Planting *Vallisneria americana* in the Freshwater Caloosahatchee River: A Dry Season Opportunity, Phase II

Task 5.1: Final Report

Submitted December 2012

by:

David W. Ceilley & Edwin M. Everham, III,

Kory M. Ross, John A. Ferlita, II and Geoff G. Rosenaw

Florida Gulf Coast University

Submitted to:

South Florida Water Management District

Coastal Ecosystem Sciences Division

List of Figures

Page

Page

List of Tables

INTRODUCTION & STUDY LOCATION

Drought conditions have caused salinities lethal to freshwater *Vallisneria americana* in the upper Caloosahatchee Estuary, where this plant once thrived. In order to establish a potential seed source for future populations in the upper estuary, *Vallisneria* was replanted upstream of structure S-79. The selected restoration sites have the following characteristics; sandy substratum, shallow depths, and low densities of submerged vegetation. Past experience indicates that for young, replanted *Vallisneria* to survive it needs to be protected from herbivores (Ceilley and Bortone 2003). Freshwater turtles of the genus *Pseudemys* have been observed grazing on *Vallisneria* in aquaria and in small ponds on the Campus of Florida Gulf Coast University. To protect newly planted *Vallisneria* in the Caloosahatchee River, the 2011 replanting effort focused exclusively on protected plants –and- post planting monitoring was extended beyond previous studies to assess both the durability of exclosure cages and the growth of planted material. *Vallisneria* planted at the end of the 2011 dry season will remain protected through the end of the 2013 dry season (June 2013), to determine if transplanted *Vallisneria* can establish large, sustainable grass beds if grazers are excluded for at least the first two full growing seasons following the transplant. Large and small exclosure cage designs were tested in the Caloosahatchee River in 2008 (E. Milbrandt personal communication) and in 2009-10 respectively (Ceilley 2009) with mixed results. Smaller cages tested in 2009-2010 were removed after one year and patches of *Vallisneria* remain at those locations. A single 1m² cage planted in August 2009 remains and contained several mature plants as of August 25, 2011. Results from these replanting efforts and from recent field inspections by SFWMD staff and FGCU researchers were used to select two initial replanting locations hereafter referred to as Site 1 and Site 2.

Phase I Methods

A kickoff meeting was held at the SFWMD offices in West Palm Beach on May 26, 2011 (Task 1.1) and a presentation was made describing the project modifications that included using 24 small exclosure cages with replicated treatments of different donor material, planted at different densities and at two locations (Table 1). In addition, a prototype of the exclosure cages was constructed and shown to District staff and the Project Manager for their approval. At the conclusion of the meeting there was verbal agreement on cage design, experimental design, and when to deploy them in the field. Modifications to the Work Plan were drafted by the Contractor and sent to the Project Manager for review and comments on June 9, 2011 (Task 1.2). Comments and suggested revisions were received from the District on June 15, 2011 and revisions were made by the PI/Contractor and returned the same day.

Table 1: Planting locations for Site 1 (VP1) and Site 2 (VP2).

Site	Latitude	Longitude	Notes
1	26.72281	-81.68731	Protected by island, moderately soft sandy sediment
2	26.71910	-81.66628	North shoreline near creek mouth, firm sediment

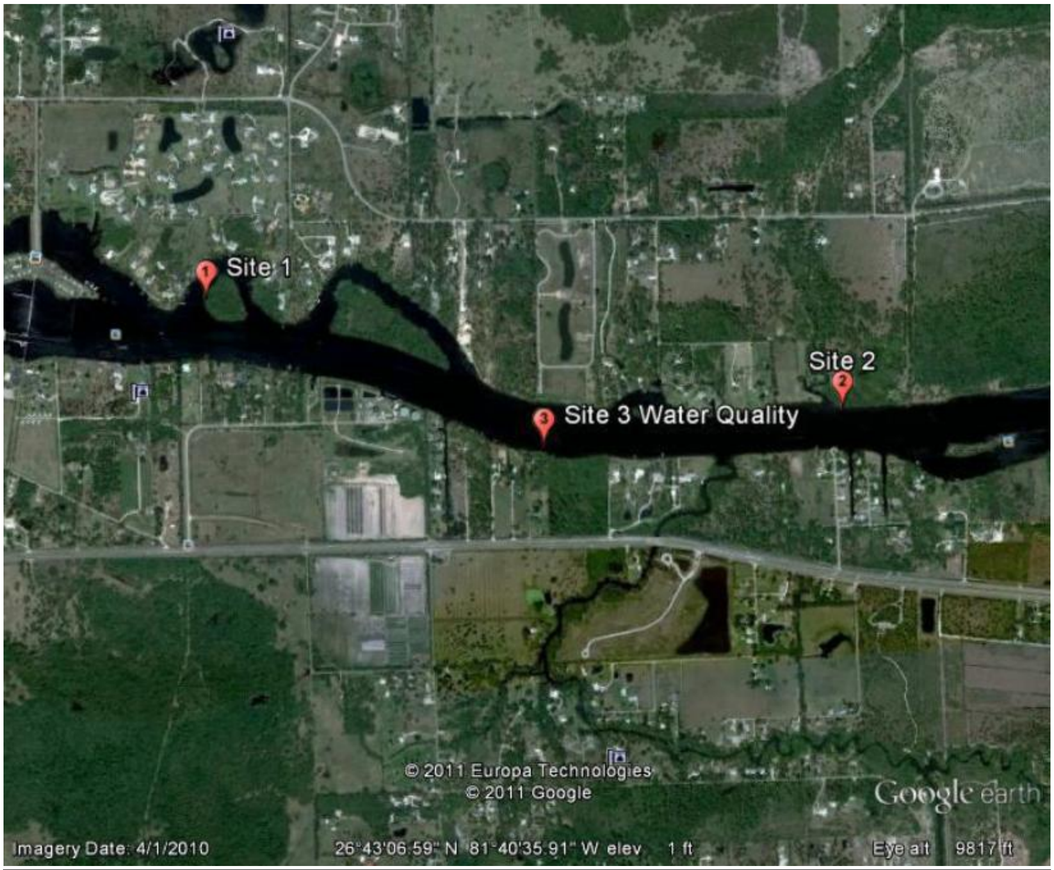

Figure 1. General location map of Phase I study Sites 1 and 2 with ambient water quality also collected at Site 3

2.0 a: OBJECTIVES: Phase 1 (Completed in FY2011)

1. To create a viable *Vallisneria americana* seed stock in the upper Caloosahatchee River;
2. Determine which of two planting densities will result in greatest survival of replanted *Vallisneria*;
3. Determine if planted Vallisneria will grow enough to survive grazing pressure if protected by exclosures for at least three (3) months.

The small exclosure experiment included two genetic strains of Vallisneria planted at two densities (10/meter vs. 20/meter) with replicate samples of three for each treatment for a total of 12 exclosure cages at each location for Sites 1 and 2 (Figure 2).

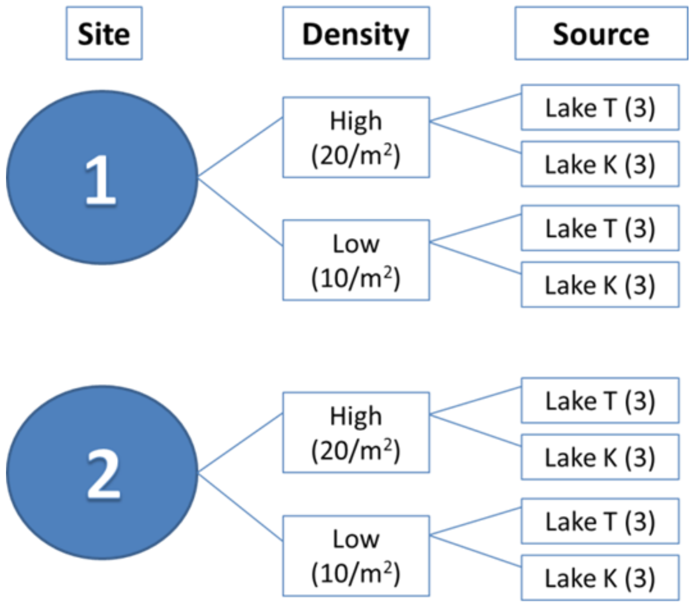

Figure 2. Graphic depicting deployment of cages at their respective sites with number of treatments (2 sites x 2 densities x 2 strains).

Figure 3. One of the 24 completed 1m² exclosure cages to be installed in the Caloosahatchee River and planted with *Vallisneria americana* strains from Lake Trafford and Lake Kennedy.

Phase I Results

Phase I of this project included the planting of two strains of *Vallisneria americana* in replicated small exclosures at two locations and at two planting densities (Figure 2). Since *Vallisneria americana* is a dioecious plant, we planted both male and female plants in this study to ensure that seed production is possible within the study area. The source site of the *Vallisneria americana* donor plants was Lake Kennedy in Cape Coral Florida and from Lake Trafford in Collier County, Florida. These donor locations were found to contain genetically different strains (Lyn Gettys, Ph.D, personal communication) with Lake Kennedy being dominated by male, possibly clonal plants. Lake Trafford has been recently restored through a multi-year $21.35 million de-mucking project and now contains an abundance of dense *Vallisneria*. Donor plants from Lake Trafford were mostly female plants and many with mature seed pods. As of August 25th, 2011 at the completion of Phase I, all cages were intact and no major breaches were documented.

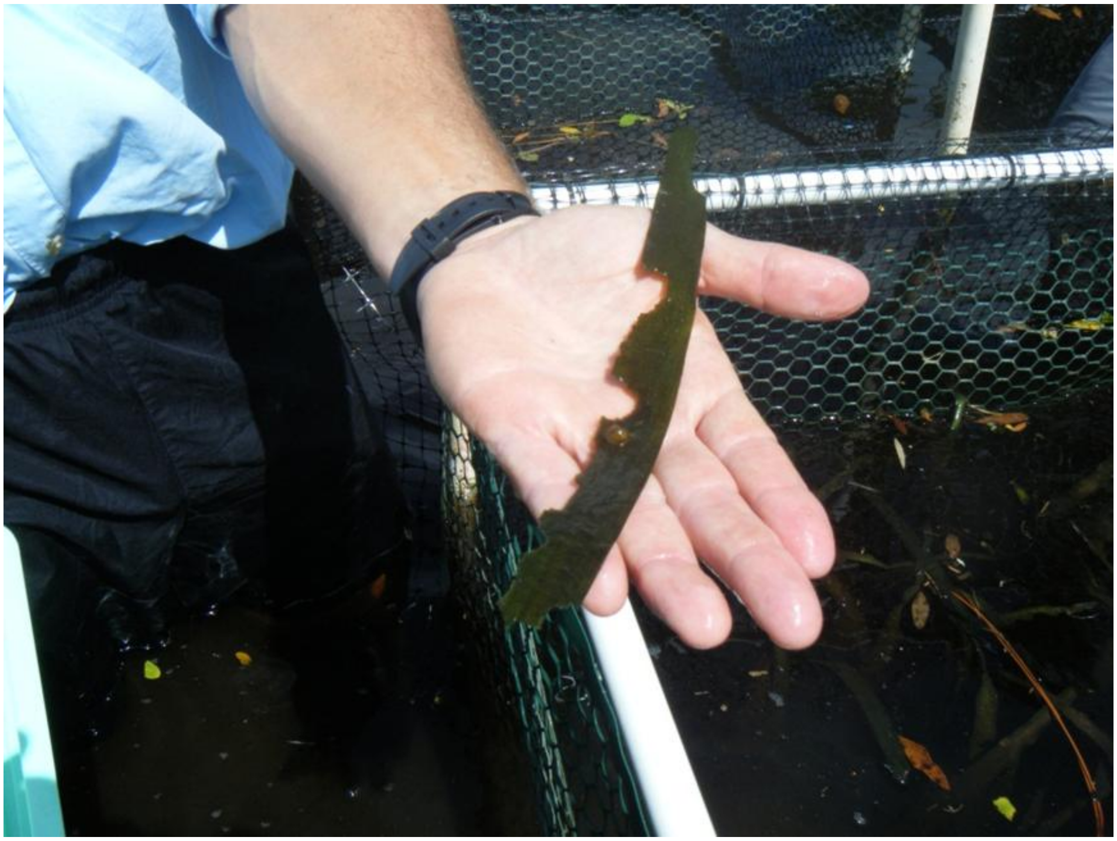

Figure 4. Observed grazing marks on *V. americana* with juvenile *P. insularim* on the blade during Phase I.

During Phase I, we observed we observed extensive herbivory and found that the invasive apple snail, *Pomacea insularim* appeared to be responsible for most of the grazing (Figure 4). In an effort to quantify the relative levels of grazing observed, we proposed the categorizing of *P. insularim* grazing intensity into three general categories; zero to minimal, moderate, and high grazing (FGCU 2011). Grazing intensity was also observed on several occasions on the outside of the exclosures where new stolons and rosettes were grazed heavily as they protruded through the plastic mesh. This grazing appeared to be the primary factor that prevented *V. americana* colonization outside of all the exclosure cages.

At the completion of Phase I planting study on September 1, 2011, *V. americana* growth metrics from subsamples taken from each plot were analyzed. Results indicated that 22 of 24 quadrat subsamples contained rooted *V. americana.* At Site 1, one of the Lake Trafford low-density samples did not contain any plants and there were not any plants at all in the exclosure, even though it was intact and anchored. At Site 2 there was one Lake Trafford high-density sample did not contain any plants but there were plants in the exclosure that did not fall within the 25cm quadrat sample. All 24 cages were intact with no evidence of being compromised or disturbed after three months of deployment.

Growth metrics for Site 1 during Phase I indicated that most of the 1m² plots showed substantial growth regardless of planting density (FGCU 2011). The two highest total biomass treatments (19.3 grams and 16.6 grams) were Lake Kennedy low-density plantings followed by a Lake Kennedy high density sample with 12.0 grams. There were strong positive correlations between the number of blades and total biomass, number of shoots and number of blades and, between number of shoots and total biomass.

Growth metrics for Site 2 were similar and most of the 1m² plots showed substantial growth independent of planting density. Total number of shoots ranged from 0-12 with a mean of 6.33 and if scaled up to the entire exclosure would equal about 58 shoots per exclosure, or about three times the high density plantings (20), and nearly six times the low density (10). As was the case at Site 1, the two highest total biomass treatments (16.6 grams and 13.4 grams) were Lake

Kennedy low-density plantings followed by a Lake Kennedy high density sample with 10.8 grams. There was a strong positive correlation between number of shoots and number of blades (Table 3). There was a much weaker positive correlation between number of blades and total biomass and between number of shoots and total biomass (FGCU 2011).

Based on the quantitative measurements of shoots, blades, and total biomass, the Site 2 plantings showed higher growth rates overall than plantings at Site 1. However, based on the qualitative assessment of grazing intensity, Site 2 plantings were impacted more and snail density appeared to be higher at this location. We attributed this to the proximity of Site 2 cages to the cattail lined shore with high densities of egg clusters observed on several occasions. The small exclosure cages constructed and installed for this Phase I pilot study remained intact and well anchored with no evidence of being compromised after 12 weeks. A more complete results and discussion of the Phase I study can be found in the 2011 Final Report (FGCU 2011) included with this Draft Final Report for Phase II.

Both sources of donor plants appeared to tolerate fluctuations in water quality (FGCU 2011) based on our grab samples of physical parameters. Specific conductance and light (PAR) availability did not appear to limit growth at the shallow depths (<1.0 meter) found at Site 1 and Site 2. The small cages were maintained throughout the remainder of the study period through November 2012 with a final quantitative assessment of percent cover within each cage.

Monitoring Bimonthly Status Report for Small Cages:

The small cage monitoring has been ongoing on a monthly basis with monthly searches for herbivores in May and June 2012. On August 22 and again on September 24, 2012 each of the small exclosure cages were inspected by removing the top mesh and then visually and tactilely assessed percent coverage by *Vallisneria*. Cover was estimated and recorded into one of four categories based on approximate cover where; 0 = none present, 1 = 1-33% cover, 2 = 34-66% cover and 3 = 67-100% cover. The small cages were also searched for herbivores including turtles and snails. By the end of the study period on November 21, 2012 the small cages remained intact with two exceptions. One cage at Site 1 was damaged by vandalism or a boat collision (Figure 5) and one cage at the north end of the array at Site 2 was destroyed at (Figure 6). Results of the small cage experiment are are summarized in Table 2.

Figure 5. Research assistant, Kory Ross checking cages at Site 1 for snails and percent coverage by *Vallisneria* on 21 Nov. 2012. Note damaged cage at top right of photo.

Figure 6. Research assistant John Ferlita inspecting cages at Site 2, 21 Nov. 2012

Figure 7. Destroyed cage and two damaged cages still intact at Site 2, 21 Nov. 2012

Table 2. Small cage: percent cover of *Vallisneria* and number of snails and egg clusters.

Site 1

Treatment	# of Snails	# Egg Clusters	% Cover Code
LT LOW	0	1	0
LT LOW	0	0	0
LT LOW	0	1	0
LT HIGH	0	0	0
LTHIGH	0	0	0
LT HIGH	0	0	0
LK LOW	0	2	0
LK LOW	0	0	2
LK LOW	0	0	3
LK HIGH	0	0	0
LK HIGH	1	0	0
LK HIGH	0	0	0

Site 2

Treatment	# of Snails	# Egg Clusters	% Cover Code
LT LOW	1	0	3
LT LOW	0	0	2
LT LOW	0	1	3
LT HIGH	0	0	3
LTHIGH	0	0	2
LT HIGH	0	1	1
LK LOW	0	0	2
LK LOW	0	1	2
LK LOW	0	0	0
LK HIGH	0	0	2
LK HIGH	0	1	3
LK HIGH	0	0	0

Cover Code legend: 0=0%, 1 = 1-33%, 2 = 34-66%, 3 = 67-100%

Only two cages (17%) at Site 1 retained any tape grass more than 18 months after planting. Both were replicates of the Lake Kennedy low-density plantings at 10 shoots/meter. Coveage in those two cages was 34-66% and 76-100% respectively. We believe that the muddy sediment conditions and herbivory by non-native *Pomacea insularum* are responsible for the poor survival and coverage at this location. Site 1 is somewhat protected from heavy wave action because of its location along the side of an oxbow island but also because it is situated in slow speed zone near the Franklin Locks and Receation Area. Snails and muddy sediments may tend to accumulate at Site 1 as a result of the quieter water. Damage to cages may also have allowed freshwater turtles to graze on exposed plants.

Site 2 showed much better survival and percent coverage with 10 of the 12 (83%) cages containing tape grass and nine (75%) of these had between 34% and 100% coverage after 18 months. Female and male flower production was documented in November 2012 and seed pods were forming at Site 2 indicating that sexual reproduction was occuring (Figure 8). This seed production indicates that the pilot study was successful and is currently producing an upstream seed source for potential transport to the estuary. We do not know if the two genetic strains are cross pollenating but future genetic testing may answer that question. Site 2 was exposed to heavy boat wakes during the entire study and some damage to small exclosures was documented and repairs were necessary. However the growth and survival was much better at this location and we attribute this to wave action, sediment suitability, and southern exposure with greater exposure to sunlight. Wave action may also serve to clear ephiphytic growth on the mesh of the exclosures resulting in greater flushing inside the cages and increased light transmittance. We did not attempt to quantify PAR levels inside and outside the cages to measure any differences.

Figure 8. Pistillate flowers documented at Site 2 on 21 Nov. 2012 on the Caloosahatchee River.

Summary of Phase I

- **Low density plantings (10/m^2) did as well or better than high density (20/m^2)**
- **Site 2 showed much greater survival and growth overall than Site 1.**
- **Small exclosure cages were effective for protecting *V. americana* but non-native apple snails were able to enter cages as juveniles and graze on plants to adulthood and lay eggs in the cages.**
- **We documented staminate and pistillate flowers and successfully produced seed pods using the small cages.**

2.0b: OBJECTIVES: Phase II (Completed in FY2012)

1. Determine which of two (2) sizes of herbivore exclosures are most effective for protecting plants will result in greatest survival of planted *V. americana* and successfully produce seeds upstream of the Franklin Locks and upper Caloosahatchee Estuary.
2. Continue to maintain and monitor 1m² cages deployed during Phase I, in June, 2011.

Phase II Planting & Monitoring Results

On May 22, 2012 and May 24, 2012 the large (6 x 2 meters) exclosures were deployed at each site in the Caloosahatchee River (Figure 9). Each exclosure was planted with *Vallisneria americana* from Lake Trafford and Lake Kennedy which had been identified by Dr. Lyn Gettys at the University of Florida Center for Aquatic Plants as different genetic strains. For each strain, 60 plants were planted (per cage) using a meter-squared quadrat (ten plants per square meter) to ensure an even planting effort. For each strain a subsample of 60 plants were collected to document plant morphology and biomass. On May 25, 2012 each exclosure was secured with rebar along the perimeter to better anchor each exclosure and prevent potential herbivorous breeches.

Figure 9. General location map of large exclosure cages to be planted with *V. americana*.

Figure 10 Large cage constructed and planted with two genetic strains of *V. americana* strains in May 22, 2012.

Figure 11. Damage to large cage at on June 7 (top) and subsequent repairs on June 8, 2012 (bottom). Additional PVC pipe with reflective tape were installed around the perimeter with 3/8” rebar attached to the bottom of Vexar mesh.

Phase II: Maintenance of Large Cages

On May 29, 2012 FGCU researchers completed a weekly check-up of the large exclosures. All of the exclosures were intact and in good condition. In addition to the check-up water quality was collected. The following week, on June 7th, 2012, an additional weekly check-up as well as water quality sampling took place. Upon reaching the first site (Fig. 11) it was apparent the exclosure had suffered damage from vandalism or wave action. The other three exclosures were also compromised. On June 8th, 2012 the four large exclosures were repaired with additional supports and 3/8" rebar attached to the bottom of Vexar™ mesh. Weekly monitoring of cages and water quality at all locations continued through July with monthly monitoring thereafter. Damage to large exclosures appears to be mainly due to large vessels created massive waves that break in shallow water where the cages are located and partly exposed above the surface. PVC frame connectors were occasionally disconnected from the frame as a result of large boat wakes. The cages remained intact through reinforcement and anchoring and with the addition of PVC pipe to the outside of the cages. This maintenance became a monthly routine throughout August, September, October and November but was successful at protecting tape grass from excessive grazing.

Methods: Large Cage Monitoring for Percent Cover and Herbivores

On August 22, September 24, November 21, and December 20, 2012 the large cages were inspected for herbivores and for assessing percent cover by *Vallisneria* in each cage. The large cages were opened to allow a snorkeler into the cage. Visibility and photographic documentation was problematic due to poor water clarity and occasional boat wakes that stirred up sediments on the bottom. Therefore we decided to use a tactile and visual method for assessing percent cover and growth. Using a one-meter square quadrat, subdivided into nine subplots with nylon line, the quadrat was placed on the river bottom beginning at the upper left corner of the cage and moving back and forth from one end of the cage to the other until the entire cage bottom was assessed. When the quadrat was placed on the river bottom each subplot was inspected for the presence/absence of *Vallisneria* shoots. Frequency of occurrence (x/9) of *Vallisneria* shoots from the subplots was reported verbally by the diver to the recorder outside the cage. The number of subplots with shoots present was then recorded in the log book. Then a random number between 1 and 9 was given to the diver for counting the total number of shoots within the randomly assigned subplot. In this manner, a total measurement of percent cover for each square meter of the cage was recorded along with a subsample of shoot density for each quadrat. The percent cover for Lake Trafford and Lake Kennedy strains of *Vallisneria* was obtained along with six measurements of shoot density for each treatment. This method allowed us to obtain a non-destructive but quantitative measurement of both the percent coverage and shoot density.

At the same time the quadrats were being assessed for presence/absence of shoots, the diver searched by hand for non-native apple snails, *Pomacea insularum.* In addition, the exclosure itself was searched for presence of apple snail egg clusters. The total number of adult apple snails and egg clusters was recorded at each location and cage. Egg clusters were removed and discarded into the River water where they will drown. Adult snails were thrown as far as possible onto land to minimize likelihood of survival. The total number of non-native apple snails and egg clusters collected and removed are included in the attached scanned field data log along with the results from quadrat and subplot monitoring.

Results: Large Exclosures at Site 4 and Site 5

Only two of the four large cages were assessed on November 21, 2012 due to time constraints and water quality sampling. For comparative purposes, Site 4 (Figures 12 and 13) is on the south shoreline and Site 5 (Figures 14 and 15) is on the north shoreline. The data were recorded and transferred into an Excel data sheet for analysis and future comparisons (attached to this report). The following is a summary of the tape grass coverage in two of the large cages. Additional morphometric data is being collected and analyzed for each of the treatments as part of the M.S. Thesis work by Kory M. Ross including the total number of blades/shoot, length of blades, width of blades, and dry weight biomass. These data are not included in the final report for this contract but will made available on the completion of the thesis defense or publication of this work in the peer-reviewed literature.

Figure 12. Site 4 exclosure cage intact with dense *Vallisneria* inside on 21 Nov. 2012. Note reinforcement bracing of PVC pipe added to the exterior during the study period.

Figure 13. Dense tape grass, *Vallisneria*in large exclosure at Site 4 on 21 Nov. 2012.

Figure 14. Site 5 exclosure cage intact with dense *Vallisneria* inside on 21 Nov. 2012. Note reinforcement bracing of PVC pipe added to the exterior during the study period.

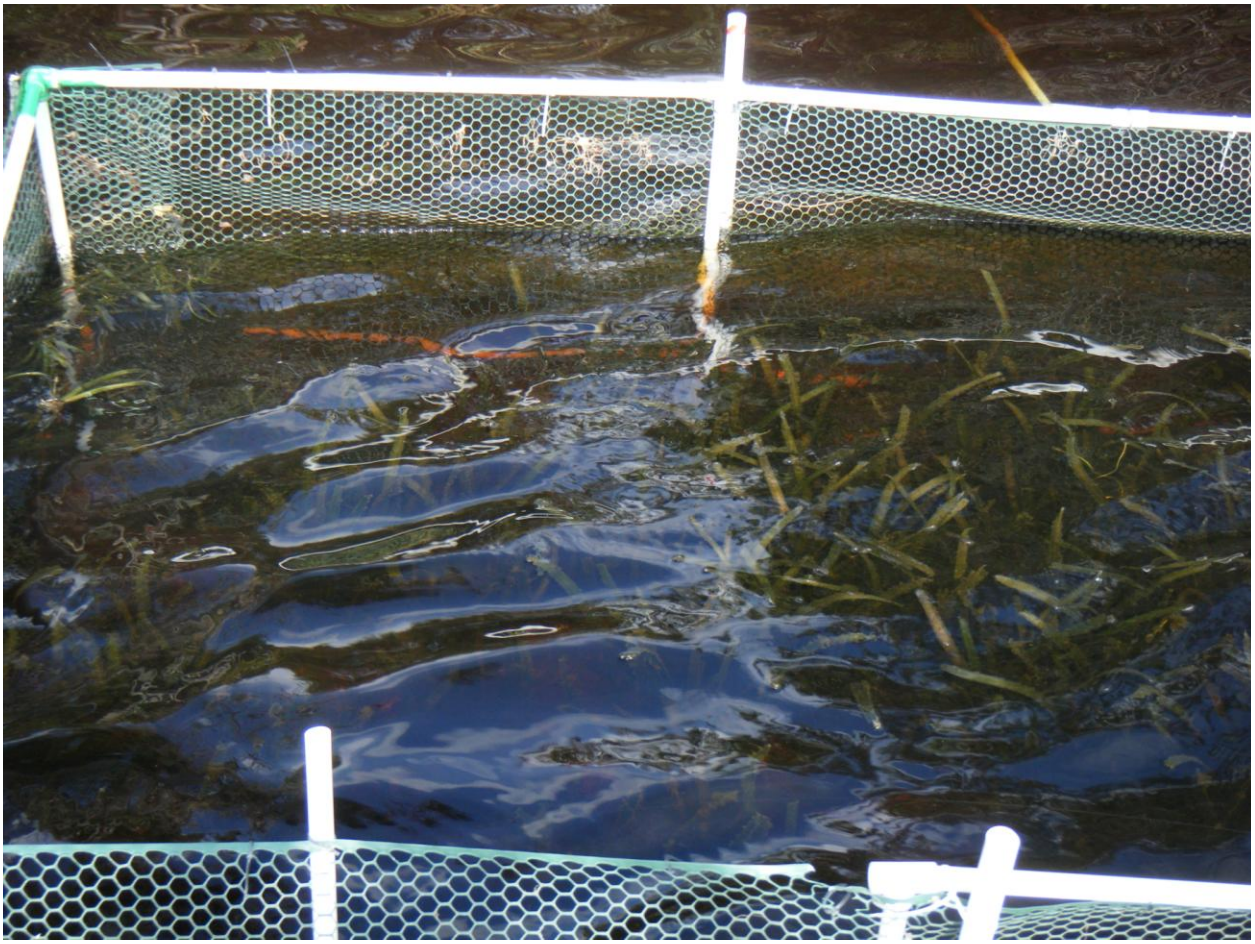

Figure 15 Dense tape grass, *Vallisneria americana* in large exclosure at Site 5 on 21 Nov. 2012.

Figure16. Successful seed production at Site 4 (top) and Site 5 (bottom) was documented in November 2012.

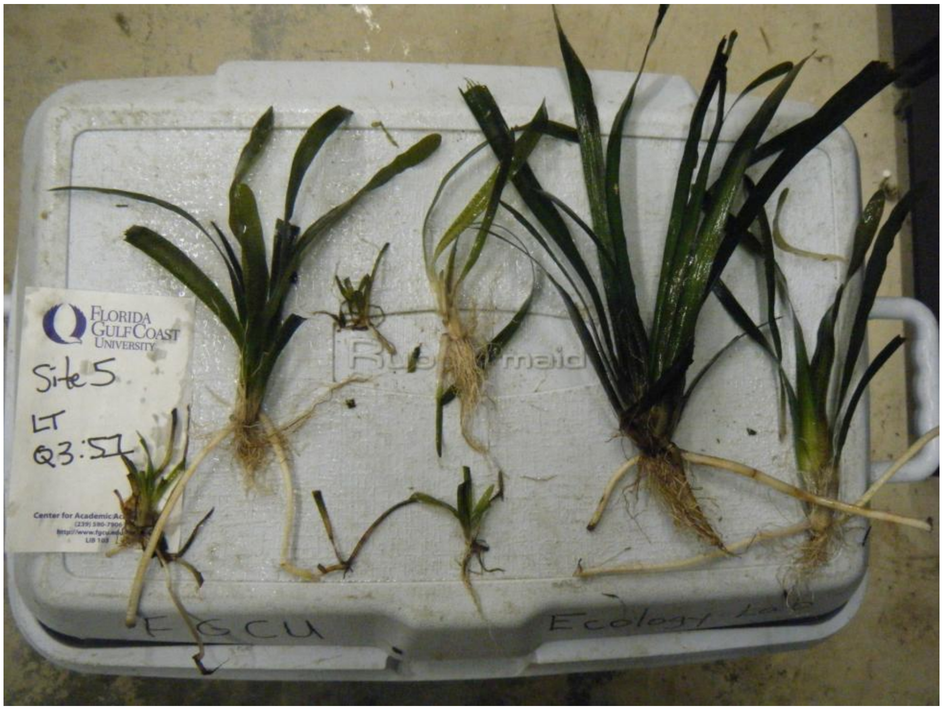

Figure 17. Typical quadrat sub-samples from Site 5 Lake Trafford planting 21 Nov. 2012.

Table 3. Phase II, Site 4 and 5 summary statistics for *Vallisneria* coverage on 21 Nov. 2012.

Site 4 Lake Kennedy							
Quadrat	Total Subplots	Subplots with *Vallisneria*	Frequency of Occurrence	Subplot Number	Count of Shoots	Count of Adult Snails	Count of Egg Clusters
1	9	9		8	12	0	0
2	9	9		9	24	0	0
3	9	9		1	15	0	0
4	9	8	89	2	0	0	0
5	9	9		3	33	0	0
6	9	9		4	39	0	0
Mean			**98**		**20.5**	**0**	**0**
Site 4 Lake Trafford							
Quadrat	Total Subplots	Subplots with *Vallisneria*	Frequency of Occurrence	Subplot Number	Count of Shoots	Count of Adult Snails	Count of Egg Clusters
1	9	7	78	5	4	0	0
2	9	8	89	6	4	0	0
3	9	8	89	7	8	0	0
4	9	9	100	8	15	0	0
5	9	6	67	9	18	0	0
6	9	9	100	1	8	0	0
Mean			**87**		**9.5**	**0**	**0**
Site 5 Lake Kennedy							
Quadrat	Total Subplots	Subplots with *Vallisneria*	Frequency of Occurrence	Subplot Number	Count of Shoots	Count of Adult Snails	Count of Egg Clusters
1	9	2	22	2	0	0	0
2	9	7	78	3	0	0	0
3	9	3	33	4	0	0	0
4	9	9	100	5	19	0	0
5	9	6	67	6	12	0	0
6	9	8	89	7	0	0	0
Mean			**65**		**5.2**	**0**	**0**
Site 5 Lake Trafford							
Quadrat	Total Subplots	Subplots with *Vallisneria*	Frequency of Occurrence	Subplot Number	Count of Shoots	Count of Adult Snails	Count of Egg Clusters
1	9	8	89	8	3	0	0
2	9	9	100	9	5	0	0
3	9	6	67	1	7	0	0
4	9	9	100	2	3	0	0
5	9	9	100	3	9	0	0
6	9	9	100	4	6	0	0
Mean			**93**		**5.5**	**0**	**0**

Figure 18. Site 4 Lake Trafford strain sample harvested 11/21/12 for morphometrics and biomass estimates. Note the length of blades and two mature seed pods.

Figure 19. Site 5 Lake Trafford sample harvested for morphometrics and biomass estimates.

Methods and Result from Large Exclosures at Site 3 and Site 6:

The remaining large exclosures were quantitatively sampled by co-PIs Ceilley and Everham on 12/20/12 to gather additional information as part of the research and also to assess the condition of all cages, large and small at the completion of the contract.

The large exclosure at Site 3 is located only a few meters to the east of small exclosures at Site 2 from Phase I of this study and therefore subject to the same water quality, wave energy, depth and photoperiod as the small cages. Site 6 is also located on the northern shoreline of the C-43 several kilometers to the east but with similar exposure to waves and photoperiod. We also conducted visual inspections for tape grass growth and herbivores at Site 4 and Site 5 even though data had already been collected from these sites in November 2012.

All of the large exclosures required some repairs and reinforcement to secure their perimeters. The frame of the large exclosure at Site 3 had been damaged and was partly off center where vertical PVC posts had become disconnected (Figure 20). Repairs were made by reconnected pipe fittings and adding vertical supports with cable ties to mesh. After repairs were made, we completed the sampling of the entire cage using the 1-meter square ($1m^2$) quadrat which is divided into nine equal subplots for assessing evenness of distribution -or- % presence across the quadrat. Using snorkel gear, the quadrat was laid down starting in one corner of the cage and each subplot was checked visually and tactilely for presence of *V. americana* and non-native herbivorous snails. The treatment and quadrat number were recorded along with number of subplots (cells) that contained any *V. americana*. The diver was then given a random number between 1 and 9 to survey and report on the number of shoots (plants) within that subplot. In this way we assessed and recorded the coverage and relative density of plants throughout the entire cage.

Results from Sites 3 and 6 are summarized in Table 6. The Lake Trafford strain produced greater percent coverage at both locations ranging from 85.2% at Site 3 to 98.1% at Site 6. Lake Kennedy cover ranged from 70.4% at Site 3 to 79.6% cover at Site 6 (Table 6). Our observations of growth patterns indicated that the Lake Trafford strain produced larger plants in general with longer and wider blades than the Lake Kennedy strain. Biomass data will confirm if these observations were accurate. The average number of Lake Trafford plants per square meter was much higher (7.0) at Site 3 compared to Lake Kennedy (1.8) but not at Site 6 where the Lake Kennedy strain averaged 14.3 plants per square meter while the Lake Trafford strain averaged 12.7 plants per square meter. However, the number of plants (shoots) per square meter does not appear to reflect biomass or size and maturity of individual plants. Biomass estimates from above and below ground will be calculated as part of the Thesis research and manuscript preparation and are not available as of this final technical report on the cage experiment.

\

Table 4. *Vallisneria americana* density at Sites 3 and 6 based on frequency of occurrence and number of plants in subplots.

Site 3 Lake Trafford Strain

Quadrat	Presence	Subplot #	# Plants	# Snails
1	7	5	9	0
2	9	4	8	0
3	6	7	0	0
4	9	8	10	0
5	6	5	6	0
6	9	4	9	0
Mean %	85.2		7.0	
SD			3.7	

Site 6 Lake Trafford Strain

Quadrat	Presence	Subplot #	# Plants	# Snails
1	8	3	13	0
2	9	4	12	1
3	9	1	3	0
4	9	5	19	0
5	9	6	7	1
6	9	5	22	0
Mean %	98.1		12.7	
SD			7.1	

Site 3 Lake Kennedy Strain

Quadrat	Presence	Subplot #	# Plants	# Snails
1	4	3	1	0
2	6	7	2	0
3	5	9	3	0
4	9	3	3	0
5	6	1	0	0
6	8	8	2	0
Mean %	70.4		1.8	
SD			1.2	

Site 6 Lake Kennedy Strain

Quadrat	Presence	Subplot #	# Plants	# Snails
1	7	7	0	0
2	9	1	25	0
3	5	6	27	1
4	9	7	24	2
5	4	2	3	1
6	9	8	7	1
Mean %	79.6		14.3	
SD			12.3	

As part of our research we also collected 10 plants from each of the treatments to get measures of average blade length, blade width, and biomass (above and below ground). Plant blades were then separated from below ground roots, turions and stolons for drying and dry-weight biomass measurements for each treatment (Figure 21). These data on biomass are not included in the final report for this contract but will made available on the completion of the thesis defense or publication of this work in the peer-reviewed literature.

Figure 20. Site 3. Caloosahatchee River 12/20/12 before (top) and after (bottom) repairs were made by FGCU researchers. Some repairs were necessary on one or more cages each month during the the study.

Figure 21. Site 6 Lake Trafford sample with blades separated for above and below ground biomass estimates. Note the staminate flowers in yellow on top of blades.

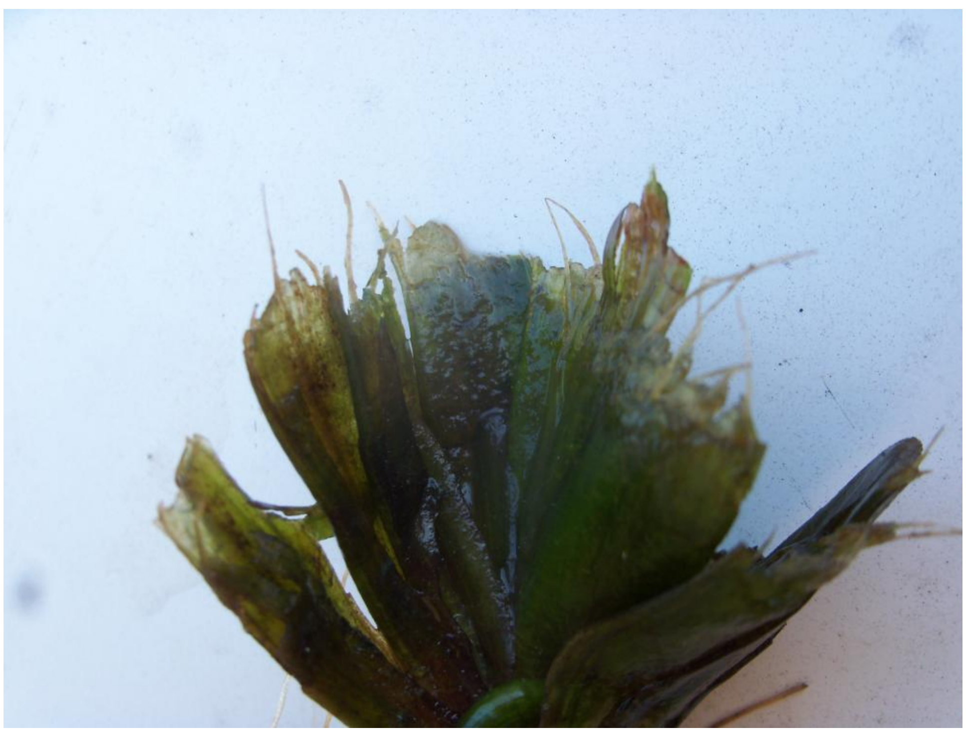

Figure 22. Site 4 grazing effects on unprotected tape grass after damage to exclosure. The grazing pattern was different than turtles and snails and we attribute to manatees.

Table 5. Phase II, Sites 3 and 6 *V. americana* coverage on 21 November 2012.

Source	Density (plants/m^2)	SD
3-Trafford	63	33.190
3-Kennedy	16.5	10.521
6-Trafford	114	64.062
6-Kennedy	129	110.618
Mean Overall	80.6	

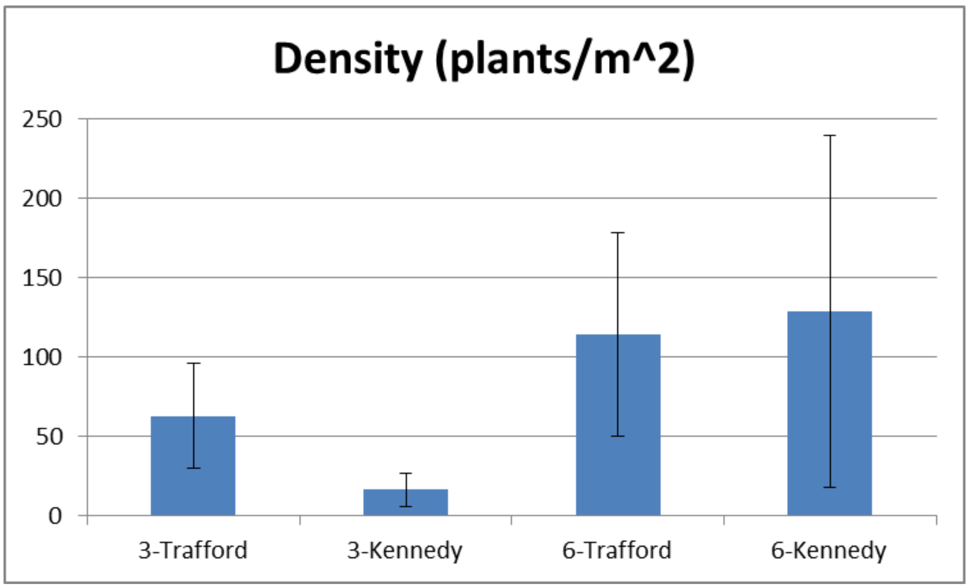

Figure 23. *V. americana* density for Sites 3 and 6 plotted with error bars to show significance. Note that Site 3 Lake Kennedy plantings were significantly less than all other treatments but still greater than initial planting of $10/m^2$.

Table 6. Phase II Sites 3 and 6 abundance (% presence in subplots) of *V. americana* throughout the large exclosure cage after six months

Source	Abundance (% presence)
3-Trafford	85.2
3-Kennedy	70.4
6-Trafford	98.2
6-Kennedy	79.6
Mean overall	83.3

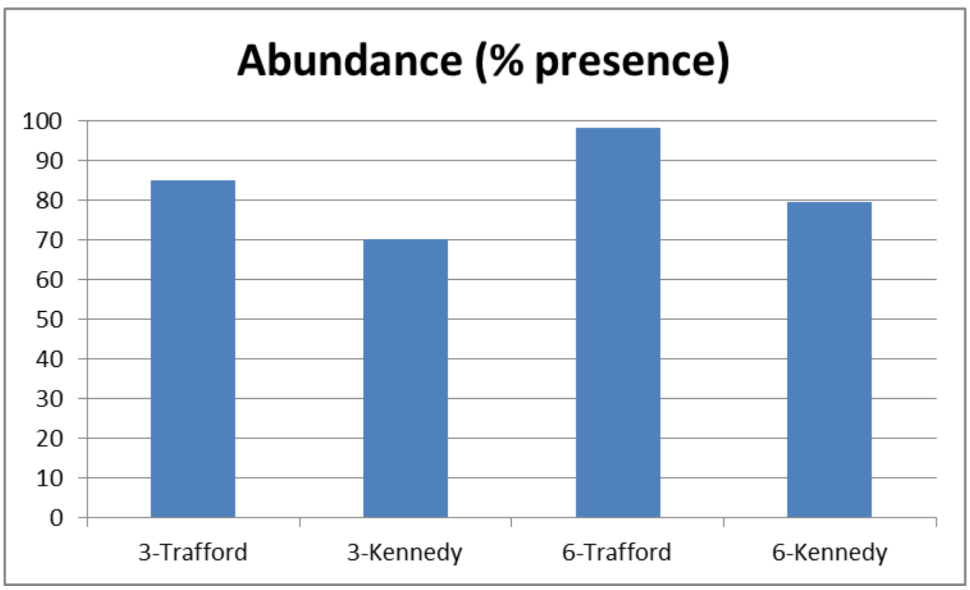

Figure 24. *V. americana* abundance as measured by % presence (frequency of occurrence) in each subplot/m² for Sites 3 and 6. Lake Trafford had slightly higher % presence in each exclosure.

Problems Encountered

The problems encountered in early June 2012 included damage to cages as a result of vandalism or extreme wave action. The problem was mitigated by reinforcing large cages with additional PVC pipes and adding rebar to the bottom of the Vexar mesh. Subsequent damage was also documented in August and early September and repairs were made within 24 hours of discovery. The large wakes created by large vessels traversing the main channel of the C-43 appears to be main contributor to the damage and weakening of the large cages. Public education and awareness of the importance of submerged aquatic habitat and the restoration projects is needed to reduce the likelyhood of vandalism and obtain public support and recruit advocates and volunteers. Local law enforcement officers who patrol the C-43 could be a part of this public education and awareness.

Herbivory by non-native apple snails, *Pomacea insularum*, remains a concern and all cages were being monitored and snails were removed on a monthly basis. Some *P. insularum* adults were collected for forage preference and consumption rate experiments at the FGCU laboratory which is ongoing and a part of graduate student research at FGCU.

Conclusions

In Phase I of this study we found that the invasive non-native apple snail, *P. insularum* was grazing heavily on planted tape grass inside the small cages. This was further confirmed during Phase II with the large cages. The juvenile snails were able to pass through the ¾ inch Vexar mesh and then feed on plants. As they grew larger inside the cages, the snails became trapped inside the mesh where they continued to feed and where they were also protected from predators (limpkins and snail kites). Outside the exclosure cages we document *P. insularum* having a severe impact on growth and spread of tape grass. It was not possible to quantify the impacts outside the cages due the presence of other grazers such as turtles and manatees in the river. Snail control was attempted during site inspections but the density and fecundity of non-native apples snails appears to be to great to eradicate them entirely from the areas around the cages. Openning the top of the cages above the water line may help control adult apple snails by allowing avian predators access to the inside of the cage. Limpkins are known to feed on apple snails and were regularly observed at our study sites. At least once we documented an endangered snail kite, *Rostrhamus sociabilus* feeding on an adult *P. insularum* near Site 2 in September 2012.

The large cages deployed and planted in Phase II quickly became an attractive nuisance to boaters as evidenced by frequent approaches by curious onlookers during our fieldwork. The large cages suffered vandalism and boat wake damage on several occassions beginning the first week of June after planting. However, we found that the large cages were very successful at protecting tape grass from June through December 2012 as long as they had regular maintenance (biweekly-monthly). The sturdiness of the large cages deserves further testing and requires some modifications to be cost-effective. Part of the problem is the high profile and rigid, rectangular framework of connected PVC pipe. The boat wakes and wind driven waves tend to break right at the depths most suitable for planting *V. americana* (0.2-0.8 meters deep) and where large cages were deployed. The cage design was intended to sit about the water line to prevent turtles, ducks, and manateees from entering the cage but this high profile and visibility to boaters makes these cages suscepable to wave damage and vandalism, respectively. We suggest that testing of different designs and various anchoring methods is justified at this point. Rebar anchoring was determined to be a good method of horizontally anchoring the mesh when used in conjunction with vertical PVC supports. The plastic mesh and PVC pipe were secured by ultraviolet-resistant plastic cable ties which were very effective and easy to remove and replace during monitoring events.

The upper frame appears to be the weak link in the design and suffered the most damage during the study. Based on our observations and numerous repairs, we suggest using a lower profile frame that sits below the water line or possibly a soft-engineered flexible upper frame with a securely anchored bottom so that the cage and upper body of the exclosure retain shape but move somewhat when heavy boat wakes and storm waves roll in. Finally, the cages need to be high enough to allow *V. americana* plants to grow and reach sexual maturity and produce seed pods. From our aquaculture experience, with the Lake Trafford and Lake Kennedy strains, the minimum height of the cages should be 0.7 meters. Low-profile cages below the water line have been tested for several years and the plants grow and fill the cages but never produced pistillate flowers or viable seed pods due to grazing on plant shoots that protrude through the top mesh (Ceilley et al. 2003, Ceilley 2009).

Summary of Phase II

- **Growth and survival at all large exclosure sites was good and plants colonized a large percentage of the bottom of the exclosures ranging from 70-98% cover.**

- **The large cages successfully protected tape grass from over-grazing after six months and density of plants ranged from 16.5 to 129 plants/m^2.**

- **The Lake Trafford strain showed greater overall spread and plant density than the Lake Kennedy strain at Sites 3 and 6.**

- **Large exclosure cages were effective for protecting *V. americana* but non-native apple snails were able to enter cages as juveniles and graze on plants to adulthood and lay eggs in the cages.**

- **We documented staminate and pistillate flowers and successfully produced seed pods using the large exclosure cages. Seed production appears to be greatest in winter months based on our observations in the C-43 and Lake Trafford.**

- **Larger exclosures appear to be a more cost-effective method for protecting plantings and for producing sexually mature plants and seed pods. However, the high maintenance required in this study indicates that a better design is needed to withstand wave action.**

- **Public education and awareness of the SAV losses in the C-43 could help reduce vandalism and build support for scaled-up SAV restoration projects.**

References Used to Prepare this Report

Bortone, S.A. and R.K. Turpin. 2000. Tape Grass Life History Metrics Associated with Environmental Variables in a Controlled Estuary. Pp 65-80 in Bortone ed. 2000. Seagrasses: Monitoring, Ecology, Physiology and Management. CRC Press, Boca Raton, FL. 318 pp.

Ceilley, D.W. 2009. Tape Grass (*Vallisneria americana*) Michx. Restoration Feasibility Study: Effectiveness of Two Exclosure Designs. Final Report to Lee County and West Coast Inland Navigation District. 18 pp.

Ceilley, D.W. and E. M. Everham, III. 2008. Biological Monitoring Program Phase II, Lake Trafford Restoration Project. Annual Report to the South Florida Water Management District. Naples, FL.

Ceilley, D.W., I. Bartoszek, G. G. Buckner, and M.J. Schuman. 2003. Tape grass (*Vallisneria americana*) restoration feasibility study. Final Report to the South Florida Water Management District, 3300 Gun Club Rd. West Palm Bch, FL. 16 pp.

Ceilley, D. W. and S. A. Bortone 2003. Tape Grass (*Vallisneria americana*) Restoration Feasibility Study, Final Report to The South Florida Water Management District, Presented at Mote Marine Lab Submerged Aquatic Vegetation (SAV) Conference, March 2003. Sarasota, FL.

Rozas, L.P. and T.J. Minello. 2006. Nekton Use of *Vallisneria Americana* Michx. (Wild Celery) Beds and Adjacent Habitats in Coastal Louisiana. Estuaries and Coasts. Vol. 29, No. 2 p.297-310.

U.S. Geological Survey. 2002. American Wildcelery (*Vallisneria americana*): Ecological Considerations for Restoration. http://www.npwrc.usgs.gov/resource/literatr/wildcel/propogat.htm

Wilder, G.J. 1974. Symmetry and Development of Pistillate Vallisneria Americana (Hydrocharitaceae). American Journal of Botany Vo. 61, No.8, pp.846-866.

List of Attachments on CD

1. **FGCU Final Report for Phase I Sept. 2011**
2. **Excel File with Water Quality, Vallisneria and herbivore data**
3. **FDEP Permit Exemptions 2009 and 2012**
4. **USACOE Letter of No Permit Required; 2009 and 2012**
5. **Purchase Order for Phase II: PO4500063378**
6. **Powerpoint Presentation to SFWMD on 23 January 2013.**