Barnegat Bay Oyster Restoration: Providing water quality and habitat improvements in Barnegat Bay

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1: Executive Summary

The Tuckerton Reef was created in 2016 as a research site to pilot the feasibility of restoring disease-resistant spat-on-shell oysters in the southern area of Barnegat Bay, NJ. This is a region that has faced a dramatic decline in natural oyster populations over the last century and is impaired by water quality issues from eutrophication and suspended sediment loads. The second phase of this restoration project is to estimate the ecological services provided by this subtidal restoration site, with a focus on water quality and habitat creation.

Oysters can filter water, with filtration rates increasing with oyster size and density and affected by surrounding environmental conditions. In addition to filtration, oysters can reduce nutrient loads by accumulating nitrogen in their tissues and removing it via biodeposit burial and denitrification. An additional service provided by subtidal reefs is habitat creation, which can increase production of fish and other invertebrates around the reef as they use the created habitat for foraging and protection.

In order to estimate the contribution of the Tuckerton Reef to these services, we doubled the footprint of the existing reef (the "Old" reef with plantings from 2016-17) by planting rounds of spat-on-shell at additional locations on the research lease. The 2019 planting deployed 600 bushels of spat-on-shell on 1,151 m^2 of bottom (the "New-19" reef) and the 2020 planting deployed 728 bushels of spat-on-shell over 2,255 m^2 of bay bottom, bringing the total reef footprint to about 1 acre of bottom covered (4,446 m^2). All spat-on-shell planting used disease-resistant larvae sourced from East Coast hatcheries. Oysters were monitored twice a year for Universal Metrics of oyster demographics for habitat restoration. These surveys allowed us track density, growth and survivorship of each planted area which help determine the reef's ability to provide ecosystem services.

Throughout the study period, the New-19 and New-20 plantings maintained densities well above the 15 oysters m^{-2} threshold for ecosystem services, but the Old reef fell below this threshold after 4 years. Planting over a smaller area to achieve higher densities would increase the ability of this site to maintain higher densities over time. Dermo disease does not seem to be a strong cause of mortality within these selectively-bred oysters, but potential impacts from boring sponge infection weaking shells and cownose ray predation may impact survival.

A water quality monitoring program using continuous loggers, discrete profile data and water samples for total suspended solids (TSS) on and off the reef site allowed us to document seasonal patterns and spatial variability in parameters such as temperature, salinity and dissolved oxygen. These parameters were also used to estimate oyster filtration using published models derived from laboratory estimates. Salinity and dissolved oxygen were never low enough to limit oyster filtration, indicating that oyster filtration near the reef is impacted primarily by temperature and particulate concentrations. TSS was found to be highly variable and contributed to variability within the oyster filtration model. Further sampling of TSS at higher spatial and temporal frequencies would help further determine its potential role in regulating oyster filtration.

We combined the density and biomass estimates from oyster surveys with environmental parameters in a series of equations to estimate filtration values for each area of the reef for 2019 and 2020. Highest filtration was observed in July both years, with rates around 12-14 $m^3\ m^2\ d^{-1}$ (or 132-154 gallons per hour). The New-19 reef had the highest filtration in both years, surpassing the

old reef even during its first year of planting. In this case, the older reef with fewer, but larger oysters was unable to achieve the filtration capacity of densely planted spat on shell oysters.

To scale up these filtration rates, we calculated the capacity for Barnegat Bay oysters to filter water and reduce nitrogen loads on an estuary-wide scale. We determined that the overall filtration rate of the Tuckerton Reef is 1.1 million l hr^{-1}, less than 1% of the total bay volume. We found that the nitrogen removal rate was low, at 1.078 kg N yr^{-1} for the 2020 reef. Thus, the Tuckerton Reef can sustain high filtration rates on a per square meter basis, and a larger footprint could have a significant impact on bay wide filtration. At the current biomass and footprint, however, the reef cannot obtain significant nitrogen removal based on our calculations. Therefore, we do not consider the Tuckerton Reef to have a large impact on nitrogen reduction in Barnegat Bay at the current scale of restoration activities, yet efforts to increase the reef's footprint would also enhance nitrogen removal.

The Tuckerton Reef has shown promise for enhancing fish habitat in Barnegat Bay, though differences with reef location and seasonal patterns obscured significant findings. Overall, we saw a 40-60% increase in nekton species and abundance on the oyster reefs compared to the control site for all fish trap samples during this study. Habitat enhancement for smaller, reef-dependent species was harder to determine due to experimental design issues in 2019 making it difficult to determine any enhancement of the reef versus the control site. In 2020, a new experiment was developed to test the ability of different reef substrates to support a mobile reef-dependent community, which showed that different species groups preferred different substrate types. Overall, oyster shell and natural shell clusters which had the most shells per unit area supported more species, but any type of hard substate capable of sustaining live oyster clusters should be considered in subtidal restoration projects in Barnegat Bay.

This report further details the methods and results for each outcome of the study: Outcome 1: maintaining the reef at a target density for ecosystem services, Outcome 2: model water quality improvement in Barnegat Bay, Outcome 3: contribute water monitoring data for Barnegat Bay and Outcome 4: assess habitat enhancement. This study represents the first attempt at quantifying ecosystem services for a restoration site in Barnegat Bay. We anticipate the methods developed in this study and the results produced from it will be applicable and comparable to the currently increasing number of oyster restoration activities in the bay.

2: Background

Loss of oyster reefs and their associated ecosystem function has been a critical issue for many U.S. estuaries (Baggett et al. 2015). The northeast Atlantic coast has seen one of the more severe declines in the country, with many estuaries containing less than 6% of historical extent of oyster beds (zu Ermgassen et al. 2012). Declines of the Eastern oyster, *Crassostrea virginica*, in Barnegat Bay, NJ have been driven by a combination of overharvest, water quality degradation and oyster disease. A recent growth in commercial oyster aquaculture and attention to water quality and habitat degradation within the Barnegat Bay has created an opportunity for oyster restoration programs to address these issues. Oysters provide ecosystem services such as water column filtration, nutrient removal and habitat provision (Grabowski and Peterson 2007).

As filter-feeders, oysters can contribute to water quality improvements through filtering and removing suspended particles and phytoplankton. When oysters feed, they remove nutrients such as nitrogen and phosphorus from their food source and assimilate it into biomass (Newell

2004, Newell 2007). Additional removal of nutrients and seston can be achieved through feces and pseudofeces (i.e. biodeposit) production. Pseudofeces are mucous-bound, undigested particles which settle into the sediment where microbial denitrification processes can remove additional nitrogen by converting it to N_2 gas. Additionally, burial of biodeposits can result in nutrient removal from the water column (Kellogg et al. 2013). Although *in situ* measurements of oyster filtration and denitrification can be difficult to estimate and require expensive analytical techniques (Grizzle et al. 2008, Ray et al. 2021), these rates can be estimated through a series of equations based on oyster biomass and water quality parameters from laboratory studies (Beseres Pollack et al. 2013, Ehrich and Harris 2015). These equations can be scaled up to determine filtration capacity of an entire reef or make projected estimates of filtration with reef expansion, which can have additional value for estimating costs of nutrient removal through nutrient trading programs (e.g. Bricker et al. 2020, Rose et al. 2021).

Habitat enhancement is another goal of many oyster restoration projects and is one that is often easily met after reef construction. Coastal and estuarine fishes and invertebrates, many of which have commercial importance, use oyster reefs for shelter, feeding and/or reproduction. Usage can range from transient species, which may frequent oyster reefs for foraging but are not dependent on them (e.g. blue crabs, summer flounder), to resident species which often live within oyster shells, feed primarily on reef-associated species, or use oyster shells to deposit eggs (e.g. black sea bass, oyster toadfish, gobies and mud crabs). Additionally, encrusting fauna on oyster shells can further contribute to water filtration benefits (Kellogg et al. 2013). Habitat provisioning is one of the first services to appear after oyster reef creation and can even be met with substrate planting before any significant oyster set or growth may appear (La Peyre et al. 2013, De Santiago et al. 2013). Reef age may matter, however, as some studies have shown that oyster density and size can impact habitat enhancement (Boudreaux et al. 2006, Brown et al. 2013), but other studies have found no effect between reef height and habitat (Gregalis et al. 2009, Humphries et al. 2011). Assessing actual benefits of habitat enhancement can be a challenge, as multiple sampling methods are often required (e.g. traps vs nets), reference sites may not exist or fully represent the restoration area and control sites may not be a comparable representation of the unrestored area. As estuarine habitats can be thought of as a mosaic within a larger system, oyster reef proximity to functionally redundant habitats such as salt marshes and SAV beds can confound data interpretation (Grabowski et al. 2005, Gilby et al. 2020).

In 2016, the Tuckerton oyster reef was created in the Little Egg Harbor region of Barnegat Bay with the goal of creating a permanent no-harvest oyster reef to restore habitat. Two methods of oyster seeding were used on the 1-acre subtidal site: a spat-on-shell method using whelk substrate and disease-resistant oysters and oyster seed transplanted from a neighboring natural population in a connected, adjacent system. Although both populations grew and survived after two years of planting, the transplanted oysters died off after three years, attributed to a high Dermo disease prevalence in the that population (Thompson et al. *in press*). A local oyster shell recycling program added additional disease-resistant oysters to the site in 2017 using a combination of restaurant recycled oyster shell and whelk shell as substrate. Local shell recycling programs and private funding sources may be a source to help maintain the small scale site in future years while restoration managers seek larger funding sources.

Although oyster restoration activities have substantially increased over the last decade along the eastern U.S., monitoring programs and evaluation of ecosystem services associated with

these programs have lagged, typically due to costs of restoration and maintenance costs of monitoring programs. In order to create standards for oyster restoration monitoring, a working group developed specific guidelines for oyster restoration practitioners that included the best practices for oyster monitoring and evaluation of ecosystem services (Baggett et al. 2015). A main component of these guidelines was establishing Universal Metrics that should accompany each oyster restoration project including: (1) reef area, (2) reef height, (3) oyster density, and (4) oyster size-frequency distributions and environmental variables such as (1) temperature, (2) salinity and (3) dissolved oxygen. Additionally, best practice methods for assessing ecosystem-service restoration goals were presented.

The goal of this project was to double the size and footprint of the Tuckerton Reef in Barnegat Bay and implement a robust monitoring program for ecosystem services following these best practices. The objectives of this program included (1) oyster reef surveys incorporating Universal Metrics, (2) a water quality monitoring program and (3) habitat monitoring for reef-associated species. The outcomes of this project will quantify the impact of this restoration site on the Barnegat Bay ecosystem and enable comparisons to other restoration projects. Additionally, it can inform future directions and monitoring programs at this site and at new restoration sites within Barnegat Bay and surrounding New Jersey coastal bays.

3: Methods

3.1 Site and Planting Methods.

Oysters were planted in 2019 and 2020 in the permitted area of the Tuckerton Reef Research lease (Fig. 3.1.1). This two-acre lease was expanded to four acres in 2020. Areas of the reef targeted for monitoring included the older plantings, or "Old" reef containing 2016 and 2017 plantings of spat-on-shell and the plantings for this project, the "New" Reef. As the plantings for this project occurred over two years, the New Reef was split into two planting areas or locations: New-19 representing the 2019 planting, and New-20 representing the 2020 planting. Table 3.1.1 gives the area of each location after the 2020 planting. Additionally, this project used a "Control" site for water and habitat monitoring, which is an unrestored area of similar substrate and water depths approximately 0.6 nm southwest of the Tuckerton reef.

The 2019 and 2020 plantings were placed in separate and previously bare areas of the lease to monitor individual cohorts (Fig. 3.1.1). Remote-set, disease-resistant oysters were used for both plantings. Hatchery-bred oyster larvae were set onto shell cultch in 3,000 gallon circular tanks at Parsons Mariculture at Great Bay Marina in Little Egg Harbor, NJ. The specific larval strain used was subject to timing and availability at east coast hatcheries, but strains selected for disease-resistance were exclusively used. In 2019, Rutgers Aquaculture Innovation Center provided disease-resistant NEH (Northeast High-Survival) oysters, and in 2020 DEBY (Delaware Bay line) oysters were purchased from Oyster Seed Holdings, LLC in Virginia. After a 24-hour period of aeration-only to allow for larval attachment, flow-through was initiated allowing larvae to feed and grow from ambient waters in Great Bay. Shell cultch type varied based on availability, with the majority of shell used being recycled oyster shells from the Long Beach Township Shell Recycling program. To estimate spat-set ratios, a subset of spat-on-shell was sampled from randomly selected cages from each tank representing top, middle and bottom regions of the tank. Table 3.1.2 summarizes the plantings and spat set ratios for each year of this project. Prior to planting, cages were unloaded

from tanks and placed onto a barge. Oyster spat-on-shell were deployed onto the designated areas of the site via pressure hose from the barge (Fig. 3.1.2).

3.2 Oyster surveys

Oyster monitoring on the Tuckerton Reef occurred in May and October each year to represent the start and end of the oyster growing season in the mid-Atlantic. Oyster surveys targeted the individual planting areas of the reef and were designed to assess the Universal Metrics of oyster demographics for oyster habitat restoration (Baggett et al. 2015): density and size frequency distributions.

Oyster demographic assessments were performed using hydraulic 1 m^2 patent tongs operated from a boat platform. Samples were obtained from each planting area with 3-4 replicates for each location. Each sample was brought up onto the deck, and all oyster and shell material were rinsed to separate it from other fouling material (e.g. sponge, macroalgae). All live and dead articulated oysters were enumerated recording predation by oyster drills when present. Shell length (umbo-margin) were recorded in mm for all living oysters. Settlement and recruitment were assessed during October surveys for cohorts in Year 2 after planting and determined to be oysters < 25 mm. The rest of shell material (e.g. unarticulated oyster shell, fragments, or pieces of substrate with no living oysters) was separated and volume measured by water displacement.

Subsamples of oysters from each reef area were obtained for condition index assessments and disease. Disease tests were performed after the first year of planting. Condition index assessments were made using the procedures of Rainer and Mann (1992) using the formula (Tissue dry weight x 100)/(Whole wet weight – shell wet weight). Oyster dry weights were obtained after shucking and drying at 60° C for 48 hours. Histopathology tests for Dermo (*Perkinsus spp.*) were performed by Haskins Shellfish Research Hatchery using the Ray's Fluid Thioglycollate Medium (RFTM) incubation method following standard histological procedures (Dungan and Bushek 2015). Microscopy tests for MSX infection (*Haplosporium nelsoni*) were performed in Fall 2020 and Spring 2021.

Additional metrics important for oyster restoration monitoring include reef footprint and reef height. Estimates of reef height were not attainable due to our sampling methods, and the project design of placing shell on bare bottom is not intended to create high-relief reefs due to permitting restrictions. Reef footprint was estimated from side-scan sonar surveys in 2019 and 2020. Side scan sonar surveys were conducted on board Stockton's *R/V Petrel* using an EdgeTech 6205 (ET6205) multi-phase echo sounder which produces co-registered side scan imagery alongside the generation of bathymetric maps. Sonar data collection was completed using 1600kHz sonar frequency with a range was set at 20 meters for a 40-meter swath. Surveys were conducted over a series of planned survey lines established over the study area and were spaced at 35 meters to allow for full coverage of the sea floor. Data was recorded using Discover Bathymetric software where positioning data was also assigned using the *R/V Petrel's* vessel mounted Hemisphere VS330 Global Navigation Satellite System (GNSS). The VS330 GNSS provided Real Time Kinematic (RTK) positioning.

The side scan data was post-processed using Chesapeake SonarWiz software to create a side scan mosaic. The mosaic was exported to Google Earth (GE) where borders were drawn around the boundaries of the oyster reefs using GE's polygon measuring tool. Boundaries were delineated based on planting year by visual interpretation of the sonar image by the sonar operator

comparing differing image characteristics of the sea floor and shell piles. The polygon tool in GE also calculated the area of sea floor coverage by the planted shell.

Statistical tests were performed to compare oyster metrics within each reef site for each monitoring period. Statistical tests were run in RStudio (v 1.3.959, 2020) and SPSS (IBM v27, 2020). T-tests and ANOVAs were performed independently for each monitoring period due to the addition of plantings each year. Data were checked for normality and heteroscedasticity and log-transformed if necessary. Non-parametric tests (Mann-Whitney and Kruskal Wallace) were used in cases where variances were unequal after transformation.

3.3 Water Quality Monitoring

A water quality monitoring program was created at the Tuckerton Reef site to monitor universal water quality metrics for oyster restoration sites (Baggett et al. 2014) and to be used as parameters to estimate oyster filtration and denitrification. We targeted the months when water temperatures would be above 10° C, roughly April – November. Continuous loggers were placed on the reef sites as early as April and recovered in October or November for the season (Fig. 3.3.1). In 2019, one HOBO U24-002 conductivity-temperature logger and one HOBO U26-001 dissolved oxygen logger (Onset Corp.) were deployed on the reef site at approximately 0.25 meters above the bed. In 2020, HOBO conductivity-temperature and dissolved oxygen loggers were deployed on the control site and a YSI EXO2 multiparameter sonde was deployed at 1 meter below the surface over the reef site. The YSI sonde allowed us to obtain continuous measurements of chlorophyll and optical turbidity (NTU) to compare to discrete measurements. All loggers used a 10-minute sampling interval.

Data QA/QC procedures were applied to the continuous series as outlined in the project QAPP document. Data series from HOBO sensors were graphed to visually check for sensor drift. A median filter with a 30-minute window was applied across each time series to remove outliers and ensure internal consistency of the data. Additional periods of unrealistic values, as determined from typical parameter ranges at a long-term Barrel Island monitoring site, were manually removed from the series as needed. Gaps shorter than 3 hours were filled by linear interpolation; longer gaps were omitted from monthly averages. Salinity and dissolved oxygen time series in 2019 were impacted by biofouling in June and October, resulting in removal of 7% of the salinity series and 9% of the dissolved oxygen series. In 2020, 11% of the salinity data was removed (in April-May only) and 28% of the dissolved oxygen (in September-November). The dissolved oxygen logger also flooded in April 2020 and had to be replaced, resulting in a gap in oxygen data prior to June 2020. Salinity and turbidity data measured by a YSI sonde were removed in August 2020 due to sensor drift (3% of seasonal time series).

Discrete measurements were used to enhance the spatial coverage of sampling and to verify logger data. Replicate YSI profiles (EXO or ProDSS instruments) were collected monthly at the control and reef sites (Table 3.3.1). The YSI multiparameter sonde used for profiling was lab calibrated prior to each sampling event and fitted with temperature, conductivity, dissolved oxygen, pH, turbidity, and chlorophyll-a fluorescence sensors. Chlorophyll and turbidity data were not corrected by in situ calibration samples, and therefore represent relative changes in these parameters over time. Additionally, chlorophyll data were not collected in May and October 2019 or in April and October 2020. Water samples (250 mL) were collected for Total Suspended Solids

(TSS) measurements which were filtered and weighed in the laboratory following EPA Procedure 160.2.

A relative standard deviation threshold of 10% was used to screen for excess variability in the discrete measurements. YSI data recorded near the surface, middle, and bottom of the water column were averaged across the three replicates and by depth. None of the temperature, salinity, dissolved oxygen, or pH profiles exceeded 10% RSD in either year. In contrast, chlorophyll and turbidity profiles exceeded this threshold in half of the months (Table 4.3.1).

3.4 Habitat Enhancement

This project targeted three areas of habitat enhancement: (1) nekton using mesh fish traps on the reef and a control site, (2) small fish and invertebrates using substrate baskets, and (3) fouling species coverage.

To sample nekton, unbaited semi-oval mesh fish traps (26" x 19" x 9" with 3/8" mesh) from Memphis Net & Twine Co., Inc. were used to assess fish usage between the reef areas and an unrestored control on the oyster reef in June, July and September in 2019 and 2020. These months were targeted as peak months for fish usage. Traps were soaked for 48-72 hours. In 2019, four traps were placed on the "Old Reef" area and four on the control site. In 2020, three traps were placed on the Old Reef, three on the New-19 area, and three on the control site. When traps were recovered, all fish and invertebrates larger than the mesh openings were identified and enumerated. Fish length and carapace width of crabs were measured in mm. Data was summarized as richness (total species per trap) and abundance (total individuals per trap) and total abundance of fishes and decapods. To adjust for varying deployment lengths in 2019, metrics for both years were divided by the number of days deployed and standardized as catch-per-unit-effort (CPUE).

To assess usage of small reef-dependent fish and invertebrates, 0.09 m^2 plastic crates lined with ¼" aquaculture mesh were filled with equal volumes of shell substrate and placed on and off the reef. In 2019, baskets were placed on the reef ($n = 5$) and in the control area ($n = 5$) in June and recovered in July and then replaced and recovered in August. In 2021 (delayed a year due to COVID-19), this survey was resigned to test habitat use of different substrates, and four baskets each of oyster, whelk, and natural reef clusters cleaned of fouling species were placed on the New-19 area of the reef and recovered after six weeks. Data was assessed as richness, abundance, and total abundance and biomass of the most dominant taxonomic groups (Fish, Decapods and Gastropods).

Fouling species were assessed in Fall 2020 on each reef planting from 8 shell clusters (with or without live oysters) were randomly selected from each sample. Solitary encrusting individuals (e.g. limpets, barnacles, and oysters) were enumerated across the total area of each cluster. For colonial species that cannot be enumerated as individuals, a 10x10 square piece of ¼" mesh equivalent to an area of 15 cm^2 was placed over a haphazardly selected area of the shell cluster. The number of mesh squares occupied by each species was enumerated as a % cover (out of 100).

Statistics methods were used to assess significant differences between site and date (if applicable) for all community metrics. ANOVA or Kruskal-Wallis tests were used depending on if data were heteroscedastic. Additionally, non-metric multidimensional scaling analysis was performed using the vegan Package in R (v 2.5-6). A PERMANOVA test was run on Bray-Curtis similarly indices to determine any significant differences between sites and dates.

4: Project Outcomes

4.1 Outcome 1: Maintain reef at target density for ecosystem services

Oyster population metrics. Oyster size and density differed among areas of the reef each year (Fig. 4.1.1A). The "Old" Reef, made up of 2016 and 2017 spat-on-shell plantings, had the lowest density of the sites and by Spring of 2020 remained below the target density of 15 oysters m^{-2}. Although we set 15 oysters m^{-2} as a target for ecosystem services, other published literature has suggested a limit of 10 m^{-2} (Powers et al. 2009). The "New" plantings associated with this project had mean densities well above the target densities throughout the monitoring period. These plantings had significantly higher densities compared to the Old Reef sites for each monitoring period (Fall 2019: $p < 0.001$; Spring 2020: $p = 0.002$; Fall 2020: $p = 0.001$). The highest mortality for the New-19 plantings occurred in the first season after planting. The New-19 density decreased by approximately 65% from Fall 2019 – Spring 2020, but only decreased by 20% from Spring 2020 – Fall 2020.

The high salinities at the Tuckerton Reef provide excellent growing conditions for the oysters with oysters reaching market-size (around 60-80 mm) after their first year. The oysters remaining from the Old Reef had the largest oysters with consistent mean shell lengths above 80 mm, but overall means did not increase with time due to the presence of spat on some of the shells (Fig. 4.1.1B). Size frequency distributions reveal the presence of spat (< 25 mm) on the older oysters, which were about 25% of the Fall 2020 sample (Fig. 4.1.2). The New-19 plantings grew steadily, and mean sizes in these areas were not significantly different from the Old Reef in Fall 2020 in post-hoc pairwise comparisons (Fig. 4.1.1B). Oyster biomass was consistently highest in the Old Reef sets each year (Fig. 4.1.1C).

Mortality estimates and causes of mortality differed among reef areas (Table 4.1.1). The highest percentages of boxes were found in the Old Reef samples in 2019, corresponding to the low survivorship seen at that location. Percent of new boxes were higher in the New-19 and New-20 reef samples ($p = 0.001$), this would be expected as those plantings were less than a year old during the time of monitoring. Oyster drill predation was also significantly higher among the New-19 and 20 reef areas ($p = 0.002$), accounting for 15-16% of deaths in the New-19 and 52% of deaths in New-20 oysters. Drill scars were less prevalent on the Old reef oysters, only representing 0-3.6% of deaths. This suggests that oysters will eventually grow out of being susceptible to drill predation after a few years and it is not a likely impact of mortality in older oysters.

Planting density can affect oyster survivorship and mortality. Oysters planted at higher densities will be expected to have a greater chance of maintaining ecosystem services. Due to different monitoring methods prior to 2019, we cannot directly compare these metrics with the planting densities for the Old Reef areas. Despite the strong growth and recruitment potential, we estimate the window for ecosystem services at this site to be around 4 years. We will continue to monitor the New Reef plantings and evaluate recruitment potential at the Tuckerton Reef site to further determine the longevity of oyster reef services. Enhancement efforts planting either spat-on-shell or bare shell deployed during the peak period of larval settlement may be necessary in areas that have dropped below threshold density to maintain the ecosystem services at this site due to low natural recruitment.

Condition and disease. In addition to the Universal Metrics, condition indices and disease prevalence was monitored for each reef area. Condition Index showed typical seasonal variation,

which higher values in spring compared to fall ($p < 0.001$). Condition indices in 2020 were higher than in 2019, suggesting environmental conditions were superior for growth in 2020 (Fig. 4.1.3). In Fall 2020, the lowest Condition Indices were seen in the New-20 samples compared to the Old and New-19 reef. Seasonal fluctuations in oyster condition have been seen in oysters with other studies in this area (Fitzgerald et al. 2020) and can reflect both food availability and spawning. Overall, the higher values (> 10) seen among all the cohorts at this site in 2020 indicate healthy populations.

Pathogen testing for *Perkinsus spp.* (Dermo) and *H. nelsoni* (MSX) was performed in Fall 2019, Fall 2020, and Spring 2021. Overall*, Perkinsus sp.* prevalence was very low and only detected in a few of the Old Reef samples (Table 4.1.2). On a scale of 0-5, infection intensity ranged from 0.47-0.69. No *Perkinsus spp.* was detected in the New Reef samples. MSX was very low but detected in a few samples in the Old and New-19 reef and minimal values (< 0.1). No statistical analysis was done due to the high prevalence of zero scores among all data sets. Overall, disease prevalence is moderate among the Old reef samples but due to low infection intensity is likely not a significant source of mortality. Additionally, Dermo intensity is lower for the Old reef samples compared to their intensities in the first years of planting (Thompson et al. *in press*), showing an improvement over time.

4.2 Outcome 2: Model water quality improvement in Barnegat Bay

As ecosystem engineers, oysters provide water quality benefits through filtration and nitrogen removal. It can be difficult to measure filtration rates *in situ*, as it often requires low flow estuaries or narrow tidal channels where changes to water quality can be readily observed. For the Tuckerton Reef project, the reef footprint is too small compared to the overall area of Barnegat Bay to be able to observe a reduction in suspended particles. A goal for this project was to use available equations derived for oyster filtration in laboratory settings and apply the parameters of oyster size and density and environmental factors known to affect oyster bioenergetics to estimate the filtration rate for oysters on the Tuckerton Reef.

Model Development. After reviewing available oyster filtration models in the literature, we chose two models to develop our oyster filtration model and to compare. The first model (Eq. 1) was based on an equation from Cerco & Noel (2005) formulated for oysters in the Chesapeake Bay. This model incorporates a temperature-based adjustment and calculates the maximum rate oysters can filter water as $m^3\ g^{-1}$ oyster C day^{-1}:

Equation 1: Cerco & Noel (2005)

$$Fr = 0.55\ e^{-0.015(T-27)^2} \qquad (1)$$

Where Fr = filtration rate ($m^3\ g^{-1}$ oyster C day^{-1}) and T = temperature in °C.

The second model from zu Ermgassen et al. (2013) uses the same temperature-based filtration rates as the Cerco & Noel (2005) model but incorporates oyster biomass directly into the equation (Eq. 2). This model has been fitted for oyster filtration rates *in situ* (Grizzle et al. 2008). This model computes filtration rates as:

Equation 2: zu Ermgassen et al. (2013)

$$Fr = 8.02\ W^{0.58} e^{-0.015(T-27)^2} \qquad (2)$$

Where Fr = filtration rate ($l\ hr^{-1}$), W = dry weight of oysters (g) and T = temperature in °C.

Both models allow for incorporating adjustments to the maximum filtration rate based on the effects of salinity (*S*), dissolved oxygen (*DO*), and seston (*TSS*) on oyster filtration. These adjustments are based on Ehrich & Harris (2015) referencing Fulford et al. (2007):

- *Salinity*. Oyster filtration increases with salinity. If salinity (*S*) is below 12, the model will adjust as follows following the equations in Fulford et al. (2007). For $f(S)$:

$= 0$ when $S < 5$

$= 0.0926 * S - 0.139$ when $5 \leq S \geq 12$ (3)

$= 1$ when $S > 12$

- *Dissolved Oxygen (DO, mg l^{-1})*. Higher filtration is observed with higher DO. Maximum filtration occurs when DO is greater than 3 mg l^{-1}. The equation, based on Fulford et al. (2007) is based on responses to bivalve molluscs to low oxygen:

$$f(DO) = \frac{1}{1 + e^{1.1 * \frac{1.75 - DO}{0.25}}} \quad (4)$$

- *Seston (TSS, mg l^{-1}).* Oyster filtration increases with TSS > 4 mg l^{-1} and decreases with higher loads of suspended solids. A step function is used to adjust for seston (Fulford et al. 2007), $f(TSS)$:

$= 0.1$ when $TSS < 4\ mg\ l^{-1}$

$= 1$ when $4 \leq TSS \geq 25\ mg\ l^{-1}$ (5)

$= 10.364 * \ln(TSS)^{-2.0477}$ when $TSS > 25\ mg\ l^{-1}$

The average biomass of oysters in each sampling area was incorporated into each equation. For equation 1, filtration rates were multiplied by the average biomass of oysters in each sampling area. For equation 2, average biomass was incorporated in the initial filtration rate calculation. We used biomass calculated for oysters sub-sampled from each years' oyster surveys to create a length:biomass regression which was then applied to all shell length measurements for each year. Mean and standard error of biomass are shown in Fig. 4.1.1C. Each planted area was treated separately due to different planting conditions and substrates used. A linear regression showed the best fit to each length:biomass data set (OLD REEF $R^2 = 0.58$; NEW-19 $R^2 = 0.46$; NEW-20 $R^2 = 0.70$). This is more accurate than using published values from other locations or bays since those oysters are typically from natural reefs or single oysters. The long, thin oysters that grow from the whelk shell substrate may have distinctly different shell length:biomass relationships compared to the oysters from natural reefs.

Steps for each model were implemented in Matlab (Mathworks, 2019). Filtration rates were calculated using monthly water quality parameters (Tables 4.3.1 & 4.3.2; Figs. 4.3.2 & 4.3.3) averaged for each month (continuous data) or from monthly discrete profiles (discrete data). For temperature, which was collected both ways, filtration rates were calculated and compared for averaged continuous and discrete data.

Filtration Rates. We ran the filtration models for each combination of filtration rate equation (equation 1 or 2) and water quality data (profiles or logger data). Equation 2 (zu Ermgassen et al. 2013) was determined to be the best equation to use moving forward as it has

been suggested in multiple restoration methods guidelines and makes these rates best for comparisons (Baggett et al. 2015, zu Ermgassen et al. 2016). We compared filtration rate and nitrogen removal outputs from each water quality data set.

To determine the impact of water quality sampling method on filtration rate, we compared the differences in model output using temperature data from the logger and profile each month using equation 1, which does not depend on oyster biomass (Appendix Table A1). The differences arise from the timing of profile collection relative to the monthly temperature trends. Discrete monthly profile data deviated from monthly average logger temperatures by as much as 3°C in September through November, when temperatures tend to decrease sharply. As a result, filtration rates modeled with profile data were comparable to continuous logger rates in Summer 2019 but a factor of 0.3-0.6 lower than continuous logger data in Fall 2019. In contrast, September 2020 filtration rates predicted from profile temperatures, which were collected early in the month, were a factor of 1.4 larger than the filtration rate based on logger data. We tested the effect of sampling on a consistent interval by subsampling temperature on the 15th of each month from the continuous series; this evenly spaced sampling scheme did not improve the match between discrete monthly profiles and averages of high-resolution continuous data. We recommend that future studies sample more often in transition months with large temperature change (early Spring and Fall) if continuous monitoring is cost prohibitive. However, use of monthly profiles would not qualitatively change our study conclusions about seasonal trends in oyster filtration.

The general trends for the filtration rates were similar between both the continuous logger and monthly profiles (Figs. 4.2.1 & Appendix Fig. A1). Monthly filtration trends follow temperature trends, with increased filtration rates in the warmer months (June–September) compared to May and October. The effects of TSS can be seen between 2019 and 2020. TSS values were higher in 2019 impacting the Old reef filtration rates, and also in August of 2020. Given that many summer months have TSS values above the 25 mg l^{-1} threshold (Equation 5), it is expected to negatively impact oyster filtration during these months.

The filtration values also demonstrate the impact of oyster size and density on this ecosystem service. The Old reef site maintained larger sizes, but lower densities, and the New-19 reef was able to surpass the Old reef site in filtration capacity almost immediately due to the high densities at planting, despite being of smaller size (Figs. 4.1.1 & 4.1.2). These estimates are on the high end, as the October sizes were used for the months of July-September when the planted oysters would have been smaller. Lower planting densities of the New-20 cohort resulted in less filtration compared to the New-19 reef in 2019. These are important considerations to make when planting reefs- more densely planted oysters will result in a smaller reef footprint but greater filtration per unit area.

The question of how TSS may affect oyster filtration in Barnegat Bay remains unresolved and provides an opportunity for potential follow-up studies. Given there was little correlation between TSS measurements and optical turbidity from profile measurements (see Outcome 3), it is unknown how representative the monthly TSS data are of the general monthly trends at the site. It has been shown that filtration models can be particularly sensitive to TSS functions as well (Keohane et al. 2019), though we did not explore other f(TSS) functions in this study. Future sampling efforts on the reef will increase the spatial coverage of TSS samples by sampling separately over each cohort. Suspended sediment concentrations in Barnegat Bay appear to be patchy both spatially and temporally, which can drive locally impaired water quality. Southern

Barnegat Bay experiences inconsistent, intermittent peaks of turbidity which can be related to phytoplankton species composition and thus oyster food quality (Ren 2014, Ren 2015). Thus, it is important that this factor be considered when developing oyster water filtration estimates in Barnegat Bay.

Nitrogen Removal. Oyster filtration and nitrogen removal are related through (1) clearance rates of phytoplankton, (2) assimilation of nitrogen into oyster biomass and (3) biodeposit burial and denitrification. Clearance rates of phytoplankton can be converted to nitrogen uptake based on ambient Chlorophyll *a* measurements and Chl:N ratios in the food (Grizzle et al. 2008). The Chl:N ratio used was 11.76 g Chl:g N based on published values (Parsons et al. 1984).

Table 4.2.1 compares total yearly N removal between clearance rate calculations from both the continuous logger and monthly profile data. Chlorophyll *a* data were used from the corresponding monthly YSI profiles or supplemented with nearby continuous monitoring data for months when profile data were missing. N clearance rates (gN m^{-2} d^{-1}) were multiplied by the area of each reef to obtain approximate daily clearance for the reef footprint (gN $reef^{-1}$ d^{-1}). The reef clearance rates were then integrated daily for the period of active filtration (May – October) to obtain an annual estimate of nitrogen removal (kgN yr^{-1}, Table 3.1.1). Comparing the methods of filtration rate calculations, the differences between each N removal estimate were small in 2020 (less than 1% difference between values) but were larger (5.4-7.7%) for 2019 data. In 2019, the logger data showed higher N removal, which corresponds to the higher clearance rates from the logger data in 2019. Overall, these differences were small and do not suggest that either method would severely over- or under-estimate N-removal given the many assumptions required to make these calculations.

Total yearly nitrogen removal was estimated to be higher in 2020 (~1 kg) compared to 0.58 kg in 2019, which reflects growth of the New-19 cohort and the addition of the 2020 planting (Table 4.2.2). Despite having lower filtration rates per unit area (Fig. 4.2.1), the New-20 reef had similar modeled N removal to the New-19 reef in the first year (0.371 to 0.387, respectively). This demonstrates that a larger reef footprint can compensate for lower filtration rates in terms of total clearance. The annual nitrogen load of Barnegat Bay has been estimated as much as 857,000 kg N yr^{-1}, with about 16.6% of this loading coming from the Little Egg Harbor area (Baker et al. 2014). Although the Tuckerton Reef's nitrogen removal estimates are small relative to the total loading of Barnegat Bay, it is notable that this project essentially doubled the contribution of oyster-mediated nitrogen removal at the Tuckerton Reef site from 2019 to 2020. The model results provide a rough estimate that 1 acre of oysters planted in Barnegat Bay is proportional to 1 kg of Nitrogen removed per year (either assimilated or removed as biodeposits). By these estimates, it would take 142,000 acres of oysters to remove the total nitrogen load in the southern Barnegat Bay.

Comparisons and Scale-Up. To scale-up our data to make it comparable to other sites, we converted nitrogen removal to kg km^{-2} and broke it down into biodeposits (50% of assimilated N), denitrification (20% of biodeposits) and burial (10% of biodeposits) based on calculations similar to those used in Beseres-Pollack et al. (2013). The summer clearance rates (July – September) were used for these calculations which represents full coverage of oysters planted at all sites each year. Total values for denitrification based on these clearance rates are 51.77 kg km^{-2} for 2019 and 65.09 kg km^{-2} for 2020 (Table 4.2.2). This value is on the low range for restoration sites. In Mission-Aransas Estuary, TX, Beseres Pollack et al. (2013) estimated denitrification using similar methods with a range of 85.20 kg km^{-2} (denitrification winter low) to 165.73 kg km^{-2} (denitrification fall

high). Overall, Barnegat Bay oysters will remove less N on an annual basis given the colder winter temperatures resulting in winter dormancy.

A recent interest in estimating nitrogen removal from aquaculture practices as a nitrogen reduction strategy has led to model developments to estimate nitrogen removal from harvest as well as denitrification (Rose et al. 2021). Models such as the Farm Aquaculture Resource Management (FARM) model (Ferreira et al. 2007) and the Harris Creek Oyster Restoration model (Kellogg et al. 2018) have been able to estimate N-removal through harvest and scaled to denitrification using basic water quality parameters. The estimates provided by these models for areas such as the Chesapeake Bay, Long Island Sound and Great Bay Piscataqua River Estuary, NH have produced orders of magnitude higher rates for assimilation and denitrification compared to our study. For example, restored reefs in NH estimated denitrification at 1,827 kg km^{-2} yr^{-1} (Bricker et al. 2020). These efforts have also suggested that reef-associated denitrification on restoration sites has a greater contribution to nitrogen removal over assimilation and harvest alone (Bricker et al. 2020, Kellogg et al. 2013). Further work needs to be done to compare methods used from this study to these models to determine the potential limitations of this site or methods used.

Finally, we ran an estuary-level simulation to determine the filtration capacity of oysters at the Tuckerton Site based on the volume of Barnegat Bay using the water filtration calculator from the Nature Conservancy (https://oceanwealth.org/tools/oyster-calculator/, zu Ermgassen et al. 2016). We input oyster reef and temperature parameters from 2020 (Table 4.2.3) to determine a current filtration capacity of 1.1 million L h^{-1} (1,100 m^3 h^{-1}) This represents less than 1% of the full bay volume. Correspondingly, it would take about 12.3 years to filter the volume of the bay (~118.3 million m^3). Scaling this up, one hundred acres of oyster reef would filter 9% of the bay's volume. In order to filter 50% of the bay volume about 600 acres (241 ha) of oyster reefs would be required, a 600% increase. Historically, the extent of Barnegat Bay oyster beds was estimated to be at 5,261 ha with an approximate density of 18 oysters m^{-2} and average size of 67 mm (Nelson, 1889, as referenced in zu Ermgassen et al. 2012). Historical filtration was estimated to be 3.5 billion L h-1, or 287% of the bay volume.

4.3: Outcome 3: Contribute water monitoring data for Barnegat Bay

Continuous time series were obtained from June through November in 2019 and May through November in 2020 (Fig. 4.3.1). Both years demonstrate seasonal trends in temperature and dissolved oxygen, with episodic variability in salinity. These trends were consistent with a nearby continuous YSI time series collected by Stockton University at Barrel Island, Little Egg Harbor. Salinity remained above 20 PSU and oxygen concentrations remained above 3 mg l^{-1} during the monitoring periods, indicating that oyster filtration near the reef is impacted primarily by temperature and particulate concentrations (see Outcome 2).

Monthly distributions of continuous data (Figure 4.3.2 & 4.3.3) and discrete YSI profiles (Table 4.3.1) confirm seasonal patterns in some water quality variables. Temperatures peaked in July-August above 25°C in both years and dropped below 10°C in April and November. Dissolved oxygen concentrations reached minima of 5-6 mg l^{-1} in August. Monthly salinity profiles varied, presumably due to differences in weather and tide stage on the sampling dates, but mean salinity tended to increase from June to October. Chlorophyll concentrations were highly variable over time scales of a few days, but also tended to decrease over the course of the summer on monthly time scales (Table 4.3.1).

TSS replicate water samples were collected monthly at the reef and control sites simultaneously with YSI profiles. Boat and personnel availability (especially during Covid-19 protocols in 2020) did not permit resampling during months in which TSS replicates exceeded the 10% variation threshold, which occurred in two months in 2019 and 3 months in 2020 (Table 4.3.2). TSS concentrations did not significantly differ between the two sites (paired t-test, df = 38, $p > 0.05$). There was an apparent seasonal trend, with lowest TSS in May and highest TSS in August-September; however, additional years of sampling are required to determine whether this is a repeatable pattern. No consistent correlations were found between TSS concentrations and turbidity data measured by YSI, which meant that turbidity time series could not be used to predict continuous variability in TSS for the oyster filtration model.

4.4 Outcome 4: Assess habitat enhancement

Nekton surveys. We used mesh traps deployed three times during the sampling season to assess how the planted areas of the reef enhanced habitat for nekton. Traps were compared with the mud-bottom control site. There were a total of 11 fish species and 4 decapod species found throughout the surveys. Overall, four species dominated the traps at both the reef and control sites (Table 4.4.1). These species were *Libinia emarginata* (spider crab), *Callinectes sapidus* (blue crab), *Centropristis striata* (black sea bass), and *Opsanus tau* (oyster toadfish). Overall, most of the crabs did not seem to show a habitat preference, however fish were generally more abundant on the reef sites over the control sites.

Summary metrics used to evaluate habitat enhancement were total abundance, richness, and abundance of fish and decapod groups. In 2019, no strong or consistent differences were found between habitat metrics and reef or date, as metrics varied both between sampling date and location (Fig. 4.4.1). A few significant differences were found between richness, where the highest richness was found on the reef in June, significantly different from the lowest value on the control site during September ($p = 0.041$). Decapods were significantly more abundant on the reef in September compared to July at both sites ($p = 0.003$).

In 2020, traps were placed at two locations within the reef to compare the older plantings to the new 2019 plantings. The 2020 trap data revealed more of a difference between reef sites and the control, but the reef sites varied from each other (Fig. 4.4.2). Less abundance and fewer decapods were consistently found on the old reef compared to the new plantings. However, fish abundance was higher on the reef sites compared to the control, where fish were only found in July 2020.

Non-metric multidimensional scaling plots were created to visually compare the communities on the different reef sites in 2020 and determine if groups could be determined by location or date (nMDS could not be generated from the data in 2019 due to insufficient data). For the 2020 data (Fig. 4.4.3), there were significant differences with date ($p = 0.001$), but not location ($p = 0.133$). Similar community structure seemed to be driven by decapods *L. emarginata* and *Cancer irroratus* (rock crab) in June, *C. striata* in July, and *Bairdiella chrysoura* (silver perch) and *C. sapidus* in September.

Despite a lack of significant difference between the metrics and the reef site versus control, there is qualitative evidence of fish habitat enhancement on the Tuckerton reef after combining data for all traps and sampling dates (Table 4.4.2). Higher richness and abundance were consistently observed at reef sites with the exception of abundance for the old reef in 2020. This

indicates that the species that commonly abundant species at both sites may be diluting the significance between the sites, and that enhancement of nekton, especially fish, at the Tuckerton site is apparent with an overall 40-60% increase in richness and abundance on the reef sites.

Substrate baskets. Crates filled with reef shell substrate were deployed to assess habitat enhancement of smaller, reef-dependent fish and invertebrates. In 2019, baskets filled with oyster shell were deployed on the reef and at the control site and resampled in July and August (a third replicate was impacted by Hurricane Dorian in September 2019 with most baskets having emptied due to sea-state conditions).

In 2019, community metrics assessed included total abundance, richness, and the dominant taxonomic groups (fish, decapods, gastropods) as well as biomass (dry weight) of the decapod and gastropod groups (Fig. 4.4.4). Statistical results varied for each metric, but the overall trend showed increased abundance on the control sites compared to the reef with some differences between sampling months. Total abundance (Fig. 4.4.4A) and abundance of fishes and decapods (Fig. 4.4.4C-D) were significantly higher in the control baskets (Fish: $p = 0.009$; Decapod: $p < 0.001$). Additionally, significant effects of sampling date were seen with decapods and gastropods higher in August compared to July (Fig. 4.4.4E-F, Decapod: $p < 0.001$; Gastropod: $p = 0.001$). Biomass was not significant for decapods (Site: $p = 0.381$; Month: $p = 0.940$), which indicates that crabs and shrimp were relatively smaller at the control site, as many small Xanthiid crabs (mud crabs) were found in these shells.

A hypothesis of habitat limitation explains the trends seen for the substrate baskets in Barnegat Bay. This hypothesis states that when appropriate habitat is a limiting factor for species, any substrate will show enhanced abundance compared to an area where habitat is non-limiting or redundant, such as areas of oyster reefs near functionally equivalent habitats like seagrass beds or salt marshes (Grabowski et al. 2005, Humphries et al. 2011). The reef itself can be viewed as a functionally equivalent habitat when the substrate baskets are placed within it, therefore, reef-dependent species like gobies, blennies and mud crabs can find suitable habitat in and around the oyster shell surrounding the baskets. When baskets are placed with shell on a bare bottom control, this becomes the only source of refuge in an otherwise barren location and will attract more species. Despite not achieving intended results, this experiment shows the need for habitat within Barnegat Bay as open waters do not provide functionally equivalent habitat compared to areas near shore. Our experimental methods did not allow for placement of empty baskets in the control area, which would need to be further secured on the bottom, however, it would be expected that lack of substrate in the units would have resulted in fewer species.

The second round of this study occurred in 2021 and was re-designed to test habitat provision for different shell types used in remote setting: bare oyster shell (OYS), bare whelk shell (WLK) and natural shell clusters dredged from the reef and cleared of fouling species (NSC, living oysters on either oyster or whelk shell). Due to tipping and substrate loss, only three out of four oyster shell baskets were recovered with $> 50\%$ of shell remaining. Total abundance of mobile species and decapods were significantly less in the whelk shell baskets compared to oyster and natural shell clusters (Fig. 4.4.5A, E, Abundance: $p = 0.005$, Decapod: $p = 0.008$). There were no significant differences between shell type and total richness, fish, gastropod, or biomass of any groups (Fig. 4.4.5). Although the data were not significant, larger fish were found in the whelk and natural shell clusters. This implies that the oyster shell, where 100 shells are equivalent to 10 whelk shells in terms of volume displacement, provides better provision to smaller invertebrates like

Xanthid crabs and shrimp, but larger resident species and fish find better refuge in the larger gaps provided by whelk and oyster shells.

Non-dimensional scaling of the communities found in the substrate basket studies revealed some of these community-level differences. In 2019, significant species groupings emerged between site and dates (Fig. 4.4.6, Site: $p = 0.014$, Date: $p = 0.002$). In 2021, there were significant grouping with substrate types ($p = 0.021$), with some species being found more commonly with different types (Fig. 4.4.7): Gobies (*Gobiosoma sp*) and blennies (*Hysoblennius hentz*) were more associated with the natural shell clusters and oyster types, but shrimp (*Palaemotetes sp*) and oyster drill (*Eupleura caudata*) were more common on whelk shells.

In conclusion, there are unclear trends with habitat provisioning for small resident species giving caution to creating any broad conclusions applicable to the restoration site. Significant differences between treatment dates further confounded our interpretations for some of the habitat metrics. Future experiments with baskets reflecting the substrate in a given area (natural shell clusters vs mud baskets) would more accurately replicate the natural habitat on the Tuckerton Reef. Although the differences between substrate types were not strong in the 2021 study, there is some evidence that mixed shell substrates may support different communities. The densely packed oyster shell in the oyster basket treatments are not representative of the shell planting methods which results in shell more spread out on the bottom. Studies on other reefs looking at habitat provisioning have found positive associations with densities of live oysters and differences in species preferences with rugosity of substrates (De Santiago et al. 2019, Karp et al. 2018). For instance, Karp et al. (2018) found mud crabs preferred substrates with higher rugosity, but not small fish like gobies. This trend was observed in our studies, with increased decapod abundance in oysters and natural shell clusters, which had more shell per basket, and increased fish abundance and biomass in natural shell clusters and whelk shell. Although there may be slight differences in habitat provisioning provided by substrate types, in a substrate-limited system like Barnegat Bay, any substrate is capable of producing habitat and substrate choice should not be a high priority consideration when designing a reef (De Santiago et al. 2019).

Fouling species. Presence of fouling species, defined as species with limited to no mobility encrusting on oyster shells or hard substrates, was enumerated across all planting locations in the Fall 2020 surveys. Fouling species can be commensal with oysters, having no effect, or harmful, such as through boring sponge infections which can weaken shell and lower condition index (Watts et al. 2018) or encrusting species limiting hard surface for spat settlement (De Santiago et al. 2019). Despite these competitive interactions, encrusting species can contribute additional water filtration and denitrification benefits (Kellogg et al. 2013) even if not directly measured.

More fouling organisms were observed on the older shell clusters (Fig. 4.4.8, $p = 0.003$), with the New-20 clusters only having an average of two species per cluster. Both New-19 and New-20 clusters had higher numbers of live oysters and were dominated by macroalgae and the hard tubeworm cases (*Hydroides dianthus,* Fig. 4.4.9). Sponges were more dominant on the older plantings, with the boring sponge *(Cliona spp.*) being prevalent on the Old reef clusters at greater than 60% coverage and at about 20% on the one year old New-19 oysters. The high levels of boring sponge infections observed is a potential contributor to mortality of the older oyster cohorts. Infections result in brittle shells making the larger oysters more easily broken off to succumb to predation or siltation. It is evident that fouling species abundance will increase with time and potentially double from year 1 to year 2 of planting. Different trophic levels were observed within

the fouling community, with increased presence of the lemon drop nudibranch, *Doriopsilla pharpa*, on the Old reef shells which had the highest presence of its sponge prey. Additionally, the high presence of filter-feeding sponges such as *Microciona prolifera, Halichondria spp.*, and *Haliclona spp.* and sea squirts such as *Styela clava* and *Botrylloides diegensis* would be expected to add filtration and nutrient removal benefits to the reef, despite also reducing hard surface for spat settlement.

5: Summary and Recommendations

This project achieved its goal of doubling the size of the Tuckerton Reef in Barnegat Bay and determining water quality and habitat benefits associated with it. The monitoring methods developed by this study can be applied to future work at this site and other current or proposed oyster restoration sites in this area, and information gained from this study can be used to inform future restoration efforts.

To demonstrate a "successful" subtidal restoration reef in Barnegat Bay, we determined that oysters should achieve a minimum density of 10-15 m^{-2} for as long as possible. The data from this study suggest that disease-resistant remote-set oysters direct-planted on appropriate bottom type can survive above this density a minimum of four years. However, the type of substrate used, initial set ratios and planting method can ultimately determine the starting density, with added environmental variation affecting survival. It follows that reefs planted with more oysters and a smaller footprint should maintain higher densities longer, though this remains to be seen for the New-20 cohort as only one sampling period was monitored for the newest planting. Continued monitoring of the 2019 and 2020 sets from this study will help determine suitable starting densities for bottom plantings based on future survival of these cohorts. When planting areas fall below the target density, we recommend enhancement efforts either by adding more spat-on-shell or bare shell to catch natural recruitment.

This study also looked at the water filtration capacity of oysters for each planting location as the first step to estimating filtration and nitrogen removal benefits of oyster restoration in Barnegat Bay. Density of oysters at each site was a greater contributor to water filtration on a per area basis, but when factoring in total volume filtered and nutrient removal, oyster biomass plays a stronger role. The filtration and nutrient removal of this 1-acre planted site is small compared to the total bay volume and TMDL, however, a scale up to 10-50% of bay volume filtration would be possible with a large-scale restoration program. Efforts to create higher densities of oysters when planted would contribute to these larger ecosystem-scale goals.

In addition to helping estimate filtration rates, water quality monitoring data was used to determine spatial variability with parameters within the Little Egg Harbor area. Consistent seasonal trends were observed between temperature and dissolved oxygen at all monitoring sites, but the spatial variability in Chlorophyll *a* and TSS require further investigation, especially given the sensitivity these parameters have on filtration and phytoplankton (and thus nitrogen) clearance rates. This study determined that monthly sampling is sufficient for filtration rate estimates for most months, but multiple sampling efforts may be necessary to capture dynamics in months that experience large seasonal swings.

In attempting to use collected water monitoring data to calculate filtration nutrient removal, many assumptions had to be made which can result in over or under-estimating actual filtration rates. These assumptions include that oysters are the only organism filtering water on the reef

(underestimating), that oysters filter water 100% of the time during the growing season (overestimating), and that certain parameters (Chlorophyll, TSS) are constant throughout the monthly intervals (undetermined). We assumed that 20% of biodeposits is denitrified. Additionally, nitrogen transformations can be subject to remineralization in the water column, be temperature-dependent and denitrification can be inhibited by oxygen and thus reduced by macroalgal presence. Overall, the best management practices for measuring dentification are still in development (Ray et al. 2021). In order to more accurately represent nitrogen removal on the reef, we recommend the following:

- Collecting *in-situ* TSS and Chlorophyll *a* samples over larger spatial and time scales. Determining how both tidal periods and high and low density reefs can impact these variables can help improve estimates.
- Incorporate tissue sequestration of nitrogen. We did not incorporate this as this doesn't represent a removal process on the reef, but this can be important for aquaculture-related nitrogen removal.
- Exploring other methods for denitrification estimation that could incorporate microbial processes (gene expression), sediment transformations (C:N ratios), and N_2 flux measurements to more accurately determine how nitrogen cycling works on the reef site.

This study also suggests that different planting methods and substrate types may provide different habitat benefits. Although there was enhanced nekton presence overall on reef areas compared to the control site, high abundance of certain species common to Barnegat Bay obscured the ability to statistically determine any increase either year. Future studies should explore additional methods for documenting habitat use and perhaps add additional categories of larger nekton through trawl or video surveys.

Overall, this project has laid the foundation for future monitoring of ecosystem services with Barnegat Bay restoration projects. Additional oyster reefs have been added to Barnegat Bay since the start of this project, with potential to further contribute to habitat, filtration and nutrient removal. Monitoring of these services should be an important component and factored into the budget of any restoration program to ensure it is meeting its intended goals.

References

Baker R.J., Wieben C.M., Lathrop R.G., and Nicholson R.S. 2014. Concentrations, loads, and yields of total nitrogen and total phosphorus in the Barnegat Bay-Little Egg Harbor watershed, New Jersey, 1989–2011, at multiple spatial scales: U.S. Geological Survey Scientific Investigations Report 2014–5072, 64 p.

Baggett, L.P., S.P. Powers, R.D. Brumbaugh, L.D. Coen et al. 2015. Guidelines for evaluating performance of oyster habitat restoration. *Restoration Ecology*, 23(6): 737-745.

Beseres Pollack J., Yoskowitz D., Kim H-C, and Montagna P.A. 2013. Role and value of nitrogen regulation provided by oysters (*Crassostrea virginica*) in the Mission-Aransas Estuary, Texas, USA. *PLoS ONE* 8(6): e65314.

Boudreaux, M.L., Stiner, J.L. and Walters, L.J. 2006. Biodiversity of sessile and motile macrofauna on intertidal oyster reefs in Mosquito Lagoon, Florida. *Journal of Shellfish Research* 25(3): 1079-1089.

Brown L.A., Furlong J.N., Brown K.M., La Peyre M.K. 2013. Oyster reef restoration in the northern Gulf of Mexico: Effect of substrate and age on ecosystem services. *Restoration Ecology* 22: 127-259.

Bricker S.B., Grizzle R.E., Trowbridge P. *et al.* 2020. Bioextractive Removal of Nitrogen by Oysters in Great Bay Piscataqua River Estuary, New Hampshire, USA. *Estuaries and Coasts* 43, 23–38.

Brush M.J. and Kellogg M.L. 2018. Harris Creek Oyster restoration Model v.2. Available online at https://exchange.iseesystems.com/public/markbrush/harris-creek-model-v2/index.html#page1

Cerco C.F. and Noel M.R. 2007. Can oyster restoration reverse cultural eutrophication in Chesapeake Bay? *Estuaries and Coasts* 30(2): 331-343.

De Santiago K., Palmer T.A., Dumesnil M., and Pollack J.B. 2019. Rapid development of a restored oyster reef facilitates habitat provision for estuarine fauna. *Restoration Ecology* 27(4): 870-880.

Dungan C.F. and Bushek D. 2015. Development and applications of Ray's fluid thioglycollate media for detection and manipulation of *Perkinsus spp.* pathogens of marine molluscs. Journal of Invertebrate Pathology 131: 68-82.

Ehrich M.K. and Harris L.A. 2015. A review of existing eastern oyster filtration rate models. *Ecological modeling* 297: 201-212.

Ferreira, J.G., Hawkins A.J.S., and Bricker S.B. 2007. Management of productivity, environmental effects and profitability of shellfish aquaculture – the Farm Aquaculture Resource Management (FARM) model. *Aquaculture* 264: 160-174.

Fitzgerald A.M., Wallace W.G., and Zarnoch C.B. 2020. Spatial and temporal trends in physiological biomarkers of adult eastern oysters, *Crassostrea virginica*, within an urban estuary. *Marine Environmental Research* 161: 105122.

Fulford R.S., Breitburg D.L., Newell R.I.E., Kemp W.M., and Luckenbach M. 2007. Effects of oyster population restoration strategies on phytoplankton biomass in Chesapeake Bay: a flexible modeling approach. *Marine Ecol. Prog. Ser.* 336: 43-61.

Gilby BL, Olds AD, Duncan CK, Ortodossi NL, Henderson CJ, Schlacher TA. 2020. Identifying restoration hotspots that deliver multiple ecological benefits. *Restoration Ecology* 28(1) 222-232.

Grabowski J.H., Hughes A.R., Kimbro D.L., Dolan M.A. 2005. How habitat setting influences restored oyster reef communities. *Ecology* 86: 1926–1935.

Grabowski J.H. and Peterson C.H. 2007. Restoring oyster reefs to recover ecosystem services. Pages 281–298 in Cuddington K., Byers J.E., Wilson W.G., Hastings A., eds. *Ecosystem Engineers: Plants to Protists*. Elsevier.

Gregalis K.C., Johnson M.W., and Powers S.P. 2009. Restored oyster reef location and design affect responses of resident and transient fish, crab, and shellfish species in Mobile Bay, Alabama. *Transactions of the American Fisheries Society* 138: 314-327.

Grizzle R.E., Greene J.K., and Coen L.D. 2008. Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: a comparison with previous laboratory studies, and the value of in situ methods. *Estuaries and Coasts* 31: 1208-1220.

Humphries A.T., La Peyre M.K., Kimball M.E., and Rozas L.P. 2011. Testing the effect of habitat structure and complexity on nekton assemblages using experimental oyster reefs. *J. Exp. Mar. Biol. Ecol.* 409: 172-179.

Karp M.K., Seitz R.D., Fabrizio M.C. 2018. Faunal communities on restored oyster reefs: effects of habitat complexity and environmental conditions. *Mar. Ecol. Prog. Ser.* 590: 35-51.

Keohane I.P., Tay J., Gawde R.K., and Hood R.R. 2019. Sensitivity of a biogeochemical model to the formulation of oyster filtration. *Ecological modeling* 403: 70-84.

Kellogg M.L., Cornwell J.C., Owens M.S., and Paynter K.T. 2013. Denitrification and nutrient assimilation on a restored oyster reef. *Mar. Ecol. Prog. Ser.* 480:1-19.

Keohane I.P., Tay J., Gawde R.K., Hood R.R. 2019. Sensitivity of a biogeochemical model to the formulation of oyster filtration. *Ecological modelling* 403: 70-84.

La Peyre M.K., Humphries A.T., Casas S.M., and La Peyre J.F. 2013. Temporal variation in development of ecosystem services from oyster reef restoration. *Ecological Engineering* 63: 34-44.

Newell R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *J. Shellfish Res.* 23: 51−61.

Newell R.I.E. 2007. Top-down control of phytoplankton by oysters in Chesapeake Bay, USA: Comment on Pomeroy et al. (2006). *Mar. Ecol. Prog. Ser.* 341: 293-298.

Oksanen J. F., Blanchet G., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Szoecs E. and Wagner H.. 2019. vegan: 622 Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Parsons T.R., Takahashi M., Hargrave B. 1984. *Biological Oceanographic Processes* (3rd ed). Pergamon Press.

Paynter K.T., Politano V., Lane H.A., Allen S.M., Meritt D.M. 2010. Growth rates and prevalence of *Perkinsus marinus* in restored oyster populations in Maryland. *J. Shellfish Res.* 29(2): 309-317.

Powers S.P., Peterson C.H., Grabowski J.H., and Lenihan H.S. 2009. Success of constructed oyster reefs in no-harvest sanctuaries: implications for restoration. *Mar. Ecol. Prog. Ser.* 389: 159-170.

Rainer J.S. and Mann R. 1992. A comparison of methods for calculating condition index in eastern oyster, Crassostrea virginica (Gmelin, 1971). *J. Shellfish Res.* 22(3): 747-752.

Ray N.E., Hancock B., Brush M.J., Colden A., Cornwell J., Labrie M.S., Maguire T.J., Maxwell T., Rogers D., Stevick R.J., Unruh A., Kellogg M.L., Smyth,A.R. and Fulweiler R.W. (2021), A review of how we assess denitrification in oyster habitats and proposed guidelines for future studies. *Limnol Oceanogr. Methods*, 19: 714-731.

Ren L. 2013. Baseline characterization of phytoplankton and harmful algal blooms in Barnegat Bay-Little Egg harbor, New Jersey (Year One). NJDEP-Science and Research, 101 p.

Ren L. 2015. Baseline characterization of phytoplankton and harmful algal blooms in Barnegat Bay-Little Egg harbor, New Jersey (Year Two). NJDEP-Science and Research, 69 p.

Rose J.M., Gosnell J.S., Bricker S. *et al.* 2021. Opportunities and Challenges for Including Oyster-Mediated Denitrification in Nitrogen Management Plans. *Estuaries and Coasts* **44:** 2041–2055.

Thompson C.M., Evert S. and Baez J. *In press*. Comparing oyster population structure and disease prevalence between a disease-resistant and transplanted wild population in Barnegat Bay, NJ (USA). *Restoration Ecology*.

Watts J.C., Carroll J.M., Munroe D.M. and Finelli C.M. 2018. Examination of the potential relationship between boring sponges and pea crabs and their effects on eastern oyster condition. *Diseases of aquatic organisms* 130: 25-36.

zu Ermgassen, P.S.E., Spalding M.D., Blake B., Coen L.D. et al. 2012. Historical ecology with real numbers: past and present extent and biomass of an imperiled estuarine habitat. *Proc. R. Soc. B.* 27: 3393-3400.

zu Ermgassen P.S.E., Spalding M.D., Grizzle R.E., and Brumbaugh R.D. 2013. Quantifying the loss of a marine ecosystem service: filtration by the eastern oyster in U.S. estuaries. *Estuaries and Coasts* 36: 36-43.

zu Ermgassen P., Hancock B., DeAngelis B., Greene J., Schuster E., Spalding M., and Brumbaugh, R. 2016. Setting objectives for oyster habitat restoration using ecosystem services: A manager's guide. The Nature Conservancy, Arlington VA. 76pp.

Tables

Table 3.1.1 Footprint of each planting area in m^2 from 2021 sidescan sonar survey.

Reef location	Footprint (m^2)
Old reef	1,040
New-2019	1,151
New-2020	2,255
Total	*4,446*

Table 3.1.2 Planting summary for 2019 and 2020 planting efforts. Setting date refers to the day larvae entered the tanks and planting date refers to the date larvae were delivered to the reef site. Success ratio is the estimated number of settled spat divided by total larvae used

Setting Date	Substrate Used	Volume Set (bushel)	Genotype / Strain	Larvae Used (in Millions)	Success Ratio	Planting Date
6/3/2019	Whelk, Clam, Oyster	600	NEH	11 M	18%	6/26/2019
5/11/2020	Oyster	728	DEBY	16 M	10.4%	6/9/2020

Table 3.3.1 YSI profile and TSS water sample collection dates.

Date	YSI replicate profiles	TSS sample collected
5/30/2019	Yes	Yes
6/20/2019	Yes	Yes
7/11/2019	Yes	Yes
7/17/2019	Yes	No
8/8/2019	Yes	Yes
9/20/2019	Yes	Yes
10/25/2019	Yes	Yes
11/25/2019	Yes	Yes
4/17/2020	Yes	No
5/14/2020	Yes	Yes
6/8/2020	Yes	Yes
6/25/2020	Yes	No
7/16/2020	Yes	Yes
8/3/2020	Yes	Yes
9/2/2020	Yes	Yes
10/19/2020	Yes	Yes

Table 4.1.1 Mortality for each date and location throughout the monitoring period as estimated through intact boxes averaged for each sample. Recent deaths were determined as less than 50% of fouling inside shell or gapers (tissue present). % Boxes = Total Dead/(Total Live + Total Dead); % New Boxes = Recent Deaths/Total Dead; % Drill = Drill marks/Total Dead. † indicates significant differences in Krusal-Wallis tests (% Boxes: K = 16.135, DF = 7, $p = 0.024$; % New Boxes: K = 23.867, df = 7, $p = 0.001$; % Drill: K = 22.15, df = 7, $p = 0.002$).

Date / Location	*% Boxes ± SE*	*% New Boxes ± SE*	*% Drilled ± SE*
SPRING 2019			
Old Reef	42.0 ± 9.7†	30.2 ± 9.7	0.0 ± 0.0
FALL 2019			
Old Reef	36.3 ± 2.2†	24.4 ± 1.2	3.6 ± 3.6
New-19	9.2 ± 1.1	74.0 ± 9.5†	16.7 ± 5.2†
SPRING 2020			
Old Reef	16.3 ± 9.7	0.0 ± 0.0	2.8 ± 2.8
New-19	17.6 ± 2.0	22.0 ± 1.3	15.6 ± 4.1†
FALL 2020			
Old Reef	10.0 ± 5.8	0.0 ± 0.0	0.0 ± 0.0
New-19	6.8 ± 2.2	66.7 ± 1.7†	0.0 ± 0.0
New-20	11.6 ± 1.1	98.7 ± 1.3†	52.9 ± 18.1†

Table 4.1.2 Pathogen testing for sampling locations (± standard deviation). Intensity of *Perkinsus spp*. infection was scored on a Mackin scale of 0-5 based on cell presence and percentage of examined tissue infected. For MSX, infection rating was on a scale of 0-4 based on infection intensity. Prevalence is the percent of infected samples and infection intensity is the average of infections for the entire sample (Dungan & Bushek 2015). n/a = not tested for the sampling period.

Date / Location	Number examined	*Perkinsus sp.* prevalence	*Perkinsus sp.* infection intensity	MSX prevalance	MSX infection intensity
FALL 2019					
Old Reef	40	33.5%	0.69 ± 1.45	n/a	n/a
FALL 2020					
Old Reef	18	35.0%	0.47 ± 0.92	5.0%	0.06 ± 0.24
New-19	20	0.0%	0.00 ± 0.00	5.0%	0.05 ± 0.22
SPRING 2021					
Old Reef	12	8.3%	0.17 ± 0.58	16.7%	0.17 ± 0.39
New-19	20	0.0%	0.00 ± 0.00	0.0%	0.00 ± 0.00
New-20	20	0.0%	0.00 ± 0.00	0.0%	0.00 ± 0.00

Table 4.2.1 Total N removed from each reef location by year. Estimated using continuous logger data ("logger") and by discrete profiles ("profile").

Year	*Planting location*	*Total N removed (kg), logger*	*Total N removed (kg), profile*	*% Difference*
2019	OLD	0.191	0.180	5.4%
	NEW-19	0.387	0.357	7.7%
Total		**0.578**	**0.537**	**7.1%**
2020	OLD	0.138	0.138	0.3%
	NEW-19	0.572	0.570	0.3%
	NEW-20	0.371	0.370	0.4%
Total		**1.081**	**1.078**	**0.3%**

Table 4.2.2 Total summer (July – September) nitrogen clearance based on environmental parameters from logger data and nitrogen removal scaled up for biodeposits, burial and denitrification. See text for calculation details.

Year	*Planting Location*	*Summer N Clearance Rate ($\mu g\ N\ m^{-2}\ d^{-1}$)*	*N Removal Biodeposits ($kg\ km^{-2}$)*	*N Removal Dentrification ($kg\ km^{-2}$)*	*N Removal Burial ($kg\ m^{-2}$)*
2019	OLD	811.84	42.52	9.90	4.95
	NEW-19	3431.60	209.33	41.87	20.93
2019 Total			***251.85***	***51.77***	***25.88***
2020	OLD	888.85	54.22	10.84	5.42
	NEW-19	3326.70	202.93	40.59	20.29
	NEW-20	1119.80	68.31	13.66	6.83
2020 Total			***325.46***	***65.09***	***32.55***

Table 4.2.3 Full estuary filtration scenarios for Barnegat Bay using parameters from 2020 and The Nature Conservancy's Oyster calculator: https://oceanwealth.org/tools/oyster-calculator/ . Additional parameters were filled in using values from the calculator specific to Barengat Bay or the Virginian ecoregion.

Year / Scenario	Reef Size (ha)	Mean Temperature (°C)	Mean Oyster Length (< 76 mm)	Mean Oyster Density (< 76 mm)	Mean Oyster Length (> 76 mm)	Mean Oyster Density (> 76 mm)	Filtration ($L\ hr^{-1}$)
2020	0.44	20.57	46	63.45	101	14.5	$1.10 * 10^6$
Goal – 10%	48	20.57	46	63.45	101	14.5	$1.23 * 10^8$
Goal – 50%	241	20.57	46	63.45	101	14.5	$6.16 * 10^8$
Goal – 100%	482	20.57	46	63.45	101	14.5	$1.2 * 10^9$
Historic	5,261						$3.50 * 10^9$

Table 4.3.1. Mean and standard deviation of water quality parameters from YSI profiles collected monthly at the reef and control sites. Data marked with * had >10% relative standard deviation among the replicate samples.

	Reef site			Control site		
Date	**T (°C)**	**S (PSU)**	**DO (mg l^{-1})**	**T (°C)**	**S (PSU)**	**DO (mg l^{-1})**
5/30/2019	21.61 ± 0.35	26.84 ± 0.35	6.92 ± 0.25	21.57 ± 0.10	27.05 ± 0.04	6.86 ± 0.09
6/20/2019	24.38 ± 0.02	27.50 ± 0.01	6.68 ± 0.03	24.47 ± 0.03	27.26 ± 0.04	6.85 ± 0.02
7/11/2019	26.26 ± 0.03	28.31 ± 0.02	6.54 ± 0.02	26.19 ± 0.02	28.85 ± 0.02	6.77 ± 0.01
7/17/2019	26.97 ± 0.02	27.75 ± 0.04	6.17 ± 0.02	26.86 ± 0.05	27.71 ± 0.05	6.07 ± 0.03
8/8/2019	27.14 ± 0.13	28.75 ± 0.05	5.63 ± 0.07	27.09 ± 0.07	29.14 ± 0.04	5.78 ± 0.13
9/20/2019	19.79 ± 0.03	27.26 ± 0.05	7.29 ± 0.03	20.06 ± 0.01	28.61 ± 0.01	7.39 ± 0.03
10/25/2019	14.90 ± 0.00	29.18 ± 0.27	7.85 ± 0.08	14.96 ± 0.05	29.44 ± 0.10	7.99 ± 0.12
11/25/2019	6.75 ± 0.01	28.61 ± 0.10	10.07 ± 0.03	6.71 ± 0.01	28.88 ± 0.04	9.93 ± 0.02
4/17/2020	9.56 ± 0.07	28.03 ± 0.04	9.24 ± 0.05	9.98 ± 0.04	27.68 ± 0.04	9.46 ± 0.02
5/14/2020	13.66 ± 0.02	NaN	9.84 ± 0.17	13.93 ± 0.03	NaN	10.10 ± 0.14
6/8/2020	22.29 ± 0.09	28.82 ± 0.01	7.52 ± 0.02	22.23 ± 0.01	28.57 ± 0.03	7.53 ± 0.01
6/25/2020	25.13 ± 0.00	29.94 ± 0.00	7.07 ± 0.02	25.29 ± 0.01	29.89 ± 0.01	7.18 ± 0.01
7/16/2020	26.97 ± 0.02	28.54 ± 0.00	6.99 ± 0.03	27.03 ± 0.02	29.38 ± 0.01	6.98 ± 0.02
8/3/2020	26.11 ± 0.19	29.92 ± 0.04	5.81 ± 0.07	26.53 ± 0.14	29.88 ± 0.03	5.98 ± 0.06
9/2/2020	24.53 ± 0.12	30.57 ± 0.10	6.33 ± 0.02	24.37 ± 0.04	30.63 ± 0.04	6.41 ± 0.03
10/19/2020	16.36 ± 0.05	29.04 ± 0.05	7.87 ± 0.04	16.05 ± 0.24	28.60 ± 0.17	7.82 ± 0.04
	Reef site			**Control site**		
Date	**Chl (ug l^{-1})**	**Turbidity (NTU)**	**pH**	**Chl (ug l^{-1})**	**Turbidity (NTU)**	**pH**
5/30/2019	9.59 ± 2.68*	4.49 ± 0.65*	NaN	7.14 ± 0.82*	4.04 ± 0.44*	NaN
6/20/2019	5.56 ± 0.53	4.29 ± 0.13	7.94 ± 0.00	6.59 ± 0.69*	3.87 ± 0.55*	7.96 ± 0.01
7/11/2019	6.17 ± 0.52	3.13 ± 0.33*	NaN	4.37 ± 0.22	2.43 ± 0.17	NaN
7/17/2019	6.88 ± 0.61	4.36 ± 0.24	NaN	6.22 ± 0.78*	5.38 ± 2.00*	NaN
8/8/2019	4.75 ± 0.39	2.16 ± 0.99*	7.71 ± 0.00	4.40 ± 0.62*	1.71 ± 0.15	7.71 ± 0.07
9/20/2019	2.88 ± 0.33*	2.14 ± 0.06	7.87 ± 0.01	1.71 ± 0.24*	1.35 ± 0.05	7.74 ± 0.25
10/25/2019	NaN	0.82 ± 0.92*	7.89 ± 0.02	NaN	0.42 ± 0.38*	7.92 ± 0.02
11/25/2019	8.61 ± 0.21	30.57 ± 3.13*	7.89 ± 0.01	8.26 ± 0.35	25.61 ± 8.21*	7.91 ± 0.00
4/17/2020	NaN	3.49 ± 0.29	8.00 ± 0.01	NaN	3.44 ± 0.23	8.08 ± 0.01
5/14/2020	2.97 ± 1.40*	4.52 ± 0.94*	7.95 ± 0.00	2.53 ± 0.41*	4.58 ± 0.48*	7.96 ± 0.00
6/8/2020	4.82 ± 0.32	3.61 ± 0.23	7.88 ± 0.01	4.76 ± 0.33	3.43 ± 0.29	7.88 ± 0.01
6/25/2020	4.21 ± 0.23	2.97 ± 0.51*	8.00 ± 0.00	3.44 ± 0.20	2.47 ± 0.08	7.96 ± 0.04

7/16/2020	5.78 ± 0.68*	3.89 ± 0.26	7.96 ± 0.01	9.59 ± 0.45	6.99 ± 0.54	8.03 ± 0.03
8/3/2020	2.60 ± 0.34*	4.24 ± 0.53*	7.72 ± 0.02	2.74 ± 0.42*	3.83 ± 0.42*	7.73 ± 0.07
9/2/2020	2.41 ± 0.20	3.15 ± 0.98*	7.83 ± 0.01	2.40 ± 0.38*	2.89 ± 0.42*	7.73 ± 0.04
10/19/2020	NaN	6.47 ± 0.73*	7.78 ± 0.01	NaN	4.21 ± 0.13	7.73 ± 0.03

Table 4.3.2. Mean, standard deviation, and sample size (N) of total suspended sediments (TSS, in mg l^{-1}) measured in water samples collected monthly at the reef and control sites. Data marked with * had >10% relative standard deviation among the replicate samples.

Sampling		**Reef**			**Control**	
Date	***mean***	***s.d.***	***N***	***mean***	***s.d***	***N***
5/30/2019	26.00	2.12	2	31.50*	3.54	2
6/20/2019	99.25	8.13	2	104.0	1.8	3
7/11/2019	90.83	4.86	3	82.8	8.1	2
8/8/2019	93.00	8.67	3	105.0	4.9	3
9/20/2019	135.50	10.61	2	115.0	7.8	3
10/25/2019	169.00	9.90	2	180.00*	55.15	2
11/25/2019	60.75	0.35	2	50.8	2.1	3
5/14/2020	23.00	2.12	2	26.25	2.47	2
6/8/2020	21.00*	2.83	2	26.83	0.29	3
7/16/2020	34.50*	18.50	2	24.25*	6.01	2
8/3/2020	65.25	5.30	2	12.50	0.71	2
9/2/2020	20.83	2.02	3	11.25	1.06	2
10/19/2020	40.50*	4.95	2	29.50*	3.54	2

Table 4.4.1 Percent abundance of common species from fish traps combined for 2019 and 2020. Abundances were standardized for deployment time and divided by total abundance for all species collected at each site. Total abundance is cumulative across all sites for each year.

Species	% on Reef	*% on New Reef*	*% on Control*	Total % Abundance
2019 – Fish				
Bairdiella chrysoura	0%		3%	2%
Centropristis striata	13%		19%	32%
Chilomycterus schoepfi	1%		0%	1%
Hippocampus erectus	1%		0%	1%
Opsanus tau	4%		1%	7%
Paralichthyes dentatus	0%		1%	1%
Stenotomus chrysops	2%		0%	2%
Taugoa onitis	1%		0%	1%
2019 – Decapod				
Callinectes sapidus	6%		7%	14%
Cancer irroratus	1%		0%	1%
Libinia emarginata	14%		18%	37%
Portunus gibbesii	1%		0%	1%
2020 – Fish				
Anguilla anguilla	3%	0%	0%	1%
Bairdiella chrysoura	3%	12%	0%	7%
Centropristis striata	17%	21%	13%	18%
Chilomycterus schoepfi	7%	1%	0%	2%
Opsanus tau	20%	4%	2%	6%
Paralichthys dentatus	0%	1%	0%	1%
Sphoeroides maculatus	3%	0%	0%	1%
Syngnathus fuscus	0%	0%	2%	1%
Taugoa onitis	11%	0%	0%	1%
2020 – Decapod				
Callinectes sapidus	10%	10%	30%	16%
Cancer irroratus	0%	0%	4%	1%
Libinia emarginata	30%	51%	49%	46%

Table 4.4.2 Total species and richness throughout the nekton surveys, combined for all surveys and adjusted for deployment time.

Metric / Location	2019	2020
Richness		
OFF	6	6
OLD REEF	10	9
NEW REEF	--	7
Abundance		
OFF	18	23.5
OLD REEF	27	15
NEW REEF	--	40.5

Figures

Figure 3.1.1 Site and planting locations. Left panel - site map showing Barnegat Bay (inset) and location of Tuckerton Reef site (TKR) within the Little Egg Harbor area. Right panel - Sonar image of reef site showing planting locations and footprint delineations.

Figure 3.1.2 Oysters being planted on the reef site in 2020. Photo credits: Susan Allen, Stockton University.

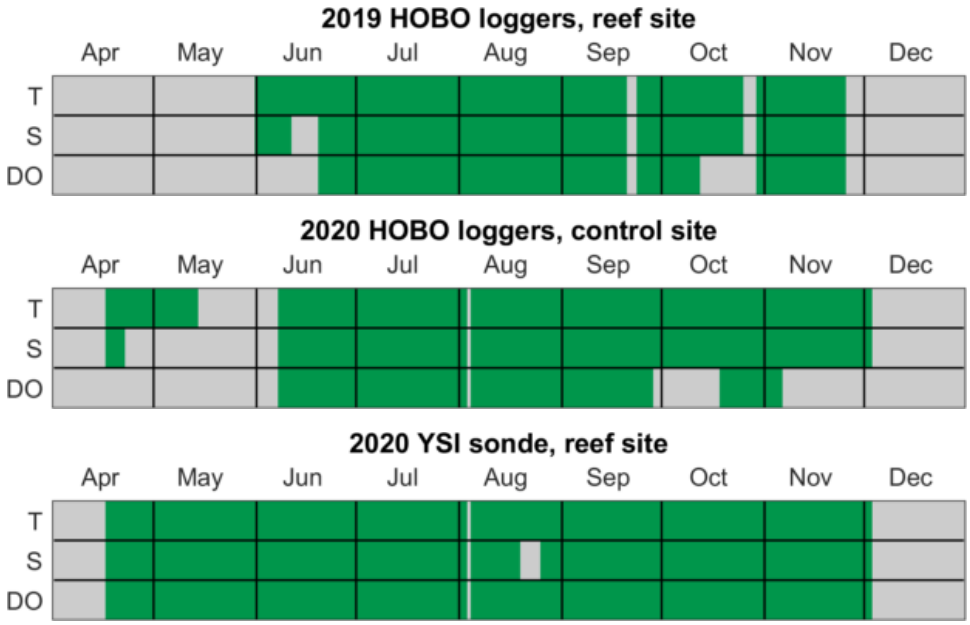


Figure 3.3.1. Continuous water monitoring inventory. Green shading indicates periods of temperature (T), salinity (S), and dissolved oxygen (DO) data collection that passed QA/QC procedures. (A) HOBO loggers deployed on the reef site in 2019. (B) HOBO loggers deployed on the control site in 2020. (C) YSI sonde deployed on reef site in 2020.

(A)

(B)

(C)

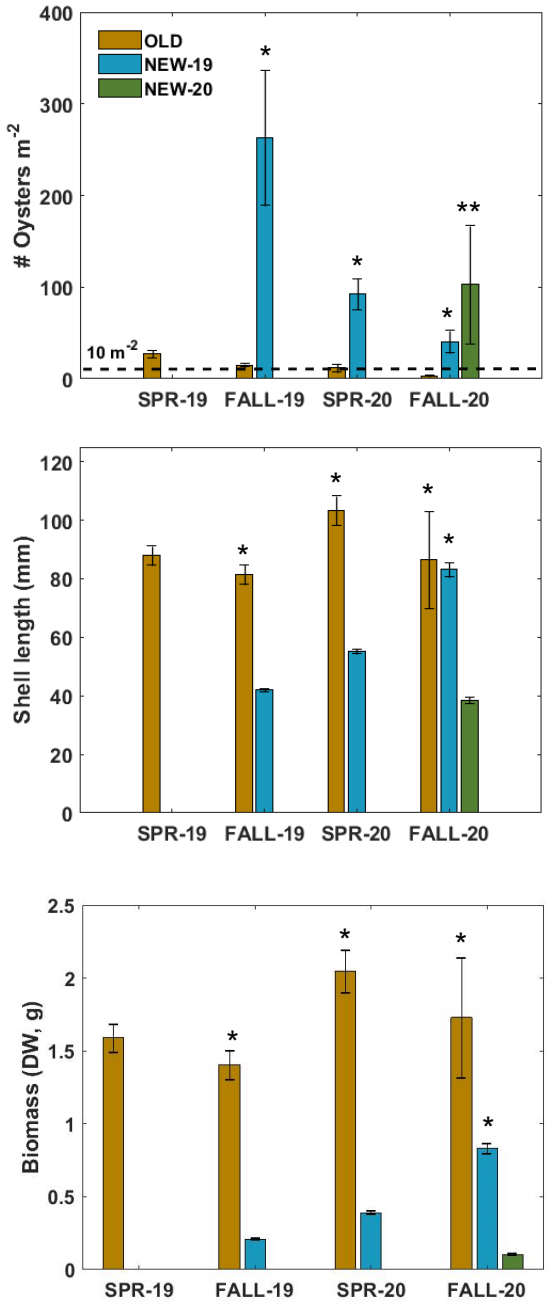


Figure 4.1.1 Oyster density, size and biomass. (A) Mean density of oysters throughout the study period. Dashed line in density plot represents the lower threshold density for ecosystem services (10 m^{-2}). Asterisks denote significant differences: Fall 2019: t = 8.548, df = 5, $p < 0.001$; Spring 2020: t = 5.063, df = 6, $p = 0.002$; Fall 2020: F = 19.606, df = 2, $p = 0.001$. (B) Mean shell length of oysters throughout the study period. Asterisks denote significant differences: Fall 2019: t = 12.23, df = 846, $p < 0.01$; Spring 2020: t = 11.419, df = 414, $p < 0.001$; Fall 2020: H = 170.3, df = 2, $p < 0.001$ (C) Mean biomass of oysters as calculated from length:biomass relationships of reef oysters (see methods section 3.3). Fall 2019: t = 12.16, df = 58.5, $p < 0.001$; Spring 2020: t = 11.39, df = 46.7, $p < 0.001$; Fall 2020: H = 201.5, df = 2, $p < 0.001$

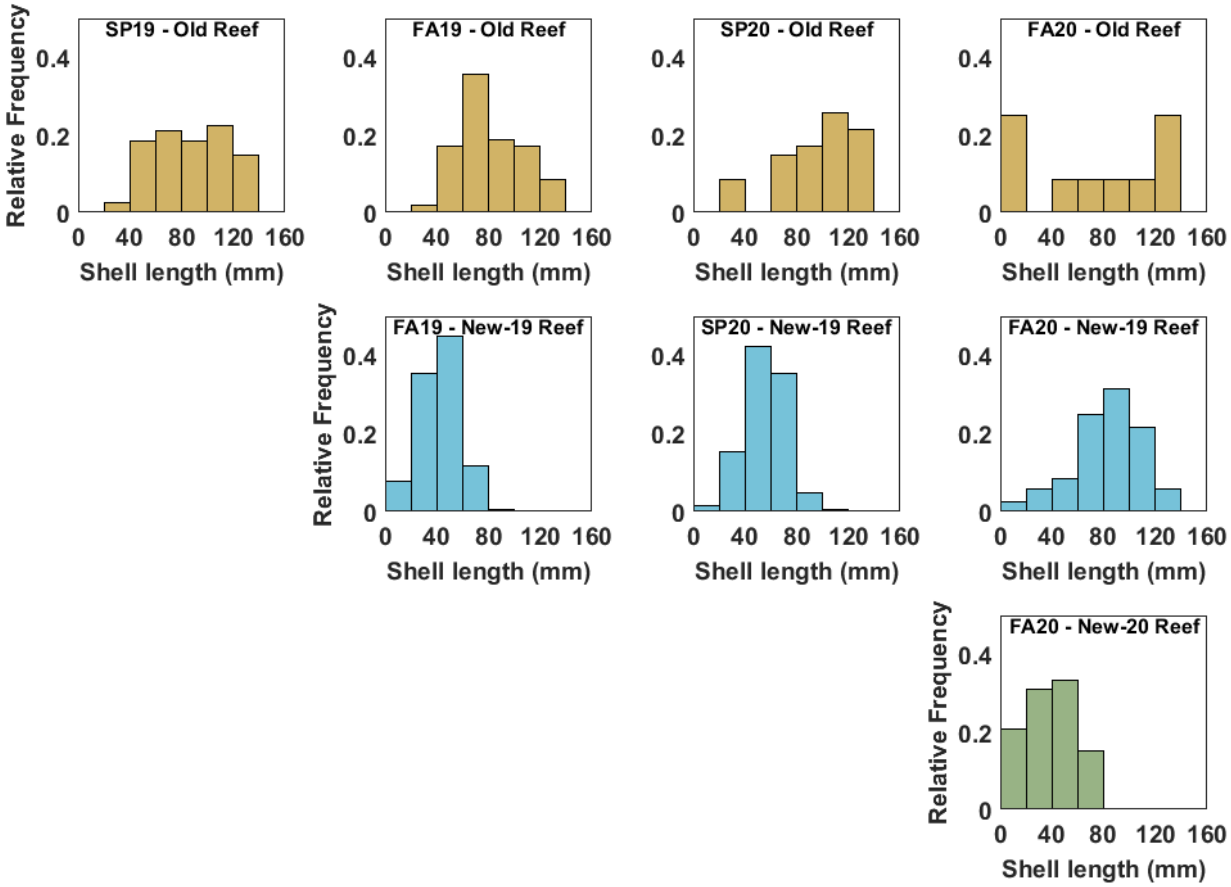


Figure 4.1.2 Oyster size frequency plots. Size frequency distribution based on shell-length combined for all samples on each planted area for each sampling date.

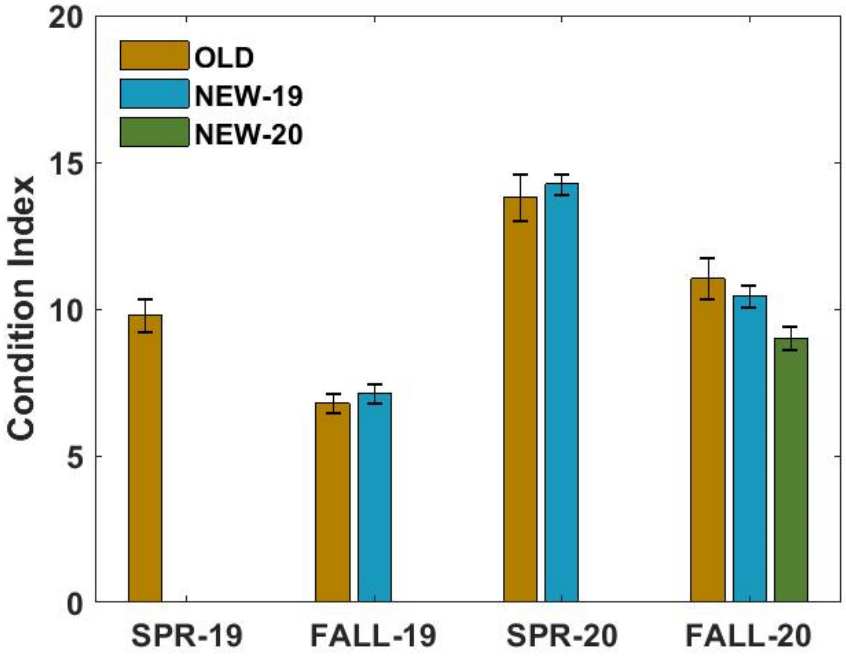


Figure 4.1.3 Condition Index from oysters subsampled from each monitoring period. Condition Index was determined using the following equation: CI = (TDWx100)/(WWW-SWW) where TDW = tissue dry weight, WWW = whole wet weight, and SWW = shell wet weight. (Old Reef: $F = 22.163$, $df = 3$, $p < 0.001$; New Reef: $F = 96.217$, $df = 2$, $p < 0.001$)

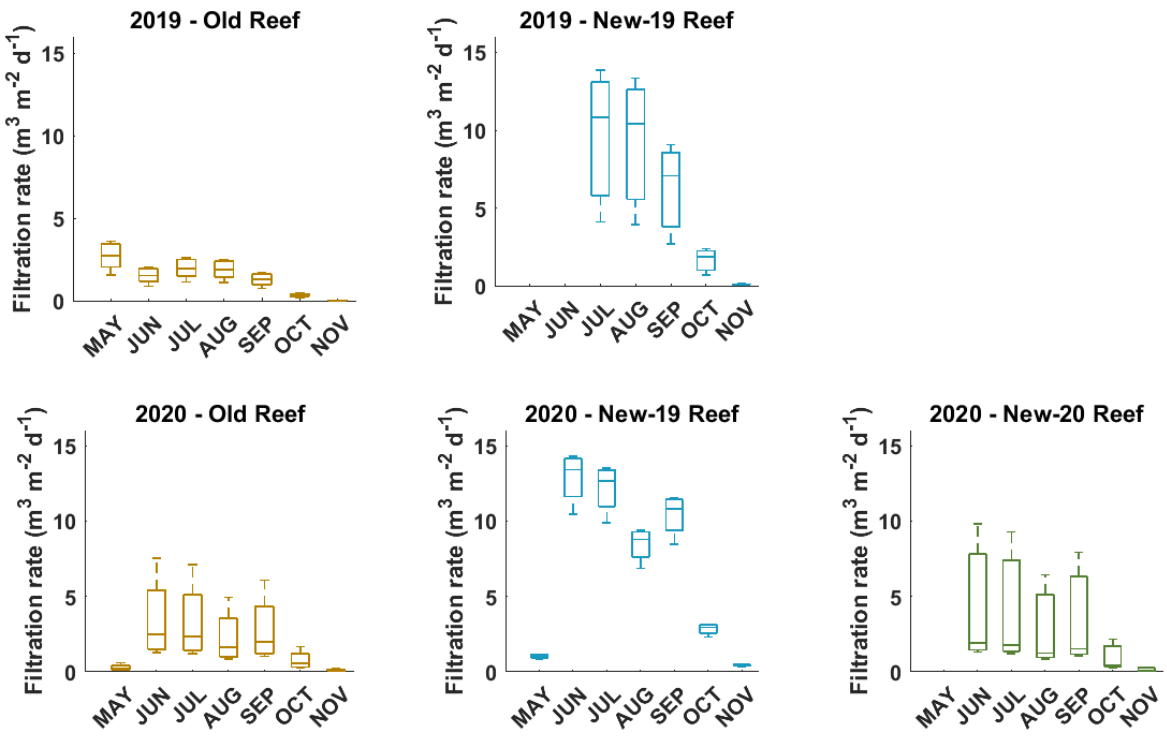


Figure 4.2.1 Monthly filtration using logger data. Box-plots showing range of filtration rates based on oyster densities for each reef cohort in 2019 and 2020 based on equation 2 (zu Ermgassen et al. 2013) and using monthly logger (HOBO – 2019, YSI - 2020) and TSS data. Filtration rates for New-19 and New-20 reef areas were adjusted for planting month (July in 2019 and June in 2020).

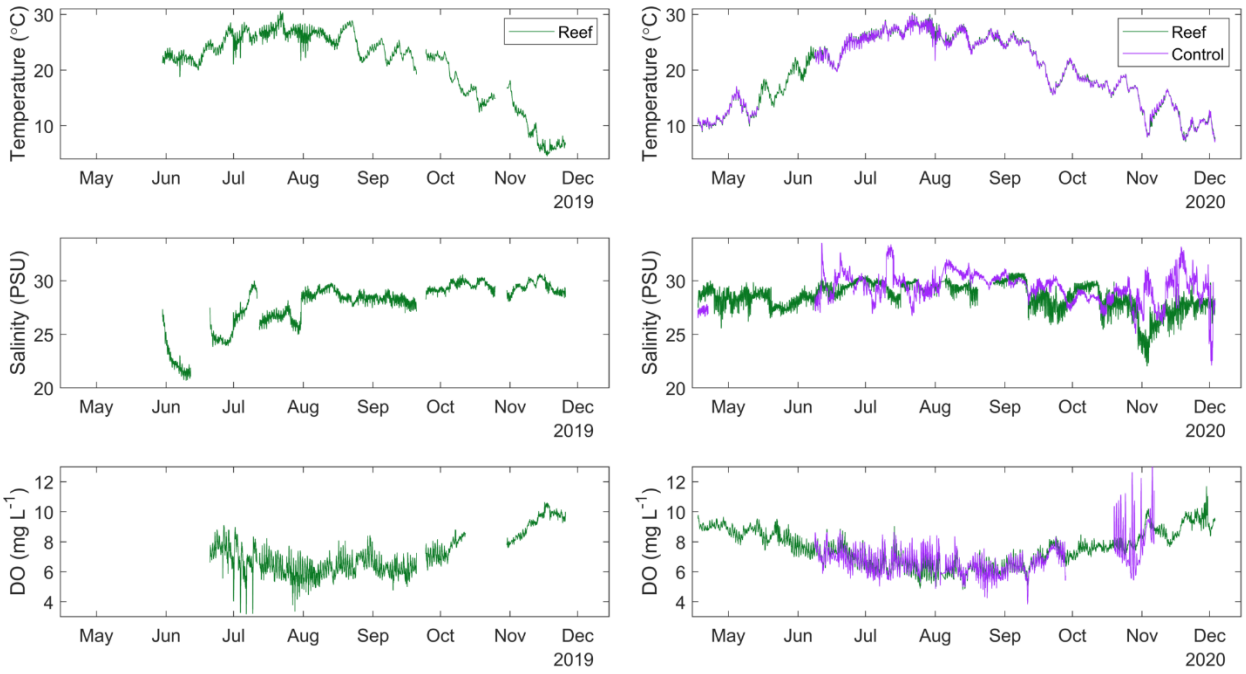


Figure 4.3.1 Continuous time series of temperature, salinity, and dissolved oxygen from (left) 2019 and (right) 2020.

Figure 4.3.2 Monthly box plots of continuous water monitoring data in 2019. (A) Temperature, (B) salinity, (C) dissolved oxygen.

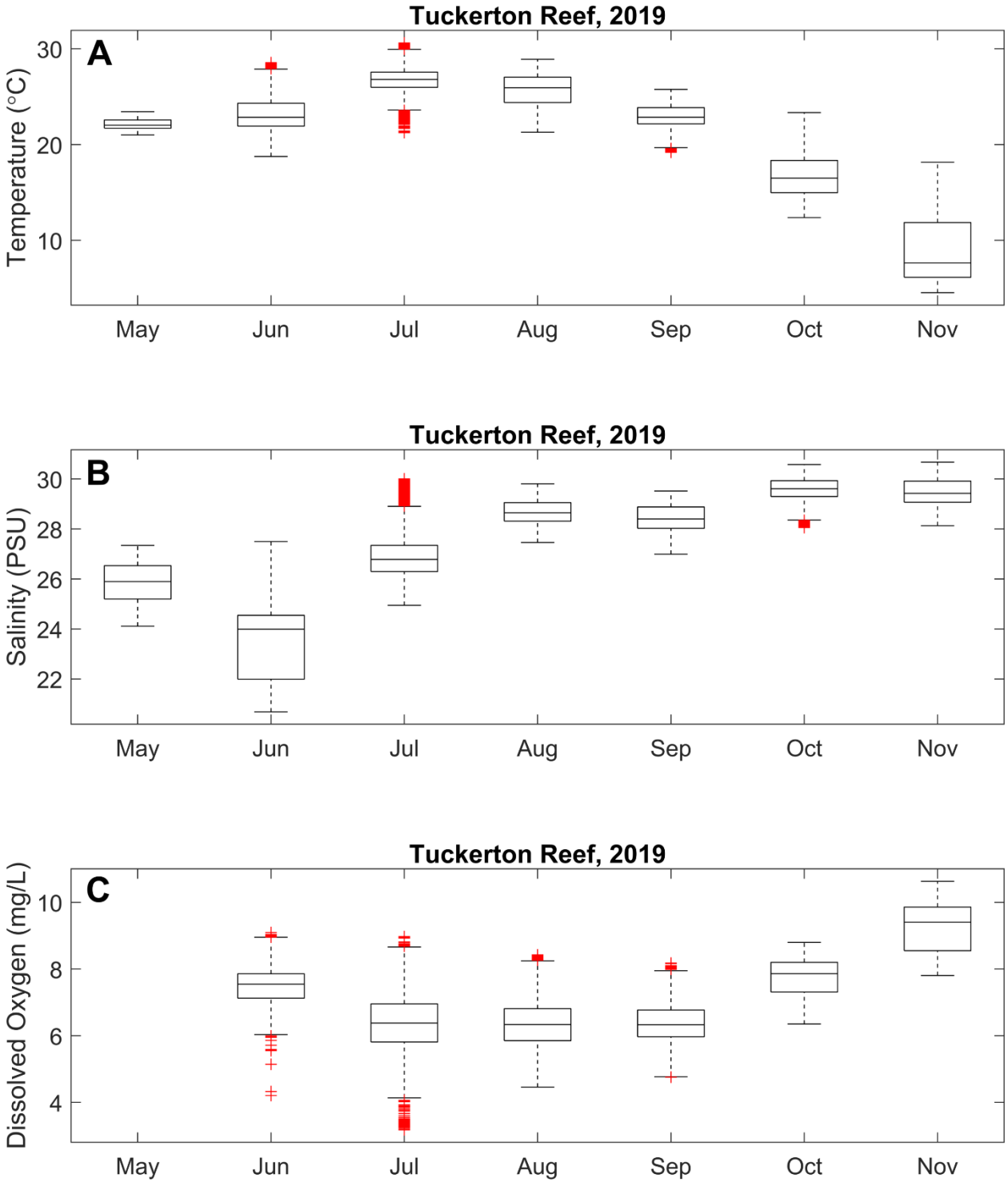
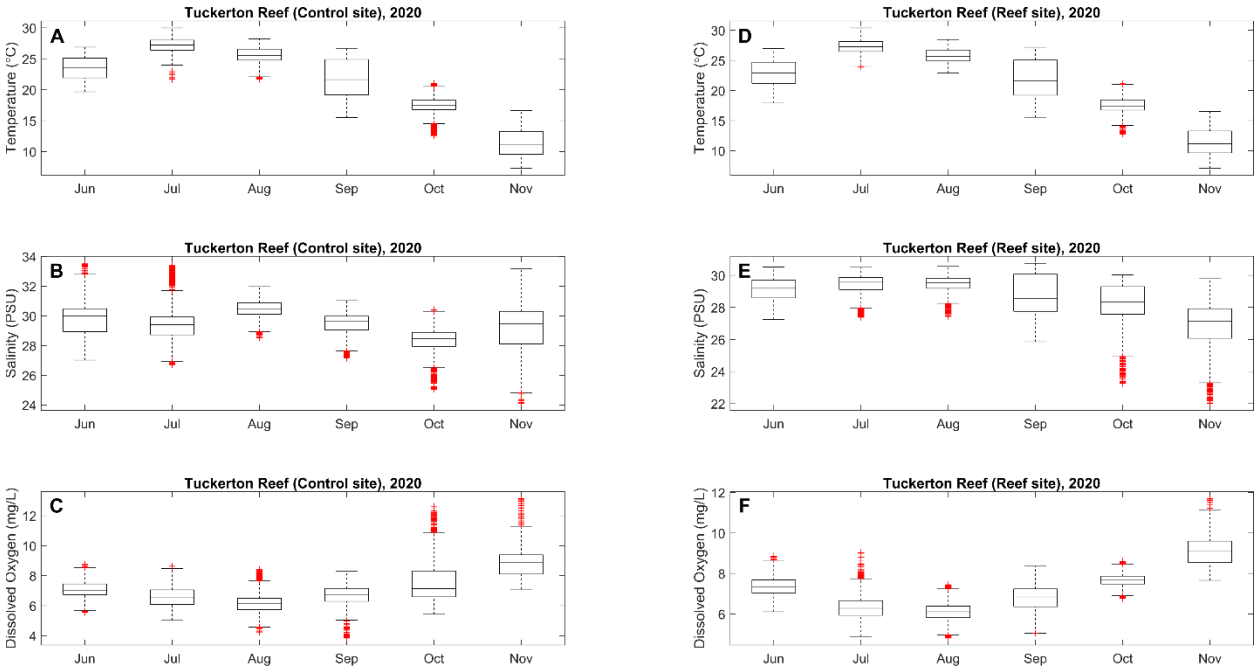


Figure 4.3.3 Monthly box plots of continuous water monitoring data in 2020 at control (A-C) and reef (D-F) sites.



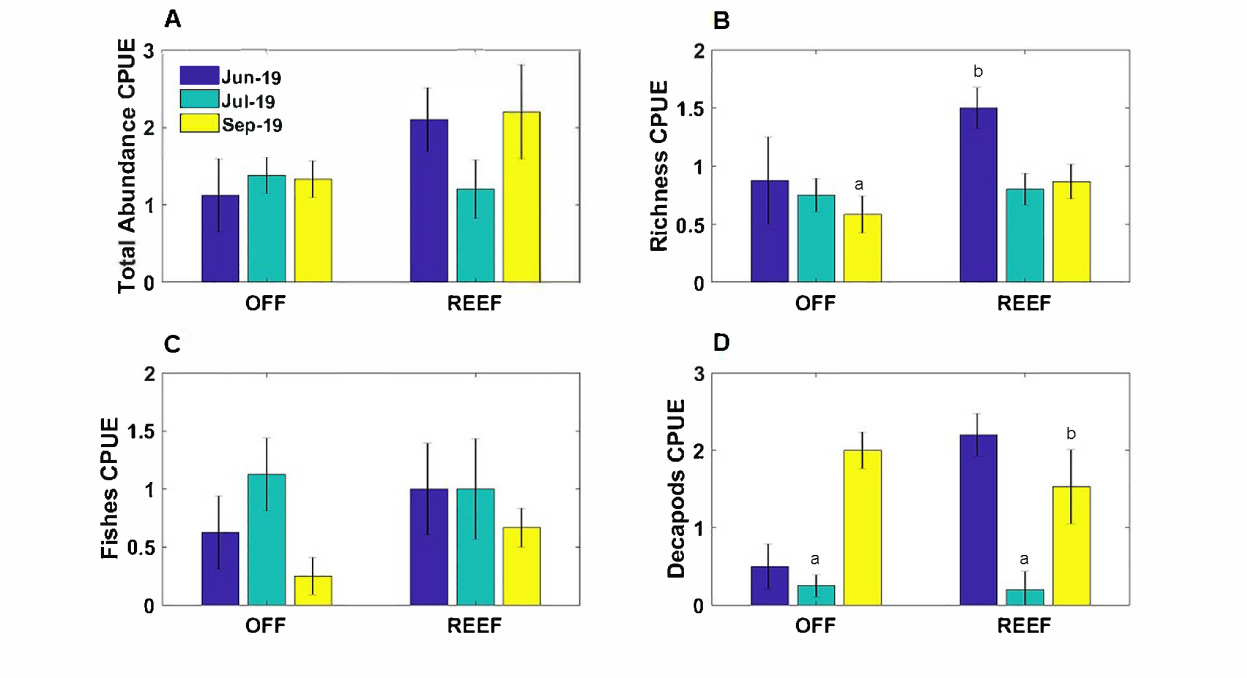


Figure 4.4.1 Nekton trap metrics for 2019 standardized by deployment time. Mean values for community metrics (± SE) are shown for each site and date. Letters denote groups that are significantly different from other groups in post-hoc tests. (A) Total abundance (Location: F = 2.89, df = 1, p = 0.10, Date: F = 0.97, df = 2, p = 0.395), (B) Richness (Location: F = 4.74, df = 1, p = 0.041, Date: F = 3.49, df = 2, p = 0.049), (C) Fish abundance and (Location: F = 0.80, df = 1, p = 0.381, Date: F = 1.83, df = 2, p = 0.184), and (D) Decapod abundance (Location: F = 2.57, df = 1, p = 0.123, Date: F = 7.72, df = 2, p = 0.003).

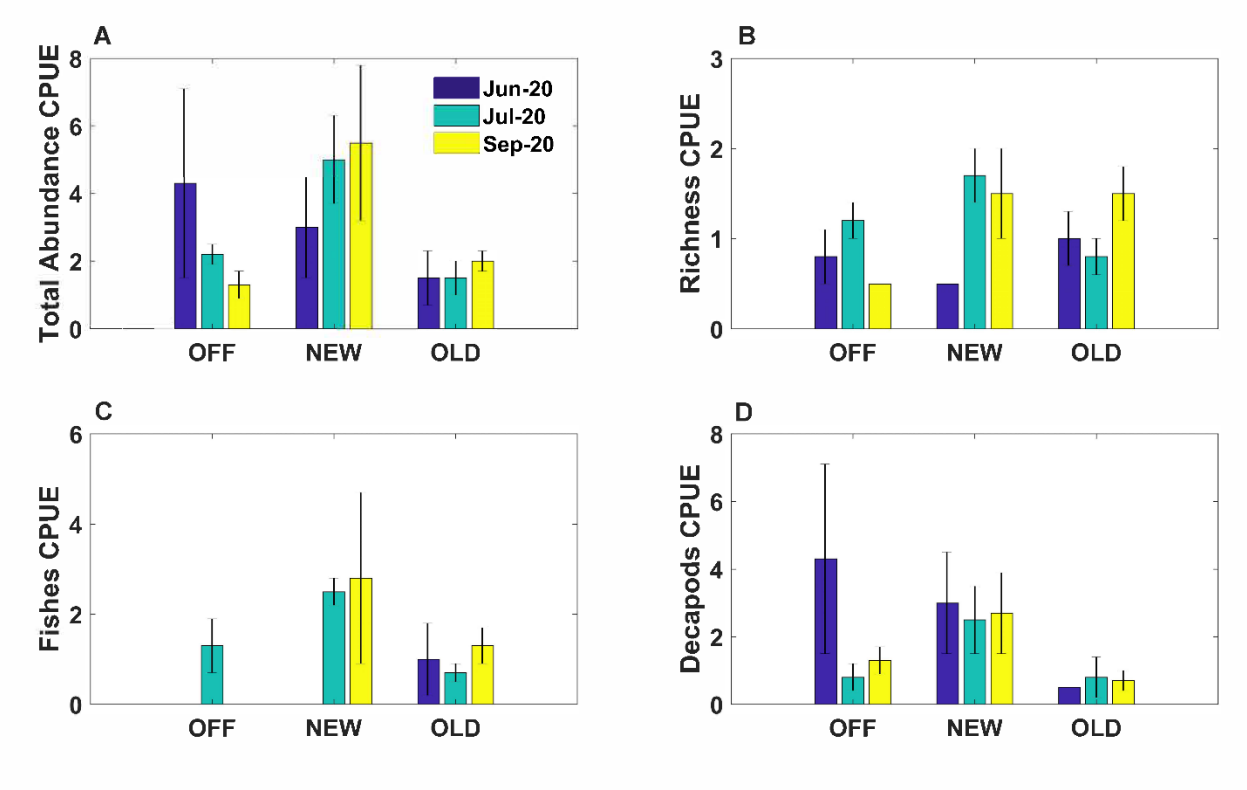


Figure 4.4.2 Mesh fish traps for 2020 standardized by deployment time. Mean values for community metrics (± SE) are shown for each site and date. (A) Total abundance (Location: F = 3.05, df = 2, p = 0.072, Date: F = 0.002, df = 2, p = 0.998), (B) Richness (Location: F = 1.56, df = 2, p = 0.237, Date: F = 2.28, df = 2, p = 0.131, Date*Location: F = 2.94, df = 4, p =0.049), (C) fish abundance (Location: F = 3.00, df = 2, p = 0.074, Date: F = 1.83, df = 2, p = 0.018), and (D) decapod abundance (Location: F = 4.19, df = 2, p = 0.032, Date: F = 686, df = 2, p = 0.516).

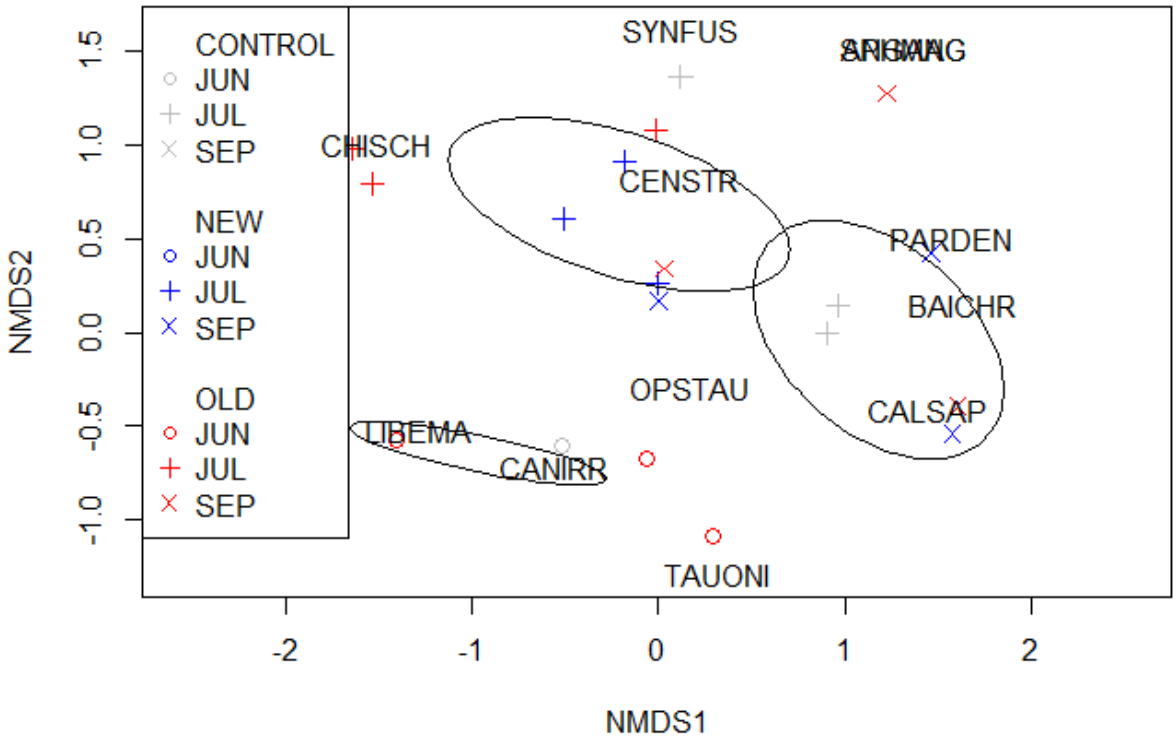


Figure 4.4.3 nMDS plot of 2020 mesh fish trap samples overlayed with species abbreviations. Ellipses show similar groupings by date. (PERMANOVA results: Date: $F = 4.20$, $R^2 = 0.259$, $df = 2$, $p = 0.001$, Location: $F = 1.59$, $R^2 = 0.116$, $df = 2$, $p = 0.133$)

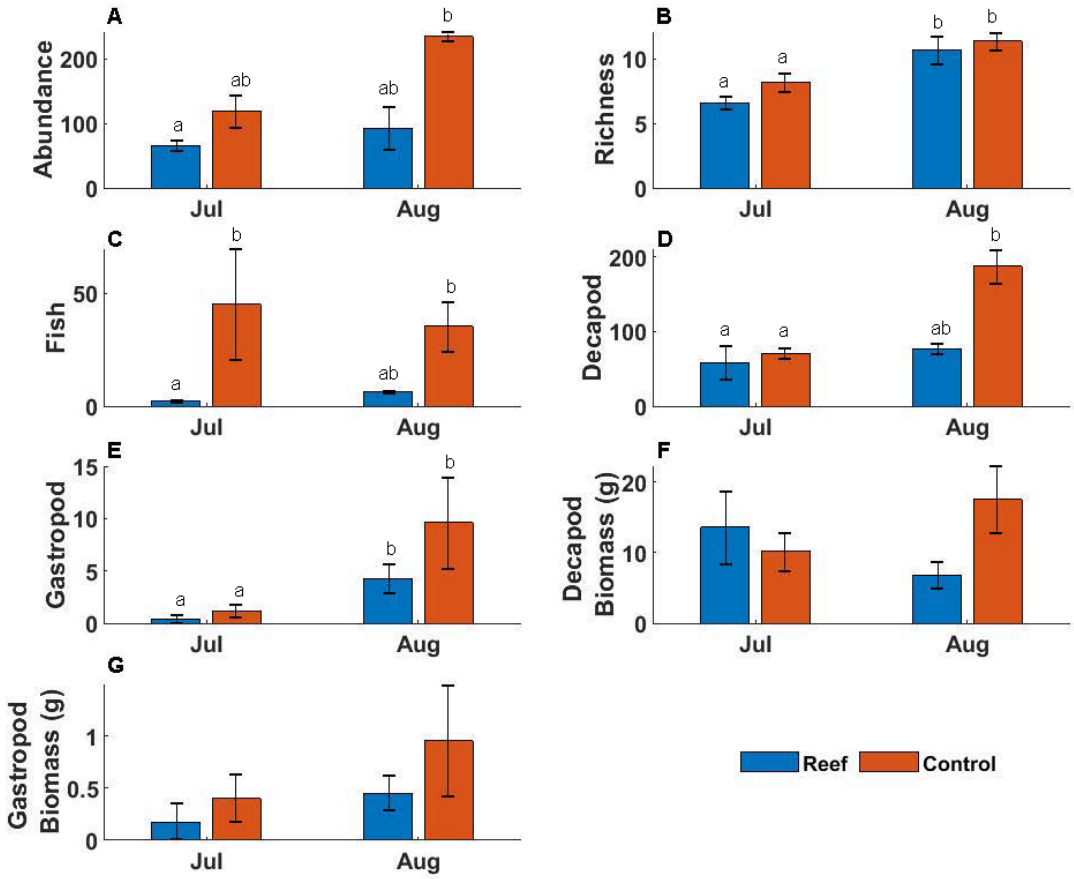


Figure 4.4.4 Substrate baskets 2019 deployments and means of community metrics (± SE) per trap and sample date. Letters denote groups that are significantly different from other groups in post-hoc tests (A) Abundance (Kruskal-Wallace test: W = 11, df = 3, $p = 0.008$), (B) Richness(GLM: Site – F = 2.236, df = 2, $p = 0.156$; Month – F = 23.86, df = 1, $p < 0.001$), (C) Total fish abundance (K-W: W = 11.643, df = 2, $p = 0.009$); (D) Total decapods abundance (GLM: Site – F = 24.849, df = 1, $p < 0.001$; Month – F = 31.501, df = 1, $p < 0.001$, Site*Month – F = 12.575, df = 1, p = 0.003), (E) Total gastropod abundance (GLM: Site – F = 2.573, df = 1, $p = 0.13$; Month – F = 17.247, df = 1, $p = 0.001$), (F) Decapod biomass as dry weight (GLM: Site – F = 0.816, df = 1, $p = 0.381$; Month – F = 0.006, df =1, $p = 0.940$), (G) Gastropod biomass as dry weight (GLM: Site – F = 0.788, df = 1, $p = 0.389$; Month – F = 3.363, df =1, $p = 0.087$)

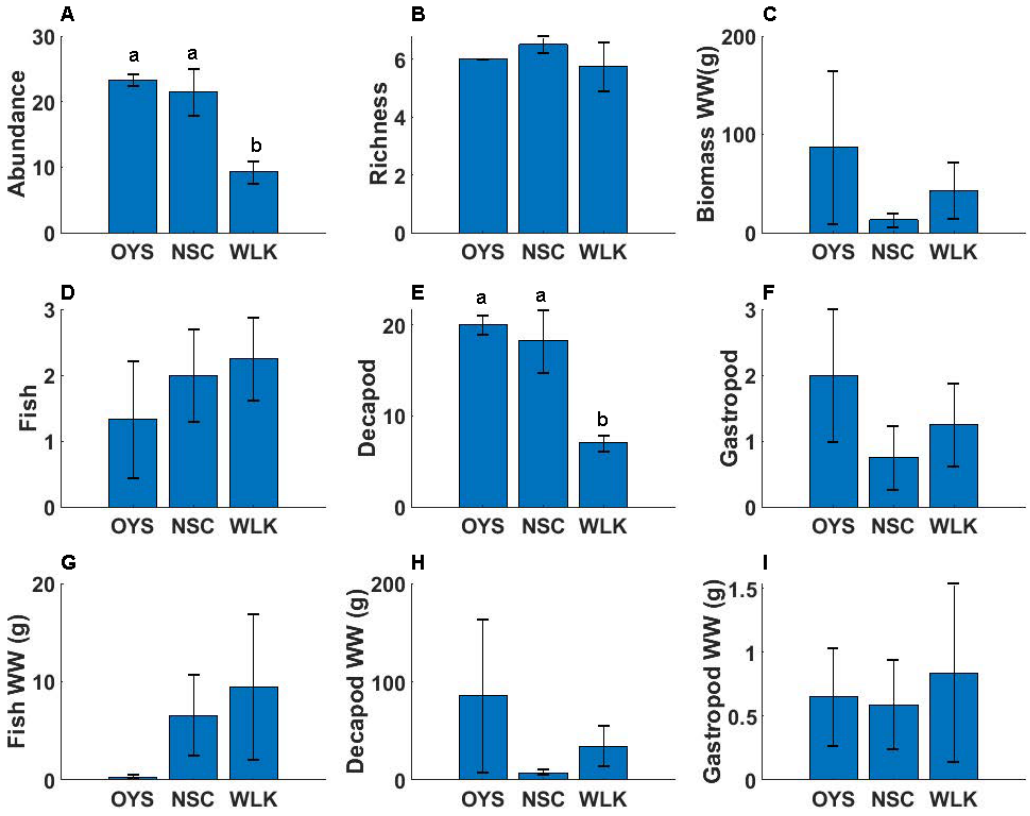


Figure 4.4.5 Substrate baskets 2021 and means of community metrics (± SE) per trap across shell types. Letters denote groups that are significantly different from other groups in post-hoc tests. (A) Abundance, or total individuals per trap, (ANOVA: F = 11.278, df = 2, $p = 0.005$), (B) Richness, number of species per trap, (ANOVA: F = 0.476, df = 2, $p = 0.638$), (C) Total biomass (WW = wet weight) (ANOVA: F = 0.440, df = 2, $p = 0.659$) (D) Fish abundance (ANOVA: F = 0.746, df = 2, $p = 0.691$), (E) Decapod abundance (ANOVA: F = 9.368, df = 2, $p = 0.008$), (F) Gastropod abundance (ANOVA: ANOVA: F = 0.795, df = 2, $p = 0.484$), (G) Fish Biomass (F = 0.683, df = 2, $p = 0.532$), (H) Decapod Biomass (F = 0.548, df = 2, $p = 0.598$), (I) Gastropod Biomass (F = 0.065, df = 2, $p = 0.938$). OYS = oyster shell, NSC = Natural shell clusters, WLK = whelk shell.

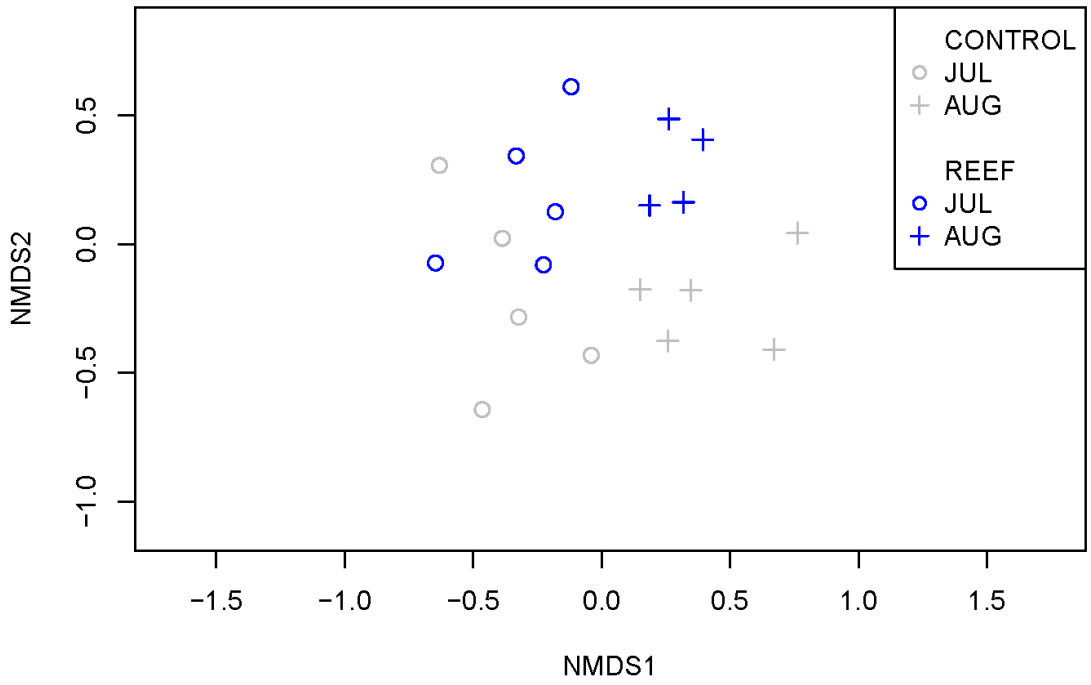


Figure 4.4.6 nMDS plot of 2019 substrate basket communities. Similar groupings are evident for both treatment (Control – gray, Reef – blue) and date (July – circles, August – crosses). (PERMANOVA Date: $F = 4.20$, $R^2 = 0.259$, $df = 2$, $p = 0.002$; Site: $F = 3.34$, $R^2 = 0.455$, $df = 2$, $p = 0.014$).

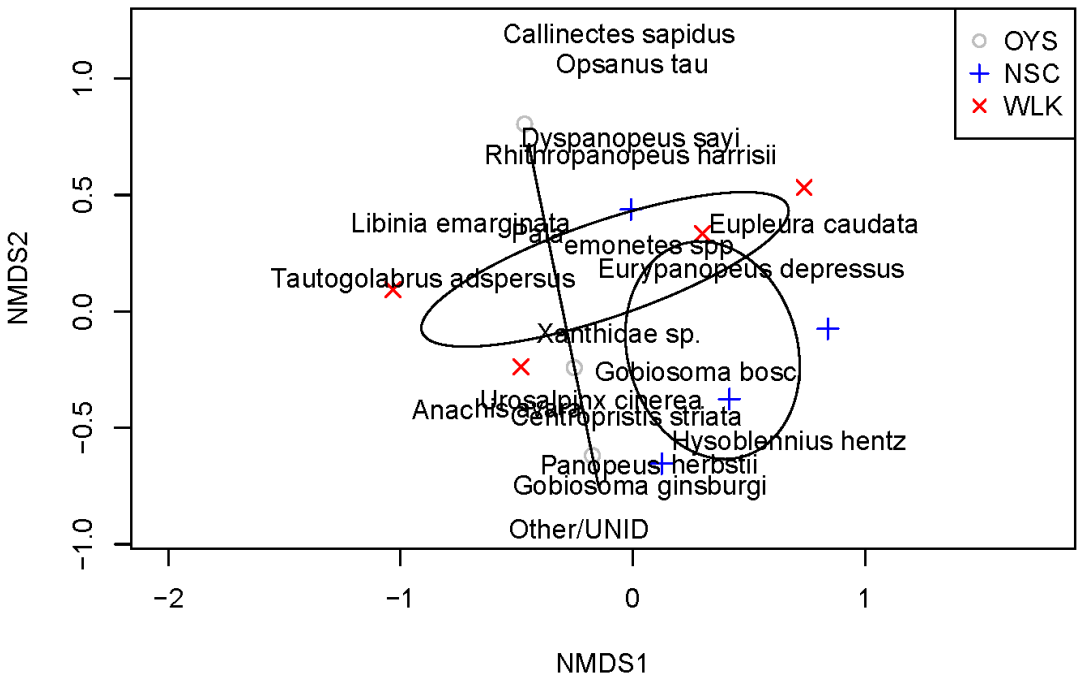


Figure 4.4.7 nMDS plot of 2021 substrate basket communities overlaid with species names. Ellipses show similar groups with shell type. (PERMANOVA Type: F = 3.34, $R^2 = 0.455$, df = 2, $p = 0.021$). OYS = oyster shell, NSC = Natural shell clusters, WLK = whelk shell.

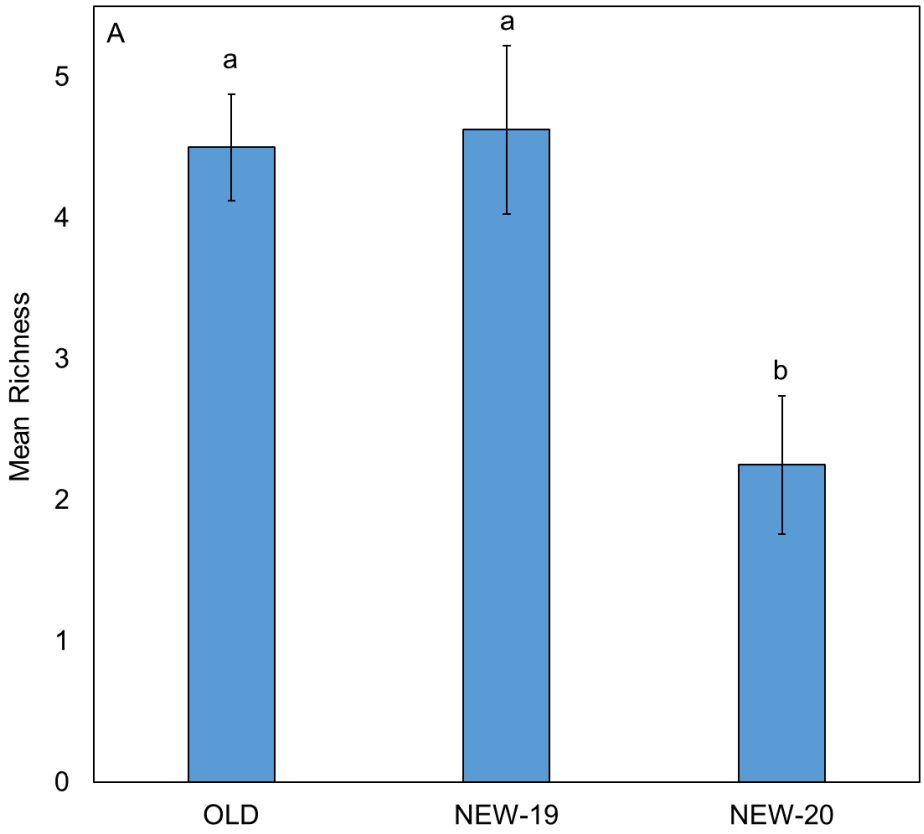


Figure 4.4.8 Mean (± SE) species richness for encrusting fauna enumerated on shell clusters from each reef location sampled in October 2020. Letters indicate significant groupings from post-hoc tests (ANOVA: df = 2, F = 7.232, $p = 0.003$).

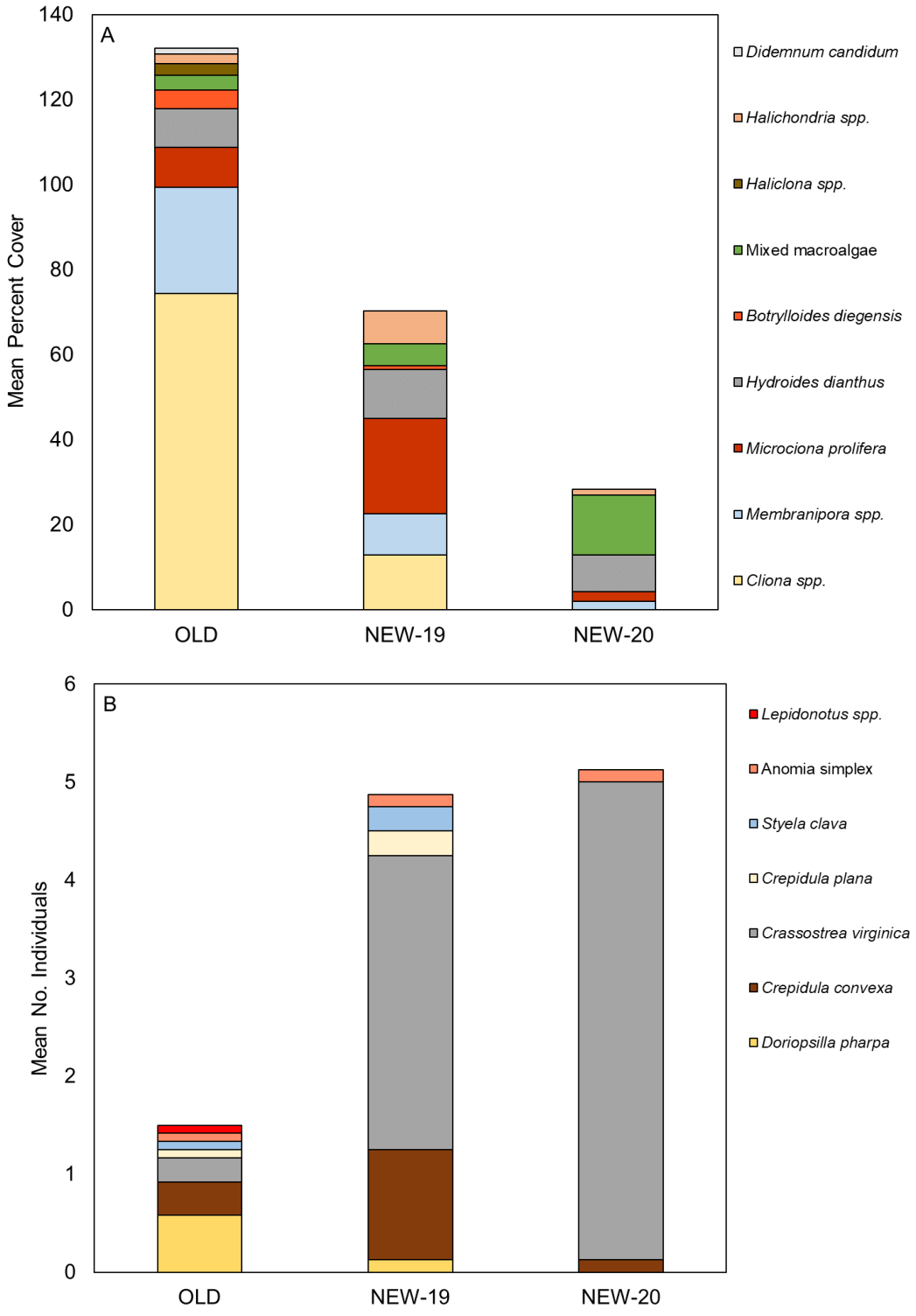


Figure 4.4.9 Fouling species composition. (A) Mean encrusting species as percent cover and (B) mean number of individuals attached to shell substrate from shell clusters from each location sampled from October 2020 surveys.

Appendix

Table A1 Comparison of monthly temperature and filtration rates calculated from continuous logger vs. discrete profiles. Positive temperature difference (ΔT) indicates that the discrete profile temperature was warmer than the monthly average. Filtration rates (Fr), in m^3 per g oyster carbon per day, calculated by Equation 1.

	2019			2020		
Month	*ΔT (°C)*	*Fr, profile (m^3 gC^{-1} d^{-1})*	*Fr, logger (m^3 gC^{-1} d^{-1})*	*ΔT (°C)*	*Fr, profile (m^3 gC^{-1} d^{-1})*	*Fr, logger (m^3 gC^{-1} d^{-1})*
May	-0.6	0.356	0.389	0.1	0.038	0.036
June	1.2	0.496	0.443	-1.1	0.394	0.453
July	-0.5	0.545	0.550	-0.3	0.550	0.550
August	1.5	0.550	0.534	0.5	0.543	0.535
September	-3.1	0.252	0.437	2.7	0.502	0.365
October	-2.1	0.061	0.123	-1.1	0.101	0.140
November	-2.0	0.001	0.004	n/a	n/a	0.015

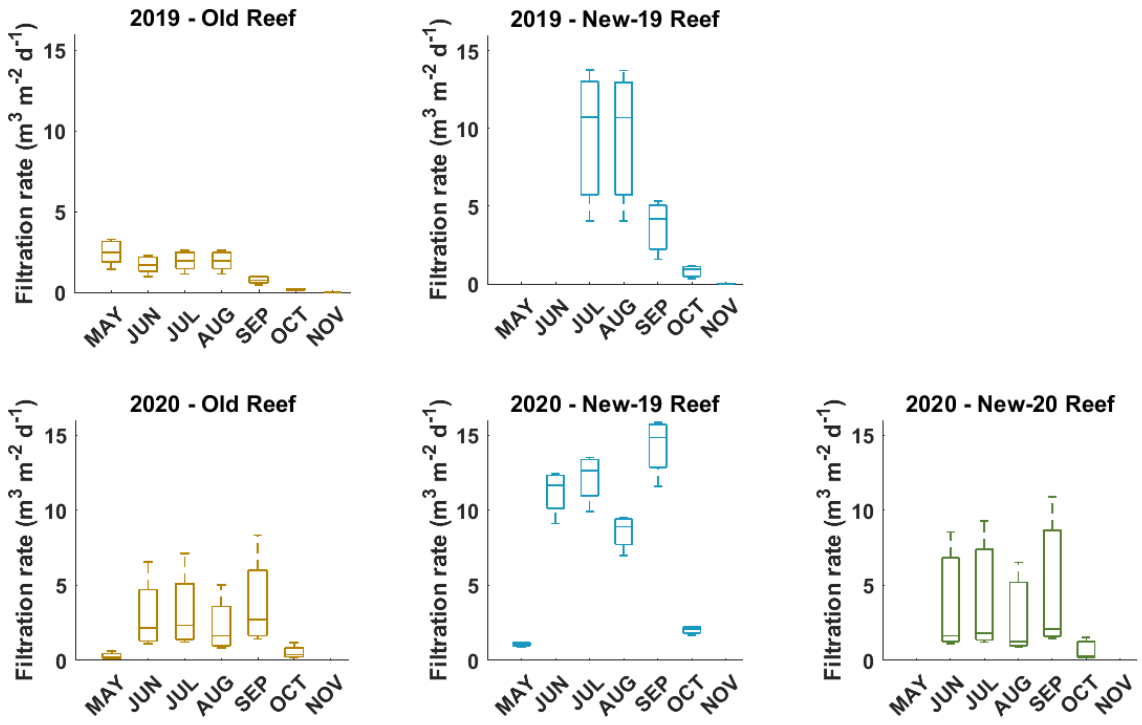


Figure A1. Monthly filtration using profile data. Box-plots showing range of filtration rates based on oyster densities for each reef cohort in 2019 and 2020 based on equation 2 (zu Ermgassen et al. 2013) and using monthly logger (HOBO – 2019, YSI - 2020) and TSS data. Filtration rates for New-19 and New-20 reef areas were adjusted for planting month (July in 2019 and June in 2020).