Brevard Zoo Clam Restoration

Contract #36524

Final Report

January 14, 2023

2021-2022 Project Summary

Between April 13th and May 18th of 2021, the University of Florida Whitney Lab and the Brevard Zoo team planted 240,000 juvenile clams at Hog Point in Melbourne Beach and 5 million seed clams were spread on existing Brevard Zoo oyster reefs in Melbourne Beach. These events provided the opportunity for Brevard Zoo staff to learn from University of Florida methods for planting clams.

Site selection for the 100 pilot clam beds began in May of 2021 and concluded in July of 2021. Site parameters included water depth, visual sediment composition, spatial application ability, and salinity. All sites were selected by Brevard Zoo staff and provided to Florida Oceanographic Society and the University of Florida Whitney Lab by the end of August. Spatial distribution of sites depended on the availability of adequate sites, defined by the selection parameters. More sites were selected in the Central Indian River Lagoon due to higher salinity values with the expectation that low salinities would limit clam survival.

Homeowners were alerted of their site selection the first week of September and signed a letter of commitment to proceed. Brevard Zoo staff held 3 in person clam workshops and 1 zoom workshop in September and October to familiarize homeowners with the project to restore clams to the Indian River Lagoon and clam bed care. Homeowners were required to attend a workshop or watch the recorded zoom workshop before the clam bed installation. Homeowners also received a hydrometer, gloves, and a field guide with a QR code to report salinity and volunteer hours via JotForm. All homeowners that were unable to perform maintenance were matched with a community volunteer to help care for their clam bed called a clam buddy.

In the weeks leading up to the first clam bed installation (November 2nd), Brevard Zoo staff collected and prepared materials and necessary instruments. Homeowners were alerted the week before their installation date. By the end of November 83/100 clam beds had been installed. Permission from FWC to plant in Indian River and Volusia County was granted December 3rd and the remaining clam beds were installed by December 9th. 1 million total clams were planted, averaging about 10,000 clams per site. The JotForm submissions from weekly salinity measures and biweekly clam bed maintenance began to be submitted by homeowners and clam buddy volunteers at a steady pace.

In December 2021, University of Florida Whitney Lab volunteers and community partners planted an additional 560,000 clams at Hog Point to expand initial clam bed footprint.

The Brevard Zoo team finalized monitoring procedures and materials for the clam project in early January 2022. The University of Florida Whitney Lab advised on quadrat size, and the Brevard Zoo team created a monitoring sheet and methods. A subset of beds was monitored starting in the last week of January and ending in the first week of March. The subset of 58% of sites were created by choosing sites that covered distance and location. All completed monitoring sheets were scanned and data recorded in Microsoft Excel.

1.2 million clams remained to be planted at suitable sites. In March 2022, Brevard Zoo reviewed the Winter monitoring data and selected 5 sites to expand and install larger, 15’x50’, clam beds. Only 3/5 sites were planted in March, April, and May due to clam nursery shortages. The remaining 106,000 clams were planted at an additional site in September 2022 in a broadcast method without the use of predatory exclusion nets. All were planted with the help of community volunteers, allowing for the community to be able to help plot out beds, spread clams and stake nets over the beds. The education opportunity left volunteers asking what else they could do to help the project the lagoon.

A second monitoring was conducted for Spring of 2022 from May-June. Brevard Zoo staff monitored all 100 original clam bed sites. The methods used in winter monitoring were kept consistent. Volunteers were immensely helpful with this process and were very eager to come out and help monitor again.

In May 2022, Florida Oceanographic Society (FOS) planted 90 burlap mats with fragments (6,720 shoots) of *halodule wrightii* seagrass (shoalgrass) at the Hog Point clam bed site. In June 2022, the FOS team planted an additional 1,728 seagrass mats (27,648 shoots) at nine of the 100 clam bed sites. Predator exclusion devices or cages were deployed on a portion of the seagrass plots to protect planted seagrass from grazers. Community volunteers maintained exclusion devices at seagrass sites throughout the project. FOS conducted 4 monitoring events of seagrass plantings between June and October 2022.

A third monitoring period took place in Summer 2022 from August-September. Brevard Zoo monitored all 100 original clam bed sites over the course of a month. The University of Florida supplemented staff and helped to make monitoring go smoothly. All monitoring data from Winter, Spring and Summer were consolidated into a single excel sheet for easy statistical analysis.

All materials used in the 2021 November and December plantings were collected roughly a year from their installation date. During the uninstallation, the beds were monitored using absence presence for clams to have a final ratio of beds with live clams and without live clams a year from their installation date. Sediment samples were also collected at 100 clam bed sites to characterize site specific sediment chemistry and its impacts to clam growth and survivorship. Florida Institute of Technology was contracted to perform LOI (loss on ignition) on site sediment samples and collect porewater salinity, alkalinity, and sediment sulfide concentration on a subset of clam beds in locations where clam survivorship was highest. All the 2022 clam bed expansion plantings were monitored, and the materials removed by December 15th.

By project’s end, 8 million total hard clams were planted at 109 sites across the Indian River Lagoon.

Methods

Site selection

All sites were volunteered by either private homeowners or local city governments. Sites were grouped by location and site evaluations were scheduled by the Restore Our Shores Office administrator. Abiotic factors at each site that were examined include water depth, shoreline type, sediment composition and salinity. Salinity was deemed to be one of the most important site conditions to consider since literature state hard clams (*Mercenaria mercenaria*) are unable to tolerate salinities below 15 for extended periods of time (Whetstone, 2005). Optimal water depth was selected to be between 20'' and 40'' due to the predation protection installation requirements. Shallower than 20'' was predicted to cause the predation protection net to be exposed to wave energy that could damage it and water deeper than 40'' was estimated to be difficult to install and monitor without SCUBA equipment.

Clam Bed Installation

Clams were received from the University of Florida Whitney Lab and categorized by size. All size classes were separated, and a subset from each size class chosen for measurement. A one-liter scoop was used to separate out clams for measurement and for dead percentage. Fifty clams from each size class were measured and more live clams were then counted out for the scale to register an accurate weight. All dead shells, found while counting, were set aside, and counted. Each individual dead shell was counted as ½ a full clam. Once counting of the dead shell within the sample, dead shells and live clams were combined and placed on the scale. The weight of the total sample and percentage of dead was recorded. All bags of clams in the same size class were then weighed. The number of live clams of the sample was then placed over the weight of the sample and set equal to 'x live clams' over the weight of the clam bags. The percentage dead found in the sample was then divided by 100 and multiplied by 'x live clams' to find the estimated percentage of clams delivered dead. This process was repeated for every size class delivered. Clams placed on clam beds were weighed to approximate the amount planted at each site. Clams were stored in coolers until planting to prevent temperature and humidity related stress.

Once arriving at a planting site, a 10'x15' predation protection net was unwrapped and held over the intended clam bed planting site. Four PVC poles were then placed outside the corners of the rectangular net and pounded into the sediment using a rubber hammer. The net was then pulled and held aside. The weighed clams were then scattered within the rectangle marked by the 4 PVC poles. Scattering was done by starting at a corner and walking in a ring that scooted around the outer edge and wound in a circular fashion until clams were deposited into the center of the bed. Once the clams were within the sediment, the predation protection net was pulled back inline with the PVC markers. The net was then pulled flush with the sediment, covering the newly planted clams, and 10'' galvanized steel landscape staples were used to hold it in place as well as 1'x1' gardening pavers placed on at least 1 corner.

Water temperature, salinity, the depth at the most seaward point of the bed, the depth at the most landward point of the bed, turbidity and a GPS point were taken at each clam bed planting location.

Clam Bed Monitoring

Before arriving at the clam bed planting site, 3 random quadrants were selected. A 1'x1' grid was mapped over each 10'x15' clam bed site. Random.org was used to generate 2 numbers, a number 1'-9' and a number 1'-14'. This avoided unrepresentative samples at the very edge of the bed, since clams were planted with a 1' buffer from the edge of the net. Three different coordinates were created for every clam bed site for every monitoring session.

Once arriving at a clam bed site, the corners of the predation protection net were located. The pavers placed on 2 of the four corners were removed and placed in a location where they could be easily found. Using gloved hands, the nets were slowly removed starting from the corners where the pavers were removed. All stakes pulled up were set aside for reinstallation post-monitoring.

At every clam bed site, field staff chose a corner of the clam bed where a 10' side of the site was on the right and the 15' side was in front at a 90-degree angle to provide reference point. The direction the net was facing geographically was noted on the monitoring sheet. Using a transect, the 1'-9' coordinate on the 10' side was measured using the transect. From there, the 1'-14' coordinate into the clam bed site was measured out. A depth measurement was taken in the measured quadrant. A modified sand flea scoop (6''x 6''x 12'') was used to pull up 36ft^3 (1.097m^3) of sediment from within the 1'x1' quadrat and placed it on the 4mm mesh sieve, this was repeated in the same spot and then gloved hands were used to feel for any live or dead clam material present in the quadrant. The mesh sieve was then brought to the aquaculture boat for further examination. All species seen in the sediment collections that were alive and not *Mercenaria mercenaria* were noted on the clam bed sheet. Live M. mercenaria were separated from shell fragments of M. Mercenaria. Using a caliper, the live M. mercenaria were measured from umbo to outer lip (length), and then outer lip to opposite outer lip (width). Up to fifty individuals were measured from a single sample and then any that remained were counted. All live M. mercenaria found in the quadrant were noted. All individual shells found belonging to M. mercenaria were tallied and divided into two, due to it taking 2 shells to create one individual bivalve. In the case of there being broken pieces of shell (shards), pieces of shell were grouped by 3 and counted as an individual shell. All quadrants with shards were noted in the monitoring sheet. The calculated evidence number of deceased M. mercenaria was noted on the monitoring sheet. Once completed, all live animals and contents of the sample on the sieve were placed back in it's original quadrant. The processes of monitoring quadrant samples were repeated for the two remaining randomly assigned quadrants.

When monitoring was completed, the net was then pulled back over the clam bed, and the 10'' galvanized steel staples used to hold it in place were reinstalled. The two gardening pavers were then placed back to their original position prior to monitoring.

If live clams were not found in the random quadrants, the entire clam bed site was searched for live M. mercenaria. Using hands to dig in the first 2'' of sediment and feel for any evidence of M. mercenaria. If no live clams were found, the predation protection net was removed. If live clams were still present, the net was re-staked and secured with pavers.

The following measurements were also documented at each monitoring event: distance to shore from clam bed sites, salinity, water temperature, the depth at the most seaward point of the bed, the depth at the most landward point of the bed, and turbidity. Seagrass species observed, predatory species present and shoreline type were also noted. Pore water salinity measurements were added to monitoring procedures in the spring monitoring period after observing varying salinities in the water column and porewater at several sites with poor clam survivorship.

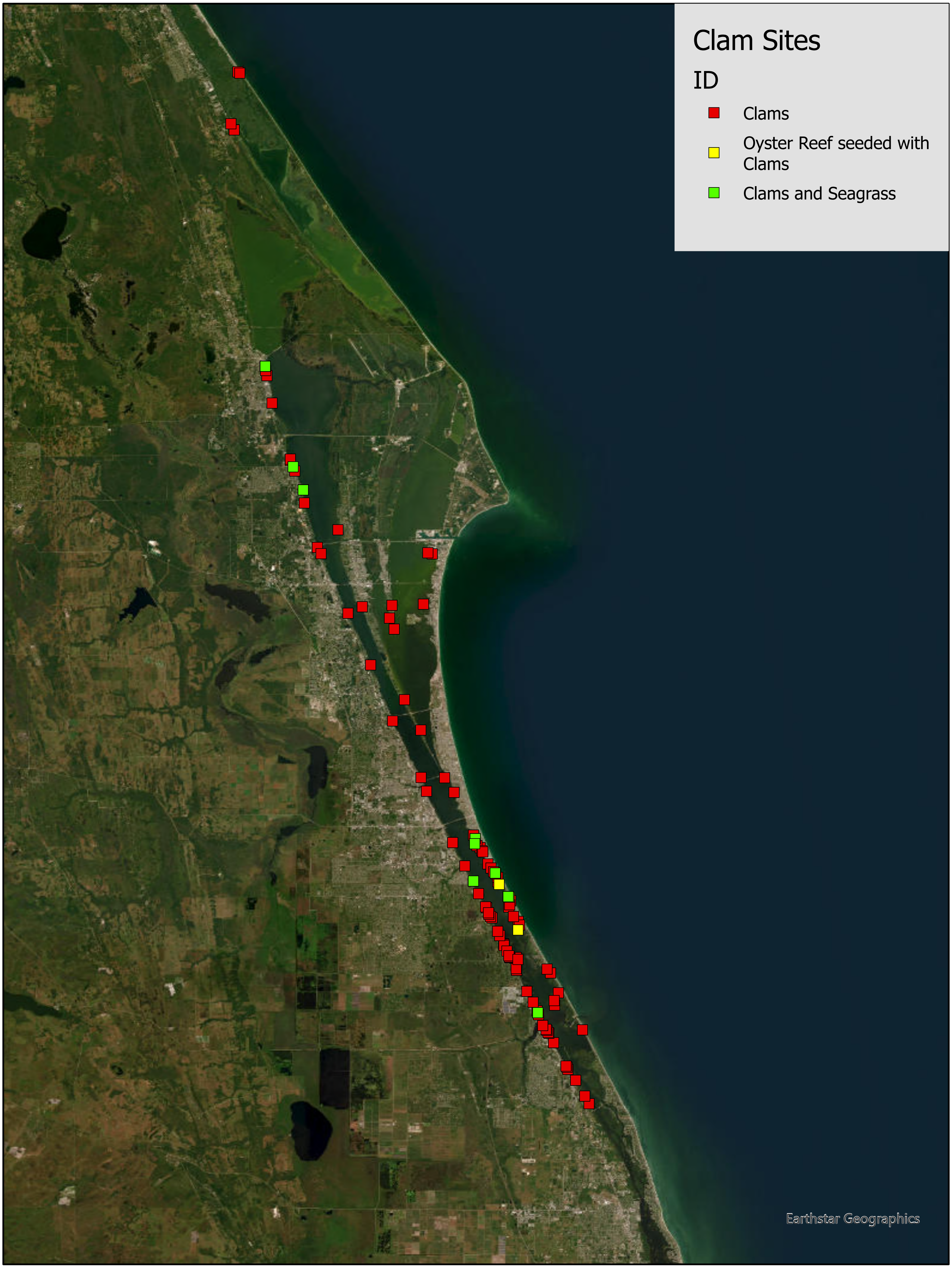
Clam Bed Uninstallation

Once arriving at the clam bed site, the pavers, the 10'' galvanized stakes, and the predation protection net were removed from the site. Staff surveyed the entire area within the PVC markers, digging with their hands to check for absence/ presence of M. mercenaria. Once absence/presence was established and documented, the PVC markers were removed.

The following measurements were taken and documented: distance to shore from clam bed sites, salinity, pore water salinity, water temperature, the depth at the most seaward point of the bed, the depth at the most landward point of the bed, and turbidity. Seagrass species observed, predatory species present and shoreline type were also noted.

Data Analysis

Once monitoring was completed, the recorded data was organized into Microsoft Excel. The clam bed sites were grouped by 21 different locations, defined by the city the site was located in. Due to its size, Melbourne Beach was separated into 3 different locations (North, Central and South) by 7-mile increments. Merritt Island clam bed sites were separated by which lagoon basin they were in (Sykes Creek, Indian River, and Banana River). Clam beds sites where the predation protection net came loose at any point in the 9-month monitoring period were categorized as "off," verses the clam bed sites where the predation protection net never came loose "on." The statistical software "R" was used to analyze the organized data.


Clam Sites
ID
Clams
Oyster Reef seeded with Clams
Clams and Seagrass
Earthstar Geographics

Results

The statistical software "R" was used to analyze the data collected for this project. The packages used were "ggplot2," "car," "forcats," "gridExtra," "FactoMineR," "factoextra," plotly," "tidyverse," "qwraps2," "nlme," and "lme4."

Winter Monitoring: 1/27/22-3/2/22

Of the 100 sites planted in Fall of 2021, a subset 58 were monitored. The subset was created due to staff not being able to visit all 100 in a timely manner in low water temperature conditions. The 58 sites chosen for Winter monitoring were spatially distributed throughout all the defined locations. Sites within less than a quarter mile from a chosen site were excluded. While winter had the largest range in salinity values, it had the highest survival ratio out of the three seasons (Table 2, PCA Graph). The mean survival rate of the clam beds monitored was 0.69 compared to the median which was 0.79. Fifty percent of the subset sites had a survival rate of over 0.79 (Table 1). The predation protection nets came loose on 10/58 of the monitored sites. No nets were lost. The minimum salinity found at a site in Winter was 15ppt and the maximum was 38ppt. Water temperatures ranged from 12°C-24°C. Locations where survival was over .88 include Melbourne Beach South and Micco. Locations where survival was over 0.75 include Sebastian, Melbourne Beach Central, Grant, and Titusville. Locations with survival rates lower than 0.25 include Cocoa Beach and Merritt Island Sykes Creek.

Table 1: Seasonal Comparison

	Winter (N = 58)	**Spring (N = 99)**	**Summer (N = 100)**
Salinity			
min	**15**	**20**	**16**
mean	**23.33**	**30.47**	**29.77**
max	**38**	**39**	**38**
Water Temperature			
min	**12**	**22**	**28**
mean	**18.04**	**28.98**	**30.85**
max	**24**	**37**	**39**
Survival Ratio			
min	**0**	**0**	**0**
median	**0.79**	**0.58**	**0.33**
mean	**0.69**	**0.54**	**0.35**
max	**0.96**	**0.95**	**0.99**

Spring Monitoring: 4/25/22-6/16/22

All 100 clam bed sites were monitored in Spring of 2022. The mean survival rate of the clam beds monitored was 0.54 compared to the median which was 0.58. The predation protection nets came loose on 30/100 of the monitored sites. No nets were lost. The minimum salinity found at a site in Spring was 20ppt and the maximum was 39ppt. Spring had the smallest range in salinity values than any other season and had a lower survival rate when compared to Winter. Water temperatures ranged from 22°C-37°C. Locations where survival was over 0.88 include Melbourne Beach South and New Smyrna. Locations where survival was over 0.75 include Melbourne Beach Central and Oak Hill. Locations with survival rates lower than 0.25 include Merritt Island Banana River and Merritt Island Sykes Creek. Cocoa Beach had a survival rate of 0.0.

Final Monitoring (Summer): 8/11/22-9/22/22

All 100 clam bed sites were monitored in Summer of 2022. The mean survival rate of the clam beds monitored was 0.35 compared to the median which was 0.33. The predation protection nets came loose on 43/100 of the monitored sites. The minimum salinity found at a site in Spring was 16ppt and the maximum was 38ppt. Water temperatures ranged from 28°C-39°C. The water temperature and salinity values were, on average, higher than the two previous monitoring seasons, while the two previous seasons had high survival ratios (PCA graph). Locations where the survival mean was over .75 include Melbourne Beach South and Oak Hill. Locations with survival rates lower than 0.25 include Cape Canaveral, Indialantic, Melbourne, Merritt Island Sykes Creek, Rockledge, and Titusville. Cocoa Beach, Merritt Island Banana River, Merritt Island Indian River, and Palm Shores all had a survival rate of 0.0. The monitoring data was subset by the categorical variable of Net. This split the summer monitoring data by whether the net had been displaced or partially removed during the project. The sites which the predation protection net stayed on through the duration of the project had a higher mean survival rate overall (*Table 2*). This difference in means was found to be statistically significant by a paired, two-sample t-test (p-value: 0.03825) Fifty percent of the sites where the net came loose

Survival Ratio

	Off (N=43)	**On (N = 57)**
Minimum	0	0
Median	0.08	0.44
Mean	0.29	0.40
Maximum	0.99	0.92

Table 2: Final Survival Ratio vs Predation net on/off

had a survival ratio of less than 0.10 (Table 2). The correlation between salinity and survival ratio in beds was found to be positive with a low p-value (r=0.3854, p-value=7.493e-05). However, the correlation between salinity and survival ratio of sites with nets on was stronger, but the p-value was lower. (r=0.4166, p-value=0.001264, Figure1, 2). Water Temperature was also tested for correlation with Survival ratio. Although the correlation coefficient was less than that of salinity and the p-value was larger, water temperature was still considered significant and negatively corelated to survival ratio (r= -0.1968, p-value: 0.04967).

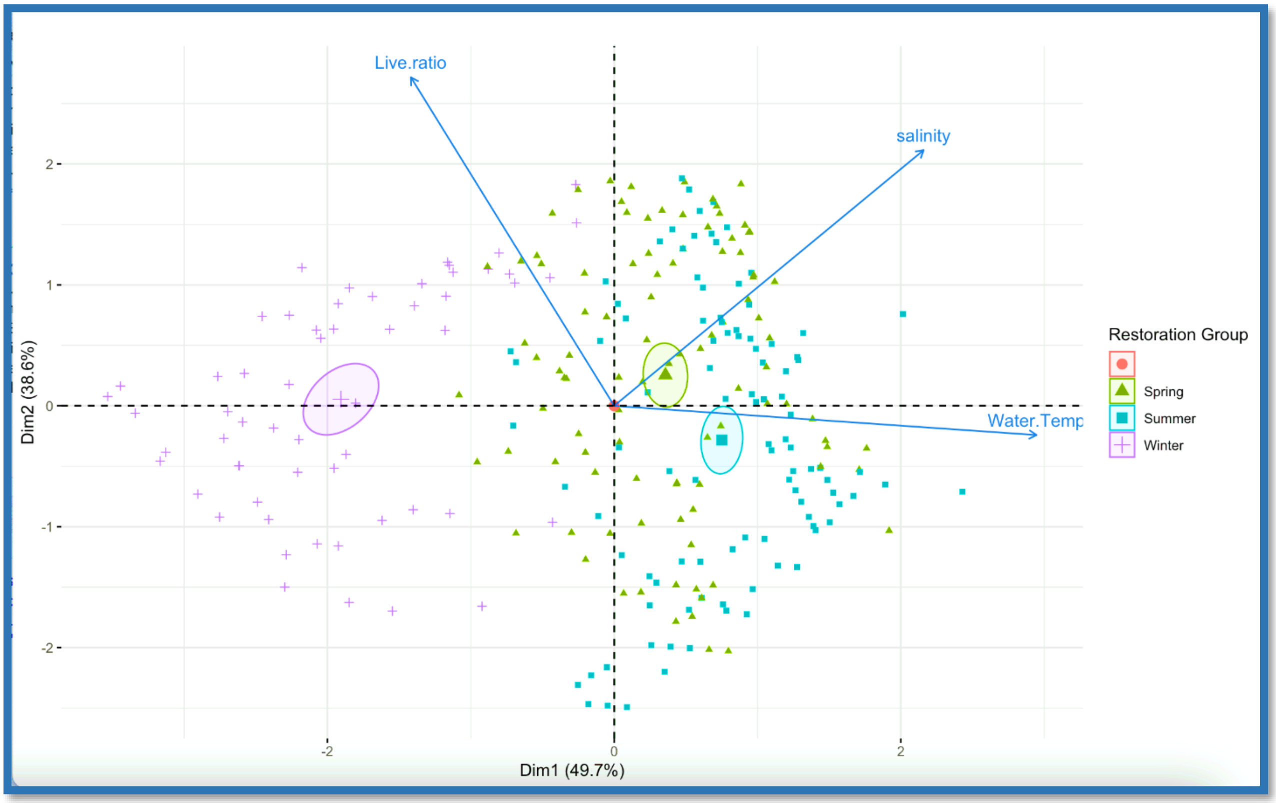


Figure 1: PCA graph comparing salinity, water temperature and survival ratio values in different monitoring seasons. (R Core Team 2021).

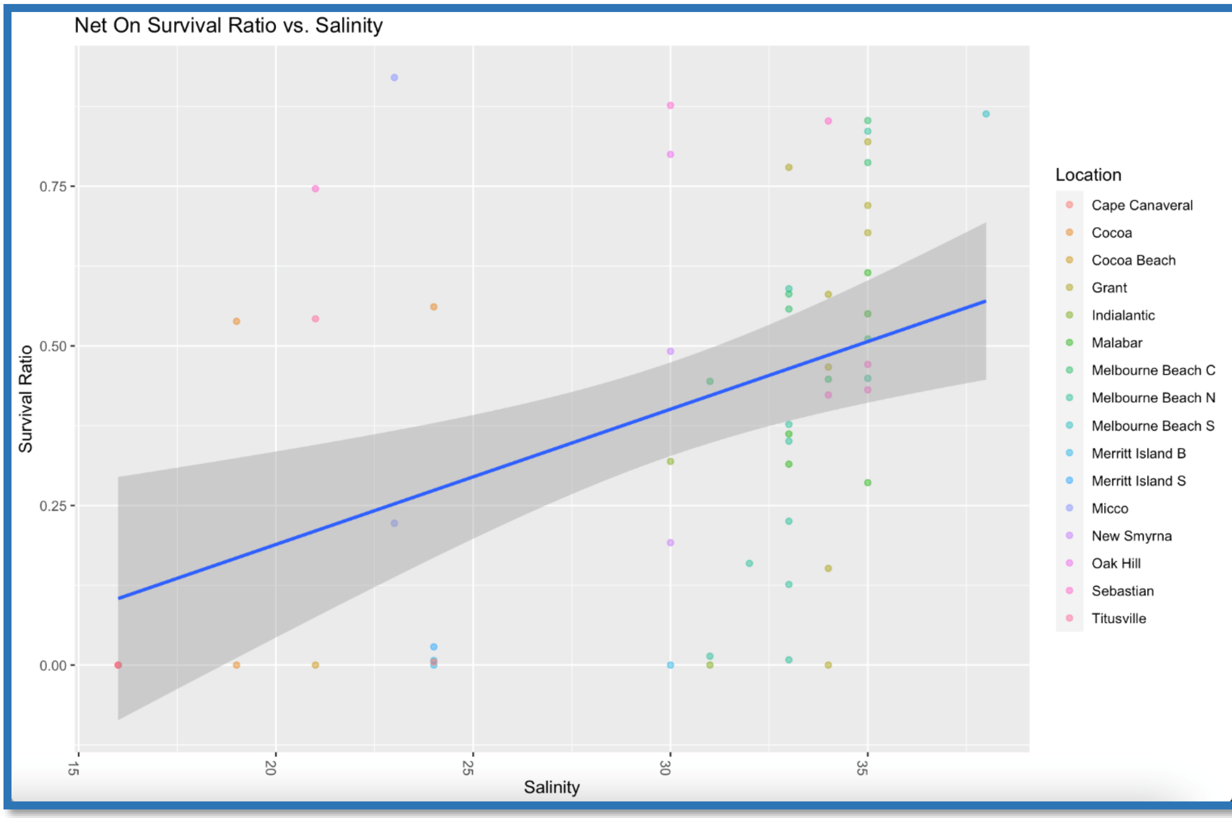


Figure 2: Net On Final Monitoring Survival Ratio vs Salinity (R Core Team 2021)

Clam Growth Measures:

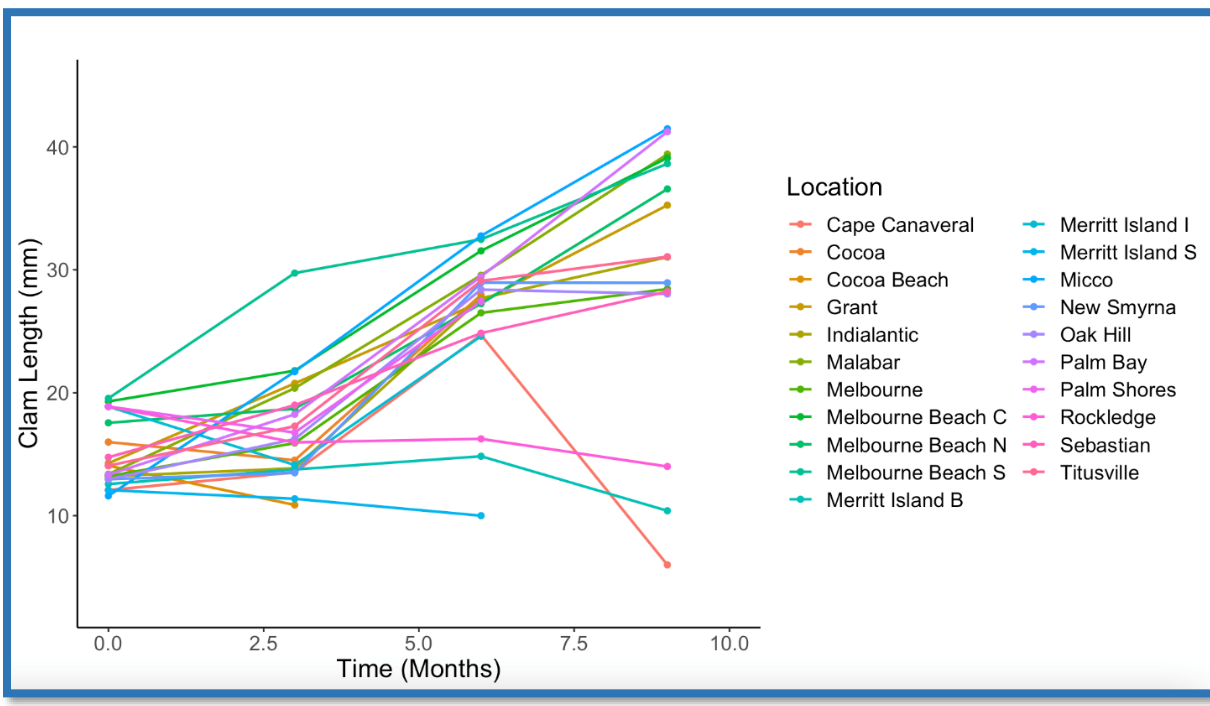


Figure 3: Clam Length vs Time by clam bed Location

Length, or the distance from the clam shell umbo to the most outer lip of the shell, was the variable chosen to define growth. The "Length" measurements, taken when the clams were planted and monitored in Autumn, Winter, Spring and Summer, were averaged by "Season" .The clam beds were then grouped by location and graphed. Location was found to be significant with the variable Length using an ANOVA model that produced a p-value of <2e-16. The basin that had the highest average slopes was the Central IRL, followed by Mosquito Lagoon, NIRL and lastly the Banana River (Figures 3-8). The area with the highest lengths was Micco and the lowest was Cape Canaveral. However, Cocoa Beach, Merritt Island (Banana), and Merritt Island (Sykes) all had negative slopes due to low survival ratios. The location with the highest average slope was Micco.

North Indian River Lagoon Locations Clam Length vs Time

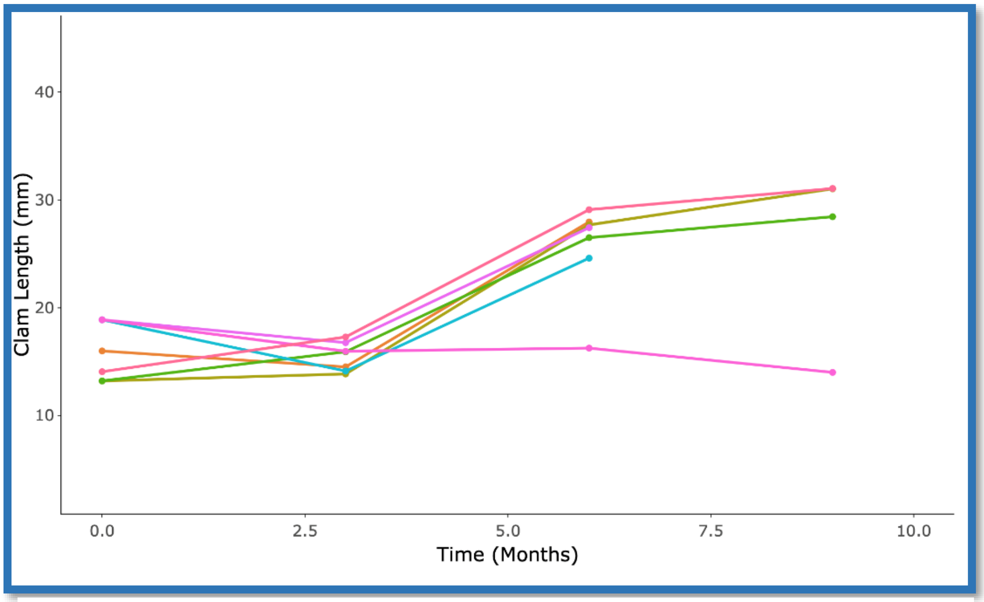


Figure 4: North IRL Locations Clam Length vs Time. From the Highest Mean Clam Length Recorded: Titusville: red-pink, Indialantic: brown, Cocoa: Orange, Palm Shores: Purple, Melbourne: green, Merritt Island Indian River: teal, Rockledge: pink.

Central Indian River Lagoon Locations Clam Length vs Time

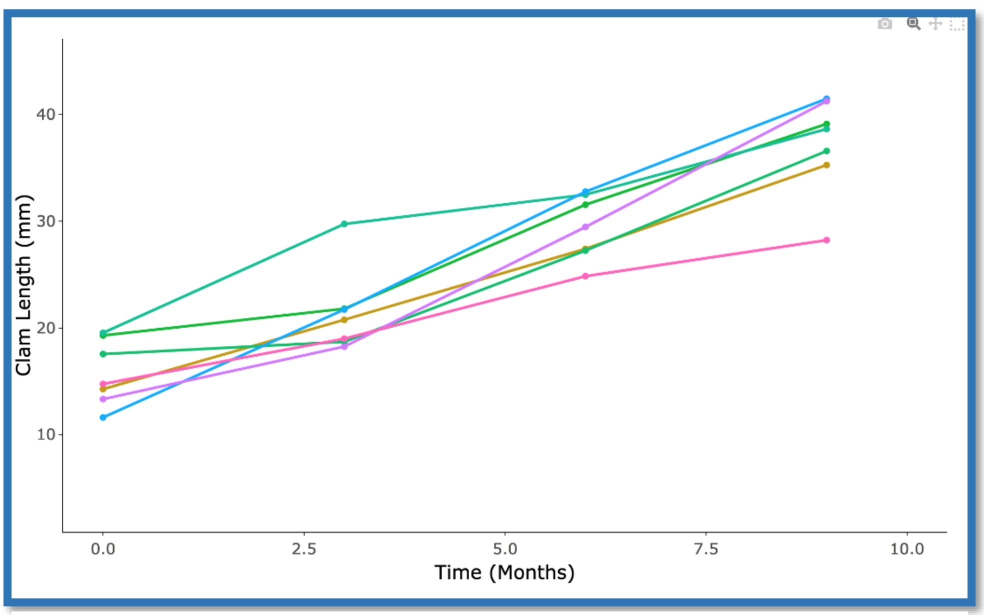


Figure 5: Central IRL Locations Clam Length vs Time. From the Highest Mean Clam Length Recorded: Micco: sky blue, Palm Bay: purple, Malabar: army green, Melbourne Beach Central: green, Melbourne Beach South: teal, Melbourne Beach North: blue-green, Grant: brown, Sebastian: pink.

Mosquito Lagoon Locations Clam Length vs Time

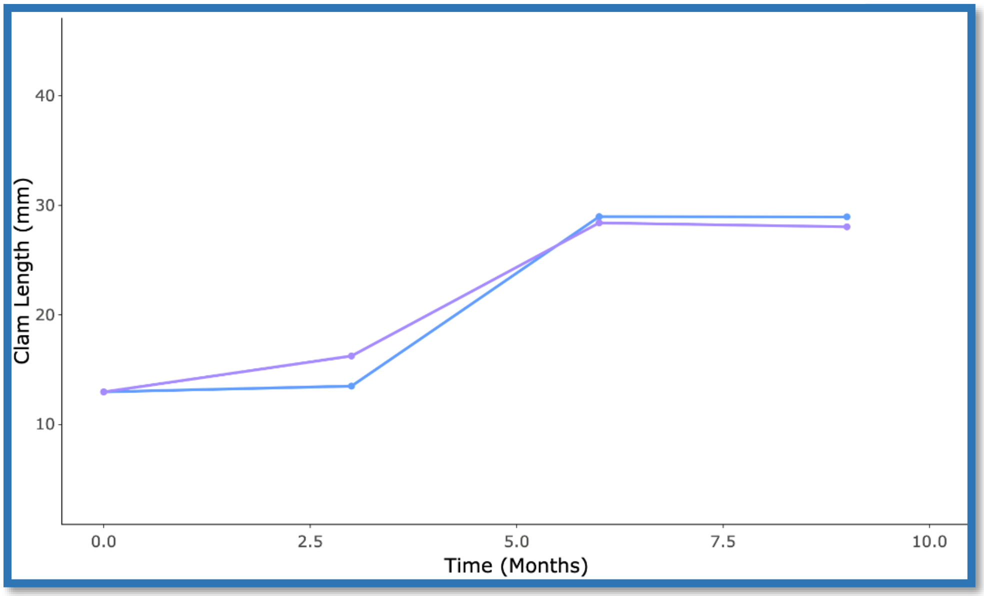


Figure 6: Mosquito Lagoon Locations Clam Length vs Time. From the Highest Mean Clam Length Recorded: New Smyrna: blue, Oak Hill: violet

River Lagoon Locations Clam Length vs Time

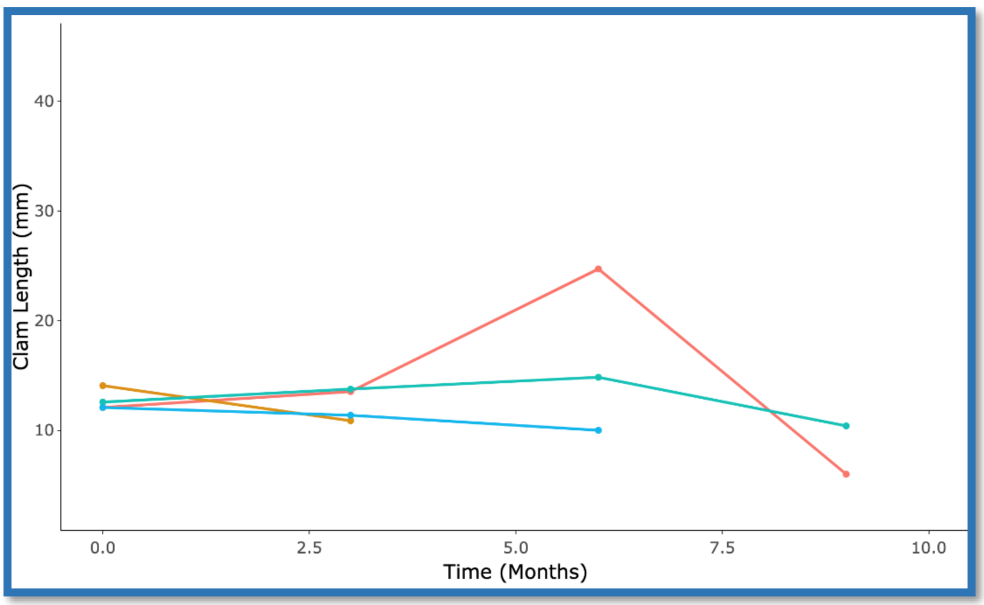


Figure 7: Banana River Locations Clam Length vs Time. From the Highest Mean Clam Length Recorded: Cape Canaveral: coral, Merritt Island Banana: teal, Cocoa Beach: brown, Merritt Island Sykes Creek: light blue.

Water & Sediment Chemistry Sampling: Sediment samples were taken at the clam bed sites at the completion of the project to determine the chemical makeup of sediments and their potential impacts to calm growth and survivorship through loss on ignition (LOI) analysis. Survival ratio was found not to be significant when correlated with Organic Matter concentration, but it was positively correlated with calcium carbonate ($CaCO_3$) with an R value of 3.1. The relationship between $CaCO_3$ and survival, however, was not significant. Of the sediment and water samples process by Florida Institute of Technology, two statistically significant trends emerged when analyzing the data. From the filtered porewater samples taken at sites in the most successful basin of the IRL for clam growth and survival, the Central Indian River Lagoon, alkalinity was found to be negatively correlated with survival ratio (r=-0.475, p-value=0.0038). pH values were found to not be significant when correlated to survival ratio. When sampling for hydrogen sulfide (H_2S), four clam bed sites were chosen to do sampling on the clam bed and off the clam bed. When comparing the H2S values taken directly in the clam bed site to those 10'-15' outside of the clam bed site, the samples outside the bed were found to have significantly higher sulfide values when performing a two-sided t test (p-value= 0.0279, Figure 8).

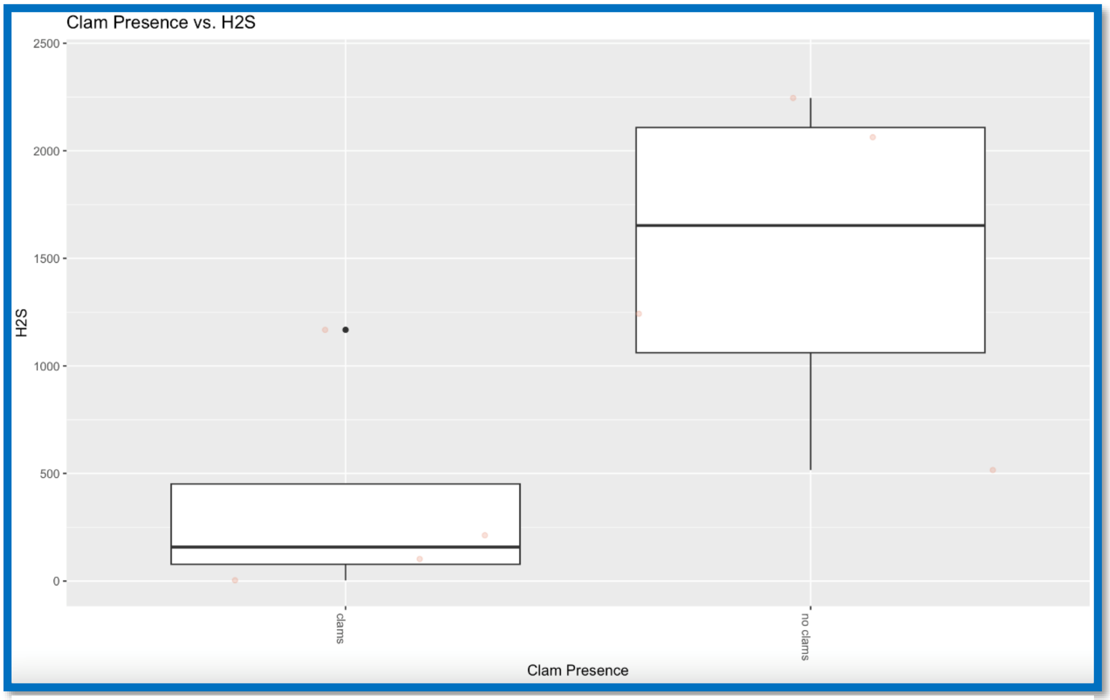


Figure 8: Hydrogen sulfide values vs Clam Presence

Conclusion

Salinity was expected to be a major driver of clam survival and weighed heavily into the Brevard Zoo's site selection process. After observing the correlation between salinity and clam survival rate during this study, it is apparent that salinity does play a role in *M. mercenaria's* ability to survive and thrive in the Indian River Lagoon. The growth averages that were the highest were all in the Central Indian River Lagoon, particularly Micco, where the Sebastian Inlet regulates and keeps the salinity higher with its constant influxes of ocean water (35ppt). Comparing the growth averages and the locations where clam survival was highest, it is clear that the southern end of Brevard County (south of Cape Malabar) is more amenable to clam restoration. Clam restoration may be more difficult in locations farther north in the Indian River or Banana River where freshwater tributaries and proximity from the Sebastian Inlet keep salinity lower. Many clams planted in the southern portion of Brevard County grew to reproductive size in under a year, suggesting that continued supplementation to adult hard clam populations through restoration could increase reproductive potential of clams in the Central Indian River Lagoon. Further investigation should be made into whether clams planted through restoration in the Indian River Lagoon are indeed reproducing and whether their larvae recruit and survive on the lagoon bottom.

In addition to salinity, predation limited clam survival. The significant difference between the survival rates of sites where the predation protection net was secured for the length of the project versus sites where the net was compromised suggests that the protection nets are necessary when planting clams in such a high density. Heavy predation of clams by species including crown conch (*Melongena corona*) and cow nose rays (*Rhinoptera bonasus*) were observed at many sites throughout the study. If nets were to be excluded from future clam restoration projects to avoid the required permitting and maintenance, the density at which clams were planted should be examined and perhaps decreased to spread predation pressure.

Finally, sediment organic matter and calcium carbonate ($CaCO_3$) concentrations at clam bed sites were not found to have significant impact on clam survival ratios in this study. However, sites with high organic matter in the sediment were avoided during the site selection process since hard clams prefer sandy sediment. The negative correlation between clam survival ratio and alkalinity observed in sediment analysis may first seem unlikely since low pH can inhibit clam growth (Ringwood and Keppler 2002), the sediment disturbance caused by the clams themselves may cause sediment reoxidation of reduced substances contributes protons (+) and therefore decreasing alkalinity (pH). Bioturbation (and reoxidation) can lead to lower alkalinity and lower hydrogen sulfide (H_2S) concentrations (Cai et al., 2017). The lower alkalinity found in the clam beds and significant difference in H_2S levels with and without clams is likely the result of oxidation reactions including nitrification. It is well established that bioturbation and bio-irrigation from in-faunal species such as hard clams promote nitrification and thereby coupled nitrification-denitrification that can remove inorganic nitrogen from the system (Figure 9). Future post hoc evaluations of clam restoration should consider sampling sediment in areas adjacent to restored clam beds prior to modification of the site with clams.

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Restoration of Clam Populations in Brevard County for Water Quality Benefits: Seagrass

FINAL REPORT
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In Collaboration with

Brevard Zoo
Whitney Laboratory for Marine Bioscience

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The primary goal of this restoration activity was to co-restore clam and seagrass populations in the northern Indian River Lagoon (IRL). The University of Florida and Brevard ZOO repatriated hardy varieties of native clams on scales that provide for reproductive success at 100 sites. At ten of these sites, Florida Oceanographic Society (FOS) planted seagrass in conjunction with clams in an effort to facilitate species success through co-restoration. To achieve that goal, the following tasks were established:

Task 1: Obtain all necessary permits related to the production, installation and monitoring of seagrass for this project;

Task 2: Conduct site evaluations at ZOO's clam sites to determine which are suitable for seagrass mats;

Task 3: Produce and install 1,000 seagrass mats to be used in ZOO's clam project, these mats will vary in size as they are site dependent – FOS will determine if large, medium or small mats are appropriate per site;

Task 4: Monitor mats at each site, two and four months after planting.

Methods

Permits

The ZOO provided FOS with a list of 58 possible sites, the majority being private homes, for seagrass restoration. From this list, FOS chose 28 sites to complete a comprehensive site assessment in September 2021. During the site assessments, multiple transects were run perpendicular to the shoreline. The submerged bottom was surveyed for the presence of seagrasses and hard bottom resources such as corals, oyster reefs, and sponges, which would potentially affect proposed restoration plans. When benthic resources were identified, sediment type was recorded and benthic resources (i.e., seagrass, oysters, coral) were quantified within a 1 m^2 quadrat by applying the Braun-Blanquet Cover Abundance sampling technique (Braun-Blanquet, 1932). Water depths (m) were recorded along each transect with a depth pole and are reported as actual depth at the time of the survey (i.e. not tidally corrected). Water quality parameters (pH, dissolved oxygen (%, mg/L), salinity (ppt), water temperature (°C), and turbidity) were collected at each site.

From the 28 sites, ten were chosen along a latitudinal gradient for clam and seagrass co-restoration. All sites were devoid of seagrass species but were known locations of historical seagrass meadows (e.g., Morris et al. 2022). One site was located on an Environmentally Endangered Lands (EEL) sanctuary and the other nine were located on private properties (Fig 1). Individual permits for each site were secured through the Department of Environmental Protection and US Army Corps of Engineers (Table 1). Permit areas were dependent on individual site characteristics.

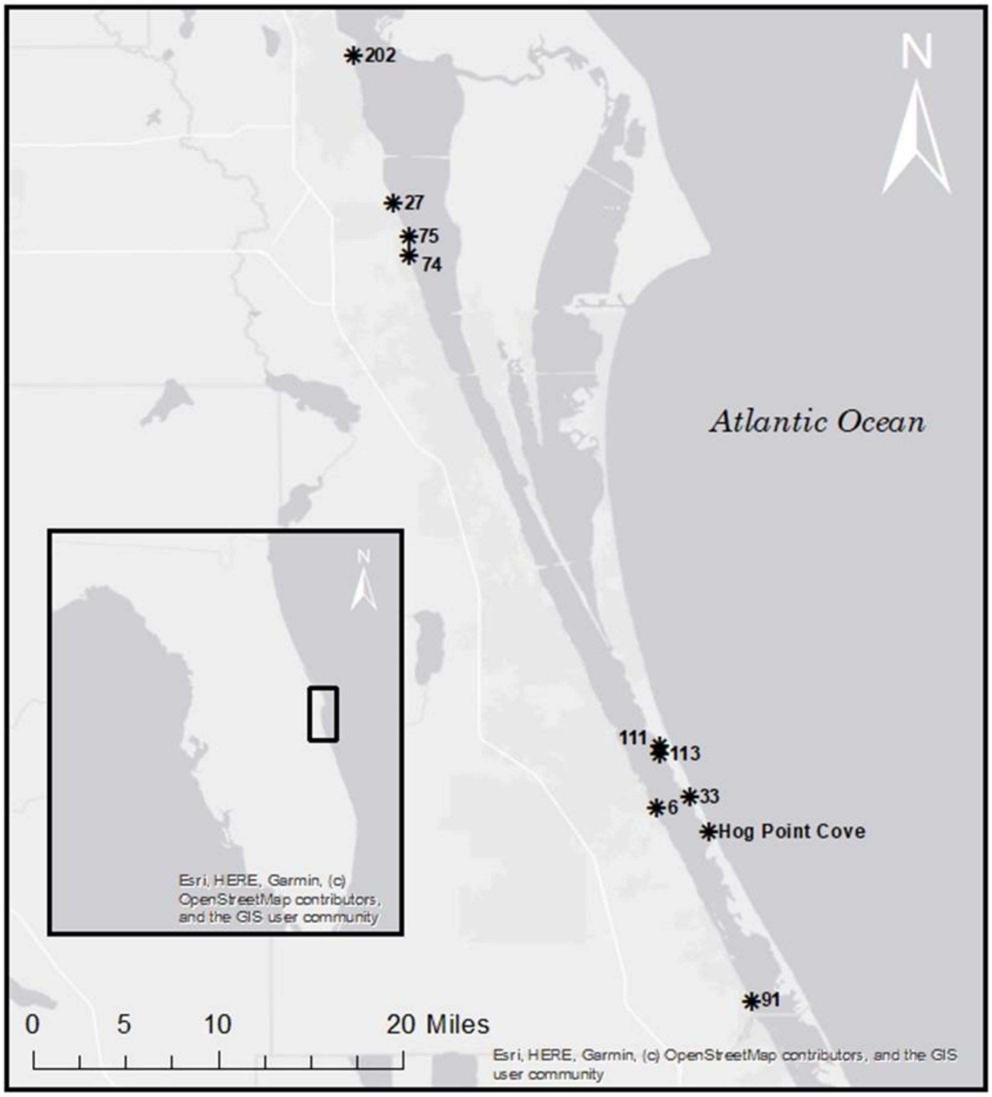

Figure 1. Site locations of seagrass plantings. Numbers denote private homes and Hogs Point Cove is an Environmentally Endangered Lands sanctuary.

Site Locations

Ten sites were chosen for seagrass planting (Fig 1, Table 1). Four of the sites (202, 27, 75, 74) were in the Northern IRL basin and six of the sites (111, 113, 33, 6, 91, Hog Point Cove) were in the Central IRL basin. All site locations were in shallow water (0.6 - 1.6 m at deepest point), had sand dominated sediments, relatively low wave action and were nearshore.

Table 1. Site overview and permit documentation

Site ID	Site Coordinates	Permit # (DEP, USACOE)
202	28.605383° N, -80.805083° W	236941-002. SAJ-2022-00152 (NW-JAZ)
27	28.490233° N, -80.773200° W	0412583-001-EI, SAJ-2022-00152 (NW-JAZ)
75	28.463771° N, -80.761621° W	412722-001-EI, SAJ-2022-00151 (NW-JAZ)
74	28.605383° N, -80.805083° W	412661-001-EI, SAJ-2022-00212 (NW-JAZ)
111	28.064207° N, -80.564405° W	0326829-002-EI, SAJ-2022-00213 (NW-JAZ)
113	28.058471° N, -80.565031° W	0412614-001-EI, SAJ-2022-00039 (NW-JAZ)
33	28.024883° N, -80.541500° W	0412547-001-EI, SAJ-2021-00585 (NW-JAZ)
6	28.015683° N, -80.566533° W	0412597-001-EI, SAJ-2021-04097 (NW-JAZ)
91	27.864933° N, -80.492683° W	0412689-001-EI, SAJ-2021-04098 (NW-JAZ)
Hogs Point Cove	27.99786° N, -80.52664° W	407037-001-EI SAJ-2019-02074 (NWP-CMM)

Seagrass Mats
Cultivars of shoal grass, *Halodule wrightii*, grown at FOS were used as the planting material. Considered a pioneer species, shoal grass is the most common seagrass found throughout the IRL and has been shown to have high tolerance to shifts in water quality parameters. The seagrass used was collected as fragments from the IRL and grown in the on-site FOS nursery for one year. Utilizing nursery grown plant stock, originally obtained from fragments found in the IRL, ensured that wild seagrass populations were not depleted and were of similar genetic stock.

For this project, we constructed and deployed 1,818 seagrass mats (34,368 *H. wrightii* shoots) across the ten project areas. Seagrass shoots were deployed on agriculture grade burlap (untreated, 100% virgin natural jute fiber) material of varying sizes. Shoot density was dependent on mat size, were 7 – 10 cm long, and were tied to the mesh mat by their roots and rhizomes with biodegradable floral wire (Fig 2). At Hog Point Cove, mats ranged in size from small (20 x 20 cm), medium (40 x 40 cm), to large (60 x 60 cm) (Fig 3). At the remaining nine sites, all mats were 20 x 20 cm. The mats were constructed by FOS staff and volunteers and were transported to the sites in buckets of brackish water.

Figure 2. Seagrass mat (20 x 20 cm). *Halodule wrightii* is attached to biodegradable burlap mat with floral wire.

Figure 3. Seagrass mats of varying sizes for Hog Point Cove. From left to right; Large (60 x 60 cm), medium (40 x 40 cm) and small (20 x 20 cm).

Seagrass Installation

Seagrass mats of three different sizes (small, medium, large) were planted over a subsection of a 50 x 25 ft clam bed on the northern end of Hog Point Cove (Fig 2) in May 2022. The clam bed contained large (~50 mm) and small (~20 – 25 mm) clams. Mats were deployed in haphazard arrays (Fig 4) and were staked into the sediment with 6-inch bamboo picks and a handful of sediment was added on top to reduce the risk of mats being disturbed by wave action. The 20 x 20 cm arrays (n = 5) contained 80 *H. wrighitt* shoots, the 40 x 40 cm arrays (n = 5) included 320 shoots and the 60 x 60 cm arrays (n = 5) contained 720 total shoots. In total, 90 mats (6,720 shoots) were planted across the site. At the remaining nine sites, seagrass was planted across 12, 0.8 m^2 plots in June 2022 (Fig 5a). Each plot contained 16, 20 x 20 cm mats (totaling 256 *H. wrightii* shoots) which were planted in a 4 x 4 configuration (Fig 5b). Units were anchored into the sediment using 6-inch bamboo picks and a handful of sediment was added on top to reduce the risk of mats being disturbed by wave action. In total, 1,728 mats (27,648 shoots) were planted across the nine sites. At each site, half of the plots (n = 6) were seeded with 30 mm clams at a planting density of 222 clams/m^2. Additionally, half of the plots were caged with herbivore exclusion devices to decrease predation from megafauna.

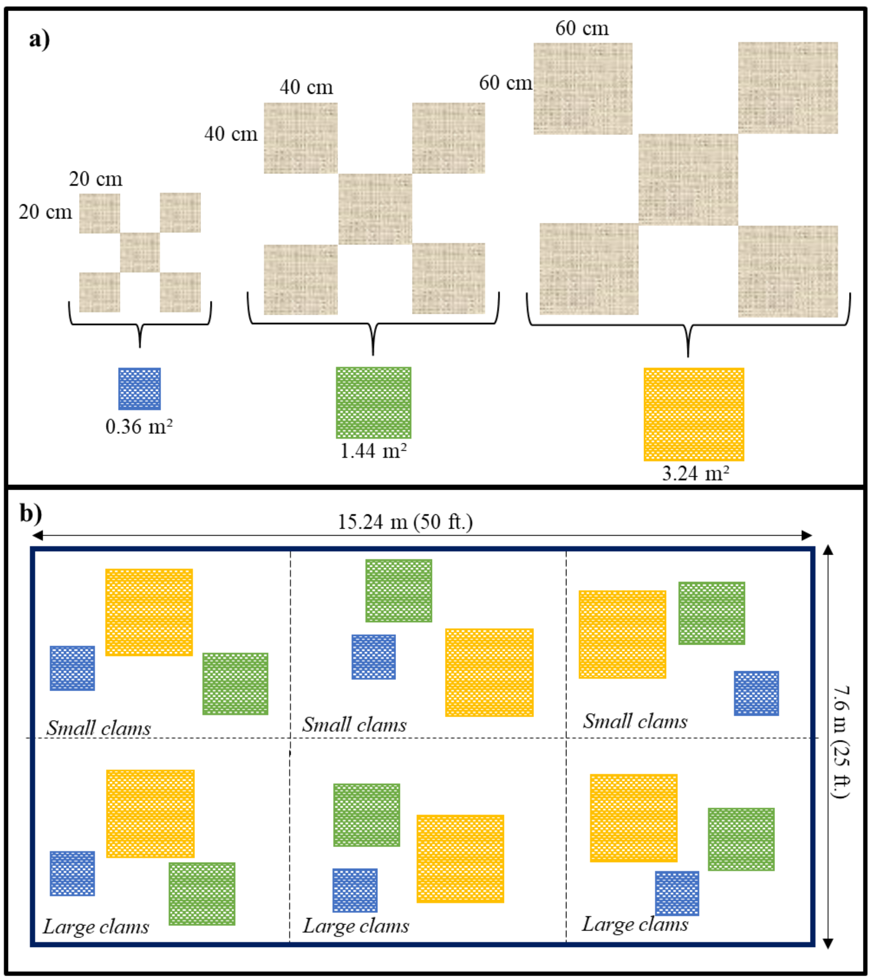

Figure 4. Site schematic for Hog Point Cove seagrass planting, **a)** arrays were composed of varying mat sizes (20 x 20 cm, 40 x 40 cm, or 60 x 60 cm). Each array had five mats of the same size. **b)** mat arrays were haphazardly deployed over a clam bed containing large and small clams.

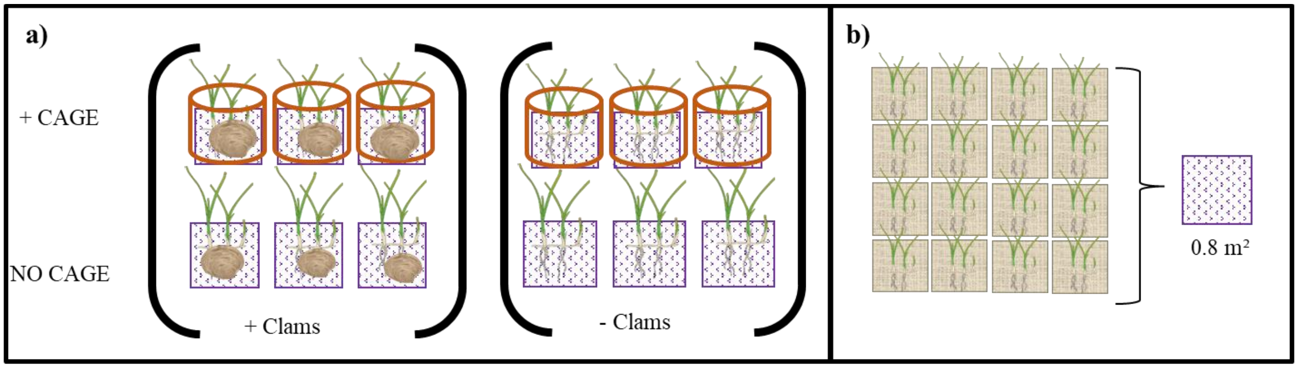

Figure 5. Site schematic for nine homeowner properties, **a)** each site had 12, 1 m^2 plots. All plots contained seagrass and half of the plots contained *Mercenaria mercenaia* clams. Half of the plots were caged with herbivore exclusion devices. Each plot had **b)** 16 (20 x 20 cm) seagrass mats, totaling 256 *H. wrightii* shoots per plot.

Exclusion Devices

Macro grazer exclusion was achieved through the construction of herbivore exclusion devices (Fig 6). These devices protected a 1 m^2 area and were 0.81 m tall. A cylinder made of 1 in square PVC coated wire mesh was held together with stainless steel hog rings and a top of the same material was added to prevent risk of wildlife entrapment. The devices were anchored to the sediment using 2 m segments of rebar placed on the outer edges of the mesh in three locations. Rebar was inserted at least 1 m into the sediment to prevent movement of the cages and the mesh cylinder was attached to the rebar using plastic zip-ties. Exclusion devices were maintained by homeowners or 'Clam Buddies' on a weekly basis which included removing macroalgae caught on the tops and sides and scrubbing the outside of the cage to remove epiphytic growth and biofouling.

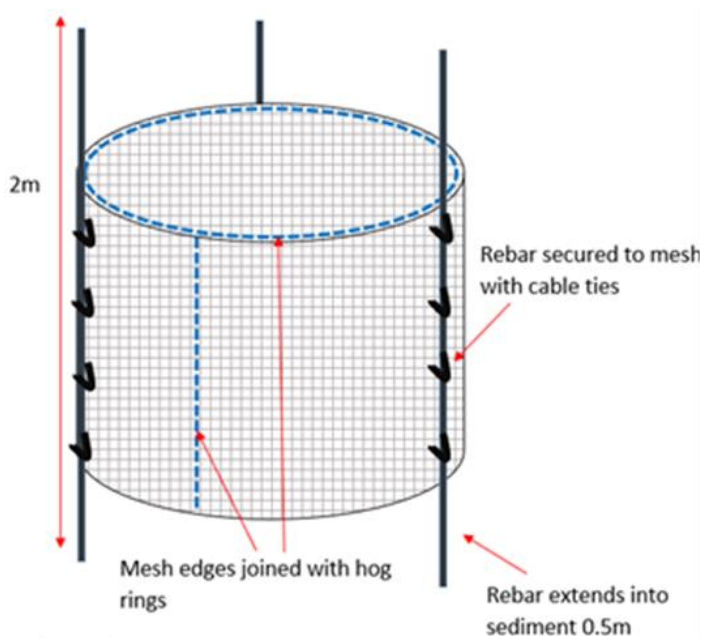

Figure 6. Herbivore exclusion device overview.

Seagrass Monitoring
Hog Point Cove was established in May 2022 (Time 0 – [T0]) and was monitored in June (T1) and July (T2). The nine homeowner sites were established in June 2022 (T0), and subsequent monitoring was completed at 1 month (July) (T1), 2 months (August) (T2) and 4 months (October) (T3).

Seagrass was assessed during all sampling events (T1, T2, T3) using a modified version of the *SJRWMD Indian River Lagoon Seagrass Monitoring Standard Operating Procedures* (see Hall et al. 2022). Modification included the use of a 0.5 m^2 quadrat to account for plot area. These methods were used to document seagrass percent occurrence, percent cover, epiphyte loading, shoot counts, and canopy height. General water quality parameters (pH, dissolved oxygen (%, mg/L), salinity (ppt) and water temperature (°C)) were also measured at all sites over the course of the experiment with a YSI ProDSS (YSI, Yellowsprings, OH).

Results

Halodule wrightii cover was 13% at T0, based on SJRWMD estimates of 100% coverage (see Hall et al. 2022). The four northern sites (27, 74, 75 and 202) showed a significant decline in seagrass coverage in T1, and 100% seagrass mortality by T2 (Figs 7,8). Sites were deconstructed and no further sampling was done. The remaining five homeowner sites were sampled at T2 and averaged 12.82% *H. wrightii* coverage. At T4, sites 113, 33, and 6 had complete mortality. Site 111 had 2.75% *H. wrightti* cover and the southernmost site (91) had 11.5% cover. Unfortunately, Hog Point Cove had 2.7% *H. wrightii* cover at T1 and 100% mortality by T2. There was no difference in mat sized mortality rate across the site.

Plots caged with herbivore exclusion devices had significantly greater *H. wrightii* cover at T2 (Fig 9) as compared to uncaged plots. Productivity in cages could not be statistically compared at T4 due to cage damage caused by Hurricane Ian (September 28, 2022).

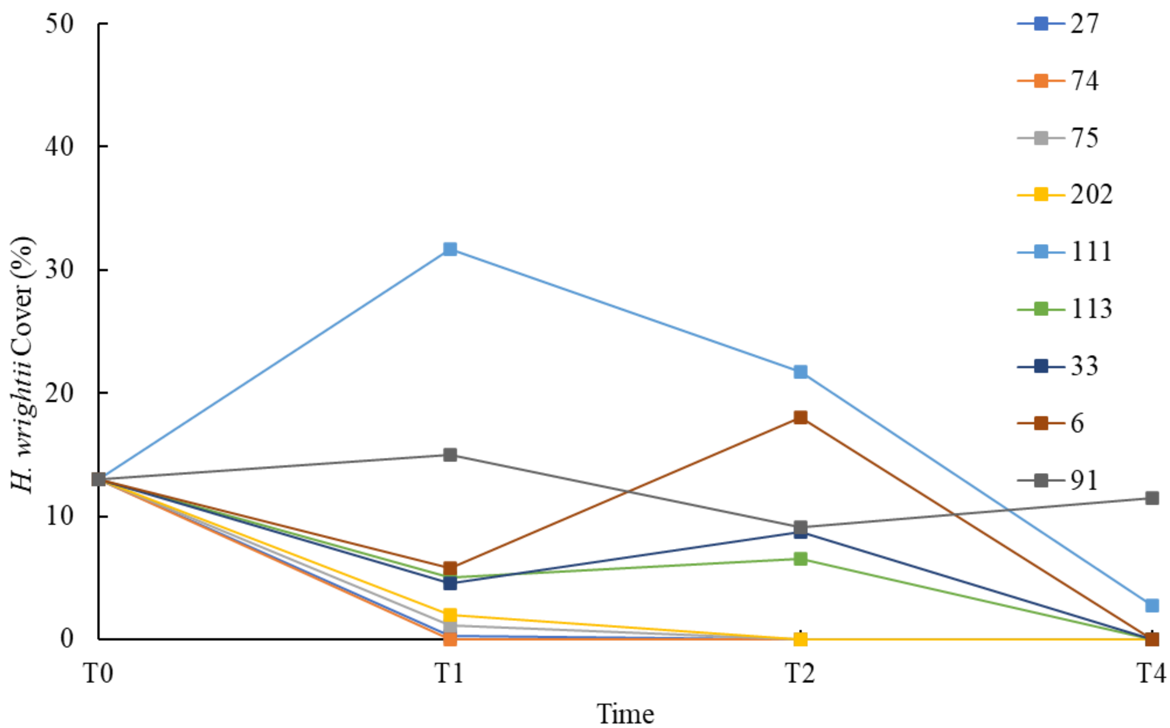

Figure 7. *Haldoule wrightii* cover (%) at the nine homeowner sites at deployment (June; T0), July (T1), August (T2) and October (T3) 2022.

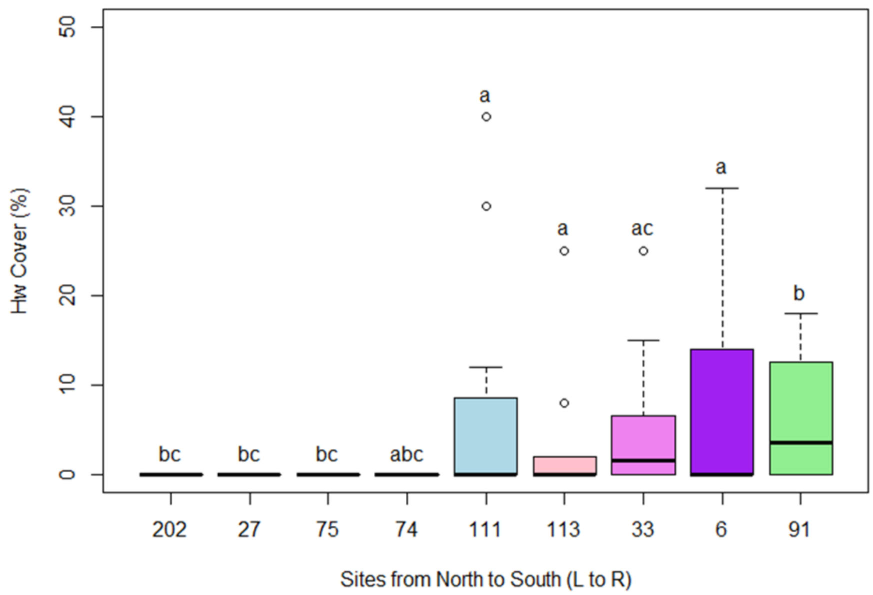

Figure 8. *Haldoule wrightii* cover (%) at the nine homeowner sites at (T1). In the box plots, horizontal lines are the medians, the edges of the boxes are the 25th and 75th percentiles, the lines are the 5th and 95th percentiles and the outliers beyond these percentiles are plotted. Different letters denote significance between monitoring events (p = < 0.0001). Data collected and analyzed by FOS staff.

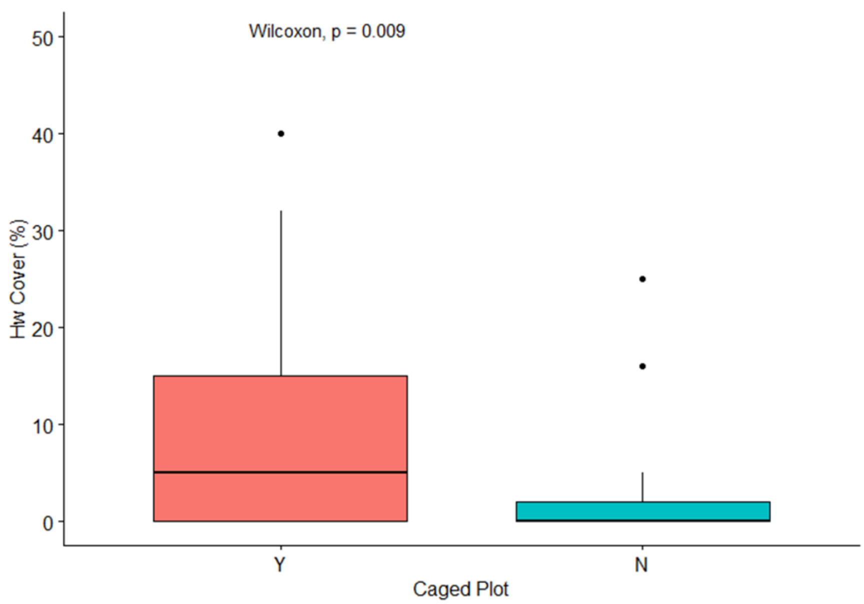

Figure 9. *Haldoule wrightii* cover (%) in caged or uncaged plots at T2. In the box plots, horizontal lines are the medians, the edges of the boxes are the 25th and 75th percentiles, the lines are the 5th and 95th percentiles and the outliers beyond these percentiles are plotted. Data collected and analyzed by FOS staff.

Conclusions

- 50% of sites experienced 100% mortality by T2.
- Seagrass growth was significantly reduced in the remaining sites (n=5).
- By T4, only two sites had seagrass growing.
- Mass mortality of seagrass restoration units is likely due to water quality in the northern lagoon.
- Exclusion devices may be beneficial to seagrass growth, especially in areas where there is adequate water flow.

Match

We held 13 seagrass matting events with a total of 271 volunteers between May and June 2022 (Table 1). These events were critical to the construction of 1,818 seagrass mats. When using the volunteer rate of $29.95, this would equate to $15,289.47 in match.

Table 2. FOS volunteer seagrass matting events

Date	Activity	Volunteers Attendance (#)	Total Hours
5/9/22	Seagrass Matting	9	22.5
5/10/22	Seagrass Matting	15	30
5/11/22	Seagrass Matting	8	16
5/31/22	Seagrass Matting	26	51.5
6/1/22	Seagrass Matting	25	27.25
6/2/22	Seagrass Matting	37	74
6/3/22	Seagrass Matting	44	77.25
6/18/22	Seagrass Matting	18	34
6/20/22	Seagrass Matting	26	52
6/21/22	Seagrass Matting	17	34
6/22/22	Seagrass Matting	21	42
6/23/22	Seagrass Matting	17	34
6/24/22	Seagrass Matting	8	16
	TOTAL	**271**	**510.5**

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