



**Facilitation of seagrass productivity in Tampa Bay using the indigenous,
suspension feeding bivalve, *Mercenaria campechiensis***

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Introduction

The importance of marine bivalves to coastal ecosystem function is now widely recognized. The feeding activity of bivalves, especially when in large assemblages such as oyster reefs, provide important ecological services. These animals are capable of filtering seston (suspended particulates) from large volumes of water as they feed. Clearance rates vary seasonally, but for oysters, rates of $5 \text{ l h}^{-1} \text{ g}^{-1}$ dry tissue weight are typical (Newell, 1988). This results in the reduction of particle concentrations by 30%-45%; chlorophyll-a concentrations by as much as 90%; and nitrogen removal up to 25% of daily load (Carmichael et al. 2012). Some of cleared particulate matter is ingested and assimilated into tissue and shell growth; some is excreted (primarily as ammonium) and is available for primary production. The remainder ends up on the bottom as biodeposits (feces and pseudofeces). The process of transferring nutrients from the water column to the sediment, or benthic-pelagic coupling, benefits both the water column (pelagic) and bottom (benthic) communities in several ways:

- 1) Nutrients contained in biodeposits can be removed from the system by burial in sediments.
- 2) Nitrogen in biodeposits can be microbially denitrified to N_2 gas and permanently removed from the system. For oyster biodeposits, this process removes 17-24% of nitrogen from the sediments (Newell et al. 2002). Kellogg et al. (2013) found that seasonal denitrification rates at a restored oyster reef ranged from 0.3 to $1.6 \text{ mmol N}_2\text{-N m}^{-2} \text{ h}^{-1}$.
- 3) Assimilation of nutrients into shell and tissue of bivalves (secondary production) effectively removes nitrogen and phosphorus from the water column (Rose et al. 2014). For oysters (*Crassostrea virginica*), the nitrogen content of dry tissue ranges from 7-9.7% and the nitrogen content of shells ranges from 0.08-0.24%; phosphorus contents are lower, ranging from 0.8-1.26% for dry tissue and 0.04-0.1% for shell (Newell 2004, Higgins et al. 2011, Carmichael et al. 2012, Kellogg et al. 2013, Reitsma et al. 2016). Data for quahogs, *Mercenaria mercenaria*, is limited, but nitrogen content of dry tissue and shell was reported to be 7.69% and 0.18%, respectively (Reitsma et al. 2016). Harvest of commercially important species thus results in a permanent removal of nutrients from the ecosystem. Bivalve aquaculture is now being embraced as a means of increasing nutrient bioassimilation and bioextraction in eutrophic estuaries (Higgins et al. 2011, Carmichael et al. 2012, Kellogg et al. 2014, Rose et al. 2014, Reitsma et al. 2016).
- 4) Filter feeding exerts “top-down” grazer control on phytoplankton, reducing water column turbidity and increasing the depth to which photosynthetically active radiation penetrates and seagrass can grow (Newell, 2004). Newell and Koch (2004) determined that even a modest density of oysters ($25 \text{ g dry tissue weight m}^{-2}$) reduced suspended particulate matter in the water

column by an order of magnitude, which in turn increased water clarity and the depth to which seagrasses were predicted to grow.

5) An additional ecological benefit provided by infaunal bivalves is the potential to increase seagrass productivity. Peterson and Heck (1999) demonstrated that biodeposition of nutrients by filter feeding bivalves (*Modiolus americanus*) increased pore water ammonium and phosphate concentrations. In a subsequent study, seagrass (*Thalassia testudinum*) leaf widths and lengths were significantly greater in the presence of *M. americanus*, demonstrating that nutrients derived from filter feeding bivalves were taken up by seagrass and resulted in enhanced seagrass productivity (Peterson and Heck 2001a, 2001b).

Unfortunately, it is now also recognized that previously abundant bivalve resources have been declining at an alarming rate due to overharvesting, disease, habitat loss, declining water quality and altered hydrology. Beck et al. (2011) estimated that worldwide, oyster resources have been reduced by 85%. In Tampa Bay, there is no way of knowing the historical (pre-dredge and fill) distribution and quantity of oysters. We do know, however, that there has been no commercial harvest of bivalves (oysters, clams, scallops) in Tampa Bay since at least 1970 (Geiger et al. 2010).

All of this has led to a greater focus on augmenting bivalve populations. Most of the effort to date has been directed toward restoring epifaunal oyster reefs (zu Ermgassen et al. 2016). However, Florida clam growers have observed enhanced seagrass growth in and around bottom leases (Aaron Welch, Two Docks Shellfish Co., pers. comm). Given the importance of seagrasses to the Tampa Bay ecosystem and the success of the Tampa Bay Estuary Program in restoring seagrass to historic levels, we proposed to investigate the relationships among the infaunal bivalve, *Mercenaria campechiensis*, water quality, sediment quality, and seagrass distribution in lower Tampa Bay. An additional goal of this project is the re-establishment of a population of native clams that will reproduce for many years.

Our objectives were to determine: 1) growth and mortality of *M. campechiensis* in lower Tampa Bay using local aquaculture practices; 2) effects of *M. campechiensis* on suspended solids, chlorophyll, nitrogen, phosphorus and sediment composition; and 3) the effects of *M. campechiensis* on the distribution of adjacent seagrass beds.

Methods and Materials

Experimental Site

Two experimental sites were established on shallow submerged land owned by Port Manatee (near Manbirtee Key) on September 24, 2016 (Figures 1 and 2). The North site (1.9 acres) was

within a large, exposed sandy area surrounded by sparse patches of seagrass (mostly *Halodule wrightii*). The South site (0.8 acres) was in a slightly deeper sand trough between well-established seagrass beds (primarily *Thalassia testudinum*). The areas were marked with PVC stakes and GPS coordinates were recorded (Table 1). Each site was divided into experimental (with clams) and control (no clams) areas.

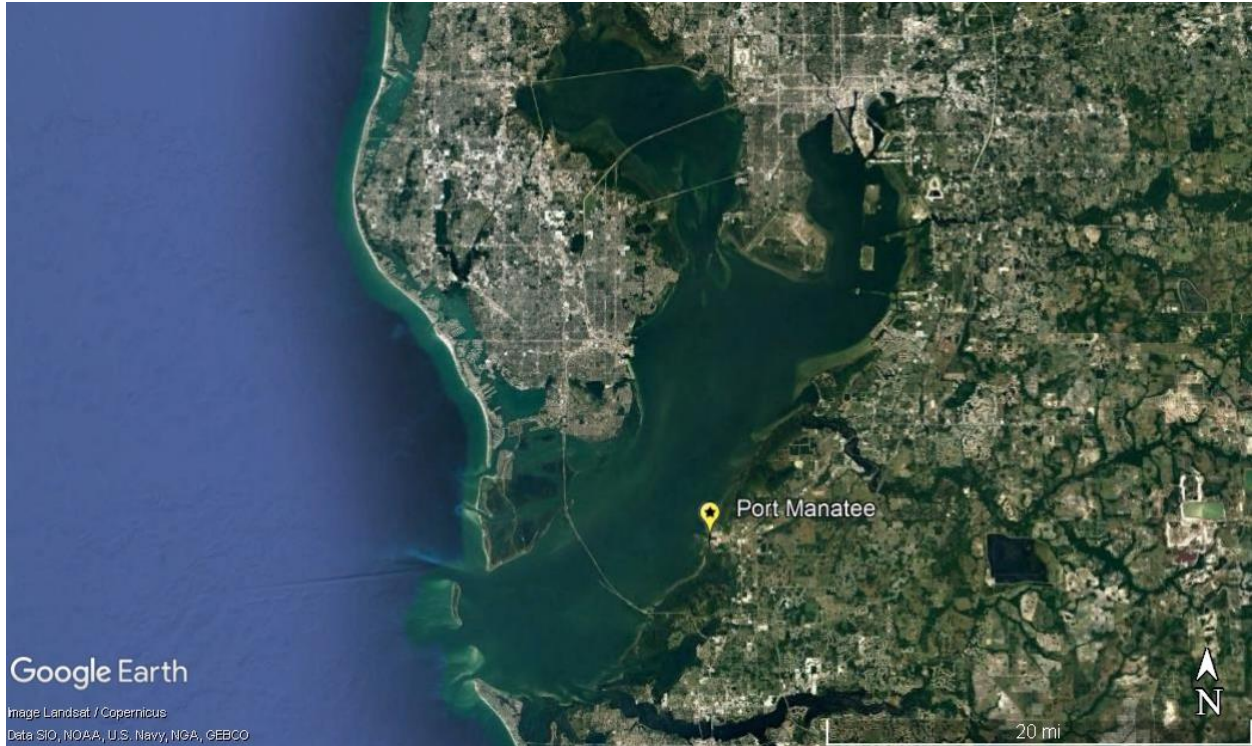


Figure 1. Aerial view of Tampa Bay showing the location of the study area at Port Manatee.

Table 1.
GPS coordinates of the stakes marking the North and South study sites, as shown in Figure 2.

North Site (Latitude)	North Site (Longitude)	South Site (Latitude)	South Site (Longitude)
27 37.984 N	82 34.024 W	27 37.765 N	82 34.039 W
27 37.981 N	82 34.062 W	27 37.782 N	82 34.015 W
27 37.979 N	82 34.100 W	27 37.802 N	82 33.993 W
27 38.019 N	82 34.024 W	27 37.782 N	82 34.045 W
27 38.014 N	82 34.062 W	27 37.799 N	82 34.030 W
27 38.012 N	82 34.101 W	27 37.817 N	82 34.006 W



Figure 2. Aerial view of Port Manatee showing locations of the North and South study sites adjacent to Manbirtee Key

Clam Production and Deployment

Broodstock *M. campechiensis* were collected locally by volunteers, donated by commercial growers, and delivered to the Bay Shellfish Co., in Terra Ceia, Florida. Clams were conditioned (induced to initiate gametogenesis) by holding them for approximately six weeks at 20 °C and providing a diet of *Isochrysis galbana* and *Chaetoceros gracilis* at a rate of 4% dry tissue weight per day.

On November 7, 2016, conditioned clams were placed on a spawning table and induced to spawn by cycling water temperature between 20 °C and 30 °C every thirty minutes. Upon spawning, clams were placed into individual containers so that eggs and sperm could be collected separately. After spawning was complete, all eggs were combined and fertilized with a mixture of sperm from all males. After fertilization was achieved, embryos were placed into an 1800 gallon larval tank and water temperature was maintained at 25 °C. Water in the tank was changed every day and larvae were fed a combination of microalgae (*Isochrysis galbana* and *Chaetoceros gracilis*) on a daily basis. Six days later, after reaching the pediveliger stage, larvae were transferred to downwellers in the hatchery for settlement and metamorphosis. Post-set clams were fed continuously (*Isochrysis galbana* and *Chaetoceros gracilis*) until a size of 2 mm was reached, at which point they were transferred to upwellers at a nearby coastal nursery until a minimize size of 5 mm (shell length) was reached. Upwellers take advantage of natural

phytoplankton by pumping seawater upward through a bin with a screen on the bottom that holds the clams. At approximately three months of age, clams had attained an average size (shell length) in excess of 5 mm.

Approximately 300,000 juvenile clams were placed at the South site on February 13, 2017 and another 300,000 clams were placed at the North Site on February 24, 2017, using proven commercial clam production methods. Clams were initially protected from predators by being placed in 4 mm mesh nursery bags. Approximately 7,500 clams (estimated volumetrically) were placed in each of 40 bags (Figure 3). After three months, when clams reached 17 mm in shell length, they were removed from the nursery bags and distributed under eight mesh covernets (6 mm mesh) (Figure 4). Again, counts of surviving clams were made volumetrically. Each covernet (15 feet by 40 feet; edges were held in place by 20-foot lengths of plastic coated rebar) thus received approximately 29,125 clams. Beginning in May 2017, the 6 mm covernets were replaced with 12 mm mesh covernets (Figure 5). Throughout the duration of the project, the clams were checked every 2-3 weeks, and the nets were cleaned of fouling organisms (mostly macroalgae) as needed.



Figure 3. Nursery bags (4 mm) at the North Site. Manbirtee Key is in the background.

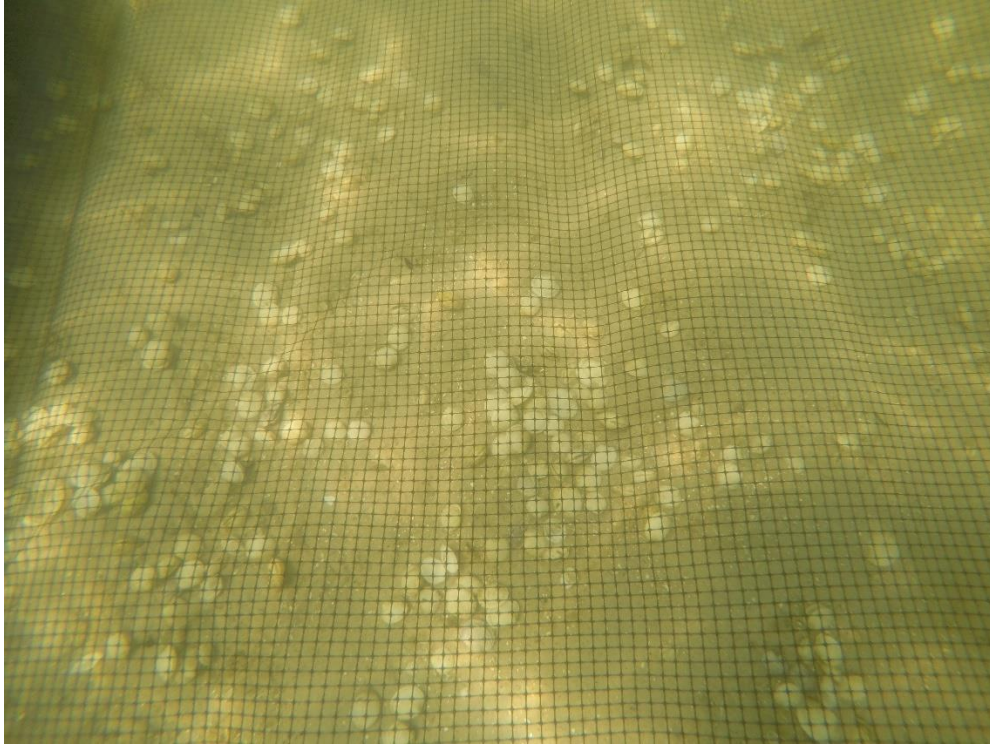


Figure 4. Clams (mean shell length = 17 mm) placed under 6 mm mesh covernets before they moved down into the sand.



Figure 5. Covernets at the South site, looking north towards Manbirtee Key. Note the seagrass beds in the background.

Data Collection and Monitoring

Water temperature was collected using HOBO Pendant dataloggers (Onset Corp.; see Appendix). Two dataloggers were placed at each study site, one in the experimental section and one in the control section. Temperature (°C) was recorded every 6 hours and daily averages were obtained from March 17, 2017 to September 6, 2018. Beginning in June 2017, salinity (psu) at each site was determined with a refractometer on each collection date.

To determine possible effects of clam filtration on water quality, samples of water (n=3) were obtained from both sites (control and experimental areas) every 4-6 weeks for determination of total suspended solids and total chlorophyll. Total suspended solids (mg l^{-1}) were calculated by filtering water (250 ml) through pre-weighed glass fiber filter; drying at 60 °C in a drying oven, and re-weighing. Total chlorophyll was determined using a Turner FluoroSense handheld fluorometer (see Appendix). This instrument has a linear range of 0-199 $\mu\text{g l}^{-1}$ *in vivo* chlorophyll and was calibrated at 0 and 100 $\mu\text{g l}^{-1}$ before each use.

At each sampling date, 20 clams were also collected from both sites and returned to the laboratory. Shell heights of clams were measured with hand-held calipers (0.1 mm). Clams from the South site were then weighed whole before being shucked for determination of wet tissue weight. Tissue and shells were then dried at 60 °C in a drying oven before being weighed for calculation of dry tissue weight and dry shell weight.

Clam survival rate after initial deployment was estimated based on counts (by volume) when clams were removed from the nursery bags and placed under 6 mm covernets and again by quadrat (0.25 m²) counts taken at the end of the study period.

Sediment cores (98 mm diameter) were taken three times throughout the study period. The first set was taken on December 16, 2016, two months before clams were planted at either site. Two cores were taken from bare sand and one was taken from adjacent seagrass beds at each site (6 cores total). On November 6, 2017, nine months after clams were planted, three cores were taken from experimental (with clam, under covernets) sections, two cores were taken from control (no clams, bare sand) sections, and one core was taken from adjacent seagrass beds at each site (12 cores total). A third set of cores was collected on August 15, 2018, eighteen months after clams were planted. On this date, three cores were taken from experimental sections (with clams, under covernets), three cores were taken from control (no clams, bare sand) sections and three cores were taken from adjacent seagrass beds at the South Site only (9 cores total).

Ten 2-mm depth intervals from each core were analyzed for grain size, percent carbonate and total organic content. Grain size was determined by initially wet sieving the sample through a 63 μm screen. The sand/gravel size fraction (>63 μm) fraction was analyzed with a settling tube (Gibbs

1974) and the percentage of each size interval within the sand/gravel fraction was calculated using the settling rate, based on Stoke's Law. The fine (<63 μm) fraction was determined by pipette (Folk 1965), which was also based upon settling rates. Data from both analyses were combined, and results were expressed as mean grain size (% gravel, % sand, % silt, % clay, and % mud). Calcium carbonate content was determined by an acid leaching method (Milliman 1974). A 10% hydrochloric acid solution was added to a pre-weighed sample. After all the calcium carbonate was dissolved, the sample was washed four times with distilled water and weighed again. The difference in weight represented the calcium carbonate fraction. Total organic matter (TOM) was determined by loss on ignition (Dean 1974). Approximately 1 g of insoluble residue (sediment remaining after acid leaching) was combusted in a muffle furnace at 550 °C for 4 hours. The sample was then re-weighed and the difference in weight from the initial weight represented the total organic matter.

Clams were planted near existing seagrass beds within each study area. On February 21, 2018, stakes were placed at the outer margins of seagrasses next to the clam nets at the South site so that over the second year of the project, observations could be made with respect to changes in seagrass distribution, density and composition.

Results

Clam samples and water samples were collected on 16 dates, from February 13, 2017 to August 15, 2018. Samples were collected at both North and South sites through October 27, 2017; after that date, clams at the North site were lost due to severe wind and wave action caused by Hurricane Irma and subsequent cold fronts moving through Tampa Bay. After October 2017, clam and water quality data was only collected at the South site, which was behind Manbirtee Key in slightly deeper water and thus protected from storm damage.

Clam growth

Overall, clams grew steadily for the first year after planting until mean shell length reached about 50 mm; afterwards growth rate was reduced (Figure 6). At the North site, mean shell length increased from 9.5 mm on February 13, 2017 to 39.9 mm on October 27, 2017. Mean shell length of clams at the South site increased from 9.5 mm on February 13, 2017 to 63.0 mm on April 19, 2018. At the South site, mean tissue wet weight ranged from 0.024 g on February 13, 2017 to 8.47 g on April 19, 2018. Mean tissue dry weight ranged from 0.005 g on February 13, 2017 to 1.56 g on June 29, 2018.

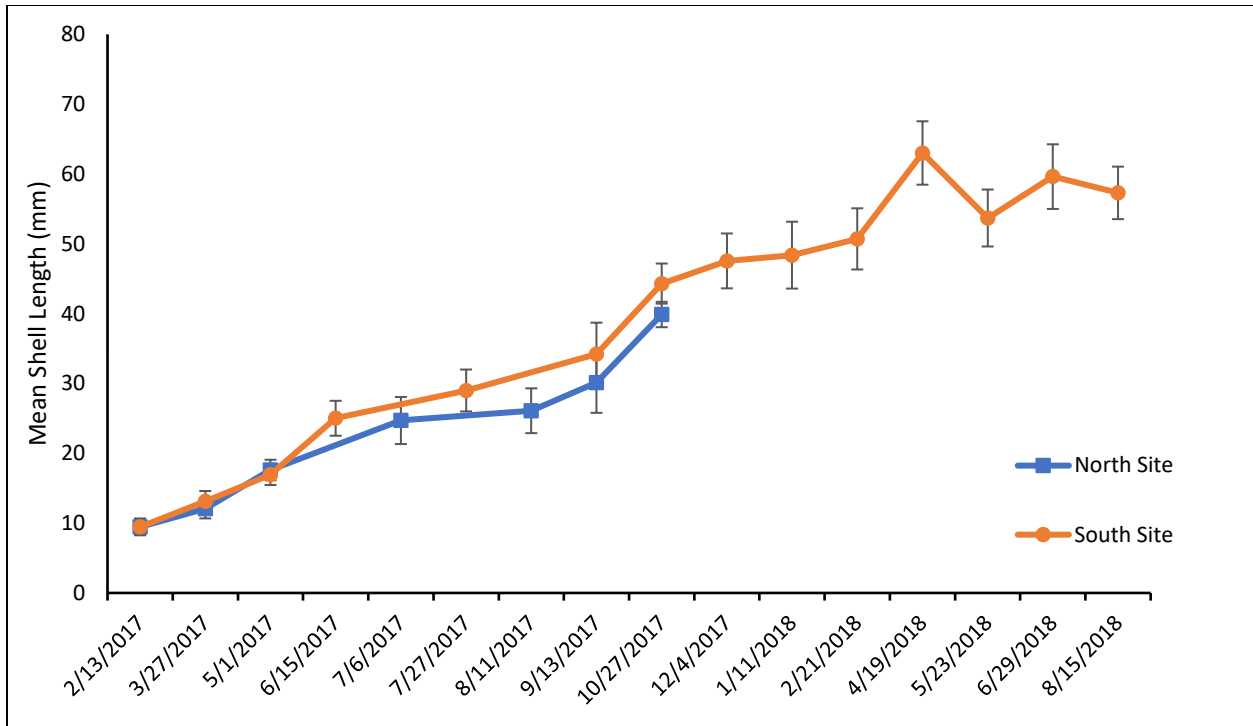


Figure 6. Mean shell height of clams at North and South sites from February 2017 to August 2018. Error bars are ± 1 SD; n=20.

Mean tissue weight (both wet and dry) was closely related to mean shell length (Figures 7 and 8). A clam with a shell length of 50 mm thus had a wet weight of 4.70 g and a dry tissue weight of 0.84 g.

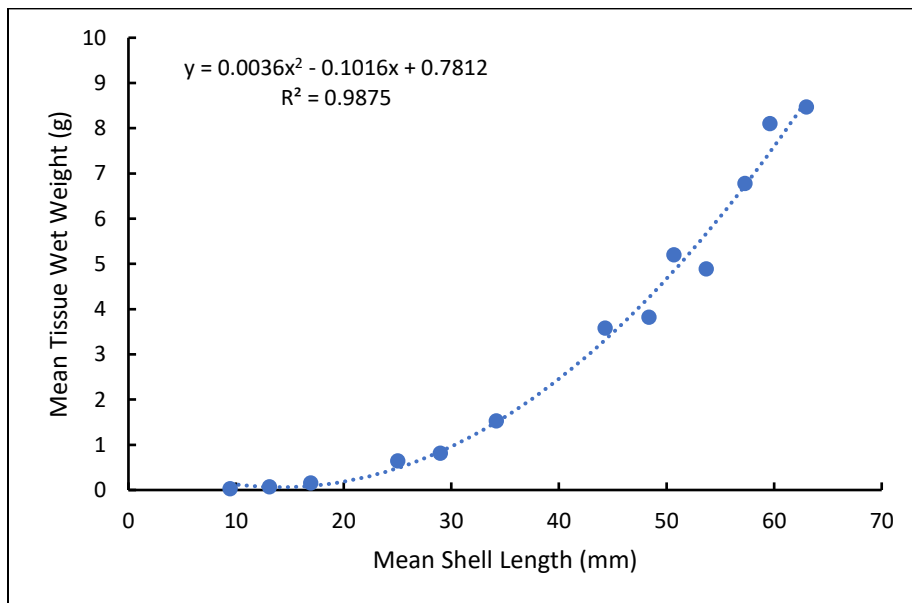


Figure 7. Mean tissue wet weight (g) as a function of mean shell length (mm) for clams from the South site (n=20).

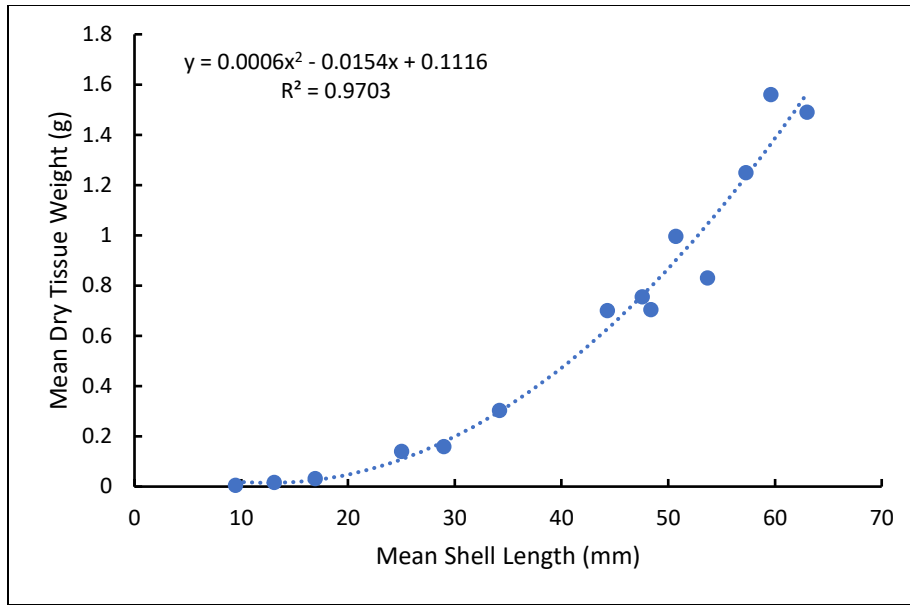


Figure 8. Mean tissue dry weight (g) as a function of mean shell length (mm) for clams from the South site (n=20).

Clam survival

Approximately 300,000 clams were initially planted at each site in nursery (4 mm) bags in February 2017. In May, when clams were transferred from nursery bags to 6 mm covernets at the South site, it was estimated that 233,000 clams remained (78% survival). When clams were transferred from nursery bags to 6 mm covernets at the North Site in July 2017, approximately 232,000 clams remained (77% survival). By October 2017, all the covernets were displaced and all the clams were lost at the North site due to a combination of physical damage, burial, and predation, as a result of Hurricane Irma and subsequent cold fronts (0% survival). At the South Site, it was estimated (based on quadrat counts of 752 clams m⁻²) that 221,389 clams were still living on July 18, 2018 (73% survival). These numbers are summarized in Table 2.

Table 2.
Counts of clams and survival rates of clams at the North and South Sites.

Date	North Site		South Site	
	Count	% Survival	Count	% Survival
02/13/2017			300,000	100
02/24/2017	300,000	100		
05/12/2017			233,000	77.7
07/06/2017	232,000	77.3		
10/27/2017	0	0		
07/18/2018			221,389	73

Water quality

Only two of the original four dataloggers were recovered at the end of the study. However, continuous water temperature data was obtained from both the North (clam) location and the South (no clam) location. As shown in Figure 9, daily mean water temperature was similar at both sites, ranging from 13.09 °C on January 18, 2018 to 33.32 °C on August 17, 2017 at the North site and 12.88 °C on January 18, 2018 to 33.28 °C on August 16, 2017 at the South site. There was very little difference in temperature between the two sites; the overall mean water temperature was 26.18 °C at the North site and 26.07 °C at the South site.

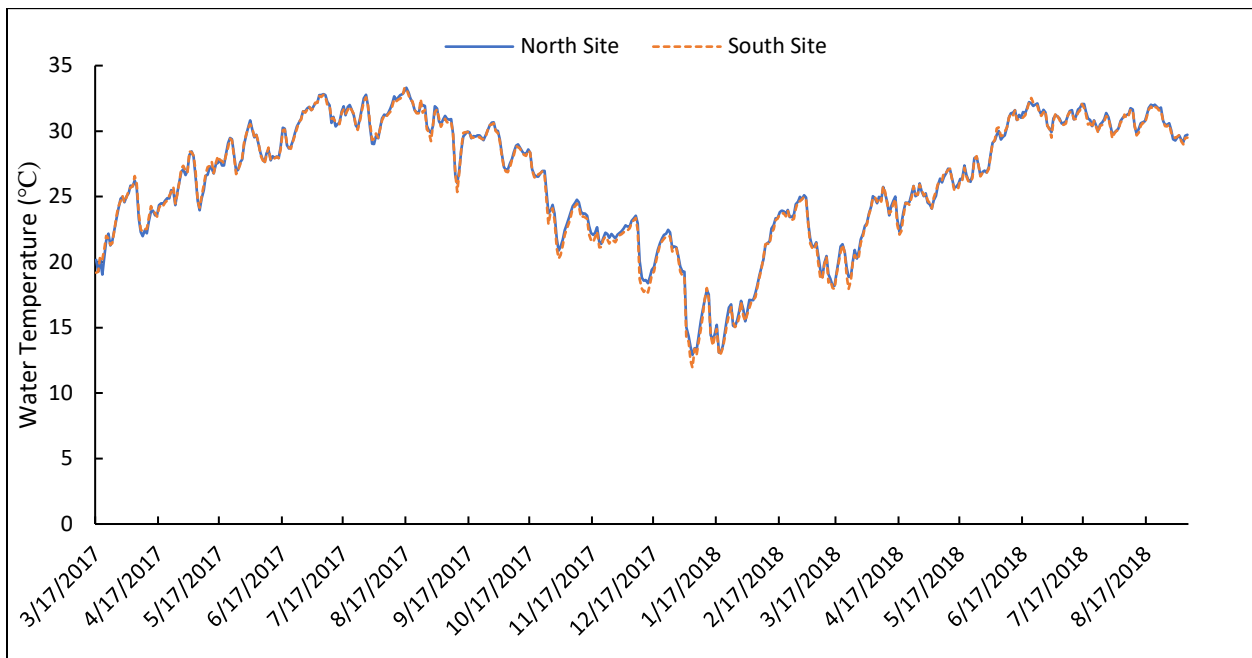


Figure 9. Mean daily water temperature (°C) at the North and South sites between March 17, 2017 and September 6, 2018.

Salinity was also similar at both sites, ranging from 26.5 psu on September 13, 2017 (three days after Hurricane Irma) to 35 psu on May 23, 2018 (Figure 10).

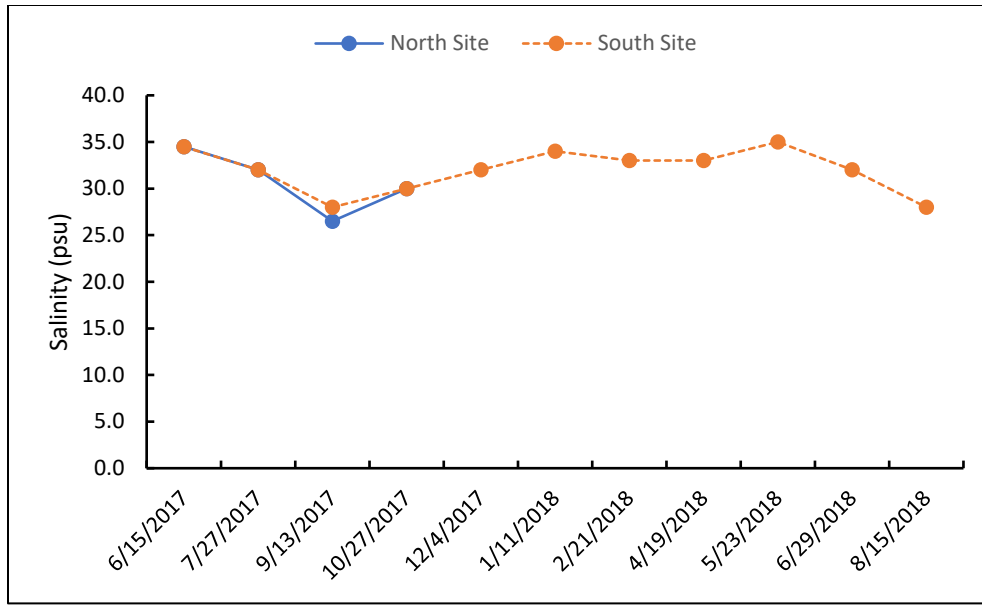


Figure 10. Salinity (psu) at both the North and South sites from May 15, 2017 to August 15, 2018.

Mean total suspended solids ranged from 1.2 mg l⁻¹ at the North site on March 27, 2017 to 34.9 mg l⁻¹ at the South site on June 15, 2017 (Figure 11). There was no consistent difference between North and South sites from March 27, 2017 through October 27, 2017. At the South site, suspended solids were consistently greater in the experimental (clams) area than the control (no clams) area.

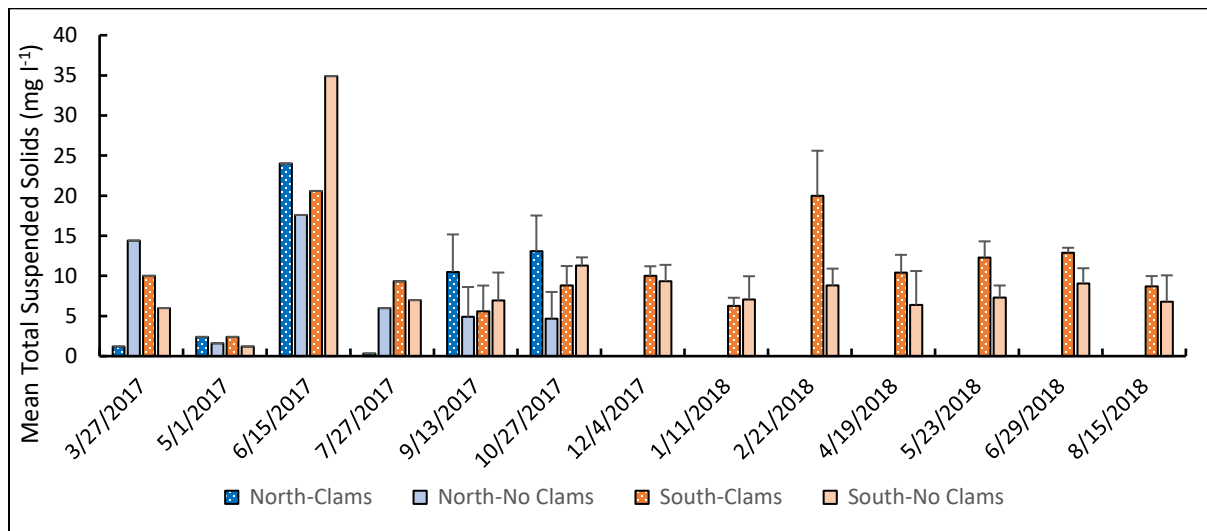


Figure 11. Mean total suspended solids (mg l⁻¹) from control (no clams) and experimental (clams) areas at the North and South sites from March 27, 2017 to August 15, 2018.

N=3; error bars are +1 SD.

Total chlorophyll increased from a minimum value of 2.0 µg l⁻¹ on March 27, 2017 at both the North and South sites to maxima of 38.3 µg l⁻¹ at the North site and 17.3 µg l⁻¹ at the South site

on September 13, 2017 (Figure 12). This was the only date on which chlorophyll values were significantly (t-test, $p \leq 0.007$) different between sites. There was no consistent difference in chlorophyll between control (no clams) and experimental (clams) areas within each site.

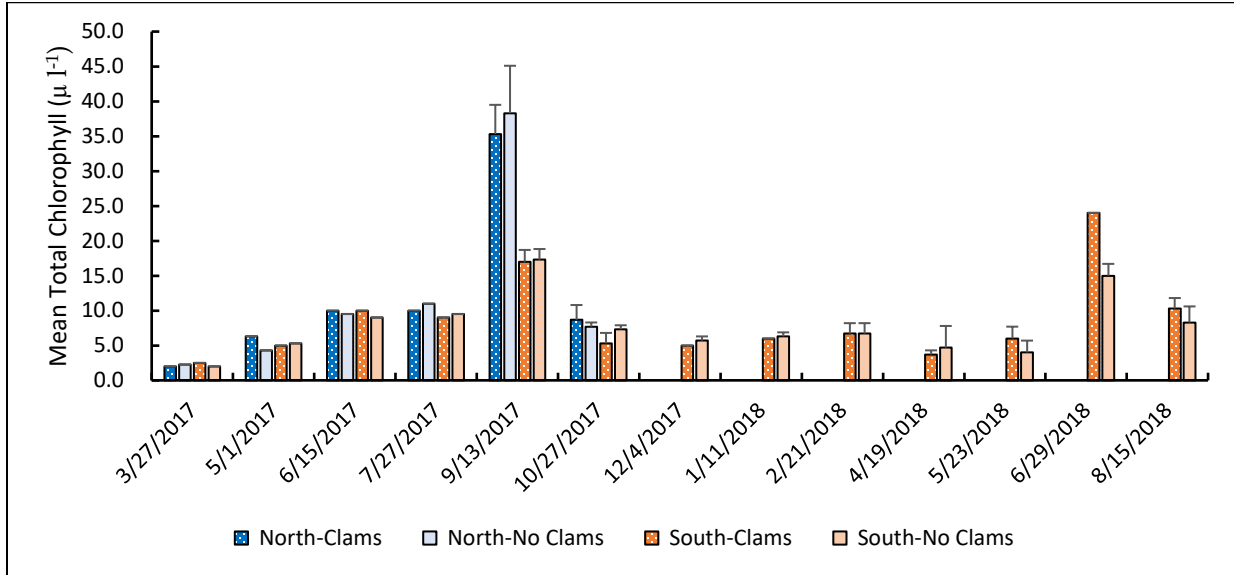


Figure 12. Mean total chlorophyll ($\mu\text{g l}^{-1}$) from control (no clams) and experimental (clams) areas at the North and South sites from March 27, 2017 to August 15, 2018.

N=3; error bars are +1 SD.

Sediment quality

Mean percent grain size and mean percent carbonate content for cores taken from sand, seagrass and clam beds are provided in Table 3. The “gravel” size fraction ranged from 0.004% to 1.13% and was generally greatest in cores taken from seagrass beds. All cores were predominantly composed of sediment in the “sand” size fraction (96.7% to 99.0%). The “silt” size fraction ranged from 0.54% to 1.48%; the “clay” size fraction ranged from 0.01% to 1.53%; and the “mud” size fraction ranged from 0.61% to 2.72%. On February 16, 2016 (before clams were planted), both clay and mud fractions were greater in the cores from seagrass beds than bare sand. On both November 6, 2017 and August 15, 2018, the clay and mud fractions were greatest in cores taken from clam beds (Table 3).

Table 3.

Percent mean grain size and mean percent carbonate for replicate cores (0-20 mm depth) taken from control areas (no clams), experimental areas (clam beds), and seagrass beds at both North and South Sites during the study period. Note: clams were deployed in February 2017.

Date		Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Mud (%)	Carbonate (%)
2/16/2016							
	Sand (n=4)	0.48	97.69	1.48	0.35	1.83	7.39
	Seagrass (n=2)	0.52	96.76	1.5	1.22	2.72	9.51
11/6/2017							
	Sand (n=4)	0.004	98.42	0.91	0.81	1.73	7.18
	Clam (n=6)	0.048	97.83	0.59	1.53	2.09	8.66
	Seagrass (n=2)	0.45	98.31	1.25	0.34	1.28	10.7
8/15/2018							
	Sand (n=3)	0.35	99.03	0.54	0.07	0.61	3.96
	Clam (n=3)	0.86	97.66	0.82	0.66	1.48	5.4
	Seagrass (n=3)	1.13	97.98	0.87	0.01	0.88	6.8

Percent total organic matter (TOM) in sediment was generally lowest in cores taken from control areas (sand) and showed little variation with depth or over time (Figures 13, 14 and 15). Cores taken from seagrass beds had the greatest percentage of organic matter, especially near the surface. On February 16, 2016 and August 15, 2018, mean TOM was significantly greater in cores taken from seagrass beds than control (bare sand) areas (ANOVA, $p \leq 0.03$). Cores taken from experimental areas (clam beds) generally had greater percent TOM than control areas throughout the depth of the entire core. In addition, the percent TOM in cores taken from clam beds (averaged for all depths) increased from an average of 0.41% on November 6, 2017 to 0.61% on August 15, 2018, approaching that of seagrass (Figure 16). Compared to control areas (sand), the mean TOM content of sediment in experimental areas (clam beds) was 37% greater on November 6, 2017 (9 months after clams were planted) and 126% greater on August 15, 2018 (18 months after clams were planted). On August 15, 2018, percent TOM was significantly greater in cores taken from clam beds than bare sand (ANOVA, $p \leq 0.02$).

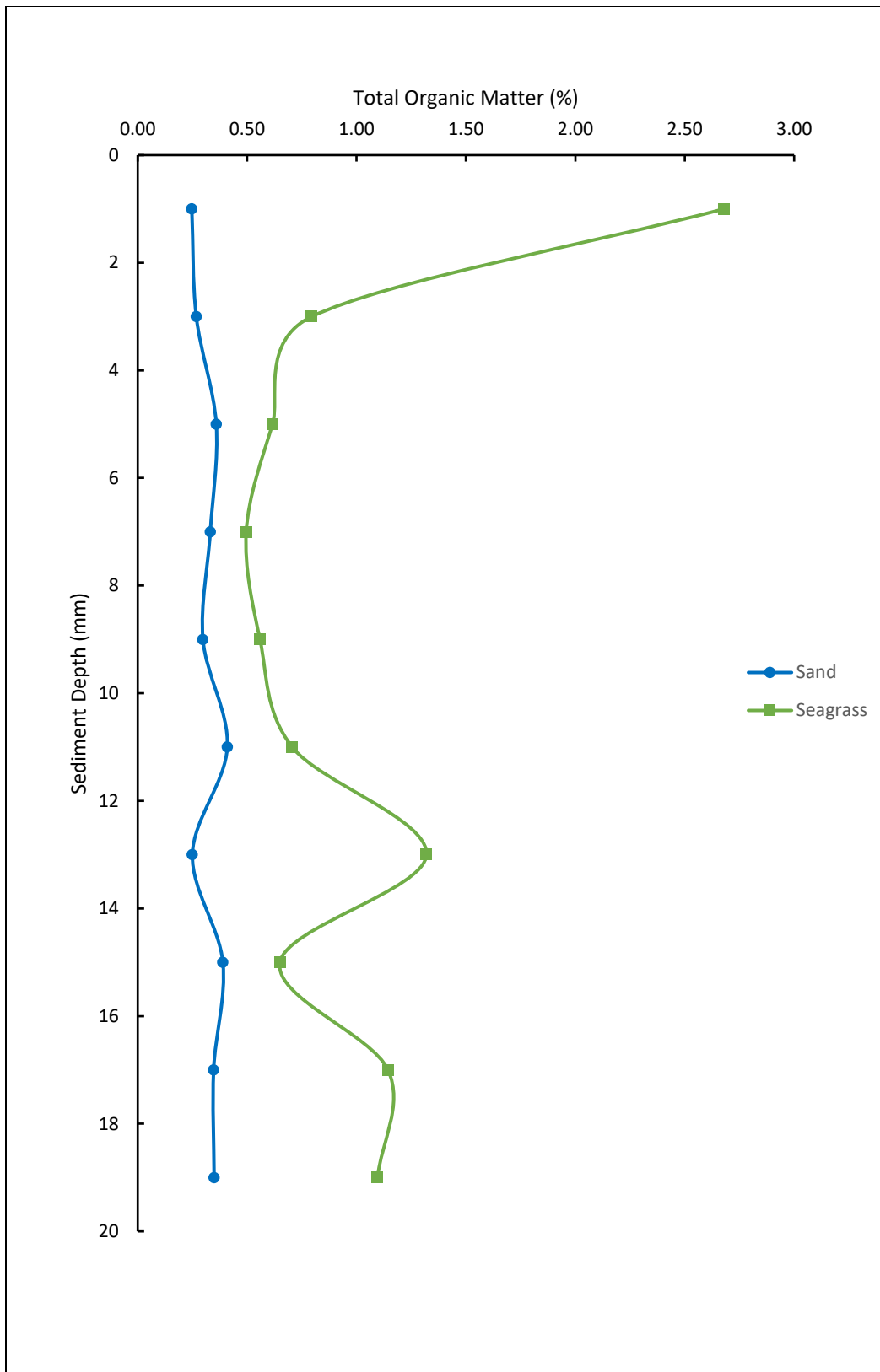


Figure 13. Mean total organic matter (%) in cores collected on January 16, 2016 from sand (n=4) and seagrass areas (n=2).

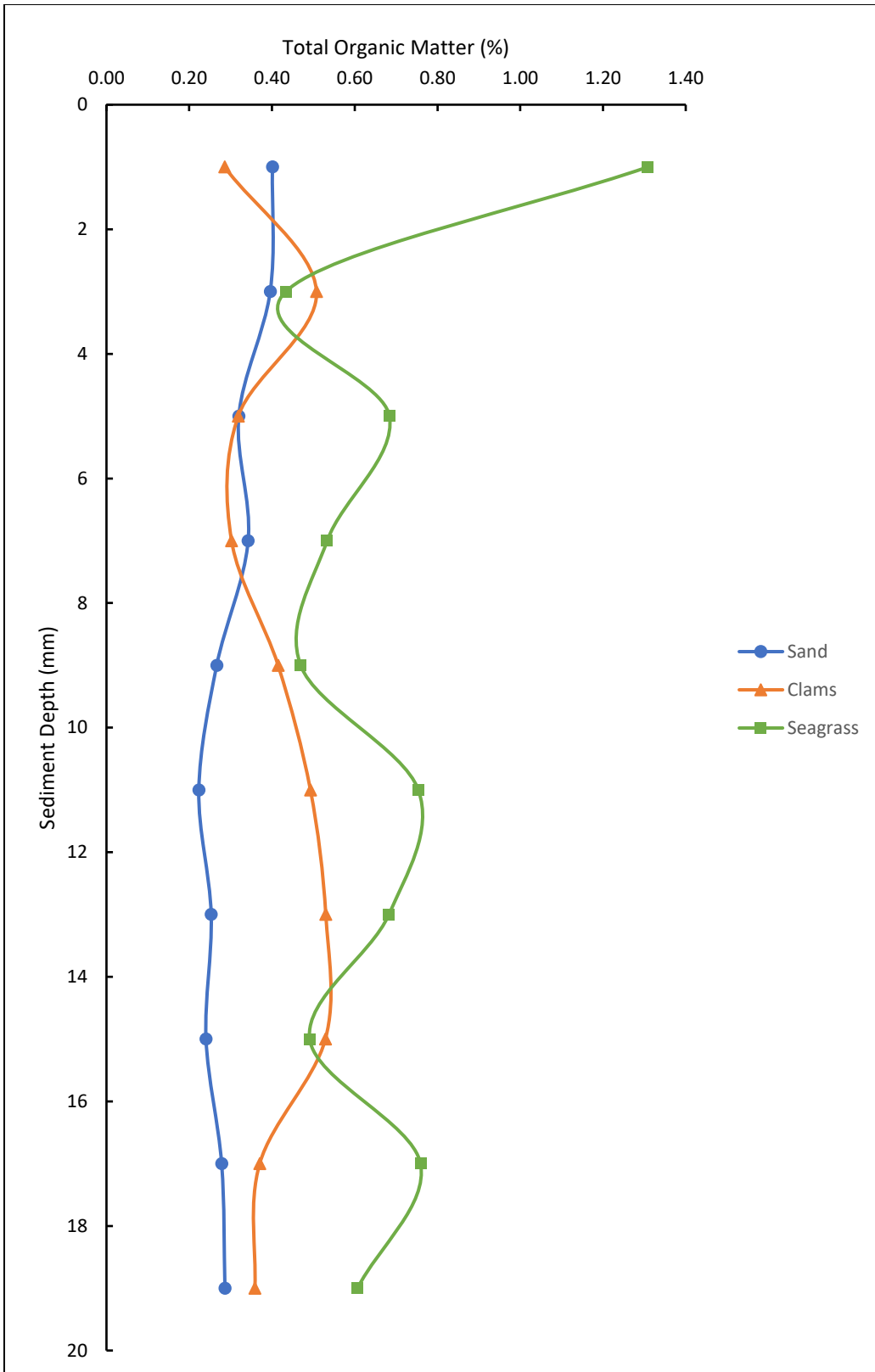


Figure 14. Mean total organic matter (%) in cores collected on November 6, 2017 from sand (n=4), clam beds (n=6) and seagrass beds (n=2).

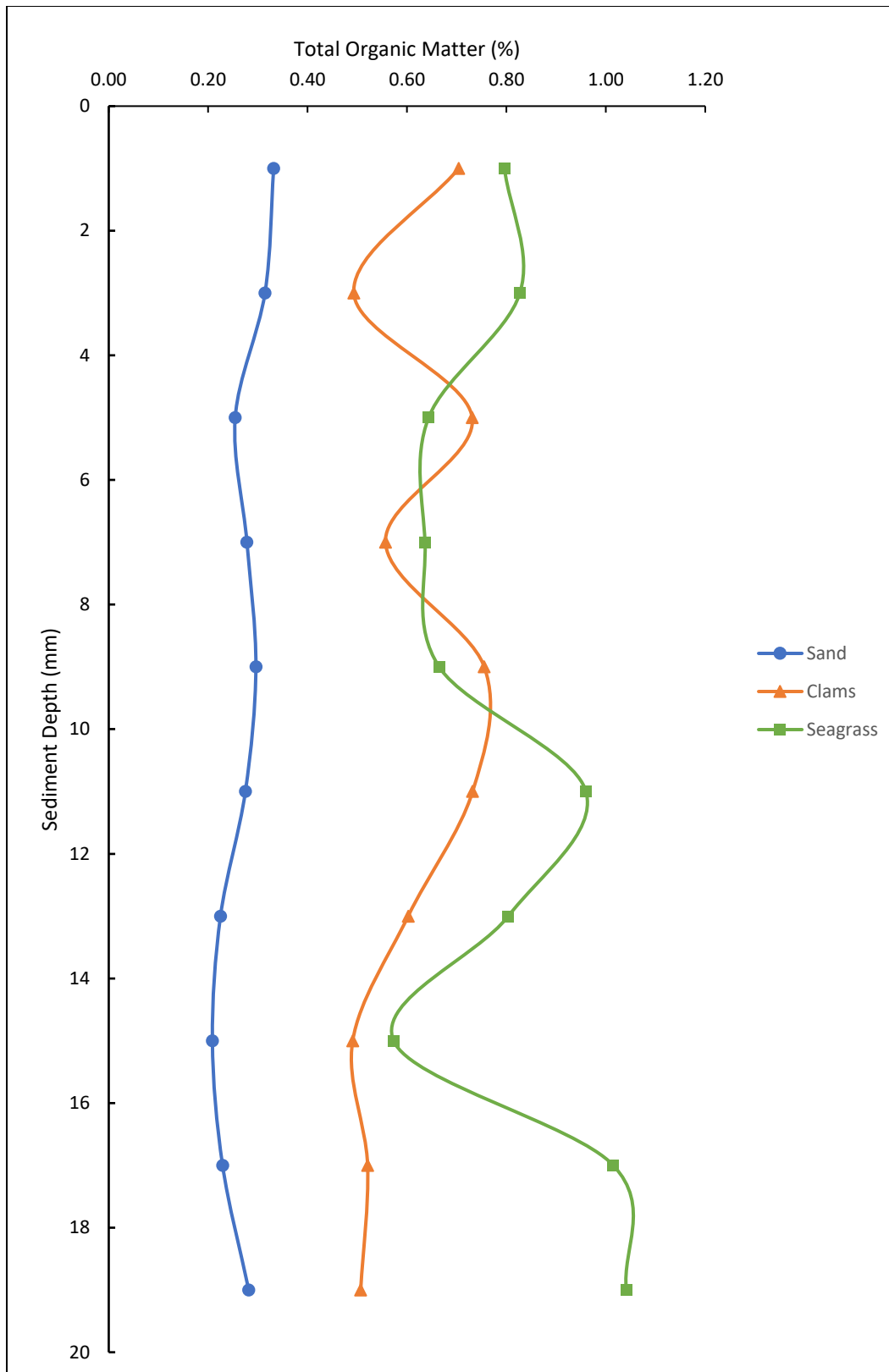


Figure 15. Mean total organic matter (%) in cores collected on August 15, 2018 from sand (n=3), clam beds (n=3) and seagrass beds (n=3).

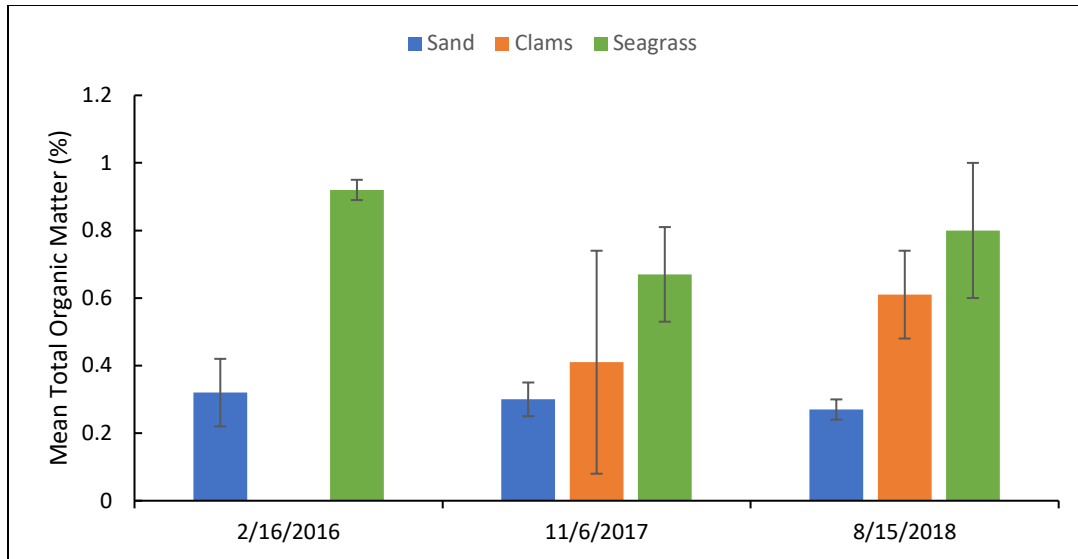


Figure 16. Mean percent total organic matter (average of all depths) from replicate cores (n=2-6) taken from three habitats on February 16, 2016 (before clams were planted); November 6, 2017 (nine months after clams were planted); and August 15, 2018 (18 months after clams were planted). Error bars are ± 1 SD.

Seagrass distribution

Clams were placed on bare sand at both the North and South sites. After clams were initially deployed at each site, we occasionally observed a few shoots of seagrass growing up through the nets (Figure 17). We also noted movement of seagrasses from adjacent established beds toward clam nets during the second year at the South site. A few shoots of seagrass from adjacent beds were observed inside of stakes marking the former edge of the bed (Figure 18). There was, however, no statistically quantified increase in seagrass distribution relative to control sites during the study period.



Figure 17. Seagrass shoots (*Thalassia testudinum*) growing through a covernet at the South Site.



Figure 18. Seagrass shoots (*Thalassia testudinum*) growing inside (to the left) of a stake marking the former edge of a seagrass bed adjacent to clam nets at the South Site.

Discussion and Deliverables

Average size and tissue weight of clams

Growth of clams depends on several factors, including water temperature, salinity, food availability, habitat and density. In this study, broodstock clams were spawned on November 7, 2016. Clam seed averaged 9.5 mm in shell length when deployed to the experimental areas of

both the North and South study sites in February 2017. Maximum mean shell height at the South Site was 63 mm on April 19, 2018, fourteen months after planting. During this same period, mean wet tissue weight increased from 0.024 g to 8.47 g and mean dry tissue weight increased from 0.005 g to 1.56 g. The growth rate of clams in this study was greater than that reported in previous studies. Menzel (1989) compared published growth rates of clams (*Mercenaria* spp.) from several locations (Canada to Florida) and found that growth rate increased with decreasing latitude. The number of days from spawning to 50 mm in clams grown in northwest Florida was 570; in this study (lower Tampa Bay), clams reached a mean shell length of 50 mm in 465 days.

Clam survival

Most shellfish restoration projects involve planting shell to provide substrate for oyster recruitment, releasing larvae, releasing seed, or planting adults to increase reproductive effort (Arnold 2001, Doall et al. 2008). Rarely do these efforts include a means to protect the bivalves from predation, and as a result most have failed or been unable to document success (Baggett et al. 2015). Unlike previous efforts, we sought to maximize survival of clams so that they could provide the ecological services we were seeking to quantify. Our approach included standard methods utilized in the clam farming industry for predator protection (i.e., the nursery bags; followed by free planting under 6 mm mesh covernets; followed by 12 mm covernets). At the end of the two-year study period, 221,389 clams out of the original 300,000 planted at the South Site were still alive. The survival rate of 73% at the South site is considerably higher than the 5% survival rate of unprotected clams planted in unvegetated sand areas in North Carolina reported by Peterson et al. (1995) but is within the upper range of survival reported by Kraeuter and Castagna (1989) for clams protected by netting. At the end of the study, when covernets were removed, the average size of clams was about 60 mm, making them resistant to all but the largest predators, including whelks, rays and crabs (Kraeuter and Castagna 1989).

Ecological Services

Clams, like other marine bivalves, process large volumes of water for the extraction of oxygen and food. Cilia on gills draw water into the mantle cavity and between gill filaments where suspended particles are trapped for ingestion and oxygen diffuses into the hemolymph. The rate of filtration (or clearance) depends on clam size, water temperature, food availability, currents and other factors. For clams at the South site, we calculated the total volume of water filtered, the total amount of suspended solids removed, and the total amount of chlorophyll removed. Hourly filtration rates were used to calculate daily rates by multiplying by 18 (rather than 24) for the following reasons: 1) it is unlikely that clams filter continuously throughout the day; and 2) published filtration rates for *Mercenaria mercenaria* were obtained at 28 °C (Riisgard 1988), which is greater than the temperatures recorded at Port Manatee for all but the summer months. Mean tissue dry weights, mean suspended solids and mean chlorophyll data were incorporated

for each sampling period. The number of surviving clams was estimated based on a linear decrease from the initial number (300,000 on February 13, 2017) to the final count (221,389 on August 31, 2018).

Similarly, the culture of marine bivalves for their “nutrient bioextraction” capacity has been proposed by Higgins et al. (2012), Carmichael et al. (2012), Kellogg et al. (2014), Rose et al. (2014), and Reitsma et al. (2016) as a possible way to mitigate coastal eutrophication. Particulate organic matter (containing nitrogen, carbon and phosphorus) is removed from the water column by bivalve filtering activity; some is rejected as pseudofeces and deposited in the sediment; and some is ingested, absorbed and incorporated into biomass (tissue and shell). Once assimilated by clams, these nutrients are no longer available to support phytoplankton growth.

Volume of water filtered by clams

Since there are no seasonal estimates available for filtration rates of local bivalves, estimates presented here are based on Riisgard (1988), who determined the filtration rate of *M. mercenaria* from Georgia at 28 °C. For clams having dry tissue weights (W) ranging from 0.017 g to 2.387 g, filtration was allometrically related to tissue dry weight by the following equation:

$$F = 1.24 W^{0.80}$$

Using this relationship, filtration rates for clams from the South site were calculated as a function of dry weight (Figure 19).

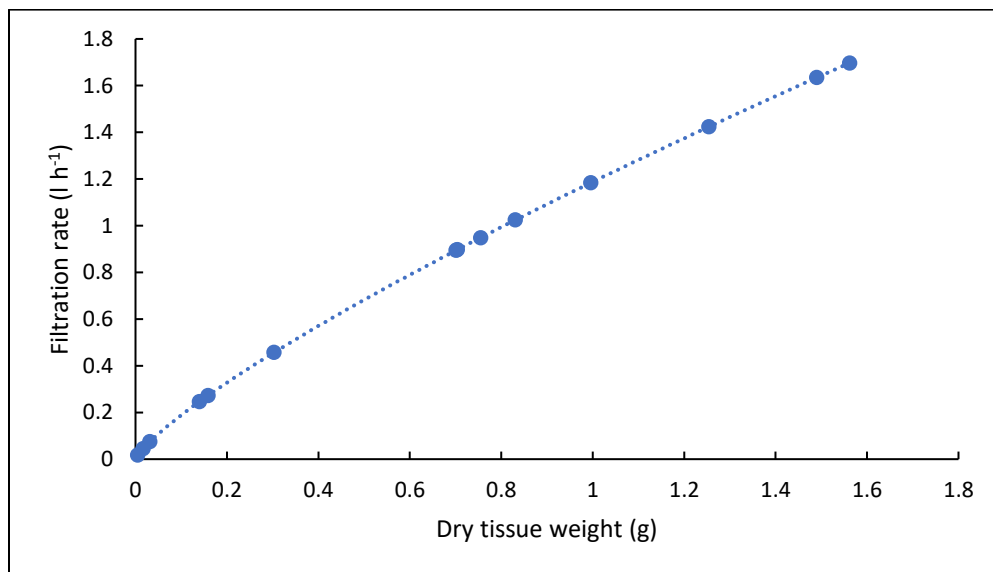


Figure 19. The relationship between filtration rate (l h⁻¹) and dry tissue weight of clams (based on Riisgard, 1988).

Accordingly, the total volume of water filtered by clams at the South site from February 13, 2017 through August 31, 2018 was determined to be 1.84 million m³ (Figure 20). The volume of water filtered increased steadily throughout the study, as increase in clam dry tissue weight more than offset clam mortality. At the end of the study period, clams were filtering an estimated 19,224 l m⁻² d⁻¹.

The estimates of total water filtered (and total suspended solids and chlorophyll removed from the water column) presented here were based on clams maintaining a constant rate of filtration for 18 hours per day, rather than 24 hours per day. Filtration rates of bivalves are determined over a relatively short time period in a laboratory at a constant temperature. Thus our knowledge of *in situ* filtration rates over daily and seasonal timeframes is severely limited.

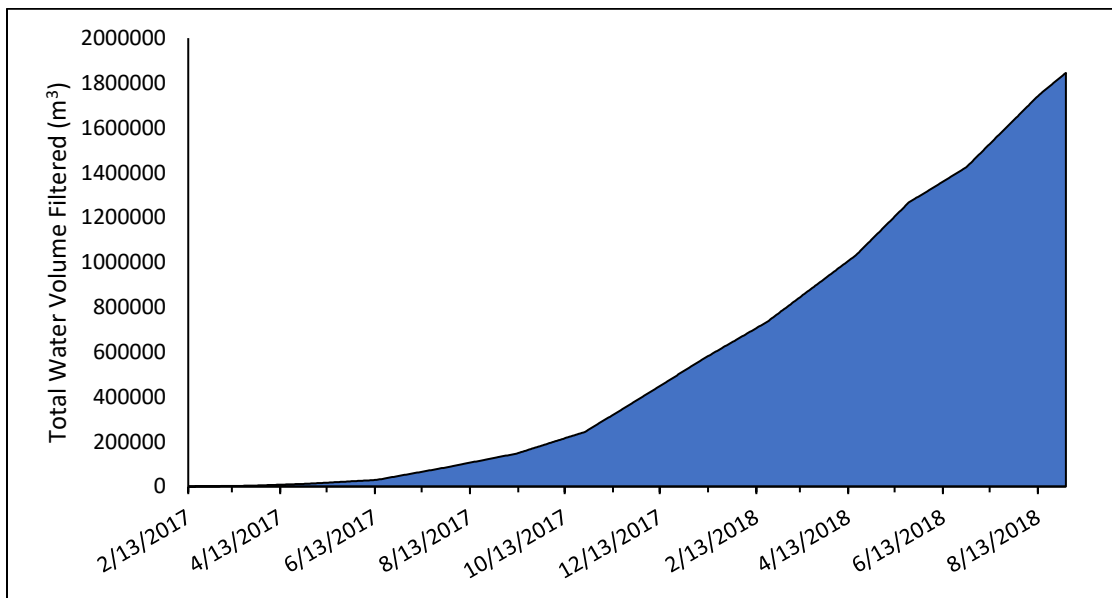


Figure 20. Total water volume (m³) filtered by clams at the South site between February 13, 2017 and August 31, 2018.

Total suspended solids removed

The total suspended solids removed by clams at the South site was calculated by multiplying mean suspended solids values (mg l⁻¹) by daily filtration rate (l day⁻¹). Total cumulative suspended solids removed by clams at the South site from February 13, 2017 through August 31, 2018 was thus determined to be 19,147 kg (Figure 21). At the end of the study period, clams were removing suspended solids at an estimated rate of 192 g m⁻² d⁻¹.

The assumption here is that clams are capable of removing all particles greater than 1.5 μm, which is the pore size of the glass fiber filters used to quantify total suspended solids in this study. According to Riisgard (1988), however, *M. mercenaria* removed 100% of particles

greater than 4 μm , but retention declined to 50% for 2 μm particles. Particle size distribution was not determined as part of this study, so we cannot estimate what portion of the total suspended solids was removed by clams. Given that retention was less than 100%, however, the estimate of total suspended solids removed presented here is probably high.

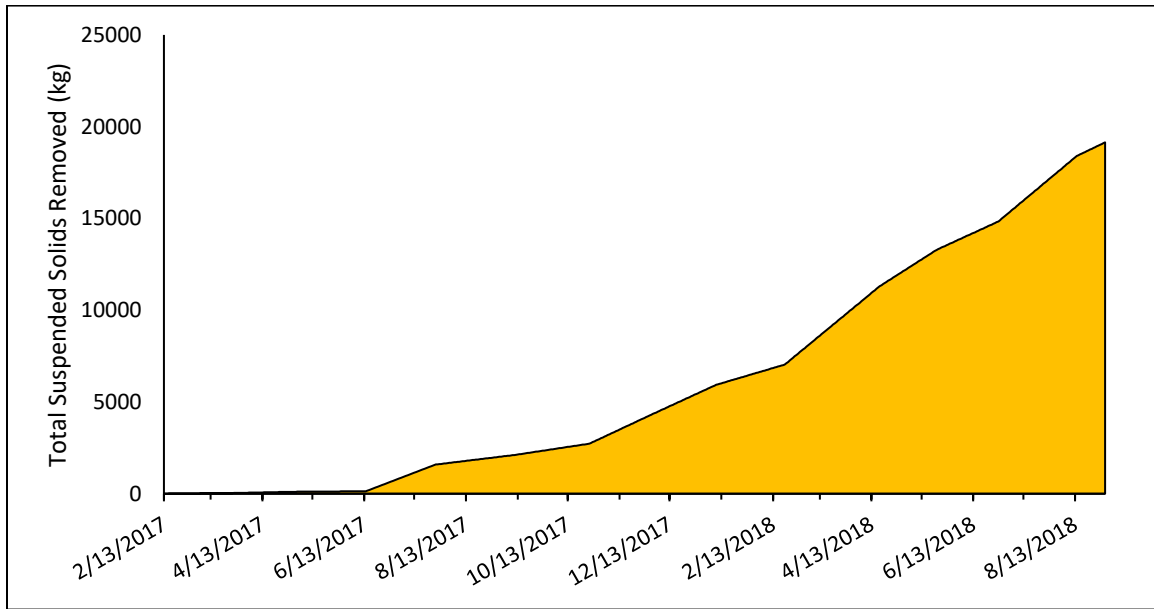


Figure 21. Total suspended solids (kg) removed by clams at the South site between February 13, 2017 and August 31, 2018.

Removal of suspended material from the water column by filter feeding bivalves decreases turbidity and reduces the attenuation of sunlight, which benefits benthic plants such as seagrasses (Newell and Koch, 2004). The fact that there was no difference in suspended solids seen between control (no clams) and experimental (clams) areas is not surprising given that the volume of water constantly moving over the site is much greater than that filtered by clams.

Total chlorophyll removed

The total amount of chlorophyll removed by clams at the South site was determined by multiplying mean chlorophyll values ($\mu\text{g l}^{-1}$) by daily filtration rate (l day^{-1}). Total cumulative chlorophyll removed by clams at the South site from February 13, 2017 through August 31, 2018 was estimated to be 16.8 kg (Figure 22). At the end of the study period, clams were removing chlorophyll at an approximate rate of $156 \text{ mg m}^{-2} \text{ d}^{-1}$.

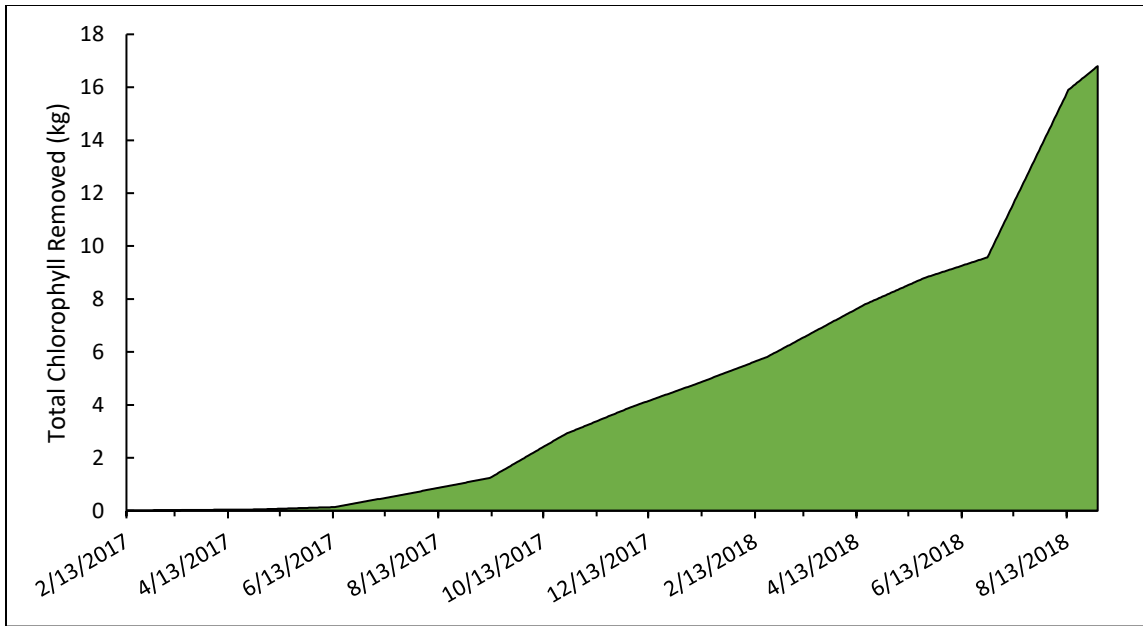


Figure 22. Total chlorophyll (kg) removed by clams at the South site between February 13, 2017 and August 31, 2018.

Most chlorophyll was contained in living phytoplankton cells, which comprises the primary diet of clams. Even though clams were continuously removing particulate chlorophyll (phytoplankton) from the water column, no difference was detected in chlorophyll levels between control (no clams) and experimental (clams) areas. Again, this is understandable given the large volume of water constantly moving throughout the bay relative to the volume filtered by clams.

Total nitrogen removed

There are few published reports of the nitrogen content of clam (*Mercenaria* spp.) tissue and shell and no information exists for local bivalve species. To calculate the amount of nitrogen removed from lower Tampa Bay by clams at the South site, we multiplied the mean dry tissue and shell weights obtained in this study by the nitrogen content of *M. mercenaria* tissue (7.69%) and shell (0.18%) reported by Reitsma et al. (2016), and then multiplied that by the number of surviving clams. As clams grew, the amount of nitrogen sequestered in both tissue and shell generally increased (Figure 23). At the end of the study, there were 21 kg of nitrogen sequestered in clam tissue and 14 kg of nitrogen sequestered in clam shell for a total of 35 kg of nitrogen in all clams at the South site.

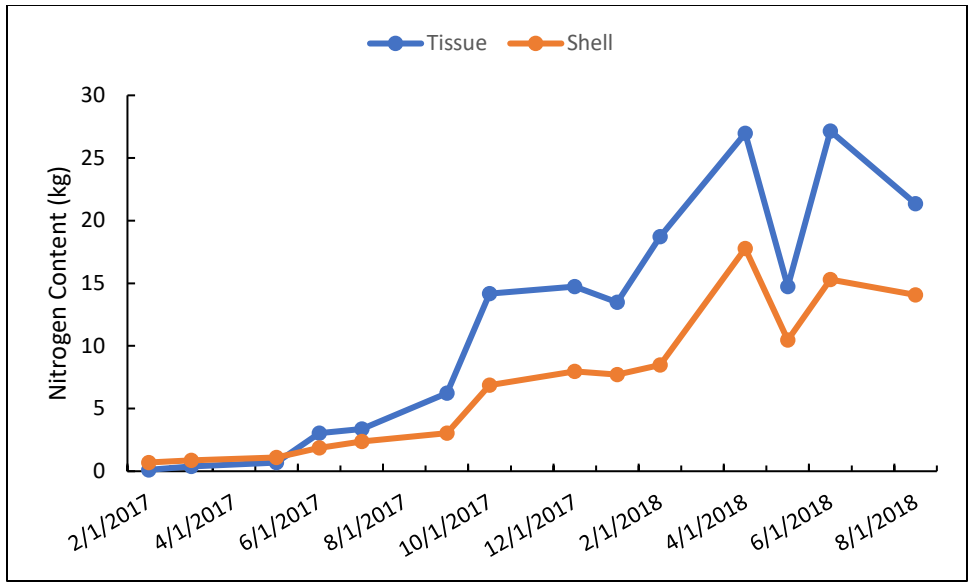


Figure 23. Amount of nitrogen sequestered in clam tissue and shell (kg) at the South site between February 13, 2017 and August 15, 2018.

Using these estimates for nitrogen content, we can also determine the amount of nitrogen sequestered in clams (tissue and shell combined) as a function of shell length (Figure 24). Thus a clam of 50 mm shell length would contain 0.12 g of nitrogen. When clams are harvested, the nitrogen they have sequestered in their tissue and shell is permanently removed from the system.

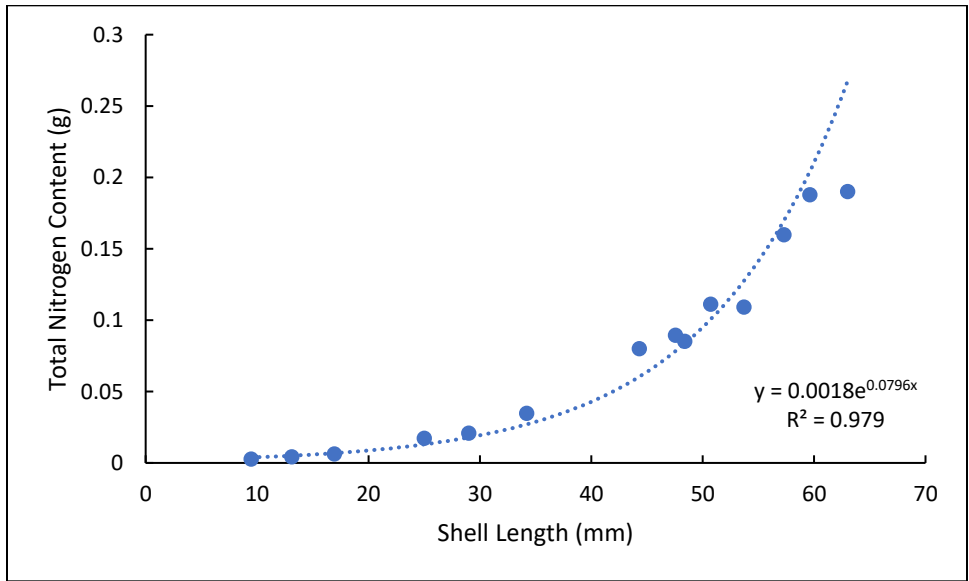


Figure 24. Total nitrogen content (g) of clams (*Mercenaria* spp.) as a function of shell length (mm).

After one year at the South site, clams had a mean shell length of 50 mm. At a density of 752 clams m^{-2} , the amount of nitrogen sequestered in tissue and shell was 86 g N m^{-2} year $^{-1}$. This is

well within the range of nitrogen removal of $12 \text{ g m}^{-2} \text{ year}^{-1}$ to $152 \text{ g m}^{-2} \text{ year}^{-1}$ modeled by Rose et al. (2015) based on estimates from shellfish farms from 14 locations in nine countries.

In addition to the amount of nitrogen sequestered in clam tissue and shell, it has been found that clam aquaculture can also significantly increase denitrification (Murphy et al. 2016). The amount of N_2 produced via denitrification processes, however, was not determined as part of this study.

Total phosphorus removed

There are even fewer published estimates of phosphorus content of bivalve tissues, and none for *Mercenaria* spp. Here we used the phosphorus content for the soft-shell clam, *Mya arenaria*, of 0.28% (tissue and shell combined) reported by Kellogg et al. (2013). Using this estimate, the amount of phosphorus sequestered in clams at the South site increased over time from 1 kg to 22.6 kg at the end of the study (Figure 25). These estimates may be high, however, since *M. arenaria* has a much thinner, lighter shell than that of *M. campechiensis*.

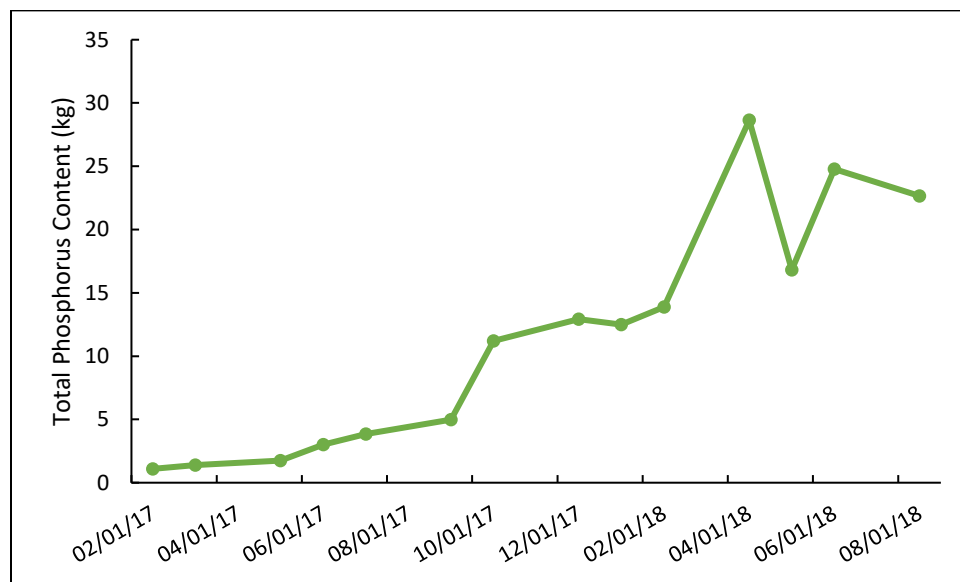


Figure 25. Amount of phosphorus sequestered in clam tissue and shell (kg) at the South site between February 13, 2017 and August 15, 2018.

The relationship between shell length and total phosphorus content for individual clams is shown in Figure 26. A 50 mm clam would thus contain 0.055 g of phosphorus in its tissue and shell combined.

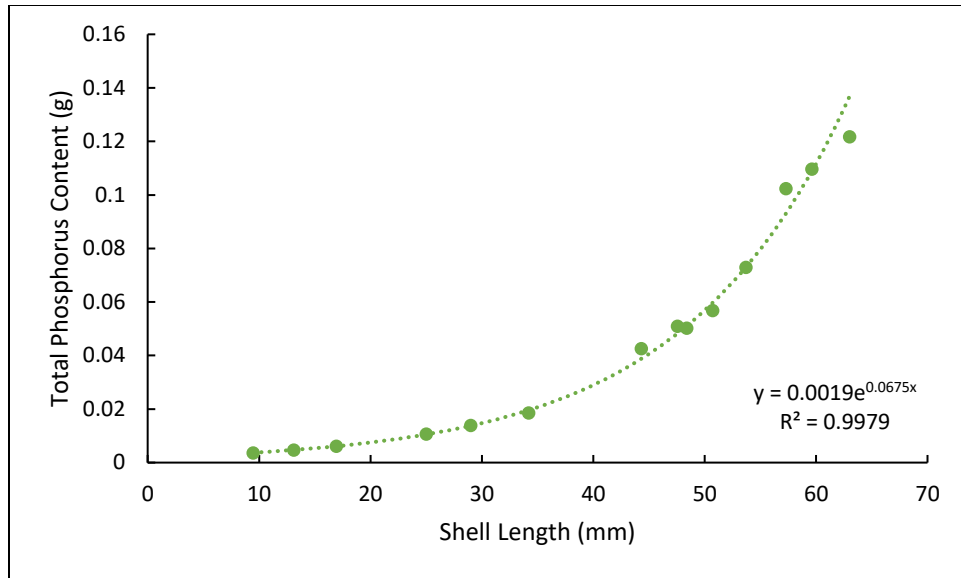


Figure 26. Total phosphorus content (g) of clams (*Mercenaria* spp.) as a function of shell length (mm).

The total impact of the ecological services provided by the clams produced, planted and maintained at Port Manatee as part of this study would have been considerably greater if the clams at the North site had not been lost. Prior to being lost, however, they were performing similar services from February through October 2017, thus adding considerably to the totals reported above for the South site.

Sediment quality

As a result of their filtration and feeding activity, clams effectively transferred suspended particulate organic matter to the sediments (benthic-pelagic coupling). Organic matter that was not ingested was deposited as pseudofeces; organic matter that was not assimilated into growing clams was deposited as feces. This process was supported by the sediment data collected in this study. The experimental area (with clams) had a greater percent TOM than control (sand) areas. As clams grew, the percent TOM in sediments from the experimental area increased, becoming significantly greater than that from control areas and more like that found in adjacent seagrass beds. Along with an increase in TOM, sediment from clam beds also had a greater percent of smaller grain size fractions (clay and mud), which also increased over time.

The ability of filter feeding bivalves to alter the geochemical characteristics of benthic habitats, as documented in this study, has been noted elsewhere. Bendel-Young (2006) compared intertidal areas in British Columbia that were used for farming the Manila clam (*Venerupis philippinarum*) with areas that were not used for farming and found that the areas that were actively farmed had a greater accumulation of organic matter and silt. The accumulation of organically enriched sediment in areas having commercial densities of clams may be enhanced

by protective netting (Spencer et al. 1997, Kaiser et al. 1998). Similarly, Nugues et al. (1996) documented changes in the benthic infaunal community associated with an increase in organic content and silt in sediments under trestles used for cultivation of Pacific oysters, *Crassostrea gigas*, in England.

Impacts on seagrass

In some situations an alteration of benthic habitat due to shellfish farming might be considered a negative consequence, but in this case, the organic enrichment of sediment by clams is a potential precursor to enhanced seagrass productivity in the region. As demonstrated by Peterson and Heck (1999, 2001a, 2001b), infaunal filter feeding bivalves convert particulate organic nitrogen and phosphorus in the water column to elevated sediment nutrient levels that are available for absorption by seagrasses.

We did not observe any notable increase in seagrass distribution adjacent to clam nets in this relatively short-term experiment. The few shoots that moved inside of stakes placed in February 2018 could be the result of normal seasonal expansion of seagrass coverage. On the other hand, the positive increases in sediment organic content documented here may be a precursor to future expansion of local seagrass beds in the area that will not be realized for several years. This aspect certainly warrants continued observation.

Clam reproduction

Obviously surviving clams will continue providing ecological benefits well into the future. One aspect that was not addressed in this study was the potential for clams to reproduce and increase recruitment of clams throughout lower Tampa Bay. By the spring of 2017, clams were large enough to begin producing gametes and spawning. Surviving clams should continue to reproduce annually throughout their life spans of up to 28 years (Jones et al. 1990).

Summary

Approximately 600,000 juvenile southern quahogs, *Mercenaria campechiensis*, were planted in February 2017 at two sites in lower Tampa Bay near Port Manatee. Water quality parameters (temperature, salinity, suspended solids, chlorophyll) were measured throughout the study period (ending August 31, 2018) in experimental (containing clams) and control (no clams) areas. Clams were sampled on a regular basis and shell length, whole weight, tissue wet weight, tissue dry weight, dry shell weight data were recorded. In addition, sediment cores were taken before clams were planted; nine months after clams were planted; and 18 months after clams were planted to compare sediment characteristics (% grain size, % carbonate, % total organic matter) among control, experimental and seagrass areas.

Clams at the North site were lost in September and October 2017 due to storm damage from Hurricane Irma and subsequent cold fronts. Survival at the South site was estimated to be 73% at the end of the study period. Growth rate was excellent; clams reached an average of 50 mm in shell length 465 days after spawning. Daily mean water temperature ranged from 55.2 °F to 91.9 °F; minimum salinity was 26.5 psu and maximum salinity was 35 psu. Total suspended solids were between 1.2 mg l⁻¹ and 34.9 mg l⁻¹, with no difference between experimental and control areas. Total chlorophyll varied from 2.0 µg l⁻¹ to 17.3 µg l⁻¹, and there was no difference between experimental and control areas. By the end of the study, total organic matter in sediments was significantly greater in clam beds than bare sand, approaching that of seagrass beds. In conjunction with the greater organic content, these sediments contained more clay and mud (finer grain size) than sediment from both bare sand and seagrass beds. Although no increase in seagrass coverage near clam beds was observed, it is likely that the increase in organic nutrients deposited by clams into sediments will be available to augment seagrass growth in the future.

Using published filtration rates and the suspended solids and chlorophyll data recorded here, we estimated that surviving clams at the South site filtered a cumulative total of 1.84 million m⁻³ water and removed a total of 19,147 kg suspended solids and 16.8 kg particulate chlorophyll between February 13, 2017 and August 31, 2018. Using published nitrogen and phosphorus contents for clam tissue and shell, and clam shell and tissue weights obtained in this study, we estimated that surviving clams at the South site sequestered a total of 35 kg nitrogen and 22.6 kg phosphorus. Based on clam growth rate and density at the end of the study, nitrogen was sequestered at the rate of 86 g m⁻² yr⁻¹.

Table III summarizes the estimated ecological services provided by clams at the South site. These services will continue throughout the life of remaining clams, in proportion to the size of the population, including clams planted as part of this study and their offspring.

Table III.

Summary of ecological services provided by clams, *M. campechiensis*, at the South site, Port Manatee from February 2017 through August 2018. Cumulative totals are for all living clams throughout the study period; individual estimates are for 50 mm (shell length) animals. Suspended solids and chlorophyll numbers are based on overall average values recorded at the site.

Service Provided	Cumulative Total	Individual clam	Individual clam (per day)
Volume of water filtered	1.84 x 10 ⁶ m ³	1.08 l h ⁻¹	19.4 l
Suspended solids removed	19,147 kg	10.8 mg h ⁻¹	194 mg
Chlorophyll removed	16.8 kg	8.78 µg h ⁻¹	158 µg
Nitrogen assimilated	35 kg	0.16 g	-----
Phosphorus assimilated	22.6 kg	0.10 g	-----

Recommendations for future research

Follow-up research that would help more completely and accurately estimate the ecological services provided by clams (*M. campechiensis*) in Tampa Bay should include the following:

1. Determine clam (*M. campechiensis*) filtration rates over diurnal cycles and seasonal time frames.
2. Determine clam (*M. campechiensis*) biochemical composition, including nitrogen and phosphorus contents of both tissue and shell.
3. Determine the net influence of clam aquaculture on an ecological scale that includes all benthic organic and inorganic nitrogen and phosphorus fluxes.
4. Monitor clam recruitment in lower Tampa Bay (to evaluate reproductive success of the clams planted as part of this study).
5. Long-term monitoring of *M. campechiensis* beds and nearby seagrass beds to obtain side-by-side controlled data for seagrass area change.

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Appendix 1: Instrument Specifications

Test Equipment Depot - 800.517.8431 - 99 Washington Street Melrose, MA 02176 - TestEquipmentDepot.com

HOBO® Pendant® Temperature/Light Data Logger (UA-002-xx) Manual



HOBO Pendant Temperature/Light Data Logger

Models: UA-002-08
UA-002-64

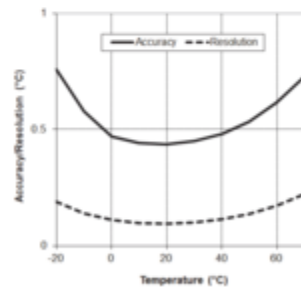
Required Items:

- HOBOWare 2.x or later
- USB cable (included with software)
- Pendant Optic USB Base Station & Coupler (BASE-U-1)
- Optic USB Base Station (BASE-U-4) or HOBO Waterproof Shuttle (U-DTW-1) & Coupler (COUPLER2-A)

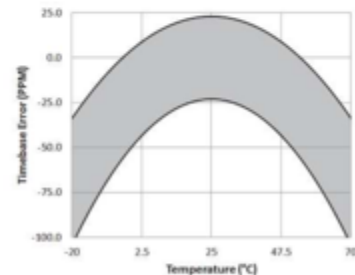
The HOBO Pendant Temperature/Light Data Logger is a waterproof, two-channel logger with 10-bit resolution and can record up to approximately 3,500 (8K model) or 28,000 (64K model) combined temperature and light readings or internal logger events. The logger uses a coupler and optical base station with USB interface for launching and data readout by a computer.

Specifications

Measurement Range	Temperature: -20° to 70°C (-4° to 158°F) Light: 0 to 320,000 lux (0 to 30,000 lumens/ft ²)
Accuracy	Temperature: ± 0.53°C from 0° to 50°C (± 0.95°F from 32° to 122°F), see Plot A Light intensity: Designed for measurement of relative light levels, see Plot D for light wavelength response
Resolution	Temperature: 0.14°C at 25°C (0.25°F at 77°F), see Plot A
Drift	Less than 0.1°C/year (0.2°F/year)
Response Time	Airflow of 2 m/s (4.4 mph): 10 minutes, typical to 90% Water: 5 minutes, typical to 90%
Time Accuracy	±1 minute per month at 25°C (77°F), see Plot B
Operating Range	In water/ice: -20° to 50°C (-4° to 122°F) In air: -20° to 70°C (-4° to 158°F)
Water Depth Rating	30 m from -20° to 20°C (100 ft from -4° to 68°F), see Plot C
NIST Traceable Certification	Available for temperature only at additional charge; temperature range -20° to 70°C (-4° to 158°F)
Battery Life	1 year typical use
Memory	UA-002-08: 8K bytes (approximately 3.5K combined temperature and light readings or events) UA-002-64: 64K bytes (approximately 28K combined temperature and light readings or events)
Materials	Polypropylene case; stainless steel screws; Buna-N o-ring
Weight	18 g (0.6 oz)
Dimensions	58 x 33 x 23 mm (2.3 x 1.3 x 0.9 inches)
CE	The CE Marking identifies this product as complying with all relevant directives in the European Union (EU).



Plot A



Plot B

FluoroSense™

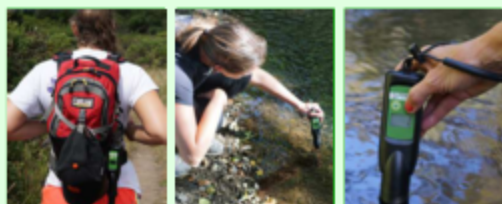
Handheld Fluorometer



FluoroSense Handheld Fluorometer

FluoroSense is a small, lightweight, highly durable handheld fluorometer ideal for quick *in situ* field measurements. Extremely simple to operate, FluoroSense displays results within seconds. Factory-calibrated for a linear range of 0-199 µg/L, the only maintenance required is simply rinsing after use. Available in two optical configurations, chlorophyll and phycocyanin (PC), FluoroSense can assist with identification of PC-containing algae typically associated with Harmful Algal Blooms (HABs). Obtaining both PC and chlorophyll estimates helps in determining whether additional testing is required to check toxicity levels in a body of water.

FluoroSense is the only portable, handheld field fluorometer on the market with an LCD displaying the measurements while the optics are submerged. Other similar meters either require an external display or require sample collection which requires training, is more time consuming, risks sample contamination, and creates excess waste (sampling bottles/containers). FluoroSense is a single-device solution with no need for peripheral instrumentation (probes) or additional calculations.



FluoroSense Specifications

Linear Range: 0-199 µg/L *in vivo* chlorophyll
0-199 µg/L phycocyanin

Resolution: 1µg/L

Power: 2 AA batteries (standard or rechargeable)

Temperature: 41° - 104°F (5° - 40°C)

Case: IP 67 standard; dustproof/waterproof

Light Source: LED

Detector: Photodiode

Weight: 4.3 oz; 120 g

Size: 8.9"x 2"x 1.75"; 22.6 cm x 5.1 cm x 4.5 cm

Ordering Information

INSTRUMENT	PART NUMBER
FluoroSense Handheld Fluorometer, Chlorophyll	2860-000-C
FluoroSense Handheld Fluorometer, Phycocyanin	2860-000-P
ACCESSORIES	PART NUMBER
800ppb Rhodamine WT Calibration Solution (Chlorophyll)	6500-220
200ppb Rhodamine WT Calibration Solution (Phycocyanin)	6500-020

Quickly assess pigment levels toward HAB identification

Highlights

- Dustproof, waterproof, highly durable
- Small size easily fits in shirt or jacket pocket
- >10,000 measurements per AA battery set
- One-button operation
- Low maintenance

Introduction to Turner Designs

Turner Designs has manufactured more than 50,000 fluorometers which are in use around the world. Manufacturing instruments since 1972, we set the standard for field, submersible, handheld, laboratory and on-line fluorometers. We specialize in providing sensitive, reliable, easy-to-use fluorometers for environmental and industrial applications. Our employees take pride in providing quality instruments and responsive technical service.

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