all observed to have been grazed to 1.5-2 cm (Fig. 7). But despite 2 months of intense grazing, all of the PUs in the protected treatment survived, and the coverage (cells occupied per m^2) was slightly higher than at the Pineda site (Table 2). Drift algae, consisting of *Gracilaria* and *Hypnea*, were observed in the plots at this site only on September 4, 2013.

Figure 6. Additional mesh was added to cages in the protected treatments at the Exxon site in an effort to deter grazing.

In contrast to the Pineda BR and Exxon sites, at the Wabasso site, all plugs in all treatments have survived and appear healthy. By September 4, 2013, about half of the PUs had begun to spread to the point that the individual integrity of the PUs was being lost (Fig. 8). The only sign of grazing in this initial phase was a slight amount on a couple of the PUs, with minimal impact, on September 4 and 19, 2013. *Halophila decipiens* had colonized some of the plots at this site and in the 2-m buffer around the plot which is monitored for natural recruitment into the study area. Drift algae (*Acanthophora spicifera*) had been present at this site, but not to the extent that it was at Pineda BR (Table 2).

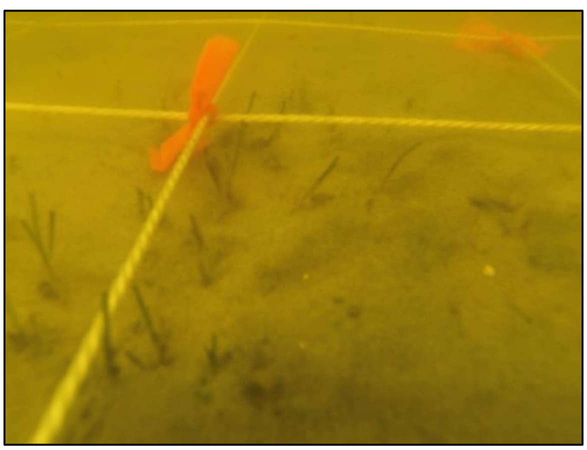


Figure 7. Although PUs at the Exxon site have been severely cropped in the protected treatments, they are still all surviving (September 19, 2013)

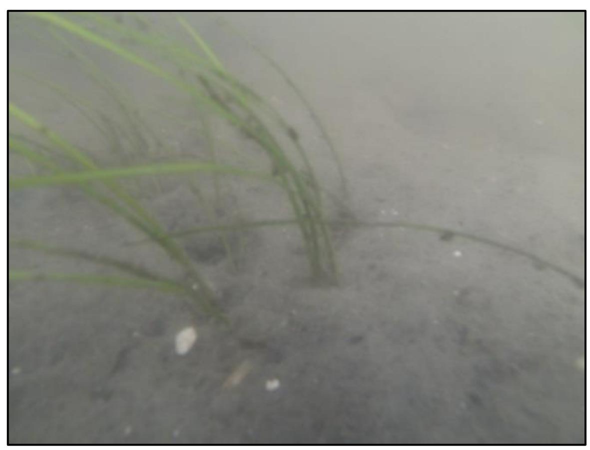


Figure 8. All PUs in both the protected and unprotected treatments at the Wabasso site have survived, with little grazing, and are beginning to spread.

In the monitoring at the Sebastian Inlet sites, considerable variation was also observed among the three recipient sites (Tables 3, 4), which will be reviewed site-by-site below. Observations at recipient sites included:

1) Good survival of PUs in both the protected and unprotected treatments at T44.
2) Stakes trapped seagrass fragments at T44; however, the fragments did not become established.
3) There was slight (1% cover) recruitment of *Halodule* into a control (bare) plot at the South Shoal site.

Table 3. Survival of the PUs (%, N = 5 per treatment) at the three recipient sites around Sebastian Inlet at five monitoring intervals.

		Unprotected Treatment				Protected Treatment			
Date (2013)	Site	Block 1	Block 2	Block 3	All	Block 1	Block 2	Block 3	All
August 4	Shoal North	100%	100%	100%	100%	100%	100%	100%	100%
	Shoal South	80%	100%	100%	93%	100%	100%	100%	100%
	T-44	100%	100%	100%	100%	100%	100%	100%	100%
August 11	Shoal North	100%	100%	100%	100%	100%	100%	100%	100%
	Shoal South	60%	100%	100%	87%	100%	100%	100%	100%
	T-44	100%	100%	100%	100%	100%	100%	100%	100%
August 14	Shoal North	100%	100%	100%	100%	100%	100%	100%	100%
	Shoal South	40%	100%	100%	80%	100%	100%	100%	100%
	T-44	100%	100%	100%	100%	100%	100%	100%	100%
August 28	Shoal North	100%	100%	100%	100%	80%	100%	100%	93%
	Shoal South	40%	100%	100%	80%	100%	100%	100%	100%
	T-44	100%	100%	100%	100%	100%	100%	100%	100%
September 14	Shoal North	100%	100%	100%	100%	60%	40%	0%	33%
	Shoal South	40%	100%	100%	80%	100%	100%	100%	100%
	T-44	100%	100%	100%	100%	100%	100%	100%	100%

Table 4. Monitoring status of key metrics at the last Year 1 monitoring visit at the Sebastian Inlet sites (September 14, 2013).

		Unprotected Treatment				Protected Treatment			
Metric	Site	Block 1	Block 2	Block 3	Mean	Block 1	Block 2	Block 3	Mean
PUs Live (Number)	Shoal North	5	5	5	5	3	2	0	2
	Shoal South	2	5	5	4	5	5	5	5
	T-44	5	5	5	5	5	5	5	5
Cells Occupied (Number)	Shoal North	22	25	24	24	8	4	0	4
	Shoal South	11	32	33	25	20	22	24	22
	T-44	36	29	13	26	28	39	43	37
Halodule (% Cover, Visual)	Shoal North	8%	8%	8%	8%	2%	1%	0%	1%
	Shoal South	2%	9%	9%	7%	8%	7%	8%	8%
	T-44	10%	9%	7%	9%	9%	10%	10%	10%
Canopy Height (cm)	Shoal North	7	7	7	7	16	16	0	11
	Shoal South	7	7	7	7	16	16	16	16
	T-44	7	7	7	7	18	18	18	18
Drft Algae (% Cover, Visual)	Shoal North	0%	0%	0%	0%	0%	0%	0%	0%
	Shoal South	0%	0%	0%	0%	5%	0%	0%	2%
	T-44	0%	10%	10%	7%	10%	10%	0%	7%

At the North Shoal site, it was observed that all of the PUs survived in the unprotected treatments, even though those PUs were grazed and did not spread significantly from the original unit. As of September 14, 2013, only 5 of the original 15 PUs survived in the protected treatments because the cages became fouled with drift algae and cyanobacteria creating habitat for hermit crabs and blue crabs. These crabs disturbed the sediment and excavated the seagrass rhizomes. In the previous monitoring events to August 28, 2013, the cages did protect the PUs from grazing (all the protected PUs had survived). In comparison, all of the unprotected PUs were grazed, but survived during this period. However, that protection has been lost since August 28 with the fouling and use of the cages as habitat by the crabs at this site. This site is also affected by the inlet related tidal currents and boat wakes from the inlet channel. Both act to erode the sediment, as evidenced by the erosion around the stakes within those treatments (Fig. 9).

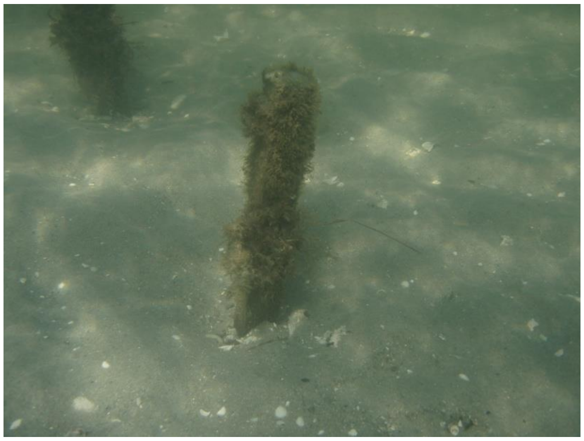


Figure 9. Erosion around stake at the North Shoal site.

At the South Shoal site, all the PUs at the protected treatments survived and three of the PUs at the unprotected treatments were lost. All of the unprotected PUs were grazed (Fig. 10). Similar to the North Shoal site, the benefit of the cages has been mitigated by the fouling on the cages. The unprotected PUs are actually spreading better than the protected PUs despite being grazed. This station does not seem to have the tidal and boat wake erosional forces that has affected the North Shoal site. As mentioned above, some recruitment is occurring in one of the bare treatments.

The greatest success of the three shoal-related stations has been achieved at the T-44 site where all of the PUs at both the protected and unprotected treatments have survived and are spreading (Fig. 11). This site has benefited from the presence of both *Halophila johnsonii* and *H. decipiens* present in many of the treatments. The presence of these small seagrasses indicate that there is minimal erosion occurring and the sediments are stable. The protected treatments are spreading faster than the unprotected treatments at this site.

Figure 10. South Shoal site unprotected treatment.

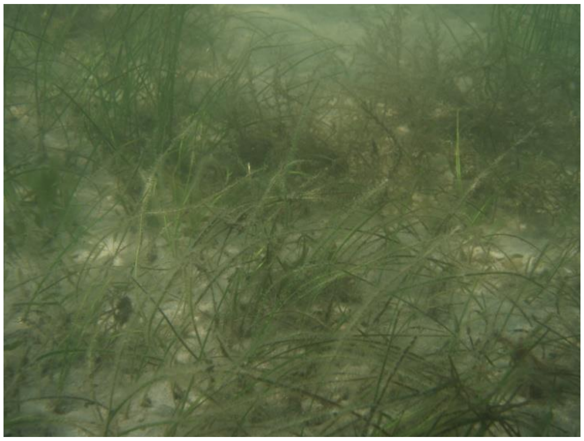


Figure 11. PUs in a protected treatment at T44.

Task 1d. Recommendations for Continued HBOI-FAU Work

Preliminary Evaluation of Performance Measures

The performance measures proposed for this study are all useful ones (Table 2). The number of PUs was certainly a useful metric to determine initial survival of the transplants. By the end of this first phase, it was clear that many of the PUs were spreading laterally as it was becoming difficult to distinguish individual PUs filling in the space between them. However, the fact that the number of cells occupied at the three sites (the average ranged from 17-22; Table 2, protected treatment), indicated that there was also some loss of the size of PUs (starting cells occupied 20 cells at these sites). Only the Wabasso site had a net increase in coverage, and interestingly was higher in the unprotected treatment (mean = 31 cells) than in the protected treatment (mean = 22 cells). The % cover visual estimate of *Halodule* indicated that the overall density of the transplant is still quite sparse, ranging from 3-7% average in all the protected treatments at the three sites; at Wabasso the visual estimate in the unprotected treatment (13%) was nearly twice that of the protected treatment (7%). Canopy height in the protected treatment was highest at Pineda BR (mean = 17 cm), followed by Wabasso (mean = 14 cm), with the heavily grazed plants at Exxon cropped quite short (mean = 1.7 cm). At Wabasso, canopy height in the protected treatment (mean = 10 cm) was less than that in the unprotected treatment (mean = 14 cm). At the end of phase 1, it is also clear that there is considerable variability in these metrics at the various sites (e.g., in the protected treatment at Wabasso, visual cover for *Halodule* ranged from 4-12% and canopy height ranged from 7-19 cm).

The abundance of drift algae, when present, was also quite variable within each of the sites. So far, the high drift algal abundance at Pineda BR does not appear to seriously impact survival or growth of the transplants. Given the potential interaction with seagrass recruitment, survival, and growth, continued observation on the drift algae might be useful during the course of this project.

The height of the stakes above the sediment is also a useful measure of accretion or erosion of sediments. During the last monitoring period, there was evidence of sediment accretion at two sites: an increase of 2.7 cm and 1.4 cm at Pineda BR and Wabasso, respectively. There has been no change in this metric at Exxon (stakes still 10 cm above the sediment).

Accordingly we recommended that all of these performance measures be continued to be used in Year 2.

Recommendations for Phase 2 Monitoring (per Year 1 Final Report, September 2013)

Based on the Year 1 results, we recommended proceeding with the monitoring plan in the SOW, as below. The only modifications that we recommended from the Year 1 work were:

1) As the donor sites showed no impact from the transplant techniques we used, that part of the experiment is over and no further monitoring was recommended.

2) It was originally proposed to begin monthly monitoring in September 2013 and to continue the Year 2 monitoring at monthly intervals. However, given the very dynamic site-specific observations we have made in a very short time, we planned to continue

twice-a-month monitoring at least through October 2013, then go to monthly monitoring through the winter.

3) We recommended considering additional plantings at these three recipient sites in spring 2014, after we see what survives over the winter. These plantings should consider what might be limiting success (e.g., the grazing problem at Exxon), but will also provide observations from transplants at the start of the growing season (light conditions for seagrasses are best in spring due to highest amount of underwater PAR being available at that time).

<u>*Phase 2 Tasks*</u>

2a) Continue monitoring recipient sites through September 2014, depending on transplant site success, and as recommended in the Phase 1 Report (e.g., a recommendation to adjust study protocol may be warranted).

2b) Produce a Final Phase 2/Year2 report that is similar in content to the Final Phase 1 report, including a monitoring schedule for Phase 3. With the completion of over a year of monitoring, the Phase 2 report also can begin to assess whether restoration in large, denuded areas can be accelerated by vegetative planting**.**

Recommendations for Phase 3/Year3 (per Year 2 Final Report, September 2014)

Based on the Year 2 results, we recommended proceeding with the monitoring plan in the SOW, as below. The only modifications that we recommend from Year 2 work were:

1) As the donor sites showed no impact from the transplant techniques we used, that part of the experiment is over and no further monitoring was recommended.
2) Having completed more than a full annual cycle at the Year 1 sites, we recommend removal of all cages in the protected treatment at those three sites in October 2014 and continued monitoring at 1-2 months intervals until July 2015.
3) For the new Year 2 sites (Pineda IR and Exxon replant), we recommend continued monitoring at monthly intervals until April 2015, in order to complete a full annual cycle of monitoring. At that time, the cages and some of the fences will be removed from the protected treatments, and monitoring will be continued at monthly intervals for the duration of the project to determine the survivorship of the *Halodule* in those plots following removal of protection.

<u>*Phase 3 Tasks*</u>

3a. Complete monitoring, depending on transplanting success, as recommended in the Phase 2 Report.

3b. Submit Final Project Report that is similar in content to the previous phase reports, but summarizes the salient final project results and provides conclusions. Included in the report will be, but not limited to: evaluation of limiting factors affecting transplant success, and final evaluation of the recovery performance measures used to determine feasibility/utility of transplanting as a recovery strategy.

Task 2a. Continue monitoring recipient sites in Year 2, depending on transplant site success, and as recommended in the Phase 1 Report.

Task 3a. Continue monitoring recipient control sites through September 2015, depending on transplanting success, and as recommended in the Phase 2 Report

Monitoring of Year 1 Sites

In Year 2, monitoring continued every 2 weeks through October 2013, after which the sites were monitored monthly throughout Year 2 and Year 3, until July 2015. Per the Year 3 research plan, the cages were removed from the protected plots during the October 2014 sampling trips.

In this report, the monthly data are presented as bar graphs with means of the three key metrics: cells occupied per m^2 plot (number, out of 100), visual cover (%), and canopy height (cm).

Stakes trapped seagrass fragments only on two occasions:

- There was a single, rooted shot of *Halodule* observed in one of the stake plots at Pineda BR in November 2014. No seagrass was observed in that plot in the following month.
- There was a single, rooted shoot of *Halodule* observed in one of the stake plots at Exxon in March, which was not observed in that plot in the following month.

As these were the only observations of any evidence of recruitment in the stake treatment, it will not be further discussed.

Pineda BR Site

Unprotected Treatment

At the Pineda BR site, in the unprotected plots, the initial transplants quickly disappeared in August 2013, assumed to be due to manatees (see Task 1). There was no seagrass observed in them during the following nine months. In June 2014, we observed *Halodule* in one of the unprotected plots (9 cells; so a mean of 3 cells for the three plots in this treatment) which persisted for three months and reached a maximum in August (25 cells in the same plot; mean of 8 cells for the treatment) (Fig. 12, top panel). The plants were sparse (highest mean visual cover was 3% in August) (Fig. 13, top panel) and had shorter canopy heights (12-14 cm) than the protected plots (Fig. 14, top panel). However, by September 2014, no *Halodule* was observed in any of the unprotected plots.

Protected Treatment

In the protected plots, the number of cells occupied by *Halodule* (Fig. 12, top panel) increased from a mean of 17 cells per plot in September 2013 to a mean of 30 cells per plot in November, and was stable, with means of 29-31 cells per plot from December through February 2014. The number of cells occupied by *Halodule* increased from a mean of 38 cells per plot in March to a mean of 46 cells per plot in April, and then rapidly increased to a mean of 74 cells per plot in May and a mean of 90 cells per plot in June. At that time *Halodule* began spreading out in all four directions from two of these plots.

Over the rest of that summer (July – September 2014), the mean ranged between 82 (August) and 92 (July) cells per plot. Thereafter, the number of cells occupied by *Halodule* fell to a mean of 53 cells per plot in October, then stabilized for several months, with means from November 2014 to February 2015 ranging from 24 (December) to 28 (February). During those months, there was some spreading of *Halodule* from the plots, with 2 small shoots outside one plot in October. In November, there were drifting fragments of *Halodule* observed, but no sign of any recruitment in the plots or in the 2-m perimeter area. During January to June 2015, there was a small amount of spreading immediately outside two of the plots. From March to June 2015, the mean number of cells was lower, but stable, ranging from 14 (May) to 17 (April) cells per plot.

Visual estimates of *Halodule* cover in the protected plots (Fig. 13, top panel) followed similar trends as those observed for cells per plot. Visual cover increased over the fall from a mean of 6% in September 2013 to 14% in November. Visual estimates of cover were stable from December 2013 to February 2014, with means of 11-12% over those months. Visual estimates of cover markedly increased from a mean of 16% in March to a peak of 81% in June, and then declined to 62% in September and more precipitously during the rest of the year (October: mean = 17%; November: mean = 7%). From December 2014 to March 2015, visual estimates of the relatively sparse cover fluctuated from a mean of 5% in December to a mean of 8% in January and February and back to 5% in March before falling to a range of 2-3% during April to June.

Canopy height of *Halodule* in the protected plots (Fig. 14, top panel) was stable (means = 15-17 cm) during September to October 2013 and declined (means = 7-10 cm) over the winter (December to February). Canopy height increased from March (mean = 13 cm) to a peak in June (mean = 26 cm), and then declined over the summer, with a mean canopy height of 10 cm in September and continuing to decline until December 2014 (mean = 4 cm). Canopy height increased during the winter ranging from a mean of 9 cm (January) to a mean of 10 cm (February to April and then declined to a mean of 8 cm in May and 6 cm in June 2105.

Site Summary

In the protected treatment, we observed a significant increase in cover and size of *Halodule*, consistent with seasonal patterns typical for this species, with growth declining over fall and winter and peaking in spring and early summer. The plants appeared quite healthy in appearance throughout the year. Removing the cages in October 2014 did not initially seem to affect the *Halodule* in the protected treatment (the sharpest declines noted above occurred prior to the removal), however during spring 2015, we saw a decline in the previously protected transplants. Observations along the nearby transect in the District's long-term monitoring program indicated that recruitment of *Halodule* is occurring at this site (Lori Morris, SJRWMD, pers. com.). We observed natural recruitment, as small individuals of *Halodule*, in the swim around the 2-m perimeter of our experimental plots in February 2014, increasing through early summer, peaking in July, decreasing in August, and disappearing by September 2014. Similarly the spread outside two protected plots in June 2014 was not subsequently observed, and the colonization observed in an unprotected plot in June did not survive until September. Collectively, these observations suggest a seasonal pattern of grazing that may limit the sustained recruitment of *Halodule* at this site, without protective measures, such as caging used in this project.

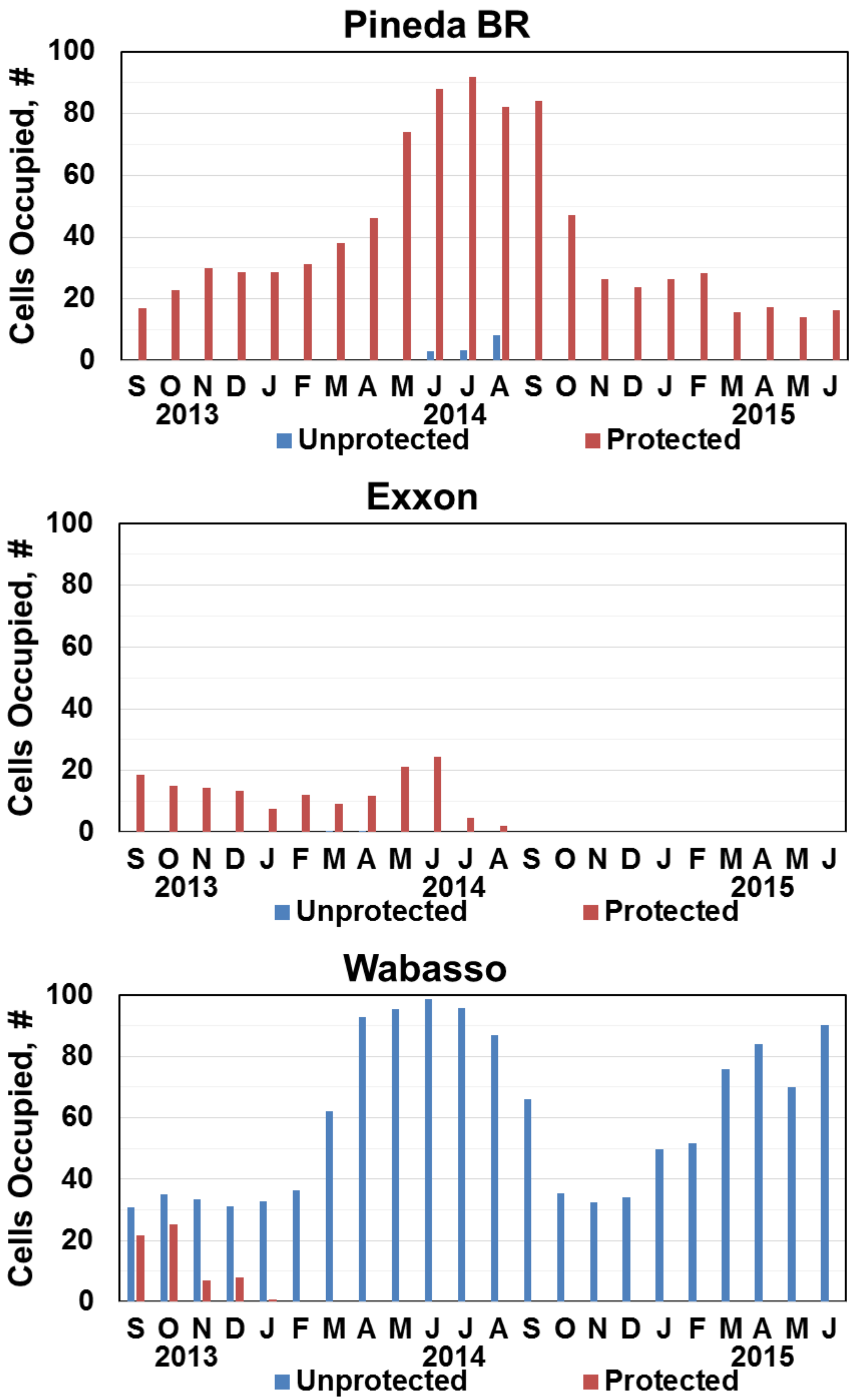


Figure 12. Mean number of cells occupied per m^2 plot at the three Year 1 recipient sites, at monthly monitoring intervals, September 2013 – June 2015.

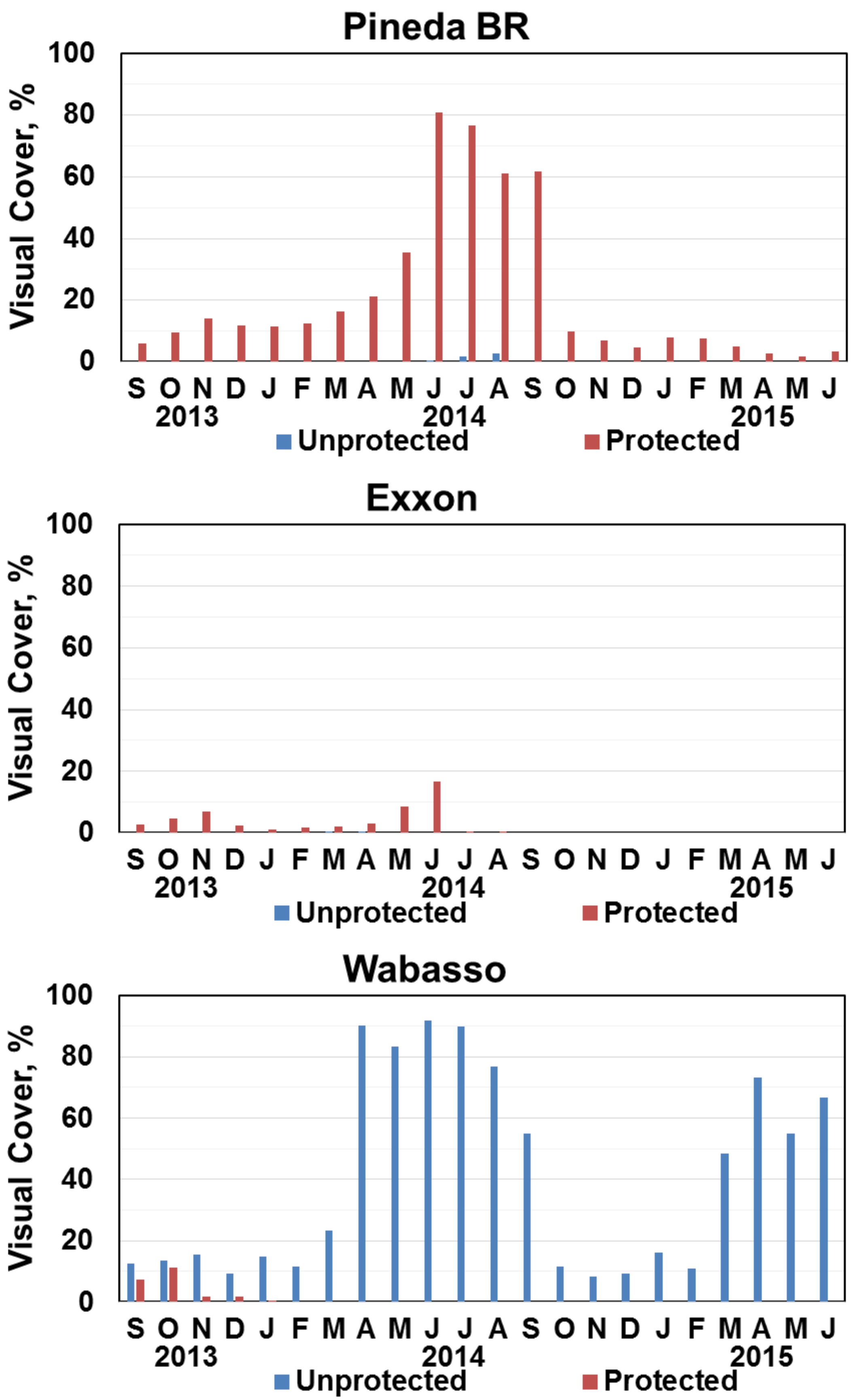


Figure 13. Mean visual cover (%) at the three Year 1 recipient sites, at monthly monitoring intervals, September 2013 – June 2015.

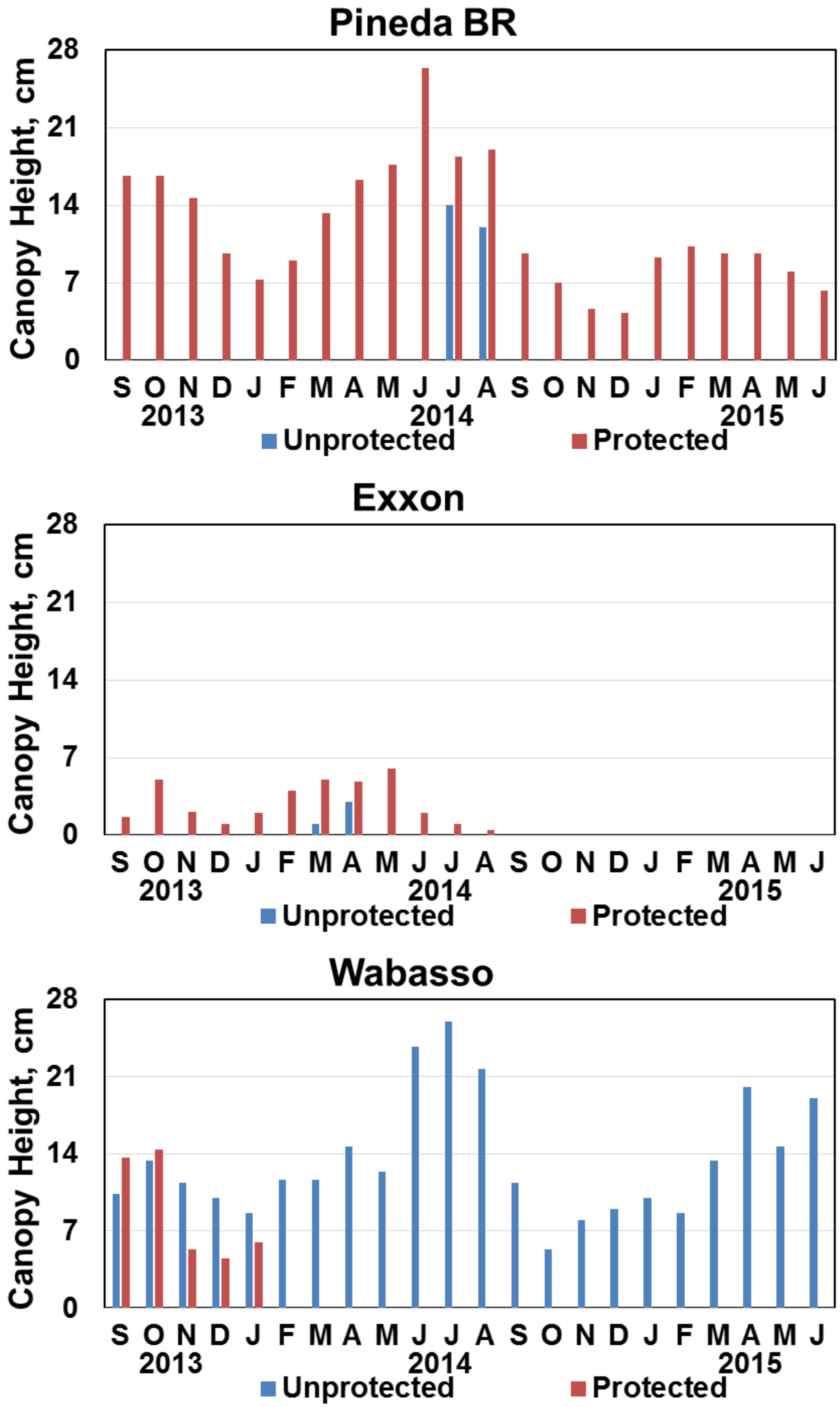


Figure 14. Canopy height (cm) at the three Year 1 recipient sites, at monthly monitoring intervals., September 2013 – June 2015.

Exxon Site

Unprotected Treatment

At the Exxon site, in the unprotected plots, the initial transplants quickly disappeared in August 2013, assumed to be due to manatees (see Task 1). Except for a single shoot of *Halodule* observed in one plot in March 2014, which appeared to be a new recruit (i.e., not from one of our adjacent plots or regrowth from a rhizome in that plot), persisting only into April 2014, no seagrass was observed in the unprotected plots during the following year.

Protected Treatment

The number of cells occupied in the protected plots by *Halodule* (Fig. 12, middle panel) declined from a mean of 19 cells per plot in September 2013 through the fall and winter to a mean of 9 cells per plot in March 2014, and then increased during the spring to a mean of 24 cells per plot in June 2014. This increase in the mean obscured the fact that 2 of the 3 plots actually went extinct (one in May 2014, one in June 2014), with the remaining plot having 73 cells occupied in June 2014. The number of cells then plummeted in that remaining plot in July 2014 (to 14 cells, equivalent to a mean of 5 cells per plot in that treatment), further declined in August 2014, and was zero in September 2014. Thus, the protected treatment at this site went extinct after 14 months.

Similarly, visual estimates of cover of *Halodule* in the protected plots (Fig. 13, middle panel) increased from a mean of 3% in September 2013 to a mean of 7% in November 2013. Visual estimates of cover decreased over winter, with means of 1-2% from December 2013 to March 2014. Visual estimates of cover increased from March (mean = 2%) to May 2014 (mean = 9%), peaked in June 2014 (mean = 17%, with the sole surviving plot having an estimate cover of 50%), then rapidly declined over the summer with the sole surviving plot having an estimate of 1% or less in July and August 2014, prior to its extinction by September 2014.

Canopy height of *Halodule* in the protected plots (Fig. 14, middle panel) increased from September 2013 (mean = 2 cm) to October 2013 (mean = 5 cm), and declined to a mean of 1-2 cm from November 2013 through January 2014. Canopy height in the protected plots increased from February (4 cm) to May (6 cm) 2014, suggesting that the grazing pressure on *Halodule* was reduced over the late winter and spring compared to previous seasons and summer (June to August 2014) when canopy height was 1-2 cm, before going extinct by September 2014.

Site Summary

We observed, even in the protected treatment at this site, obvious and sustained grazing impacts, resulting in the extinction of all transplants from Year 1. The continued severity of grazing at this site was presumed to be due to herbivorous fish. In December 2013, we observed two potential grazer species, pinfish and sheepshead, in one of the cages, which was our first observation of these species within these plots. We saw no improvement with the addition of secondary, smaller mesh to the tops of cages in an effort during September to December 2013 to reduce grazing at this site. While the transplants did ultimately go extinct after 14 months, the ability of the plants to survive sustained grazing was amazing, and points to the resiliency of

Halodule in this system. While two of the three plots barely survived the winter, the third one spread in the spring despite continued grazing.

Observations along the nearby transect in the District's long-term monitoring program indicated some recruitment of *Halodule* was occurring at this site (Lori Morris, SJRWMD, pers. com.). Natural recruitment, as small individuals of *Halodule*, was observed in the swim around the 2-m perimeter of our experimental plots only in April and May 2014, when we saw the spurt in growth of the one remaining unprotected plot, including spreading outside that plot. We observed one individual of *Halophila engelmannii* in one of the control plots in June 2014. Both the spreading *Halodule*, and the *Halophila* in one of the controls, were short-lived, gone by July 2014, concomitant with the decline in *Halodule* in the last remaining plot. However, we did see signs of natural recruitment during January 2015 (1 plant in the 2-m perimeter swim), which was not observed in February; more significantly, during March to May 2015, we observed much more natural recruitment (11 separate small patches) in the 2-m perimeter swim, and additional recruits within a few meters of the experimental site. By June 2015, there was only one individual observed. In September 2015, several small (<1 m^2) patches of *Halodule* and a few tiny (<0.01 m2) patches of *Halophila engelmannii* were found about 20-40 m offshore of the treatments in a site swim of the general area.

So, even more so than what we observed at the Pineda BR site, our results at the Exxon site point to the important role grazers (both manatees and fish) have on the successful recruitment of seagrass in the IRL.

Wabasso Site

<u>Unprotected Treatment</u>

At the Wabasso site, the number of cells occupied by *Halodule* in the unprotected treatment (Fig. 12, bottom panel) was stable between September 2013 and February 2014, with means ranging between 31-35 cells per plot. The number of cells occupied by *Halodule* in the unprotected treatment rapidly increased in March (mean = 62 cells) and April 2014 (mean = 93 cells). The mean number of cells per plot ranged from a mean of 87 to 99 cells per plot during May through August 2014, as *Halodule* spread throughout the plots in this treatment, and then declined in September 2014 to a mean of 66 cells per plot. In April 2014, using our 1-m^2 quadrat, we also counted the number of cells of *Halodule* cover, within 1 m surrounding each of the unprotected experimental plots, and those counts, combined with that of the original plot, ranged from 246 to 347 cells (mean = 301 cells); these populations continued to spread out from the plots and beyond our ability to routinely measure. By June 2014, the transplanted *Halodule* had runners up to 1.5 m from the plots in multiple directions. This spreading from our transplants made it difficult to discern if any natural recruitment occurred near our transplants.

During the fall 2014, the number of cells occupied by *Halodule* fell sharply from a mean of 66 cells per plot in September to a mean of 35 cells per plot in October but then stabilized (means of 32 and 34 cells per plot in November and December, respectively). *Halodule* continued to spread into the surrounding area from the plots at high % cover, while appearing to thin in the actual plots. Over the winter, the number of cells in the unprotected plots occupied by *Halodule* increased to a mean of 50 cells per plot in January 2015, 52 cells per plot in February, and 76 cells per plot in March, reversing the decline we saw at this site in the fall. *Halodule* continued to spread into the surrounding area (at least 6 m from the plots). Mean number of cells then fluctuated: 84 cells per plot in April, 70 cells per plot in May, and 90 cells per plot in June 2015.

The mean visual estimates of *Halodule* cover (Fig. 13, bottom panel) initially (September 2013 to February 2014) ranged between 9% (December) to 15% (November, January), increased to 25% in March, and expanded to 90% in April 2014. Mean visual cover was stable (between 83-92%) through July, decreased to 77% in August, further declined to 55% in September and to 12% October before stabilizing (means of 8% and 9% in November and December, respectively). Over the winter, visual estimates of cover markedly increased to a mean of 48% in March 2015. Visual estimates of cover then fluctuated: 73% in April, 55% in May, and 67% in June 2015.

From September 2013 to May 2014, the canopy height of *Halodule* in the unprotected plots (Fig. 14, bottom panel) ranged from a mean of 9 cm (January) to 15 cm (April). Mean canopy height was much higher in the summer (June through August) 2014, ranging between 22 and 26 cm. Mean canopy height declined in September to 11 cm and in October to 5 cm, before stabilizing at 8-10 cm from November 2014 to February 2015, then increasing in March to 13 cm. Canopy height then fluctuated: 20 cm in April, 15 cm in May, and 19 cm in June 2015.

<u>Protected Treatment</u>

In the protected treatment, the number of cells occupied by *Halodule* (Fig. 12, bottom panel) was similar in September (mean = 22 cells per plot) and October 2013 (mean = 25 cells per plot), declined to means of 7-8 cells per plot in November and December 2013 and a mean of 1 cell per plot in January 2014. No remaining shoots of *Halodule* were present in February 2014.

Visual estimates of cover in this treatment (Fig. 13, bottom panel) increased from 7% in September 2013 to 11% in October 2013, before declining to 2% in November and December 2103, then to 0.2% in January 2014 and becoming extinct by February 2014.

Mean canopy height of *Halodule* in the protected plots (Fig. 14, bottom panel) was 14 cm in September and October 2013, and then declined to 5-6 cm through January 2014 prior to the extinction of this treatment.

During the decline in this treatment, the plants were much sparser, with thin blades; chlorosis and browning of blades evident in November 2013. The decline appeared to be due to shading by the cage, during the period of year where light limitation was maximal due to high water and highly colored water at the end of the wet season. This site difference was exemplified in November when it was initially impossible to monitor the transplants because the water was too dark and turbid to see, and we had to return after conditions improved. By December, many of the populated cells remaining were reduced to single, and quite thin, shoots. It was clear that at this site, unlike the other sites, the protective cages reduced the success of the transplants.

Site Summary

In contrast to the other two sites, the transplants at this site did well, without any protection from grazers and readily spread; indeed the cages in the protected treatment ultimately were detrimental, likely due to shading by the cage. This site is the only one where the transplanted *Halodule* significantly began to spread outside the 1-m^2 blocks and continued to expand.

Observations along the nearby transect in the District's long-term monitoring program indicated that *Halodule* is beginning to recover at this site, along with *Halophila decipiens* and *H. johnsonii* (Lori Morris, SJRWMD, pers. com.). We observed natural recruitment of *Halodule*, as small individuals, in the swim around the 2-m perimeter of our experimental plots beginning in March and sustained through September 2014. We previously observed (August - October 2013) patches of *Halophila decipiens* in these surveys.

Summary of Year 1 Sites

We observed a large amount of variability among the three sites for the transplants initiated in July 2013. Two sites, Pineda BR (protected treatment until October 2014) and Wabasso (unprotected treatment), had good survival and growth, with limited spreading from the experimental plots at Pineda BR and considerable spreading at Wabasso. Grazing impacts remained an issue at the third site, Exxon. At that site, although *Halodule* showed considerable resilience to sustained grazing for over a year, eventually it resulted in the extinction of all transplants at that site. At Pineda BR, survival and growth of *Halodule* required protective caging; at Wabasso, it did not, and indeed, that treatment became detrimental at this site.

The seasonality we observed with the surviving treatments at Pineda BR and at Wabasso are consistent with seasonal patterns typical in the IRL. Following modest initial growth and expansion of the planting units in the late summer, we saw seasonal declines in the fall and winter. Plants were healthy and stable over the winter, and significantly increased in cover and size in the spring and early summer, consistent with what is generally considered the peak season during the year for seagrass growth in the IRL, which is largely driven by the seasonal patterns of the availability of underwater PAR.

The better overall performance of the transplants at Wabasso compared to Pineda BR appears to be due to the lack of significant grazing at Wabasso. The success at that site, given the slow natural recruitment of *Halodule* at some sites in the lagoon, assumed to be due to lack of a sufficient seed bank and vegetative fragments, suggests that limited transplant efforts in such areas might facilitate the natural recovery of seagrass beds in the IRL following extensive losses such as experienced in 2011 and 2012. The challenge with grazers, clearly evident at the Exxon site, and to a lesser extent at the Pineda BR site, points out the limitation that any significant transplant efforts might face elsewhere in the Lagoon. The fact that the transplants in the protected treatments at Pineda BR did survive for at least 9 months following removal of cages, suggested that grazing at these sites may be seasonal (highest in the summer), or perhaps lower than in Year 1 due to partial recovery of seagrass beds in the area. We also observed positive signs of natural recruitment at all three sites, which became more frequent over the course of our study, even at the at the heavily grazed Exxon site.

Monitoring of Year 2 Sites

In our Year 1 Final Report, we recommended considering additional plantings in spring 2014, after seeing what survived over the winter. These plantings were intended to consider what might be limiting success (e.g., the grazing problem at Exxon) and also to provide observations from transplants at the start of the growing season in the spring when light conditions for seagrasses are best due to highest amount of underwater PAR availability. Accordingly, we met with District staff on February 17, 2014, and made plans for the Year 2 transplantation to be done in March.

On March 25, 2014, the following transplants were made with collections at the same Brevard County donor site (Titusville) as the July 2013 transplants to Pineda-BR and Exxon sites:

- At the donor site:
    - As in July 2013, a control (3 unprotected plots, 2 m apart) was established to test that our transplanting methods do work and that PUs (Planting Units) survive the stress of the handling process; plugs were re-planted at this donor site prior to conducting the experimental transplants at the recipient sites.
    - In addition, a second treatment ("recovery", consisting of 3 unprotected plots, 2 m apart) was established by filling in the holes of these treatments with sand to the level of the surrounding sediment to determine how quickly these areas are colonized by the surrounding seagrass.
- A new recipient site (Pineda IRL) was established near the District's existing transect #28 (just south of the Pineda Causeway in the IRL) with the same design as the sites established in July 2013, with 3 blocks (= replicates) of each set of 4 treatments:
    - An unprotected plot (without enclosures), with 5 PUs in an X array, with each PU 28 cm apart on centers, to allow for vegetative spread.
    - A protected plot (within enclosures), also with 5 PUs in an X array and each PU 28 cm apart on centers.
    - An unprotected plot (without enclosure), with an X array, 28 cm apart, of 40-cm long 2-cm-by-2-cm wooden stakes driven into the sediment and extending 10 cm above the sediment, to test whether there are any available *Halodule* vegetative fragments which would be captured by the stakes and also to measure sediment loss and/or gain.
    - An unprotected 1-m^2 bare plot, to test for unassisted natural recruitment.
- At the existing Exxon site, a modified design from the previous one was used, with 3 blocks (= replicates) of each set of 4 treatments:
    - An unprotected plot (without enclosures), with 5 PUs in an X array, with each PU 28 cm apart on centers, to allow for vegetative spread.
    - A protected-caged plot (within cage enclosures), also with 5 PUs in an X array and 28 cm apart on centers.
    - A protected-fence plot (within plastic-mesh netting or fences, similar to what the District has used in previous experiments on impacts of macroalgae on seagrass, with 1.9-cm

openings), also with 5 PUs in an X array and 28 cm apart on centers, with the intent to see if this approach would eliminate the grazing we have seen at this site, and without the detrimental effect of shading, as there are not tops to the fences, unlike the cages we had previously used.

- A protected-fenced clustered plot with 5 PU's, all "clustered" in the center of the plot rather than spread out as described above, in an effort to simulate more of a sod approach to transplanting

Figure 15. Images of the Year 2 fenced treatment at the Exxon site, with fences replaced monthly to reduce fouling impacts: the array of fenced treatments (top, left); an individual fenced plot (top, right); heavily fouling of the fences by filamentous algae in April 2014 (bottom, left); more typical fouling of the fences in September 2014 (bottom, right).

Monitoring of the new donor and recipient treatments was conducted at two-week intervals through April 2014 as done for Year 1 transplants, with monthly sampling thereafter. In this report based on monthly monitoring efforts, we averaged the two April samplings for that month. Data are presented as bar graphs with the means of three key metrics: cells occupied per m^2 plot (number), visual cover (%), and canopy height (cm). We did not observe any recruitment in the "stake treatment" or in the controls ("bare treatment") at the two recipient sites.

Titusville Donor Site

The "control" plots, in which plugs were re-planted at this site prior to conducting the transplants at the recipient experimental sites, rapidly recovered from plug removal; during our two monitorings in April, 100% of the plugs showed no impact of our handling, and all plants were healthy. The "recovery" plots, established by filling in the holes of these treatments with sand to the level of the surrounding sediment, were rapidly colonized by the surrounding seagrass; during our two monitorings in April, 80% of the filled-in holes where plugs were taken were already colonized by healthy seagrasses from surrounding plants. Due to clear evidence of minimal impact of the transplant process, no further monitoring of the donor plots was done.

Pineda IRL

<u>Unprotected Treatment</u>

Similar to the Pineda BR site in Year 1, *Halodule* in the unprotected plots was quickly grazed, although not to extinction as fast as what was observed at Pineda BR in Year 1. PU survival was 40%. The mean number of cells occupied per plot (Fig. 16, top panel) was 9 in April 2014, declined to 6 in May and June, declined to 4 in August, and was 0 in September 2014. Visual cover (Fig. 17, top panel) from April to August was always low (1-3%) before extinction in September 2014. Canopy height (Fig. 18, top panel) decreased from 14 cm in April to 7-8 cm from May to July, and declined to 2 cm in August. Plants in this treatment showed signs of grazing, increasing in August prior to their extinction observed in September 2014.

<u>Protected Treatment</u>

Halodule in the protected plots were not initially grazed. PU survival was 100%. Seagrass spread was much more rapid than at Pineda BR in Year 1. The mean number of cells occupied per plot (Fig. 16, top panel) was 25 cells in April 2014, increased to 63 cells in May, peaked at 66 cells in June, declined to 52 cells in July and 32 cells in August, and was 0 cells in September 2014. During May and June, *Halodule* began to spread outside of the protected plots (one plot in May, all three in June); none outside the plots were seen in subsequent months. Observations along the nearby transect in the District's long-term monitoring program indicated *Halodule* was not recruiting at this site (Lori Morris, SJRWMD, pers. com.), but in June 2014 we observed three small plants of *Halodule* in the swim around the 2-m perimeter of our plots, and in July we saw three small ("hand-sized) patches of *Halodule* at those locations, which subsequently disappeared.

Mean visual cover (Fig. 17, top panel) was 9% in April 2014, increased to 53% in May, declined to 22-24% in June and July, decreased to 6% in August and 0% in September 2014. Mean canopy height (Fig. 18, top panel) ranged from 13-16 cm from April to June, increased to 25 cm in August, dropped sharply to 2 cm when heavy grazing was apparent, prior to the disappearance of all aboveground material in September 2014, although rhizomes were still present in one protected plot.

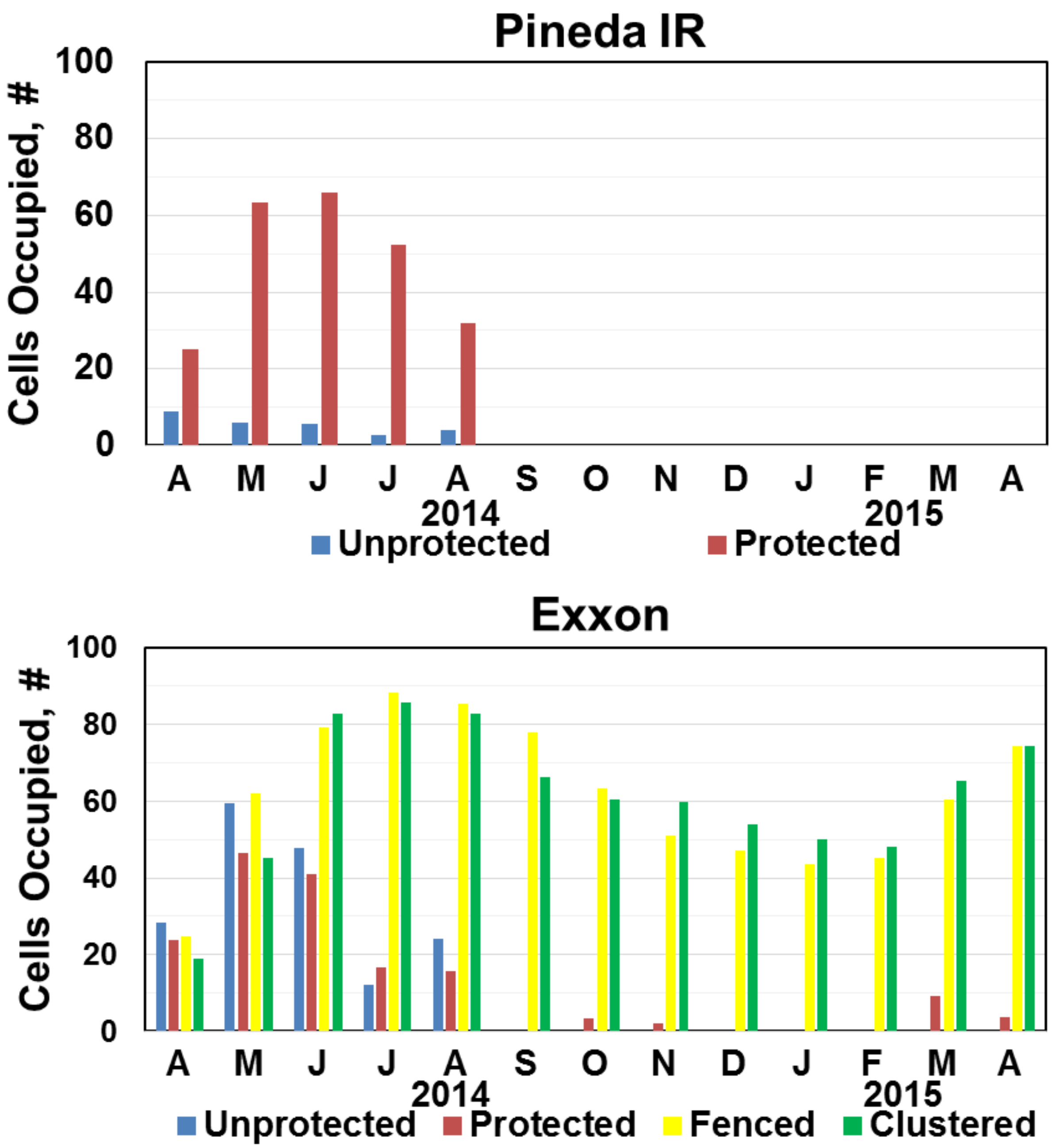
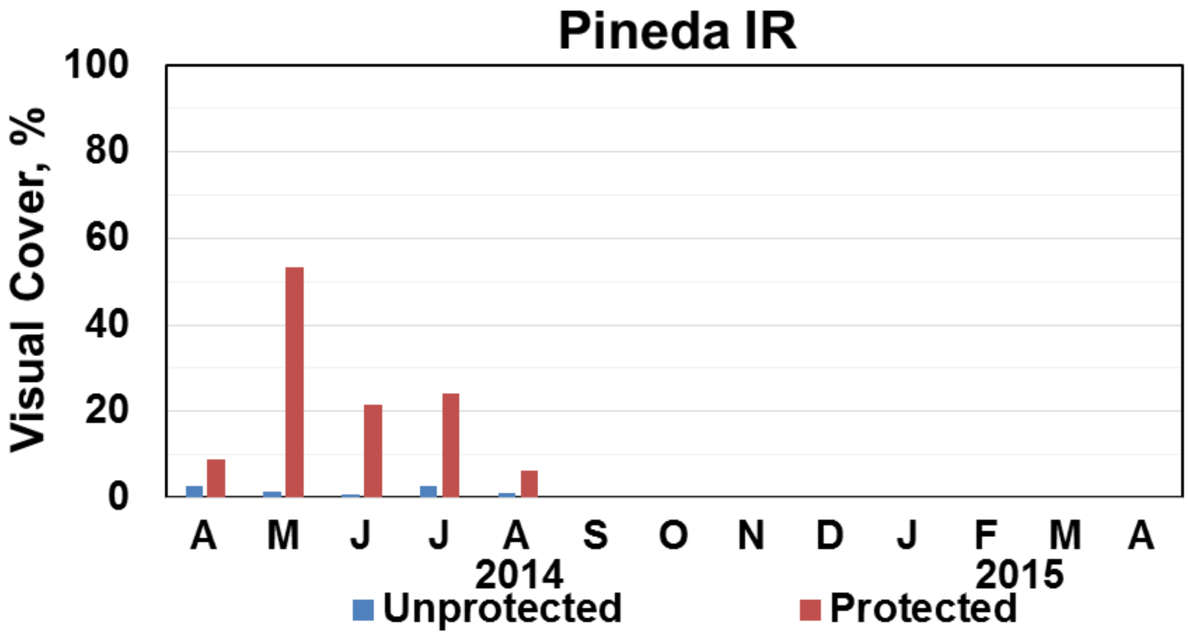


Figure 16. Mean number of cells occupied per m^2 plot at the two Year 2 recipient sites, at monthly monitoring intervals, April 2014 – April 2015. The "clustered" plots were also fenced.



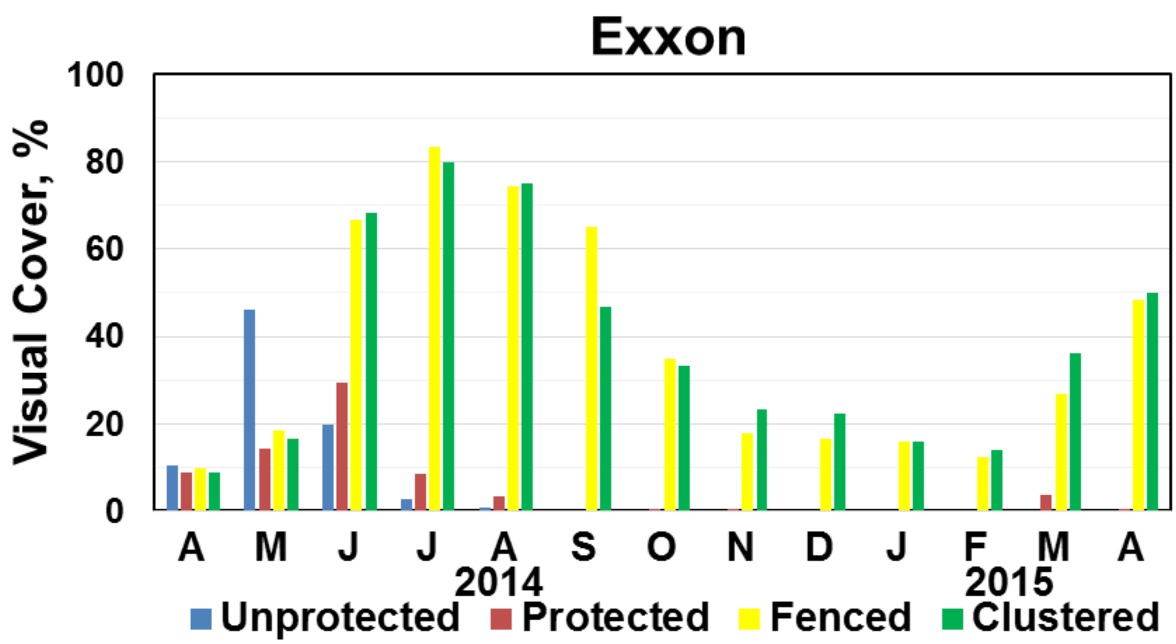


Figure 17. Mean visual cover (%) at the two Year 2 recipient sites, at monthly monitoring intervals, April 2014 – April 2015. The "clustered" plots were also fenced.

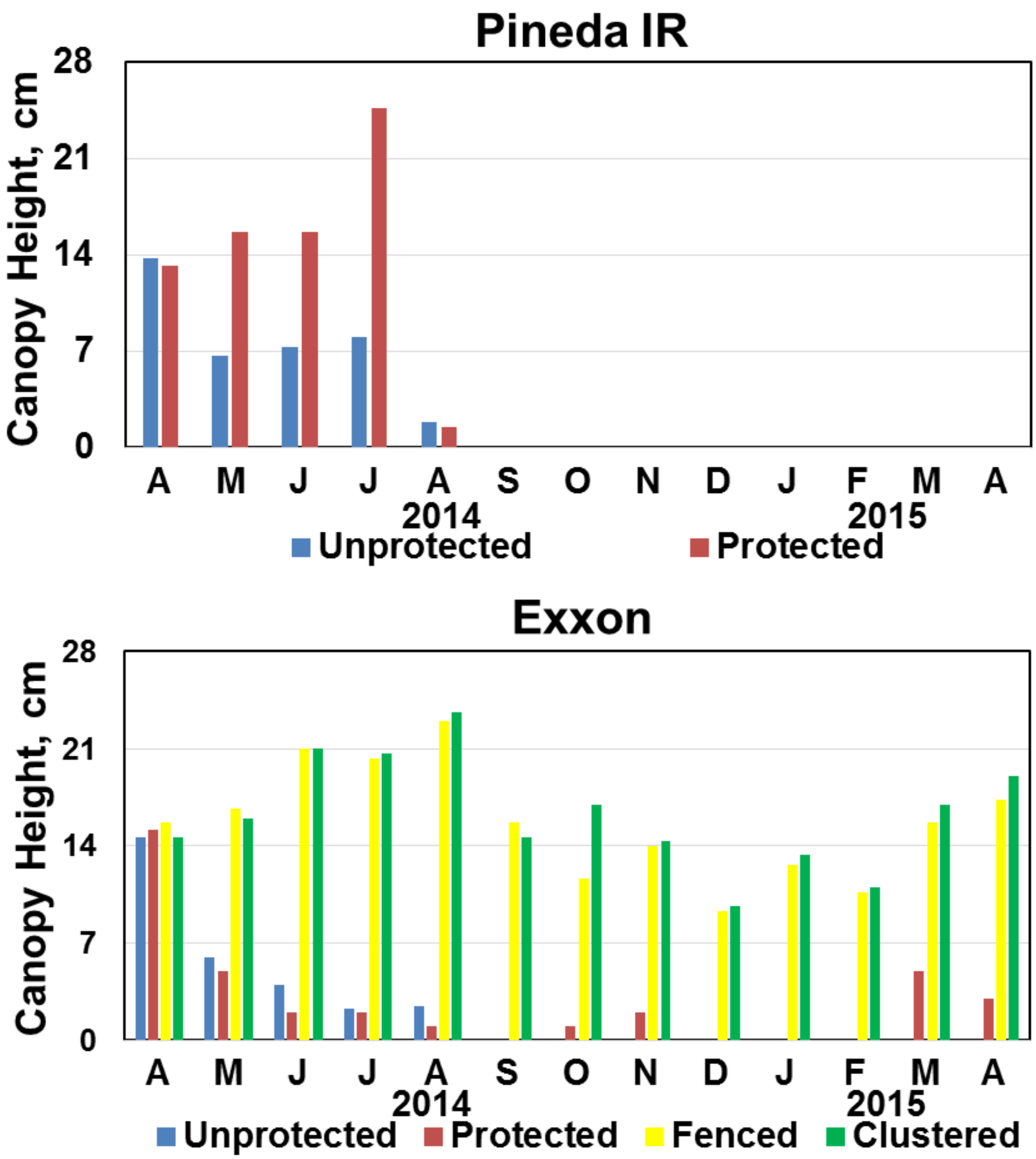


Figure 18. Canopy height (cm) at the two Year 2 recipient sites, at monthly monitoring intervals, April 2014 – April 2015 The "clustered" plots were also fenced.

Site Summary

The Pineda IRL site overall performed similarly to the Pineda BR site, with an initial better performance likely due to seasonality; however, as summer 2014 progressed it was obvious that there was more grazing pressure at the Pineda IRL site, similar to the Exxon site, and water quality issues (high turbidity = low light) may also have contributed to the demise of the protected treatment. No seagrass has been observed in these plots since September 2014. In addition, we have seen no sign of natural recruitment outside the plots at this site.

Exxon Site

Unprotected Treatment

Halodule in the unprotected plots initially fared much better than in Year 1 at this site. PU survival was 100%, and PUs quickly spread. The mean number of cells occupied (Fig. 16, bottom panel) increased from 29 cells in April 2014, peaked at 59 cells in May, declined to 48 cells in June and 24 cells in August, before becoming extinct in September 2014. Similarly, mean visual cover (Fig. 17, bottom panel) increased from 10% in April 2014, peaked at 46% in May, declined to 20% in June, and further declined to 3% in July and 1% in August, before becoming extinct in September 2014. The mean canopy height (Fig. 18, bottom panel) was 15 cm in April, 6 cm in May, and 2-4 cm from June to August 2014. While signs of grazing were observed in April, they rapidly accelerated during the following months.

No seagrass was been observed in the unprotected plots during any of the monthly monitoring after September 2014.

Protected-Cage Treatment

Halodule in the protected-caged plots had similar trends as the unprotected treatment. PU survival was 100%, and PUs quickly spread. The mean number of cells occupied (Fig. 16, bottom panel) increased from 24 cells in April 2014, peaked at 47 cells in May, declined to 41 cells in June and 16 cells in August, before becoming extinct in September 2014. Similarly, mean visual cover (Fig. 17, bottom panel) increased from 9% in April 2014 to 14% in May, peaked at 29% in June, and declined to 9% in July and 3% in August, before becoming extinct in September 2014. The mean canopy height (Fig. 8, bottom panel) was 15 cm in April, 5 cm in May, and 1-2 cm from June to August 2014. Grazing impacts, similar to the Year 2 unprotected treatment and the Year 1 protected treatment at this site, appear to be responsible for the demise of these plants.

A small cluster of grazed *Halodule* shoots was observed in one plot in October 2014 (10 cells, 1% visual cover, 1 cm canopy height) and again in November (6 cells, <1% visual cover, 2 cm canopy height), but not in December 2014. We also observed new recruitment of *Halodule* in two of the three plots in March 2015. The mean number of cells per plot was 9 (range 0 to 17 cells); the mean % visual cover was 4% (range 0 to 8%); the mean canopy height was 5 cm (range 0 to 6 cm). Those recruits persisted in one of the plots during April to June 2015. During this period, the mean number of cells per plot ranged from 4 to 5 cells; the mean % visual cover was approximately 1%; the mean canopy height varied from 3 cm (April) to 8 cm (May). No

Halodule was observed in these plots again until September 2015, when in one plot there were 4 shoots (one in each of 4 cells) with a canopy height of 1 cm.

Protected-Fence Treatment

In sharp contrast, *Halodule* in the protected-fenced plots performed much better than in the previous two treatments (Fig. 19). PU survival was 100%, and PUs quickly spread. There have been no signs of grazing. The mean number of cells occupied (Fig. 16, bottom panel) increased from 25 cells in April 2014 to 62 cells in May, and were in the range of 78-88 cells from June through September, to 63 in October, 51 in November, and 47 in December 2014. The mean number of cells was stable in January and February 2015 (44 and 45 cells, respectively) and increased in March (mean = 60 cells per plot) and April (mean = 74 cells per plot).

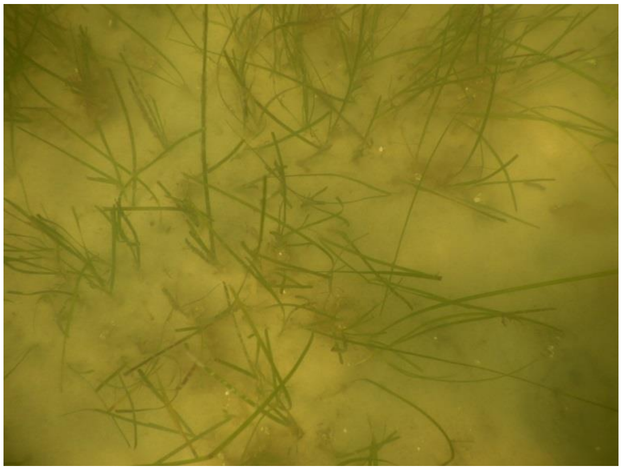


Figure 19. *Halodule* plants in both of the fenced treatments quickly spread within the plots and were not grazed (April 22, 2014).

Similarly, mean visual cover (Fig. 17, bottom panel) increased from 10% in April 2014 to 18% in May and 67% in June, peaked at 83% in July and dropped to 74% in August and 65% in September, 35% in October, 18% in November, 17% in December, 16% in January 2015, and 12% in February. Mean visual cover increased in March (mean = 27%) and April 2015 (mean = 48%).

The mean canopy height of *Halodule* (Fig. 18, bottom panel) was 16-17 cm in April and May 2014, 20-23 cm from June to August, and 16 cm in September. Mean canopy height decreased to 9 cm in December 2014 and then increased to 16 cm in March and 17 cm in April 2015.

Protected-Fenced Clustered Treatment

Halodule in the protected-fenced clustered plots performed very similarly to the protected-fenced plots planted in an X pattern. PU survival was 100%, and PUs quickly spread. There were no signs of grazing. The mean number of cells occupied (Fig. 16, bottom panel) increased from 19 cells in April 2014 to 45 cells in May, and were in the range of 83-86 cells from June through August, and decreased to 66 in September, 60 in October and November, 54 in December, 50 in January 2015, and 48 in February. The mean number of cells increased in March (mean = 65 cells per plot) and April 2015 (mean = 74 cells per plot)

Mean visual cover (Fig. 17, bottom panel) increased from 9% in April 2014 to 17% in May and 68% in June, peaked at 80% in July and decreased to 75% in August, 47% in September, 33% in October 23% in November, 22% in December, 16% in January 2015, and 14% in February. Mean visual cover increased in March (mean = 36%) and April 2015 (mean = 50%).

The mean canopy height (Fig. 18, bottom panel) was 15-16 cm in April and May 2014, 21-24 cm from June to August, and 15 cm in September. Mean canopy height decreased to 10 cm in December 2014, and then increased to 17 cm in March and 19 cm in April 2015.

As mentioned for the Year 1 sites, observations along the nearby transect in the District long-term monitoring program indicated some recruitment of *Halodule* was occurring at this site (Lori Morris, SJRWMD, pers. com.). Natural recruitment, as small individuals of *Halodule*, was observed in the swim around the 2-m perimeter of our Year 2 Exxon experimental plots in May 2014 (1 plant of *Halodule*), June (several plants of *Halodule* and *Halophila engelmannii*), July (four plants of *Halodule*), and August (two plants of *Halodule*), but none in September 2014, when all of the non-fenced plots also went extinct. We also saw spreading outside some of the experimental plots in May 2014 (two unprotected plots and one protected plot) and June (three unprotected plots, one protected plot, and two fenced-clustered plots). But none of those individuals survived into July 2014, consistent with the increased grazing observed at this site at that time. Since January 2015 we have consistently observed signs of natural recruitment in the 2-m perimeter swims (1-2 shoots each month) and additional recruits within a few meters of the experimental site.

Final Manipulations at Exxon Site

At the Southeastern Estuarine Research Society (SEERS) meeting in Jacksonville on March 13, 2015, we had a brief meeting with Lori Morris and Bob Chamberlain to discuss the removal of the fences at the Exxon. We affirmed plans previously made at a project review meeting in Palatka on December 12, 2014. On April 14, 2015, we removed two of the fences completely, ("fence-removed" treatment) in the protected-fenced and fence-clustered plots (now considered a single treatment as we did not observe any difference in success of planting units due to clustering), we removed one side (the shoreward side: the "open shore-side" treatment) of two other fences, and kept the remaining two fences intact (the "fenced" treatment). In addition, we located and deployed fences around two patches of new recruits at that site, at depths similar to our experimental plots, and identified two other control plots with new recruits. We monitored these new plots for the duration of Year 3.

In the fence-removed treatment, the mean number of cells occupied by *Halodule* initially (April to August 2015) following removal was stable, ranging from a mean of 47 (June) to 56 (July), then declined to 29 in September. In the open shore-side plots, the mean number of cells occupied by *Halodule* increased from a mean of 77 cells in April to 93 in May and 99 in June, then declined to 74 in July, 60 in August, and 50 in September. In the fenced plots, the mean number of cells occupied by *Halodule* initially declined from 92 to 57 in May and then was stable through the end of the monitoring in September, ranging from 50 in September to 60 in June).

In the fence-removed treatment, the mean visual cover of *Halodule* declined following removal, from a mean of 48% in April to 3% in September. In the open shore-side plots, mean visual cover of *Halodule* initially increased considerably from 40% in April to 95% in June, then declined through September to 26%. In the fenced plots, mean visual cover of *Halodule* fluctuated from 60% in April to 40% in September.

In the fence-removed treatment, the mean canopy height of *Halodule* declined rapidly following removal from 17 cm in April to 9 cm in May and June and continued to decline to 2 cm in September. In the open shore-side plots, the mean canopy height of *Halodule* also decreased from 17 cm in April to 12 cm in May and June and continued to decline to 2 cm in September. In the fenced plots, mean canopy height of *Halodule* remained higher than the other two treatments (means = 21 cm and 22 cm in April and May, declining to 10 cm in September.

In the control recruit plots, the mean number of cells occupied by *Halodule* increased from April (mean = 14 cells) to July (mean = 46 cells), before declining to a mean of 31 cells in August and 5 cells in September. In the fenced recruit plots, the mean number of cells occupied by *Halodule* increased more than in the control, from a mean in April of 8 cells to a mean of 67 cells in July). Unfortunately, during the August monitoring, we discovered that one of the fences had been removed from the rebar and drifted or was moved to the second fence in this treatment with heavy losses of *Halodule* in both plots (only 1 cell in one of the plots in August and one in September).

Similarly, in the control recruit plots, the mean visual cover of *Halodule* increased from April (mean = 4%) to July (mean = 18%). In the fenced recruit plots, mean visual cover of *Halodule* increased from April (mean = 3%) to July (mean = 25%). Following the impacts mentioned above, cover was less than 1% in August and 0% in September.

In the control recruit plots, the mean canopy height of *Halodule* increased from a mean of 4 cm in April to a mean of 10 cm in August 2015, then declined to a mean of 2 cm in September. In the fenced recruit plots, the mean canopy height of *Halodule* varied little, from 5 to 7 cm while plants were present from April to August.

<u>Site Summary</u>

In summary, it is clear that grazing remains a challenge to long-term recruitment at the Exxon site. The use of the fence material, with its smaller mesh size as protection against grazing has been effective in eliminating that issue. Survival at this site was much better with the fence material compared to the steel cages as protection against grazing. There was no difference in the cluster arrangement vs. the X arrangement of plantings.

The April 2015 manipulation of the 6 fenced plots suggested that removal of the fence once the plots have filled in with seagrass does result in some losses due to grazing, with greater losses when fences are fully removed versus opening on one side (all shoreward in the experiment).

It is clear that we are seeing natural recovery of this site. It is encouraging that the new April 2015 control plots (with natural recruits) were growing and it is clear that the initial fencing of these recruits resulted in greater growth.

Summary of Year 2 and 3 Sites

Survival of both the Year 1 PUs (planted in the summer) and Year 2 PUs (planted in the spring) was high, but the Year 2 transplants grew and spread much more rapidly than those in our Year 1 effort. This higher metric of success is probably due to the timing of peak growth of seagrass, which is generally in the spring and also seen in the seasonality of growth at the Year 1 sites.

Transplants at the Year 2 Pineda IRL site overall performed similarly to those at the Year 1 Pineda BR site, with an initial better performance likely due to seasonality; however, as the summer progressed, it was obvious that there was more grazing pressure at the Pineda IRL site, similar to the Exxon site, and water quality issues (high turbidity = low light) may also have contributed to the demise of the protected treatment.

At the Exxon site, where we targeted refining the protected treatment as well as testing clustering vs. traditional planting for transplants, it is clear that the fencing material is far superior for preventing grazing than the steel cages with their larger mesh. There was no difference in the cluster arrangement vs. the X arrangement of plantings within the fenced treatments. Removal of the fences once the plots filled in with seagrass does result in some losses due to grazing, with greater losses when fences were fully removed versus opening on one side (all shoreward in the experiment), but survival was high enough to be encouraging for future efforts.

We are also observing positive signs of natural recruitment at Exxon site, but no recruitment at all at Pineda IRL.

Conclusions of This Project

Evaluation of Transplant Success

We have determined that our basic transplant technique works. The proposed metrics readily demonstrate survival, growth, and expansion rates, and we believe these metrics should continue to be used in future studies.

We discovered (under the established experimental conditions) that grazing may be a major impediment to seagrass recolonization at the three northern sites, as evidenced by the initial 100% survival rate in the protected treatments. Grazing varied tremendously at our sites; in particular, the plants at the Exxon site faced a serious problem with grazing. Grazing is higher in the summer than the rest of the year.

Our results suggest that, in the absence of grazing pressure, the environmental conditions present at all sites but Pineda IRL appear favorable for seagrass recovery. Our results support the hypothesis that the lack of natural recruitment and recovery in areas of the IRL system nearly devoid of seagrass is due to the lack of seagrass vegetative stock (below and above-ground vegetative material) and vegetative reproduction.

Whether or not restoration in large, denuded areas of the Lagoon, can be accelerated by vegetative planting is still an open question. Clearly, grazing pressure may limit large-scale restoration. In areas such as the Wabasso site with little or no grazing pressure on colonizing seagrass, it does appear that fairly small efforts strategically located might be able to jump start seagrass restoration. But given the encouraging signs of initial seagrass recovery at a number of locations with severe seagrass losses in 2011-2012 (Hanisak, unpublished data; Lori Morris, SJRWMD, pers. comm.), it may be that significant natural/unassisted recovery will occur in a fairly short time frame, possibly 5 years or less. Such recovery would be more desirable than investing significant amounts of money into large-scale restoration efforts. Yet given the uncertainty of that natural recovery, the next step in considering seagrass restoration might be consider doing a pilot-scale test, drawing on what has been learned in this project which tested the initial feasibility of seagrass transplants given the limitation of natural seagrass recruitment.

Recommendations for Future Seagrass Transplanting in the IRL

1. **Conduct monitoring of natural recruitment patches**
   This effort could be done at a relatively small scale, with a few fixed "quadrats" up to about 5 by 10 m that contain some *Halodule* patches. To consider the grazing challenge to the establishment of seagrass beds, this monitoring might be done as a comparison at a pair of sites, with different levels of grazing pressure, e.g., the Wabasso (low grazing pressure) and Exxon (high grazing pressure) sites.

2. **Conduct a pilot-scale seagrass restoration test**
   These pilot plantings could perhaps be 2-4 plots of about 0.1 ha (= ¼ acre). A 0.1 ha plot could be 20 m by 50 m. Each plot would require 1,000 planting units (PUs) at a suggested planting density of 1 PU per m^2. Plots could be set up at different sites or at different depths at a particular site. This effort could be done in parallel with monitoring of natural recruitment patches at the same site(s).

3. **Develop a land-based experimental seagrass nursery facility**
   To facilitate pilot-scale seagrass restoration tests, it would be desirable to design and develop an outdoor seagrass nursery facility capable of providing donor material for transplanting seagrass as part of experimental IRL restoration efforts. The PI has secured one-year of funding to initiate such an effort at Harbor Branch. This facility will be used to produce planting units initially for research trials and subsequently in future restoration/mitigation efforts. The facility will be modular in design for further scale up. If successful, this experimental onshore seagrass nursery will provide enough material for a 1-acre test planting effort in the later part of 2016. Site selection, transplant methods, and future monitoring will be done by partnering with one or more agencies.

4. **Engage citizen scientists in seagrass restoration**
   To enhance community engagement, citizen scientists should be engaged in all key aspects of seagrass restoration projects: collecting and planting propagules, maintaining tank cultures, and assisting with restoration plantings. Such a network, similar to that being used for oyster restoration at multiple locations in the IRL, will not only enhance the involvement of citizens, but also greatly reduce the cost of cultivation and restoration efforts.

Appendix 1
Evaluating the Feasibility of Transplanting to Promote Seagrass Recovery in the Indian River Lagoon

Summary Project Kick-off Meeting
SJRWMD, Palm Bay Office
525 Community College Parkway SE, Palm Bay, FL

M. Dennis Hanisak & Bob Virnstein

Attendees: Dennis Hanisak, Bob Virnstein, Bob Chamberlain, Lori Morris, Ron Brockmeyer, Troy Rice, Don Deis (Akins North America), Hyun Jung Cho (briefly: Ed Garland, Lauren Hall, Jan Miller)

Following brief introductions and discussion of the final approval of the contract between FAU and SJRWMD, Bob Chamberlain went over the permits involved in the project and distributed them to all parties. He reviewed the project objectives in the SOW and then led a discussion on the work plan which was the focus of most of the meeting. Key points:

1. Site Selection - The team reviewed candidates from the list on the permit.

   Important criteria for recipient sites: zero or nearly zero canopy-forming seagrass cover currently, but documented to have high seagrass cover pre-bloom; encompass sites over a large amount of the IRL in Indian River and Brevard counties

   Additional criteria for recipient sites: Ongoing monthly seagrass monitoring by SJRWMD; accessibility (land access and proximity to boat ramps)

   Three Recipient Sites Selected for FAU Work:
   - SG017 (“Southwest Pineda”)
   - SG034 (“Exxon”, south of Turkey Creek, Melbourne)
   - SG048 (“ELC”, Wabasso)

   Alternate Recipient Sites Selected for FAU Work:
   - SG009 (NASA Causeway, has land access, but no monthly monitoring by SJRWMD and would require NASA clearance)
   - SG025 (SW of 528 Causeway, but no monthly monitoring by SJRWMD)

   Recipient Sites Selected for Sebastian Inlet District Work (Don Deis: Atkins):
   - SG044 (Sebastian Inlet South)
   - Shoal Areas of Sebastian Inlet (TBD)

   Donor sites:
   - SG020 (N. 406, Titusville) - This site will be used for all recipient sites north of Sebastian Inlet.
   - SE side of spoil island, just N of 17th St. Bridge, Vero Beach - This site will be used for all recipient sites at Sebastian Inlet and to the south.

2. Responsibilities – Hanisak & Virnstein are responsible for the work at the sites designated for FAU work. Don Deis of Atkins will handle gear and personnel and all aspects of sites at Sebastian.

3. The team discussed and revised the draft experimental design from FAU (the revised design will be submitted as a second deliverable)

   <u>Changes to original experimental design</u>:
   - Add a simple bare plot to the treatments
   - Need a control at both donor sites: return plugs to original holes
   - Keep the wire along the entire bottom outside edge of the cage (to keep the horizontal surface of the cage off the bottom)
   - In her future sites, J. Cho will add a treatment where a core is dug up, shaken to bring *Ruppia* seeds to the surface, then put back

4. Transplant protocol – The team:
   - Went over procedure for collecting planting units (PUs)
   - Went over procedure for planting PUs
   - Agreed on spacing and arrangement of planting units (PUs): center of quad and two diagonal squares away from the center in all 4 directions, making PUs 28 cm apart on centers.

5. Field Monitoring Data Sheet – The team agreed on priority needs for monitoring and made several suggested revisions to the field monitoring data sheet; they will be incorporated into revised data sheet. (the revised data sheet will be submitted in the second deliverable)

6. Initial Supplies – Bob Virnstein had prepared a list of what he thought was needed and will handle initial logistics for the FAU sites, while Don Deis will do the same for the Sebastian sites.

7. Review of probable schedule – The team proposed starting the field work during the week of July 22, doing as much as possible that week. FAU and the Atkins' crew will meet with SJRWMD in the field to review site set-up and layout so that all sites are set up and monitored with consistency. The team discussed upcoming schedule of personnel and potential boat use. Troy Rice offered use of an NEP boat, if available, which was gratefully accepted.

The group reviewed all action items and confirmed an initial launch of the project's field work with all key parties present on Monday July 22 at Sebastian.

Appendix 2
Evaluating the Feasibility of Transplanting to Promote Seagrass Recovery in the Indian River Lagoon

Work Plan and Experimental Design

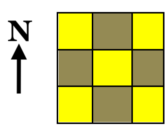
M. Dennis Hanisak & Bob Virnstein

The main question to be answered is: Will canopy-forming seagrass survive at sites in the Indian River Lagoon that previously had seagrass consistently, but do not now after the superbloom and concurrent 2011 secondary bloom, even though supportive water quality has returned? To address this question, this study will test whether transplanted seagrass will survive at these sites. If so, several possible alternative explanations for the lack of recruitment are: (1) seagrass just can't get there (seagrass is needed to get seagrass); and/or (2) grazing or physical factors are prohibiting the establishment and survival of natural recruitment.

Experimental Design:

- Exclusive use of *Halodule wrightii*, the main colonizer species and the most widespread species, which is also tolerant of fluctuating salinities
- Planting units (PUs) will be intact plugs, with roots and associated sediment
- PUs will be 12 cm in diameter, 113 cm^2 or 0.01 m^2 (a standard post-hole digger), and 10-15 cm deep, deep enough to include the rhizomes and roots of *Halodule wrightii*
- For experiment (i.e., recipient) sites north of Sebastian, the donor site for PUs will be near Transect #20 on the north side of Titusville. For sites at or south of Sebastian, the donor site will be located on the southeast side of the spoil island just north of the 17th Street Bridge at Vero Beach
- To minimize stress on PUs during the planting process, the recipient sites will be set-up before harvesting. This includes identifying plots to receive transplants, and placing flags at the exact spots within the plots.
- To minimize damage and recovery time to the donor site, all plugs will be taken with a minimum distance of 1.0 m between plugs. In addition, clean sand will be used to fill in plug holes.
- At both donor sites, plugs will be pulled, placed in 15-cm diameter plastic nursery pots, and temporarily kept in the water around the boat anchor until all harvesting is completed. Only enough PUs will be harvested for transplanting one recipient site at a time. Therefore, the number of recipient sites that a donor site supports also will be equal to the number of times that donor site is visited for harvesting.
- At the southern donor site, all PU pots will be removed from the water (anchor area) at once, placed into 11-cm deep plastic tubs filled with water, and loaded onto the boat for the short ride to the recipient site. During the boat ride, the plants will be covered and kept wet.
- At the northern donor site, the pots will be placed in coolers and filled with water to just below the rim of the pots. The boat will be trailered (with coolers) to a ramp near the recipient site.
- Upon arrival at the recipient sites, the pots will be immediately removed from the boat and placed near the pre-designated plots, awaiting planting.
- For planting, a bottomless nursery pot will be pushed by hand into the sediment and dug out by hand to the depth of the PU. The PU will then slide out of its transport pot, placed into the hole, then the bottomless sleeve removed. Some of the excavated sediment will be used to level the PUs so they were at the same level as at the donor site.

- All donor and experimental sites will be about 0.5 m water depth, within 20-100 m of the transect.
- Transplanting will initially be done during the week of July 22, 2013, at 3 sites: near transects #17 ("Southwest Pineda" Causeway in Banana River), 34 ("Exxon," south of Turkey Creek), and 48 ("ELC," south of Wabasso bridge). All of these sites have seagrass transects that are monitored monthly and nearby water quality monitoring sites that are also monitored monthly.
- An additional 3 sites will be planted near Sebastian Inlet during the beginning of the same week. One site will be located adjacent to Transect #44, south of the Inlet. The additional 2 sites will be on the Inlet tidal shoals: 1 south of the Inlet channel and one north of the channel. This additional work at Sebastian is funded by the Sebastian Inlet Tax District and conducted by Atkins North America under the direction of Don Deis. For all 6 sites, SJRWMD and Sebastian, methods are identical.
- At each recipient site, there will be 3 blocks (= replicates) of each set of 4 treatments. The position of each of the 4 treatments is randomized within a block.
- All treatments will be within a 1-m^2 plot (1 m by 1 m), marked with PVC poles on 2 diagonal corners and flagged landscape staples on the other 2 corners. The PVC poles will be flagged with colored surveyors tape to color-code all of the treatments at each site (because treatments within blocks will be randomized and the arrangement of treatments will be different at each site)
- Treatments per block at each recipient site include:
    1. An unprotected plot (without enclosure), with 5 PUs in an X array. Each PU will be 28 cm apart on centers, to allow for vegetative spread. This arrangement corresponds to the center of a m^2-monitoring quadrat (divided into 100 squares). Therefore, each PU will be 2 squares from the other along each of the 4 diagonals.

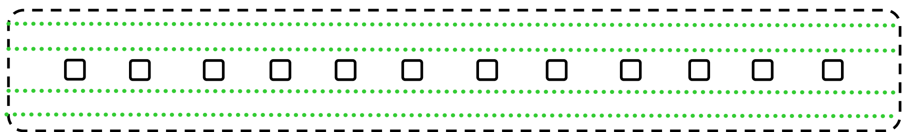
    2. A protected plot (within enclosures), also with 5 PUs in an X array and 28 cm apart on centers. The plot will be protected by a cover of 15-cm by-15-cm mesh welded wire, the kind used for reinforcing concrete, with 15 cm of all 4 edges of the wire bent down 90° and resting on the sediment surface so that the horizontal 1.2-m by1.2-m surface of the cage is 15 cm above the sediment – intended to keep out large grazers and diggers such as manatees and rays, again to increase the likelihood of survival.
    3. An unprotected plot (without enclosure), with an X array, 28 cm apart, of 40-cm long 2-cm-by-2-cm wooden stakes driven into the sediment and extending 10 cm above the sediment, to test whether there are any available *Halodule wrightii* vegetative fragments which would be captured by the stakes. Any resulting natural recruitment to these plots may indicate that the lack of structure is an inhibiting factor to natural recruitment.
    4. An unprotected 1 m^2 bare plots, to test for unassisted natural recruitment.

- Each 1-m^2 treatment plot will be 2 m apart from other treatment plots.
- At the donor sites, a control will be established to test that the methods do work and that PUs survive the stress of the handling process. Plantings at the control sites will be done in a similar fashion as the recipient sites – plugs harvested, put in pots, transferred to a holding area (in water at the boat anchor), left there while other PUs are harvested for a recipient site, then re-planted in one of the original plug holes that will be marked with 30-cm PVC poles

pushed into the sediment adjacent to the western edge and extending 10 cm above the sediment so that the PUs also can be re-located for monitoring. The arrangement of PUs within the control plots is the same as in the experimental transplants plots. At the control, 3 unprotected plots will be placed 2 m apart. There will be no protected plots established, nor plots with stakes or bare plots. All other harvesting and re-planting processes will be identical to the experimental plantings.

These treatments will allow us to compare PU survival of protected versus unprotected and assess any natural recruitment.

Physical arrangement: With the 3 blocks of 4 treatments all in a line, and with 2 m between plots, the plots will be parallel to shore along a line 34-m long. The green dotted lines (see typical site lay out below) indicate a 2-m buffer around the experimental plots that will be monitored for presence of seagrass.



Data Sheet: A data sheet was developed for recording the observations made during the monitoring of the sites (attached at end of report). Upon arrival at each of the sites, monitoring personnel will record the site name, date, personnel conducting the monitoring, and weather conditions (cloud cover, wind, wave height).

For each plot, the following data will be recorded:

- the status of the five plugs (presence or absence), using the grid provided
- the condition (healthy/green, dying/brown, spreading or not) of the surviving plugs
- the number of cells out of 100 in the 1-m^2 quadrat occupied by *Halodule wrightii*
- the visual estimate of the cover of *H. wrightii* in the entire 1-m^2 quadrat
- the canopy of height (cm) of *H. wrightii*, following District protocol (Morris et al. 2001): Blades are "combed" with fingers vertically up along a meter stick. A composite measure of the blades is made to the nearest cm. Most blades tend to reach a similar maximum length; those few extra-long blades (<5%) are excluded.
- the visual estimate of the cover of any other SAV (seagrasses, macroalgae) in the entire 1-m^2 quadrat (under “Other”)

For the plots with wooden stakes, the following data will also be recorded:

- the height of the stakes above the sediment
- notes (under “Other”) on any materials caught be the stakes

At the bottom of the data sheet, the following observations/notes will be made

- Water clarity and WQ: Estimates of water clarity and any observed water quality issues (e.g., discolored water, high turbidity) will be made.
- Water depth: Depth (cm) will be recorded at the two end PVC- poles that delineate the site.
- Drift algae abundance: Drift algae (DA) abundance will follow District protocol (Morris et al. 2001), based on the following scale from 0 to 5:
    - 0 - no algae
    - 1 - <10% cover of only single strands
    - 2 - >10% cover of only single strands
    - 3 - "tumble weed" clumps <50% cover
    - 4 - "tumble weed" clumps >50% cover, but can still see the bottom
    - 5 - "tumble weed" clumps 100% cover, cannot see the bottom..
- Epiphytes on *Halodule*: Visual estimates of epiphytes will follow District protocol (Morris et al. 2001):
    - Three shoots of *H. wrightii* will be collected from each plot by pinching off near the sediment surface.
    - The shoots will be examined by floating in hand and compared with the Epiphyte Photo Index (EPI) on a scale of 0-5 (per Appendix 6 in Morris et al. 2001). The scale is a photographic reference of different "levels" of epiphyte loading (epiphyte biomass per seagrass biomass).
    - By comparing to the EPI, the general category (1-5) will be picked first (check that both higher and lower alternatives have been eliminated).
    - Again comparing to the EPI, select a sub-category (a-e), if appropriate
    - Considerations:
        - Length and width of seagrass blades (affects seagrass biomass)
        - Entire distribution on blades, not just fuzz on tip
        - Type of epiphyte (a fine film over the entire blade may be a heavy loading)
- Fouling on cage, and algae caught on and in cage: Notes will be made on these items; cages will be cleaned of fouling and trapped material. An important consideration in the notes on fouling is if light is being transmitted through the cage (i.e., is light generally unimpeded by the algae or is there substantial or a total shading due to algae on the cage?). When drift algae are present, the sampler will provide a DA index number for the cage top as described above.
- Disturbance of site or structures: Notes will be made on any sign of natural or human-related disturbance of the site, and actions taken on site to resolve.
- Items needing attention (missing poles, etc.): Any items that require further attention should be noted and rectified at the earliest opportunity.

Lastly, seagrass naturally recruiting to the site (2-m perimeter around plots = 5 x 38 m), based on 4 parallel swims/walks, as illustrated in the cartoon above, will be recorded and the approximate location relative to the plots recorded. Beginning the second month of monitoring, a short distance (~20 m) will be surveyed along lines extending away from each end of the site along the site's axis, as well as lines toward and away from shore beginning at the ends and middle of the site.

Reference Cited: Morris, L.J., L.M. Hall, and R.W. Virnstein. 2001. Field Guide for Fixed Seagrass Transect Monitoring in the Indian River Lagoon. St. Johns River Water Management District, Palatka, FL.

Monitoring Schedule: All sites will be prepared and all initial transplants made during the week of 22 July (to be completed by 26 July). The initial plan at the 3 HBOI transplant sites and northern donor locations of weekly monitoring in August and monthly monitoring in September was modified to monitoring ca. every two weeks for the rest of this FY (through 30 September). This revised HBOI plan will result in the same number of monitoring trips as originally envisioned, but will provide better temporal resolution if PUs begin to spread. As the Year 1 project ends on 30 September, the last monitoring will likely be conducted the previous week. Details of schedule, with personnel and boat needs:

Monitoring (2 weeks) – Week of 5 August (to be completed by 9 August) – John Hart (HBOI), Frank Sakuma (NEP), NEP Boat

Monitoring (4 weeks) – Week of 19 August (to be completed by 23 August) – John Hart & Bob Virnstein (HBOI), HBOI small boat

Monitoring (6 weeks) – Week of 2 September (to be completed by 6 September) – John Hart & Bob Virnstein/Dennis Hanisak (HBOI), HBOI small boat

Monitoring (8 weeks) – Week of 16 September (to be completed by 20 September) – John Hart & Bob Virnstein/Dennis Hanisak (HBOI), HBOI small boat

Monitoring (9/10 weeks) – Week of 23/30 September (to be completed by 30 September) – John Hart & Bob Virnstein/Dennis Hanisak (HBOI), HBOI small boat

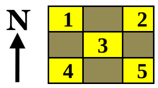
Transplant Data Sheet

Site: ____________ Date (m/d/yr): ______ Personnel: ________________________________

Conditions at site: ____

Treatment	PUs live*	Condition	#cells occupied	m^2 visual % cover	Height (cm) Canopy/Stake	Other SAV species, m^2 visual % cover
Block 1						
Unprotected						
Protected						
Stakes						
Bare						
Block 2						
Unprotected						
Protected						
Stakes						
Bare						
Block 3						
Unprotected						
Protected						
Stakes						
Bare						

*indicate location, facing north ("✓" = present and live *Halodule*; "—" = absent of *Halodule*



Observations/Notes, e.g.:

Water clarity and WQ:

Water depth:

Drift algae abundance:

Epiphytes on *Halodule*:

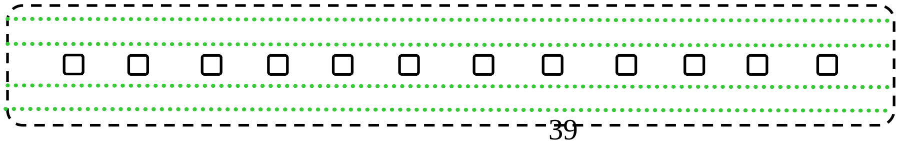
Fouling on cage, and algae caught on & in cage:

Disturbance of site or structures:

**Items needing attention (missing poles, etc.):

Our Year 1 Final Report (September 2013) included reviewing transplant methodology, reconning potential sites, acquiring supplies, scheduling field work, testing the logistics of the preferred

Seagrass naturally recruiting to site (2-m perimeter around plots = 5 x 38 m), based on 4 parallel swims/walks:



transplanting

Other: