

ANNUAL MONITORING REPORT (1 OF 3)

APRIL 2016

SAVE CRYSTAL RIVER PILOT PLANTING REPORT

FDEP PERMIT NUMBER: 09-0332123-001 ACOE PERMIT NUMBER: 2015-00363

Prepared for:

Gator Dredging, llc.

By:

Carter Henne Sea and Shoreline, Ilc. 4331 Cockroach Bay Rd Ruskin Fl, 33570

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1. INTRODUCTION

The city of Crystal River is located northwest of the center of Citrus County (28.900670, -82.593699) on the northeast side of Kings Bay and the Crystal River, an inlet of the Gulf of Mexico. U.S. Routes 19 and 98 pass through the center of the city, leading south 7 miles (11 km) to Homosassa Springs and north 46 miles (74 km) to Chief land. State Road 44 leads east from Crystal River 17 miles (27 km) to Inverness, the Citrus County seat. Crystal River is at the heart of the Nature Coast of Florida. The city is situated around Kings Bay, which is spring-fed and so keeps a near constant 72 °F (22 °C) temperature year round. A cluster of 50 springs designated as a first-magnitude system feeds Kings Bay. A first-magnitude system discharges 100 cubic feet or more of water per second, which equals about 64 million gallons of water per day. Because of this discharge amount, the Crystal River Springs group is the second largest springs group in Florida, the first being Spring Creek Springs in Wakulla County near Tallahassee. Kings Bay is routinely home to over 400 manatees during the winter when the water temperature in the Gulf of Mexico cools. Crystal River was designated as an Outstanding Florida Water under Chapter 62-302.700 F.A.C, which affords the waters special protection due under Florida State law. Crystal River is unique in that the headwaters are freshwater springs, which transition into a tidally influenced river system that spans 6 miles and over 600 acres. The springs offer many recreation activities, and provide a portion of the tourism revenue to Crystal River and Citrus County. The current project took place in 3.4 acres of upland canals as outlined in Figure 1.

Recently, the Crystal River system has suffered from declining of water quality, attributed to increased nutrient loads and invasive plant and algal species. Non-native invasion and nutrient loading are linked, with the combination of increased nutrients and lack of natural controls allowing the invasive species to flourish. It is the intent of the project to restore Submerged Aquatic Vegetation (SAV), in particular *Vallisneria Americana* (tape grass) to the upland canal systems in the Kings Bay water way. Tape grass will (1) compete with noxious macroalgae and invasive plants, (2) absorb excess nutrients and (3) bolster dissolved oxygen levels.

Prior to the commencement of the Crystal River Pilot restoration project, Sea and Shoreline assessed benthic conditions and cleaned the benthos by vacuum removal of algae and organic material. Bottom conditions were surveyed for soft-sediment depth and organic composition. The substrate on average was a mixture of organic material and sand. The thickness of the soft-sediment layer was 18 inches to 24 inches from the sediment surface to the rocky foundation. Peat pots were used as the planting material instead of pre-rooted coir mat because there were no bare rock areas. Based on stakeholder input it was decided that there needed to be anchorage areas to allow tour boat access to the newly restored areas. The canals were marked in gridlines with three "lanes" of GrowSAV Herbivory Exclusion Devices. The lanes provided room for vessel and manatee traffic to occur without impacting navigation and movement through the canals. The GrowSAV Devices were placed roughly on 15-ft centers. In areas expected to have heavy anchorage from vessels, the distances between the GrowSAV Devices were expanded to compensate for the increased vessel intensity. There were three areas, one in each canal, that were left without GrowSAV Devices to allow for anchorage. The GrowSAV Devices were loaded onto a 20-ft fiberglass barge by hand

from shore. The GrowSAV Devices were then placed into the water in the predetermined areas marked by buoys and a gridline. The gridline was based on equal distance from the necessary depth contour line to ensure that the GrowSAV device will have at least two feet of water above the planting site based on mean low low waterline. When the GrowSAV Devices were properly weighted and placed on the bottom the gridline and buoy system were removed.

Once all 360 GrowSAV devices were placed on the canal bottom, the planting began. Nursery grown *Vallisneria americana* (tape grass) peat pot units were loaded into enclosed trailers and delivered from Sea and Shoreline's land based aquaculture facility (Ruskin, Florida). Peat pot units were transported in trays to reduce disturbance, and were acclimated to the system overnight. The trays were then loaded onto the fiberglass barge and delivered to the planting site. Planting took place in November 2015 by certified divers experienced in submerged aquatic planting. Each of the 360 GrowSAV Devices were planted with 5 peat pot units.

As part of the Crystal River Pilot restoration package, Sea and Shoreline will conduct regularly spaced monitoring campaigns (4 weekly, 2 bi-weekly, 9 monthly and 3 yearly), each resulting in a report on the ecological health and survivorship of planted tape grass for a total of 18 events by 2018. During each monitoring campaign, Sea and Shoreline will evaluate the growth and proliferation of the tape grass protected by the GrowSAV Devices and in adjacent areas.

2. METHODS

2.1. Sampling Design for Monitoring and Site Description

The transplanting site is situated in the upland canal systems located in the Kings Bay basin. Accessibility to the site was made available by the presence of canals (Figure 1). Sediment inspection revealed that it was muddy-sand to sandy-mud, reflecting the protected nature of the canal system (i.e., reduced fetch). Central water depths were 0.8-2.5 m. Additionally, prior to planting, there was no tape grass observed in the restoration area, only filamentous green algae. However, small populations of *V. Americana* were recorded in nearby canals.

The transplanting/restoration sites (1, 2 and 3) were nearly rectangular polygons, oriented parallel to the canals (Figure 1). Cages were spaced approximately 3.0 m apart in rows oriented parallel to the shoreline. Between November 9 and 16, 2015 (7 working days) Sea and Shoreline Team transplanted more than 1,800 PUs, into 360 GrowSAV cages (nominally 5 PUs per cage). Once planting was completed, Sea and Shoreline delineated the perimeter of the transplant area using a handheld DGPS unit with sub-meter accuracy (Trimble GeoExplorer 6000). Each cage was physically tagged for future identification, and 10% of the 360 units were selected for monitoring. As reference, a randomly located position was selected no less than 5 m from each monitoring point. All monitoring and reference locations were permanently marked with a DGPS. A small waterproof label was securely attached to the looped onto the cage. Each point (72 total points) was monitored during each sampling campaign. WGS coordinates of the 36 sampling points are presented in Table 1 while that of the 36 reference points are presented in Table 2.

In order to assess the survival, health and growth of the planting units at the planted site, a comprehensive suite of biological attributes was quantified and compared to the reference sites. These include benthic community composition, assessments of planting unit survival, *V. americana* shoot density, areal coverage (frequency, abundance, and density), canopy height, epiphyte cover, macroalgal cover and general notes on site condition. Physicochemical water and seabed properties were also measured at each site to provide environmental context for any observed changes in benthic cover or PU performance.

2.2 Biological Parameters

Benthic community composition was monitored using 0.25-m² quadrats by Sea And Shoreline biologists using surface supplied air. Supplementary photo-quadrats of the seafloor were collected at each of the monitored planting and reference locations. All imagery was reviewed in the laboratory to verify SAV species composition and will serve as archival evidence of project performance.

2.2.1. Survival of Planting Units

Survival of planting units within tagged cages was assessed by noting the presence or absence of healthy *V. americana* (Fonseca et al., 1998). Survival was defined as the presence of a single shoot, as even a single shoot indicates association with a growing rhizome.

2.2.2. V. americana Shoot Densities

V. Americana shoot density was estimated at each of the 72 individual permanent sampling points by placing a 10 x 10 cm quadrat in the center of each Braun-Blanquet monitoring quadrat and manually counting all of the shoots present (Figure 2). Shoot count data were then multiplied by 100 to obtain shoot densities in the number of shoots per square meter (shoots m^{-2}). Total grass shoot density is reported the sum of all species counted in a quadrat (shoots/ m^2). The data are reported as mean densities per treatment: planted (N = 36) and reference (N = 36).

2.2.3. Visual Assessment of Braun-Blanquet Frequency, Abundance, and Density

The coverage (frequency, abundance and density) of each SAV species, total SAV community, macroalgae and total macroalgal community in the planting and reference sites were evaluated using the Braun-Blanquet visual assessment method in 0.25-m² quadrats (Table 3; Braun-Blanquet 1965, Kenworthy et al. 1992, Fourqurean et al. 2001). In each quadrat, all observed benthic plant and algal species, total SAV and total macroalgae were visually scored and recorded inside the quadrat by Sea and Shoreline biologists using surfaced supplied air. Braun-Blanquet scores corresponded to coverage ranges reported in Table 5. Three variables (frequency, abundance, and density) were then calculated from the scores according to the following formulas:

E	1	/1 \
Frequency = number of occupied anadrats \div total is	number of anadrate	1 I I
T T C G	namber or audurais	\1

Abundance =
$$sum\ of\ B-B\ score\ values\ \div\ number\ of\ occupied\ quadrats$$
 (2)

Density =
$$sum\ of\ B-B\ score\ values\ \div\ total\ number\ of\ quadrats$$
 (3)

2.2.4. Benthic Plant Community Canopy Height

In the same 10×10 cm quadrats used for shoot counts, the canopy height of the benthic plant community (SAV and/or macroalgae) was measured *in situ* by the observer using metric ruler. All values were rounded to the nearest 0.5 cm. For sites with *V. americana* these data represent blade length, for those without, algal thickness. Data are reported as a mean values per treatment: planted (N = 36) and reference (N = 36).

2.2.5. V. americana Epiphyte Cover

The cover of epiphytes on V. americana leaves in the relocated and reference grasses was assessed inside each Braun-Blanquet quadrat (0.25-m²). Observers used a visual estimation technique based on a scale ranging from 0 (clean) to 3 (heavy; Table 4). The data are reported as mean epiphyte cover per treatment: planted (N = 36) and reference (N = 36).

2.3. Physical Parameters

2.3.1. Water Quality

Water temperature, pH, salinity, dissolved oxygen (DO) and turbidity (NTU) were measured at the surface and bottom at four (4) stations once during each monitoring event (two stations per treatment)

using a calibrated YSI 6600 V2 sonde or a YSI Professional Plus multi-parameter water quality logger (Figure 3). The water quality values for each individual parameter at each depth are reported as mean values for the two stations at the planted and reference sites, respectively. Water quality measurements were further evaluated for compliance with DEP/EPA Standards for environmental protection.

2.4. Permanent Archive

Video recordings of the seafloor along longitudinal/diagonal transect and/or photographs of the "tagged" sods/quadrats in the monitoring and reference sites were collected during each monitoring period. These recordings were electronically archived and will serve as a permanent record of project performance.

3. RESULTS

3.1 Biological Parameters

3.1.1. Survival of Planting Units

During this 1st monitoring campaign, 5 months post-planting, survival of planted cages was 97.2% (Figure 4)..

3.1.2. Mean V. americana Shoot Densities

V. americana was observed in both the planted and reference sites (Figure 5). Shoot densities within the planted zone were highly variable, ranging from 0 to 2000 shoots m⁻² with a mean (+/- 1 s.d.) value of 867 +/- 549 shoots m⁻². Reference densities were considerably lower at 31 +/- 62 shoots m⁻² with a range of only 0 to 200.

3.1.3. Braun-Blanquet Frequency

In Campaign 1, the frequency of total SAV was higher (0.97, planted; 0.78, reference) and total macroalgae lower (0.69, planted; 0.94, reference) in the planted versus the reference sites (Figure 6). *Lyngbia* was more than half (0.41 x) as frequent in the planting area than adjacent reference locations, while the transplanted species, *V. americana* was 2.06 times more frequent. Interestingly, the other two SAV species: *Hydrilla* and *Najas* were less abundant in the planted cages (0.14 and 0.00, respectively) than references (0.36 and 0.19, respectively).

3.1.4. Braun-Blanquet Abundance

In the quadrats occupied *V. americana* in campaign 1, abundance values were higher in the relocation sites (3.48) then in the reference sites (0.95; Figure 7). Total SAV abundance was also higher in the relocation (3.56) than the reference sites (2.15), while total macroalgae was lower (1.34 and 2.80, respectively). Within the macroalgae community, *Spirogyra* was less abundant (1.73 vs. 2.71) despite being more frequently recorded in planted rather than reference quadrats. Overall, however, frequency and abundance patterns were similar.

3.1.5. Braun-Blanquet Density

During Campaign 1, Braun-Blanquet densities for total SAV were 2.07 times higher within the planted cages (3.46) than in adjacent reference stations (1.67; Figure 8). This was driven almost entirely by *V. americana* (3.49) that was nearly absent from the reference (0.45). As with other Braun-Blanquet derived metrics, total macroalgal densities were much higher outside of the planted cages than within (2.64 and 0.93, respectively), with *Lyngbia* driving much of that pattern (2.36, reference; 0.43 planted).

3.1.6. Mean Coverage of Total SAV in the Relocation Site.

The coverage of total SAV in the relocation sites ranged from 0 to 5 with a mean Braun-Blanquet score of 3.46+/-1.33 (Figure 9). This was much higher than in reference locations, which ranged from 0 to only 4 with a mean coverage of 1.67+/-1.42.

3.1.7. Benthic Plant Community Canopy Height

Canopy heights ranged from 0 to 68 cm within GrowSAV cages and 0 to only 13 cm in adjacent references (Figure 10). Mean values were 35.4+/-20.4 and 4.4+/-2.9 cm, respectively. The disparity was due to the much larger leaf lengths of *V. americana* relative to other SAV and macroalgal components of the benthic plant community. However, this only applied to protected *V. americana*, because reference locations that contain *V. americana* were only marginally taller at 4.75+/-2.81 cm relative to 4.25+/-2.93 cm for quadrats that did not. This indicates that grazing maintains shorter canopy heights in the absence of cage protection.

3.1.8. Mean Epiphyte Cover

Epiphyte cover was indistinguishable between the planted cages and adjacent controls with mean values of 1.69+/-0.91 and 1.86+/-0.67, respectively (Figure 11). Based on the epiphyte scale values (Table 3) both treatments had clean to heavy coverage with mean values approximating light epiphytic loading. This is consistent with idea that the primary epiphyte grazers (most likely small invertebrates) were smaller than the GrowSAV mesh aperture, leading to equivalent grazing pressures between treatments.

3.3. Physical Parameters

3.3.1. Discrete Water Quality Data

The discrete water quality data for each individual station are presented in Table 6. Water temperatures at all 4 sites (2 reference, 2 planted) were 74° F. Also identical were turbidity readings at 1.6 NTU. Dissolved oxygen (DO) varied slightly with mean values of 6.035+/-0.007 and 6.030+/-0 mg/L. Repeated depth profiles of salinity, temperature and dissolved oxygen were conducted during Campaign 1. Based on these readings, it was determined that there was no evidence for vertical stratification in the water column, therefore only the surface readings are presented here.

4. DISCUSSION & SUMMARY OF RESULTS

The results of this first monitoring campaign indicated that nearly the entire PU deployment survived the transplanting process (97% survival). This survival rate is better than the average global success rate for transplanting seagrasses, considered to be 50% (Fonseca et al. 1998). The initial biological response of transplanted *V. americana* indicate that the relocation methods were successful in alleviating undue stress and, at one year, the survival rate of relocated plants exceeds the target rate of 80%.

Qualitatively, the PU's appear to be in very good condition. In the restoration sites, mean SAV density is higher than when first transplanted, as vegetative runners were observed beyond cage boundaries, a clear indication that transplanted individuals have acclimated to their new location. In general, the mean total grass shoot densities in the relocation sites were comparable to densities of natural meadows reported for the Crystal River area (Shepard et al. 1992; Kenworthy et al. 1993; Erftemeijer and Shuail 2012). Over the entire relocation site, SAV cover was generally 50%. Typically, during the initial period following grass transplanting there is a stress response as the relocated grasses adjust to the physical disturbance of relocation and acclimation to a new environment. However, the results of the biological survey data from Campaign 1 show no sign of PU stress. As indicated above, shoot densities were normal and coverage values were acceptable. Additionally, the leaf epiphyte cover values were low overall and identical to adjacent *V. americana*; at present epiphytic cover is below thresholds known to inhibit the establishment of SAV.

The initial success of relocation may be attributable to the use of the GrowSAV Herbivory Exclusion Devices that provides protection from large grazers while mitigating some of the hydrodynamic influences of the site. Both relocation and reference sites were physically identical but limited *V. americana* recruitment has been observed outside of the cages. As reported in the baseline survey sediments at the relocation and reference sites consisted mostly of fine, medium and coarse sands with silty mud. The sediment conditions at both sites were typical of conditions known to support the growth of grasses in Crystal River. During this campaign we observed very little change in the measured environmental parameters compared to the baseline survey. The pH conditions were normal for spring fresh water temperatures were 74° F. Turbidity (NTU) in the water column was identical between the relocation and reference sites and low overall. With respect to water depth, all of the measured water quality parameters indicated that the water column was well mixed with no indication of vertical stratification or bottom-water anoxia that could inhibit SAV development. Should the environmental conditions we observed during this monitoring campaign continue, we expect that the PUs will continue to propagate vegetatively beyond the confines of the GrowSAV enclosures, ultimately expanding to cover a significant portion of the project site.

In summary, the biological assessments of survival, density and abundance of SAV during this monitoring campaign indicated that newly transplanted *Vallisneria americana* were healthy and thriving. There was limited evidence of plant mortality; therefore the amount of SAV present in the relocation or planting site is currently meeting project requirements. Presently, all of the physical parameters measured at the relocation site were similar to the conditions in Crystal River were *Vallisneria americana* has been shown to thrive.

5. BIBLIOGRAPHY

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6. TABLES

Table 1: Real time coordinates of tagged grass planting units (PU) cages for monitoring

Latitude	Longitude	Point_ID
28.892502950	-82.590531883	1
28.892555200	-82.590524147	2
28.892776858	-82.590543423	3
28.893062196	-82.590458280	4
28.893338166	-82.590076064	5
28.893400581	-82.589967110	6
28.893482639	-82.589843256	7
28.893606153	-82.589674322	8
28.893660283	-82.589551950	9
28.893849625	-82.589258693	10
28.893804401	-82.589403657	11
28.893949503	-82.589110507	12
28.893643942	-82.589646866	13
28.893808685	-82.589441222	14
28.893899611	-82.589292980	15
28.894017165	-82.589180425	16
28.893829972	-82.589510418	17
28.894043300	-82.589300498	18
28.893772291	-82.589085731	19
28.893597179	-82.588957463	20
28.893397637	-82.588880960	21
28.893167109	-82.588679265	22
28.893204555	-82.588698470	23
28.893280672	-82.588731238	24
28.892700445	-82.588506284	25
28.892612355	-82.588465588	26
28.892509625	-82.588412240	27
28.892354862	-82.588337215	28
28.892596090	-82.588672779	29
28.892588468	-82.588824789	30
28.892611637	-82.588896264	31
28.892589289	-82.589006155	32
28.893146216	-82.590349790	33
28.893397066	-82.589992206	34
28.893623148	-82.589660493	35
28.893698006	-82.589565070	36

Table 2: Real time coordinates of tagged reference grass quadrats for monitoring.

Table 3: Braun – Blanquet (BB) score values and corresponding grass cover.

Braun Blanquet Score	Cover Value	
0	Absent	
0.1	Solitary specimen	
0.5	Few, with small cover	
1	Numerous, but less than 5% cover	
2	5% - 25%	
3	25% - 50%	
4	50% - 75%	
5	75% - 100%	

Table 4: Epiphyte cover scale with corresponding qualitative descriptions.

Scale	Epiphytic Coverage Description	
0	Clean	
1	Light	
2	Moderate	
3	Heavy	

Table 5. Temperature, salinity, dissolved oxygen, pH and turbidity in the bottom waters at each of the two stations in the relocation and reference sites.

Temperature (°C)	Bottom
Monitoring Site 1	74
Monitoring Site 2	74
Reference A	74
Reference B	74
Salinity (ppt)	
Monitoring Site 1	0
Monitoring Site 2	0
Reference A	0
Reference B	0

Dissolved Oxygen (mg/L)	
Monitoring Site 1	6.03
Monitoring Site 2	6.04
Reference A	6.03
Reference B	6.03
рН	
Monitoring Site 1	ND
Monitoring Site 2	ND
Reference A	ND
Reference B	ND
Turbidity (NTU)	
Monitoring Site 1	1.6
Monitoring Site 2	1.6
Reference A	1.6
Reference B	1.6

7. FIGURES



Figure 1. Map showing the location of the grass relocation site (blue polygons), exclusion cages and planting units location (Yellow dots), locations of the permanent sampling sites (red dots).



Figure 2. Underwater photograph showing the shoot count quadrat (10 cm x 10 cm) placed inside the center of the larger Braun Blanquet quadrat (50 cm x 50 cm, not to scale).



Figure 3. YSI Professional Plus multi-parameter water quality logger. Inset – YSI 6600 V2 Sonde

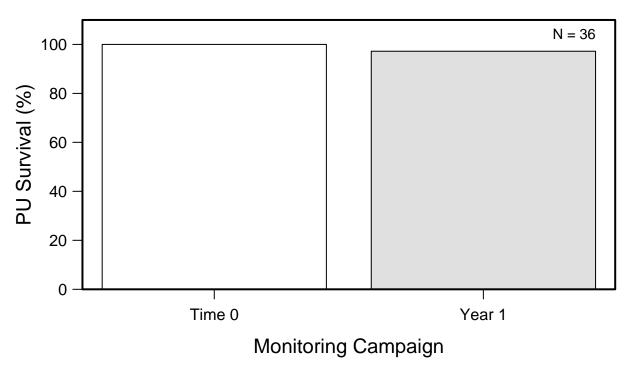


Figure 4. Survival of V. americana PUs during Campaign 1.

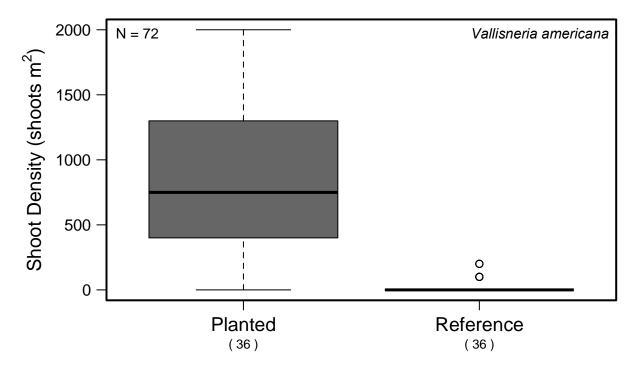


Figure 5. Boxplot of Vallisneria americana shoot density (shoots/m2) at the reference and relocation sites during Campaign 1.

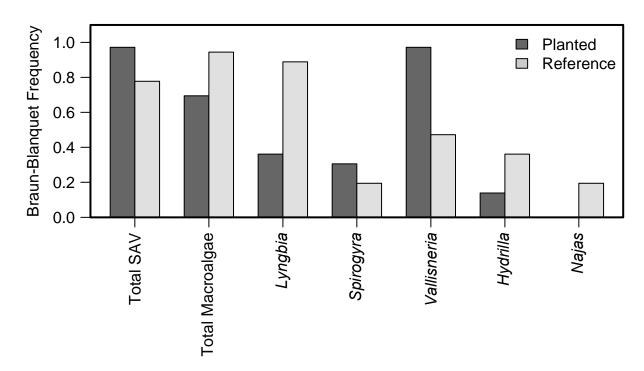


Figure 6. Braun-Blanquet frequencies for SAV and algal species at the planted and reference sites

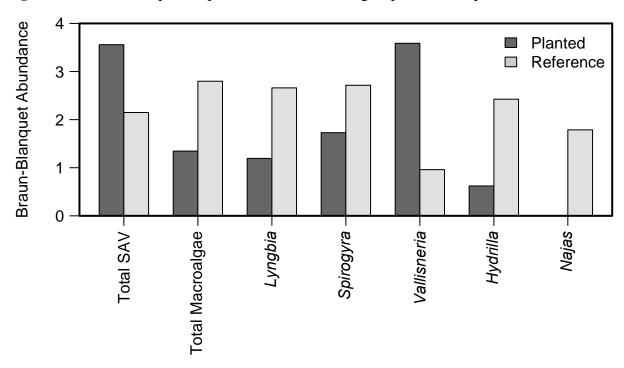


Figure 7. Braun-Blanquet abundances for SAV and algal species at the planted and reference sites

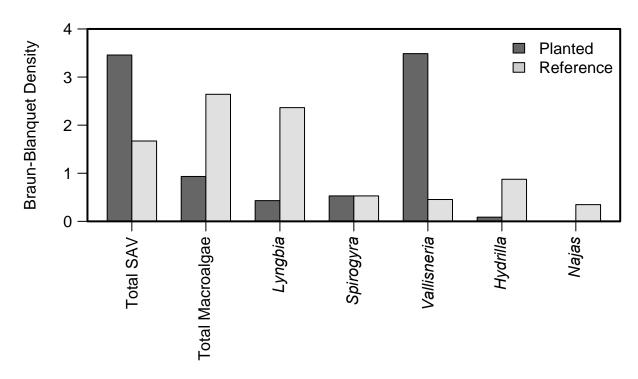


Figure 8. Braun-Blanquet densities for SAV and algal species at the planted and reference sites

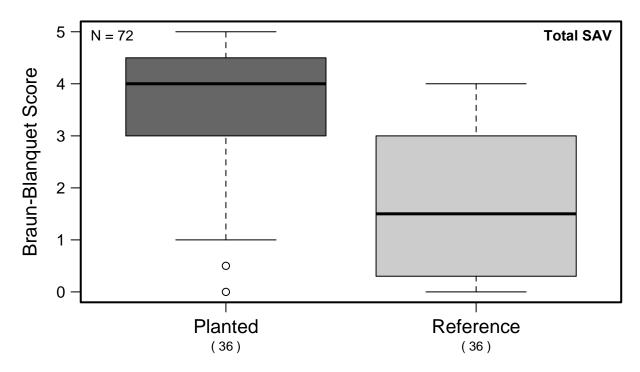


Figure 9. Boxplot of Braun-Blanquet scores for Total SAV recorded in the planted cages and reference areas during Campaign 1.

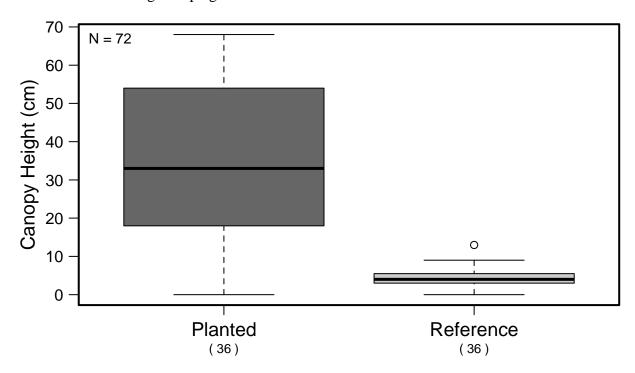


Figure 10. Canopy height (cm) for benthic plant communities at the relocation and reference sites.

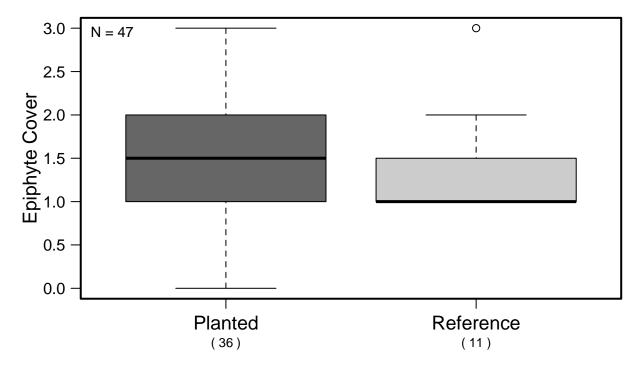
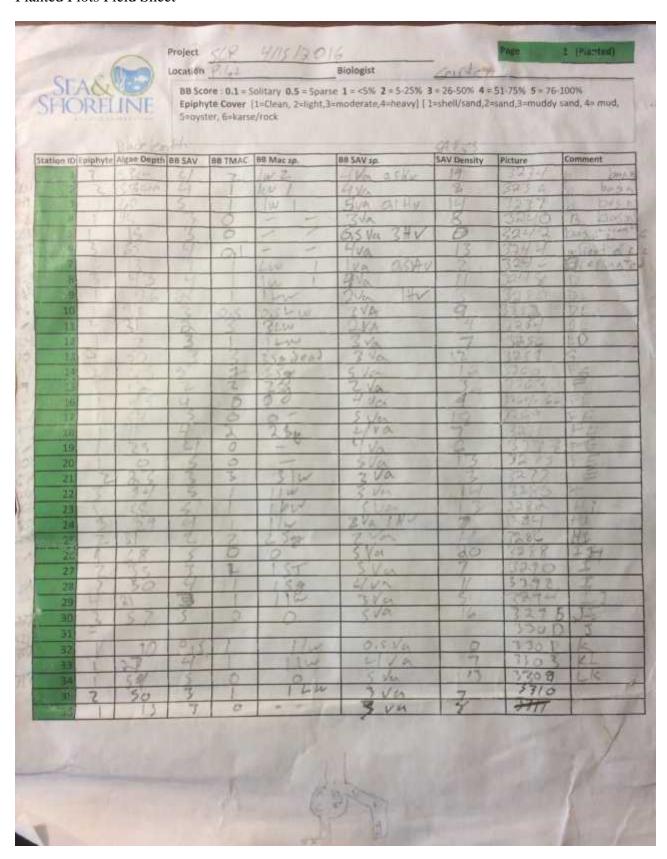
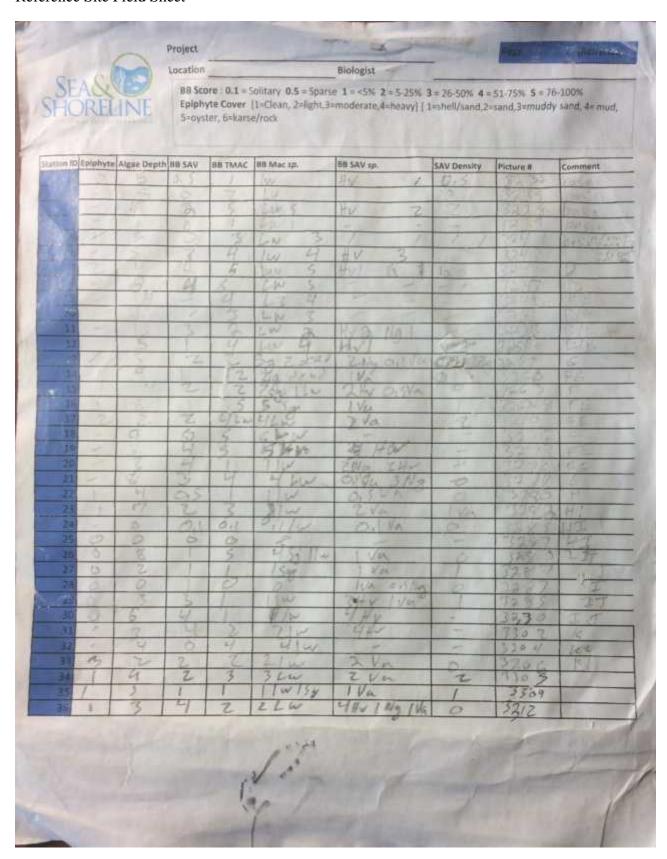


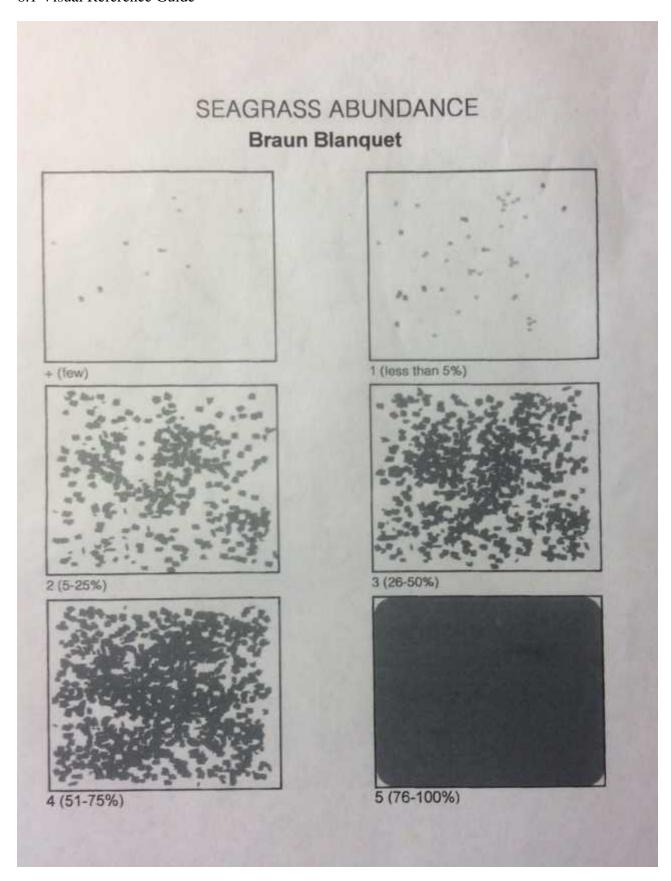
Figure 11. Epiphytic cover on Vallisneria americana at the relocation and reference sites.

8. APPENDICES

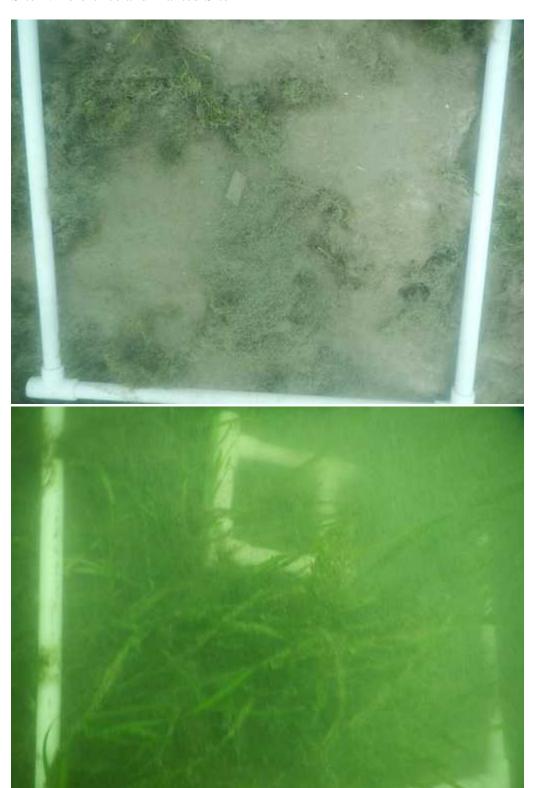
8.1 Field Data Sheets



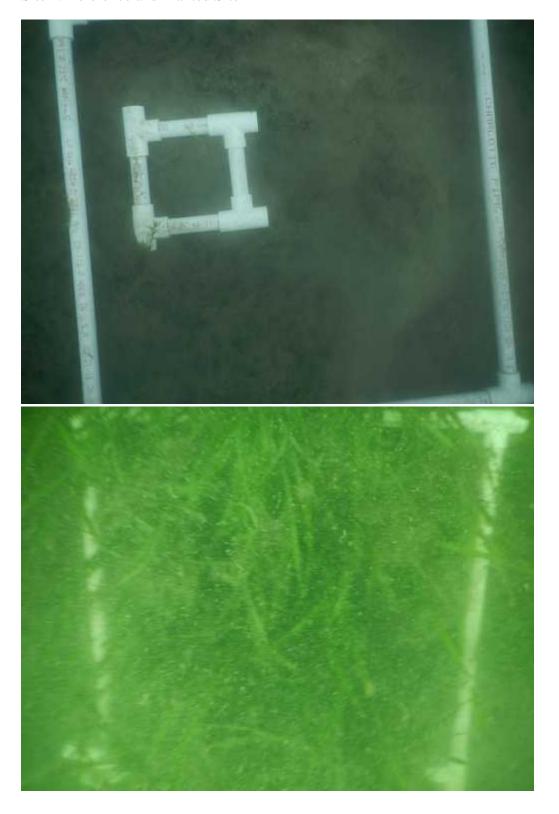




Site 1: Reference and Planted Site



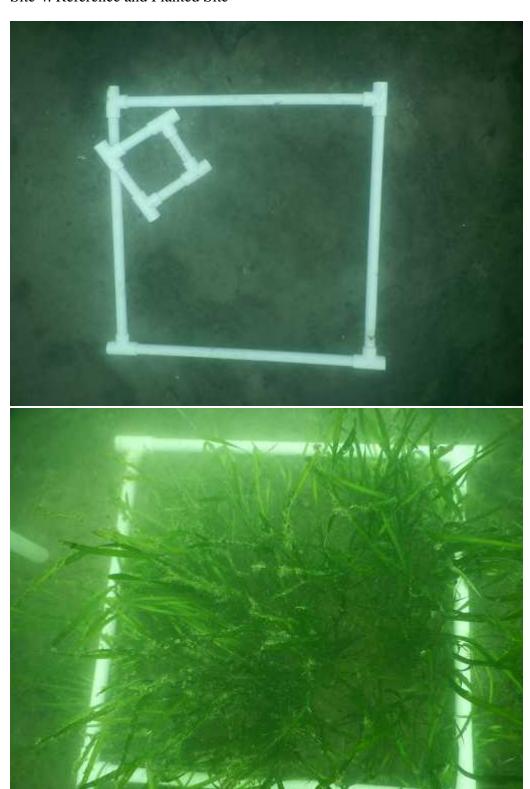
Site 2: Reference and Planted Site



Site 3: Reference and Planted Site



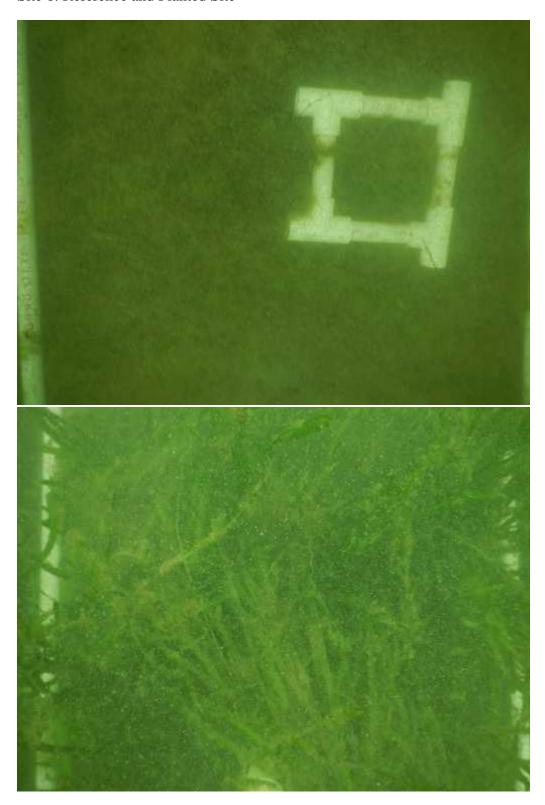
Site 4: Reference and Planted Site



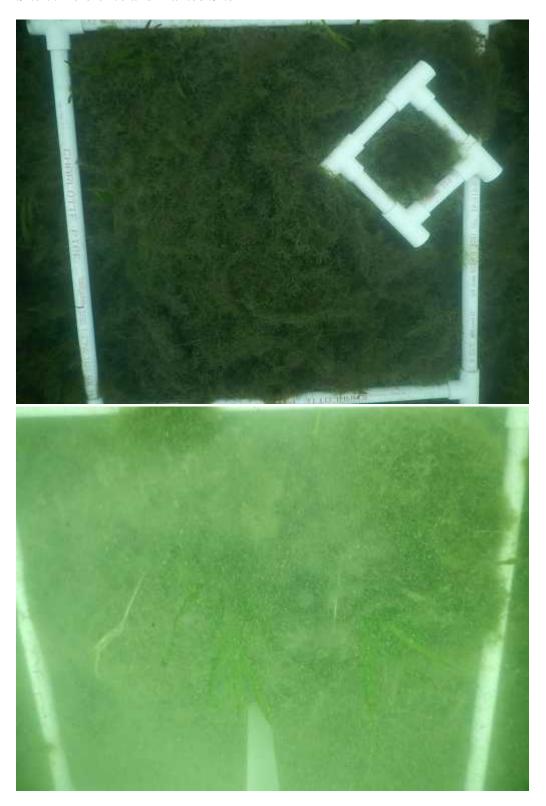
Site 5: Reference and Planted Site



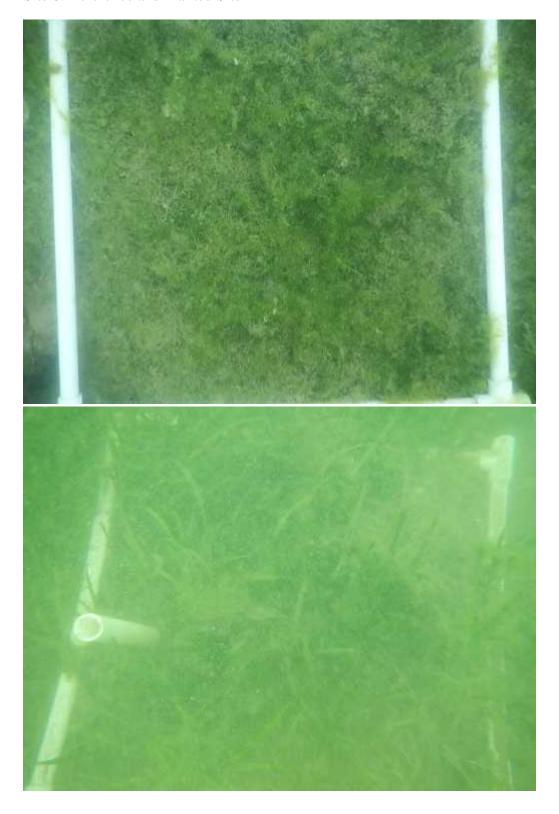
Site 6: Reference and Planted Site



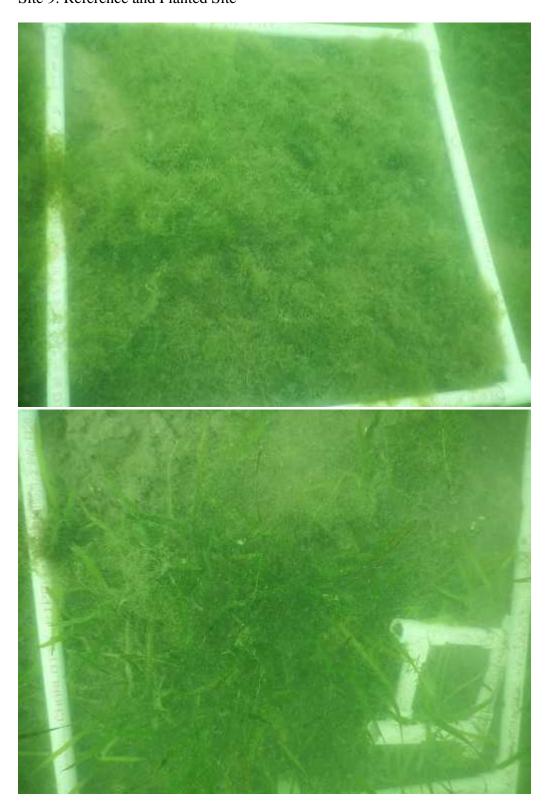
Site 7: Reference and Planted Site



Site 8: Reference and Planted Site



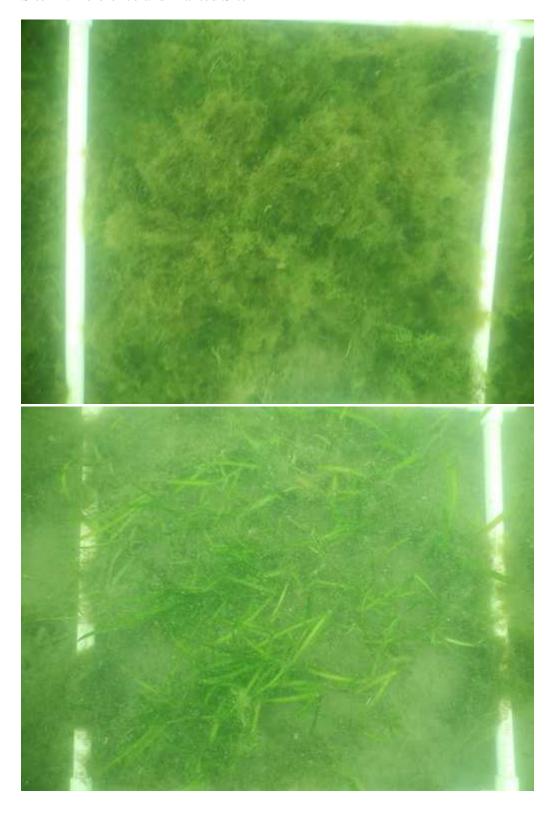
Site 9: Reference and Planted Site



Site 10: Reference and Planted Site



Site 11: Reference and Planted Site



Site 12: Reference and Planted Site



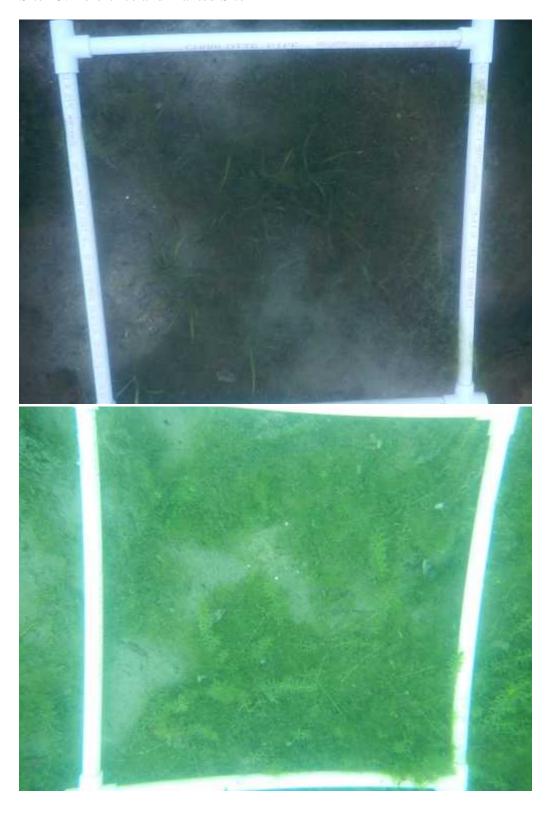
Site 13: Reference and Planted Site



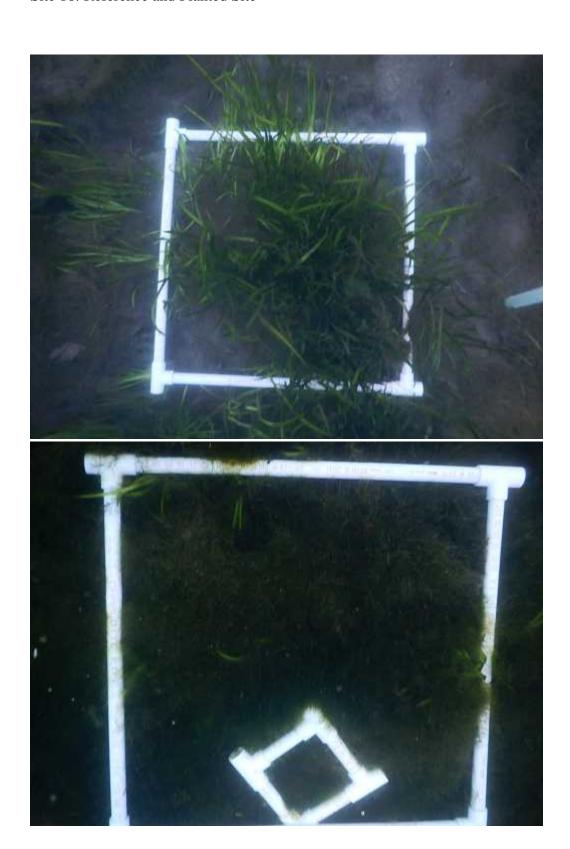
Site 14: Reference and Planted Site



Site 15: Reference and Planted Site



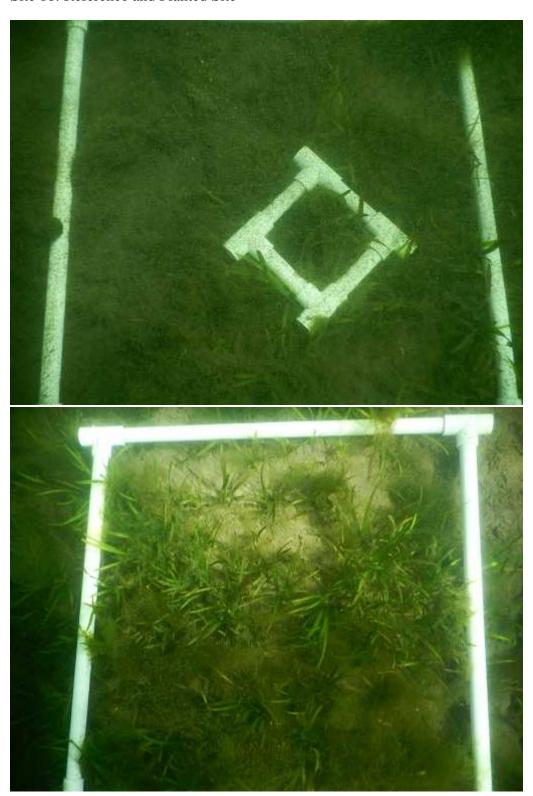
Site 16: Reference and Planted Site



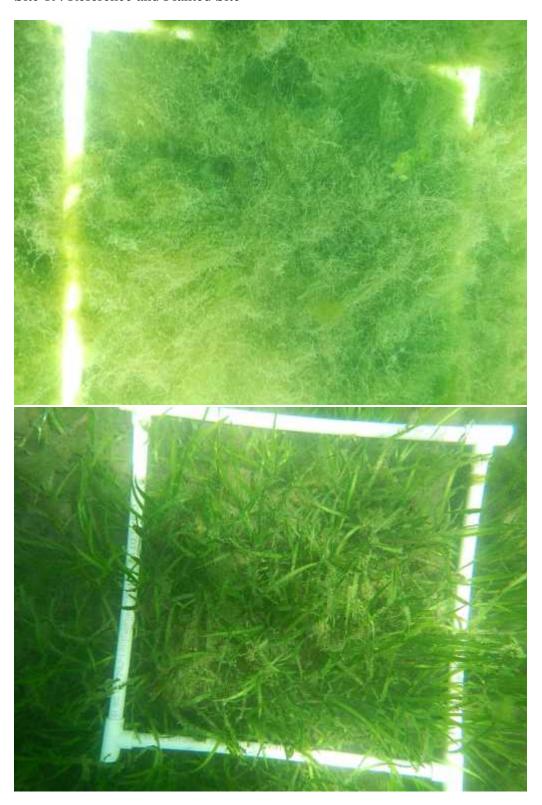
Site 17: Reference and Planted Site



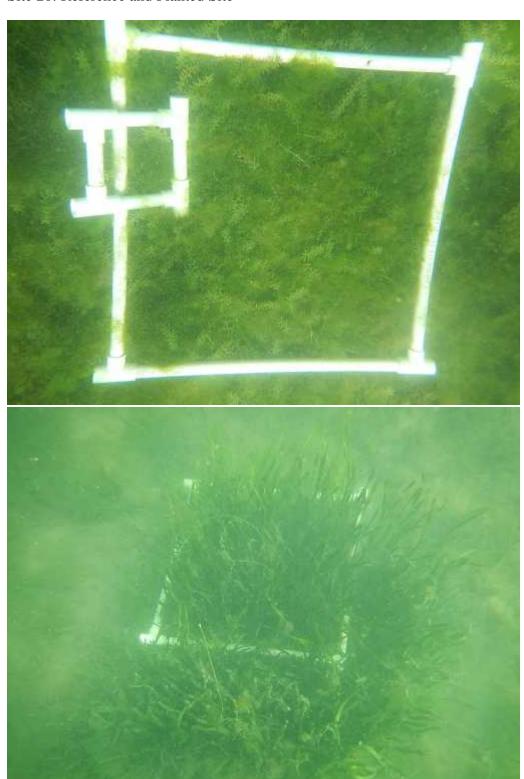
Site 18: Reference and Planted Site



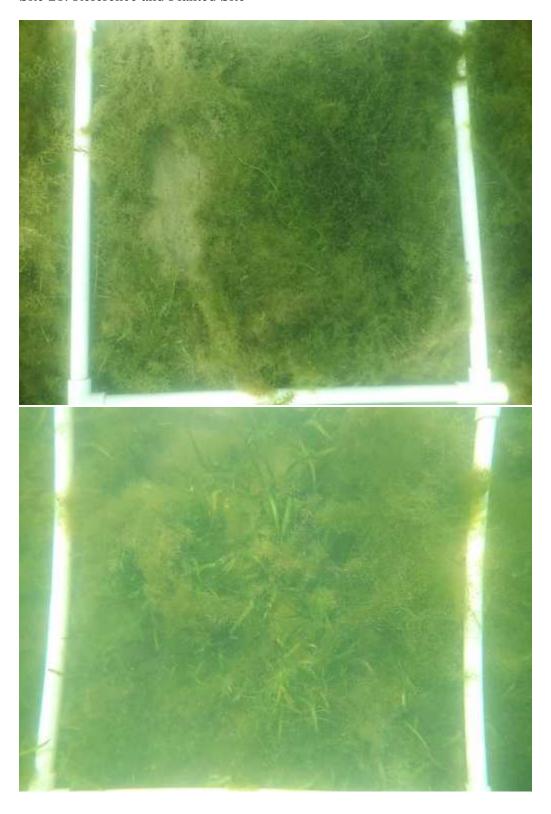
Site 19: Reference and Planted Site



Site 20: Reference and Planted Site



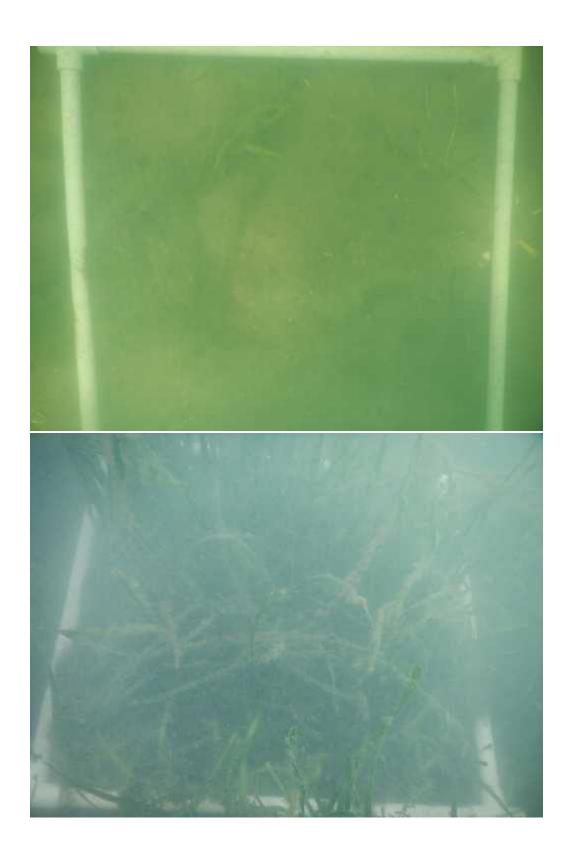
Site 21: Reference and Planted Site



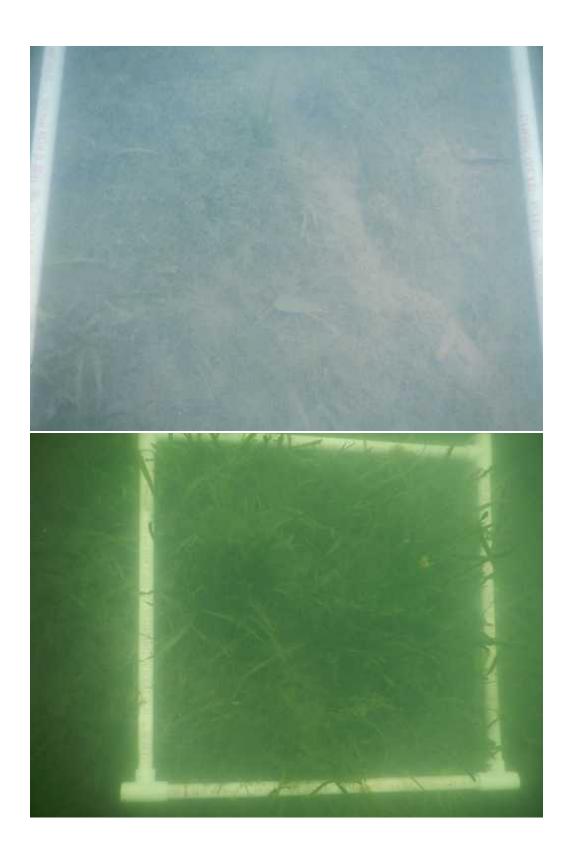
Site 22: Reference and Planted Site



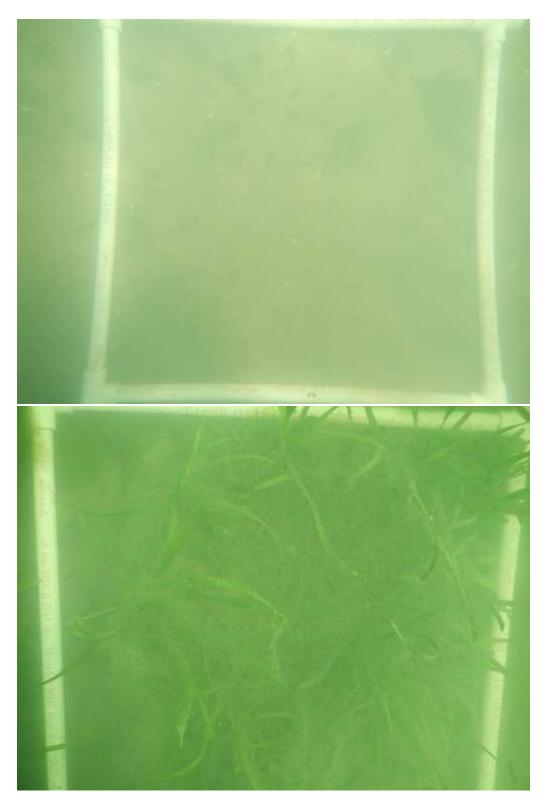
Site 23: Reference and Planted Site



Site 24: Reference and Planted Site



Site 25: Reference and Planted Site



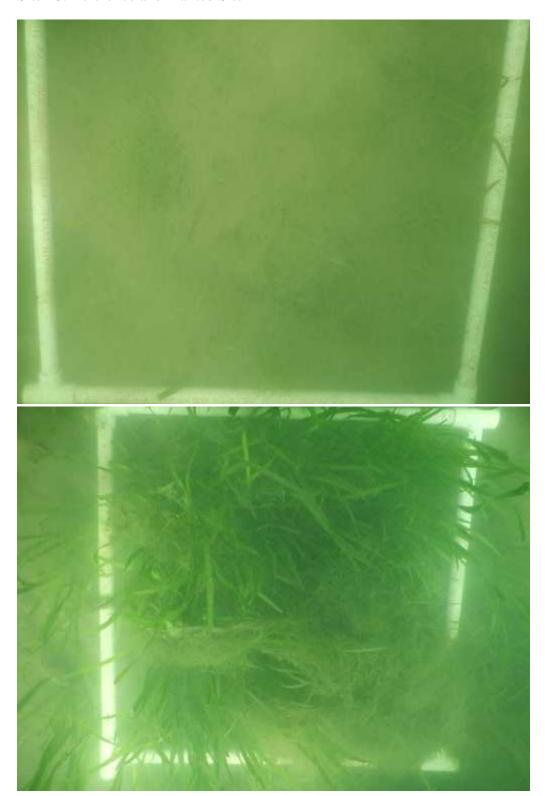
Site 26: Reference and Planted Site



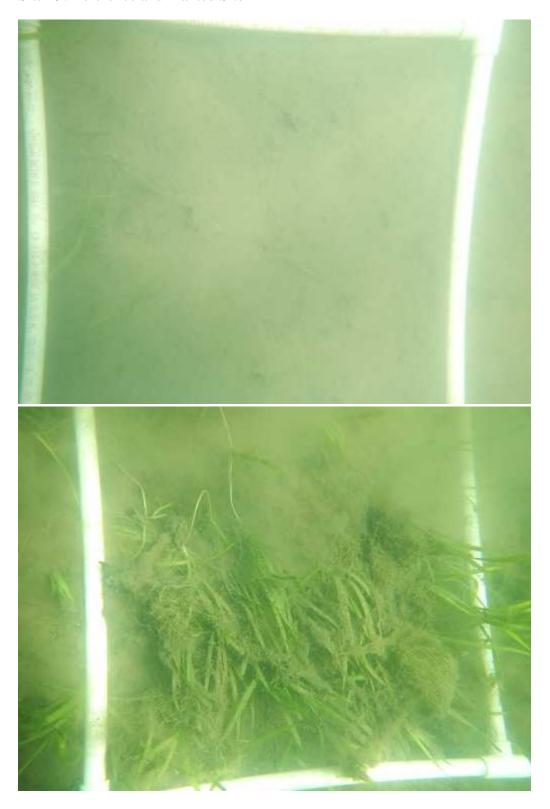
Site 27: Reference and Planted Site



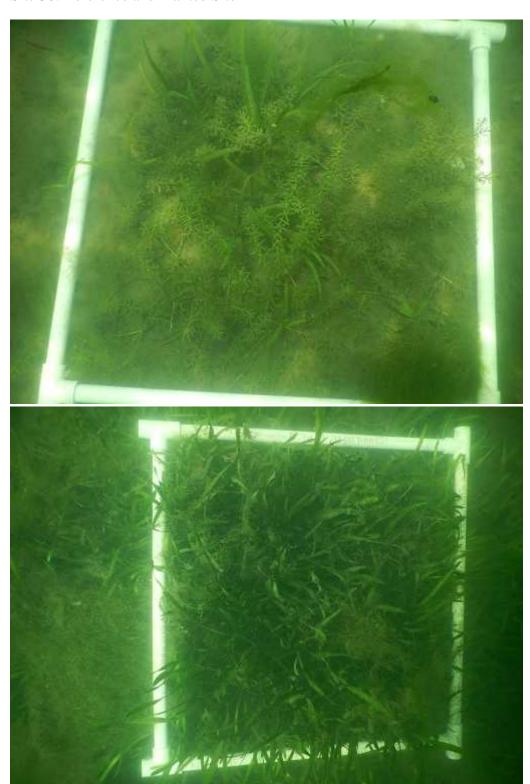
Site 28: Reference and Planted Site



Site 29: Reference and Planted Site



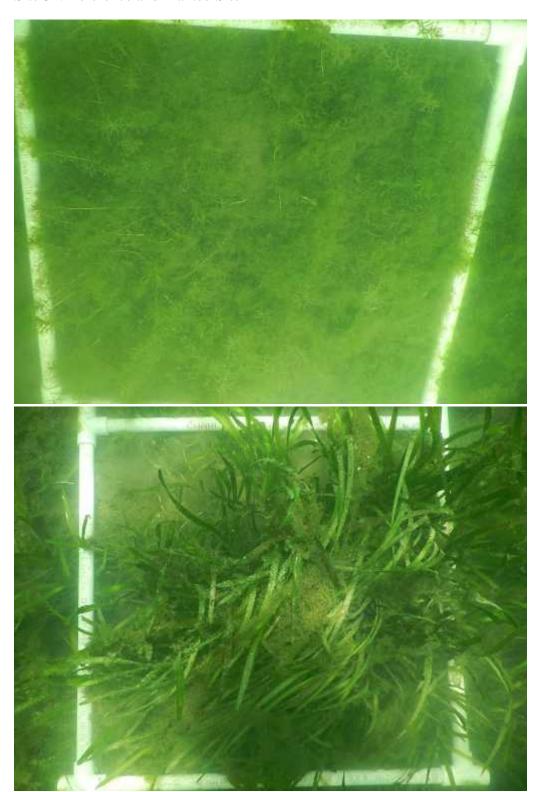
Site 30: Reference and Planted Site



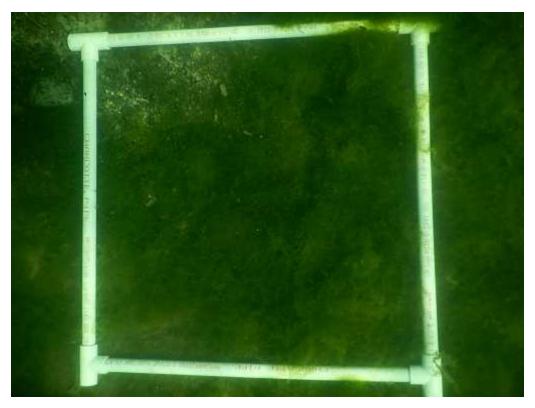
Site 31: Reference and Planted Site



Site 32: Reference and Planted Site



Site 33: Reference and Planted Site



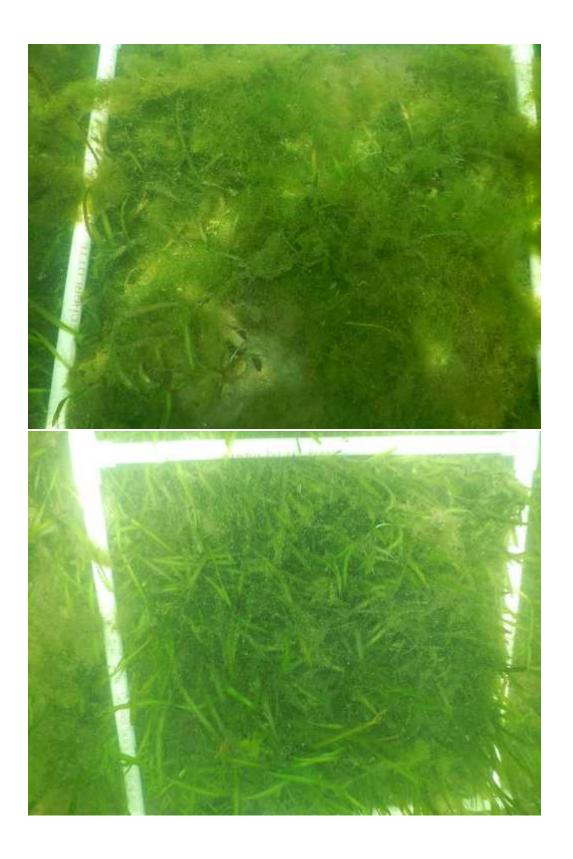


Site 34: Reference and Planted Site





Site 35: Reference and Planted Site



Site 36: Reference and Planted Site

