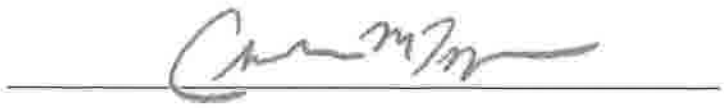
Barnegat Bay Oyster Restoration: providing water quality and habitat improvements in Barnegat Bay

Stockton University

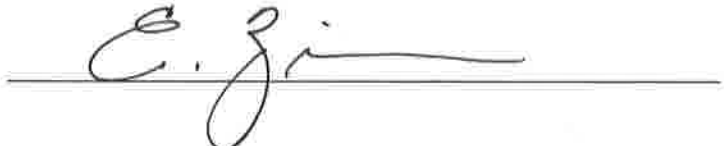
Effective May 2019 to December 2020

Stockton University:

Overall Project Manager: Christine Thompson



Quality Assurance Officer: Elizabeth Zimmerman



Field Program Coordinator: Steve Evert

Water Quality Monitoring Manager: Anna Pfeiffer-Herbert

Barnegat Bay Partnership: Jim Vasslides, Project Manager

5/23/2019 V1 FOR SUBMISSION

2.0 Table of Contents

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4.0 Project/Task Organization

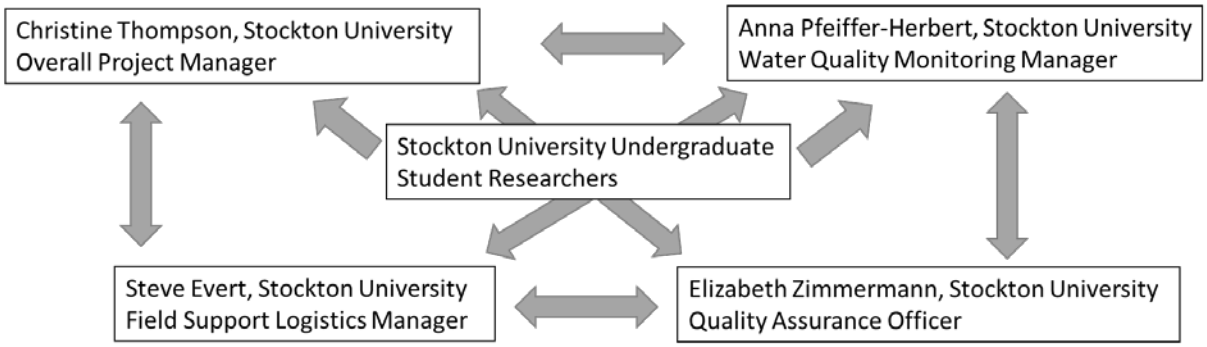
Dr. Christine Thompson, the Overall Project Manager, will be responsible for project management, data collection, and analysis for this project. Steve Evert will be responsible for coordinating all field and monitoring efforts. This includes boat time, available field technicians, equipment deployment, maintenance, and recovery, and will assist with data collection when necessary.

Dr. Anna Pfeiffer-Herbert will prepare the water quality instruments for deployment with monthly checks and downloading of data. She will work with Elizabeth Zimmerman, Stockton's quality assurance officer, for instrument maintenance and QAQC of water quality data.

Dale Parsons, contractor on this grant, will be responsible for all spat sets and planting for this project. He will coordinate with Dr. Thompson with all data collection related to spat set and planting.

Rutger's Haskins Shellfish Research Laboratory will perform the oyster disease testing for this project.

Student researchers will be supervised under Drs Thompson and Pfeiffer-Herbert for field monitoring help, data entry, water quality QAQC, and analysis.



5.0 Special Training Needs/Certification

There are no special training needs or certifications for this project except where it applies to the management of water quality instruments (see section 8.3). Students and staff working on the biological monitoring portion of the project will do so under the direct supervision of Dr. Thompson.

6.0 Project definition / Background

Barnegat Bay oysters have experienced declines over the last 100 years to numbers less than 1% of historical abundance (zu Ermgassen et al. 2012). This decline has resulted in a loss of ecosystem services provided by oysters, such as water filtration, nutrient removal, and habitat value. Further degradation of water quality and hydrological changes alongside oyster disease have led to difficulties in re-establishing a functioning oyster population in Barnegat Bay (BB). However, hydrology, bottom sediment, and connectivity between a natural population of oysters in the Mullica River-Great Bay system has made a location in Little Egg Harbor a prime site for small-scale oyster restoration in BB.

In 2016, a pilot oyster restoration reef was created in Little Egg Harbor bay (named Tuckerton Reef) and funded by the Barnegat Bay Partnership. One acre was seeded with 300 bushels of half remotely-set disease resistant oysters (using whelk shell as substrate) from Rutgers Aquaculture Innovation Center (spat-on-whelk shell or SOWS) and the other half transplanted wild oysters from the Mullica River, a site of naturally-occurring oyster populations. Remotely set oysters have established well at this site, showing survival into year two with minimal disease and over half of remote set oysters over market size, or 76 mm (Evert and Thompson 2018). An additional effort of 100 bushels of SOWS plus community-recycled oyster spat-on-shell were placed on the reef in 2017, with an additional 300 bushels of remote-set oysters to be placed in 2018 funded through a partnership with the Jetty Rock Foundation and Long Beach Township. This will total 550 bushels of SOWS (mixed with oyster shell) and 150 bushels of transplanted oysters for a total of 700 bushels of shell covering one acre of a 2-acre site.

Healthy oyster reefs can provide improvements in water quality and nutrient removal. Adult oysters can filter water and remove phytoplankton, nitrogen, and suspended material. Nitrogen deposited into sediments by feces and pseudofeces production can then be removed from the system via microbial denitrification (Newell 2004), but rates of these process can be system dependent (Kellogg et al. 2013). Effects of oyster reef filtration on water quality can be seen even in small scale restoration through downstream reductions in TSS (Nelson et al. 2004). It is anticipated that the size and density of oysters currently at the Tuckerton reef are capable of showing ecosystem services for water quality enhancement and nutrient removal through denitrification, but this needs to be quantified. Oyster filtration rate depends on many factors such as oyster size, planting density, temperature, and salinity (North et al. 2010, zu Ermgassen et al. 2013). Simple estimates of oyster density and biomass can help determine a reef's impact on filtration and nitrogen removal based on literature values.

Oysters are also important as habitat for fish and other invertebrates. Increased oyster production will increase the available trophic levels on a reef further increasing biodiversity. Oysters can enhance recruitment of early life stages of fish by providing habitat and shelter from predators (Grabowski et al. 2012). Managed species like blue crab (*Callinectes sapidus*), tautog (*Taugoa onitis*), and black sea bass (*Centropristis striata*) were found around shell planted areas during reef surveys (Evert and Thompson 2018). Smaller, resident fish such as the goby (*Gobiosoma sp*), blennies (*Chasmodes bosquianus* and *Hypsoblennius hentz*) have been found associated with the whelk shell on the Tuckerton reef and may serve as prey to larger species.

This project seeks to double the remote-setting efforts to date by adding 800 bushels of SOWS combined with spat on recycled oyster shell to the second acre of the Tuckerton reef site. This will create oyster densities throughout the site on par with expected restoration metric densities for ecosystem services: 15 oysters / m^2 or biomass density of 15 g oyster DW (dry weight) / m^2 containing two year classes and covering 30% of the restored bottom (Allen et al. 2011). Oysters will be implemented in year 1 with year 2 focused on monitoring and quantifying ecosystem services. Data collected will be able to determine the oyster reef's abilities to contribute to water quality improvements and improve habitat within the BB system. Monitoring of resident and transient fish and invertebrates will also help determine habitat value created by the oyster reefs with special attention to managed fish species.

7.0 Project/Task Description

Previous work with the BBP in creating the Tuckerton Reef has laid the groundwork for oyster restoration in Barnegat Bay. This project seeks to expand the existing reef to the second permitted acre and begins to assess the longevity of the reef and its impacts on ecosystem services. Outcomes of this project will be critical to understanding the potential for larger-scale oyster reef restoration projects to achieve water quality benefits in Barnegat Bay. This project will specifically provide the following:

1. Maintain reef at target oyster density for ecosystem services.
2. Develop a model for water quality improvement in Barnegat Bay
    a. Estimates of seston removal (TSS, chl *a*)
    b. Estimates of nitrogen removal (denitrification and burial)
3. Contribute water quality monitoring data in Barnegat Bay.
4. Improve habitat quality for other species.
5. Develop success criteria for oyster reef restoration in Barnegat Bay.

This QAPP is intended to cover the biological and data collection portions of the project beginning in or around May 2019 and continuing with monthly monitoring through November 2019. Monitoring protocols will expand onto the new reef once planted, and continue again from May-Nov 2020.

	2019												2020												PI
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	responsible
1. Water Quality Monitoring																									
Data loggers					x	x	x	x	x	x							x	x	x	x	x	x			A PH
Chl / TSS samples					x	x	x	x	x	x							x	x	x	x	x	x			A PH / CT
2. Oyster monitoring																									
Biomass / growth					x					x							x					x			CT
Survivorship / density					x					x							x					x			CT
Disease testing																						x			CT / SE
3. Habitat Monitoring																									
Substrate baskets						x		x		x								x		x		x			CT
Fish traps						x		x		x								x		x		x			CT
4. Remote set																									
Set larvae						x	x																		SE
Plant shell							x	x																	SE
5. Filtration model																									
Clearance rates											x	x	x	x	x	x									A PH / CT
Denitrification											x	x	x	x	x	x									A PH / CT
6. Outreach																									
Educational displays/events	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	CT / SE
Public spat tank						x	x											x	x						CT / SE
7. Project Implementation																									
Prepare QAPP	x	x	x																						ALL
Analyze data											x	x	x	x	x	x	x						x	x	ALL
Prepare progress/final report												x	x	x									x	x	ALL

8.0 Quality Objectives and Criteria for Measurement Data

All data sheets are found in Appendix B.

8.1 Precision. This project aims to assess the growth and survivorship of oysters at restoration site and estimate ecosystem services through biological and water quality monitoring. Oysters will be monitored twice yearly (spring and fall) and sampled from different cohorts on the reef. Precision for assessing pre-deployment spat-on-shell (SOS) densities is attainable through direct observation sampling described here. Precision in sampling for survivorship and growth is field-intensive but statistically attainable at the sample rates proposed here.

8.1a Pre-deployment oyster. Tank set SOS will be sampled prior to field deployment by randomly selecting shell from cages in each setting tank. Each tank will contain 15 cages with random arrangements of whelk shell, recycled oyster shell, or combination oyster and clam shell. As cages are dumped onto the barge prior to deployment, shells will be collected from areas corresponding to top, middle and bottom of the cages from three randomly selected cages per tank. If cage is whelk, 20 whelk shell will be collected each from areas representing the top, middle and bottom of the cages. For oyster or oyster/clam combination cages, 50 oyster shell will be collected from each region. Ten whelk shells and 35 oyster shells will be randomly selected from the samples and inspected for visible oyster spat. Spat counts will be recorded separately by two technicians and/or a project manager. Differences in spat counts that exceed 5% will be discarded and re-sampled. Only a set of two independent spat counts that result in numbers within a 5% error of each other will be accepted. This data will be used to assess initial SOS densities and spat set ratios. See Appendix B, Data Sheet 1.

8.1b Reef monitoring and post-deployment oyster. Monitoring of oysters on the reef will be performed through a combination of hydraulically-operated patent tongs (area = 1 m^2) and dredging. Use of patent tongs for oyster surveys been shown to be an accurate and precise method for quantifying oyster density on subtidal, low-relief reefs (Schute et al. 2018), however concerns for leaving divots and scars along the reef when sampling will restrict their use to density surveys only. Prior to deployment of new SOS, surveys will be performed on the three cohorts currently on the reef (2017 SOS, 2016 SOS, and 2016 transplants). Sampling will include a stratified design to ensure an even number of samples (minimum of 3) will be taken from each portion of the reef. Sampling will be performed haphazardly with side-scan sonar as a guide to ensure the boat is on top of the reef and not along the edges. If patent tong sampling is unable to achieve enough live oysters for population size estimates (at least 50 live oysters), we will resort to standardized dredging (marked with buoys to calibrate distance) to obtain greater sample sizes to ensure minimal damage to reef structure and habitat. Methods for these surveys may evolve after the first sampling event (May 2019) and will be adjusted as necessary.

Oyster metrics collected will include number, size (mm), biomass (g), as well as counts of dead oysters. All field technicians will be trained on how to measure live oysters and assess the different mortality types of oysters (box, gaper, and drill hole present) prior to recording data for each sampling event (see Appendix B, Data Sheet 2). Biomass will be measured from 20 randomly chosen oysters across the subsamples from each reef section. Each oyster will be weighed to obtain a whole wet weight (WWW), shell wet weight with tissue removed (SWW), and tissue dry weight (TDW) after drying at 60° for 48 hours (Appendix B, Data Sheet 4). The same balance will be used for all measurements and zeroed

prior to weighing new samples. Survivorship and mortality will be estimated both as through change in density over time of live and dead oysters.

8.1c Histopathology. Histopathology (disease-testing) data will be performed in the fall of each survey year. Twenty oysters will be collected from each area and sent to Haskins Shellfish Research Laboratory (HSRL) for histopathology testing. In year 1, 20 oysters will be randomly selected from each area of the current reef. In year 2 (2020), oysters will be collected from both old and new areas of the reef. See Appendix C for bias and other details contained in the approved QAPP.

8.1d Water quality measurements. Water quality measurements will include both continuous and discrete water quality sampling. Continuous water quality monitoring will be performed by HOBO water quality sensors (Onset Corporation) to measure temperature, salinity, and dissolved oxygen. Target calibration precision is oxygen within 0.5 mg/L, temperature within 0.2 deg C and conductivity within 40 uS/cm. Sensors will be placed in a central location of the reef prior to shell deployment in year 1. These instruments will be initially calibrated and checked against a discrete YSI after 24 hours of sitting in a oxygenated saltwater aquarium prior to deployment on the reef. Values of continuously logged data will be checked for precision by ensuring repeated measurements within a 15-minute window are within 10% of the median value. Any values outside this 10% range, or repeated measurements outside a normal range for this area of Barnegat Bay, will be discarded. To assess normal range values, we will compare the data collected at the reef to the Barrel Island YSI data (maintained by Stockton Marine Field Station). These continuous loggers will also be validated using the discrete data measurements described below and used to indicate precision. We will be able to tell if the data seems off if the logger measurements do not agree within 10% of the data measured during the time of the discrete sampling profiles.

Discrete samples will be taken on the reef using an EXO YSI sonde (Yellow Springs Inc) during each sampling event (see Appendix B, Data Sheet 8). YSI profiles will be taken in the middle of the reef and in an area outside the reef to measure chl *a* and turbidity (NTU) monthly each year during the oyster growing season (May-October). Three replicate profiles will be taken at each location to ensure precision and to account for patchiness in the area. We will compare the data collected at the reef to the Barrel Island YSI data to use as a non-reef comparison for chlorophyll *a* and turbidity. Data that seems anonymously high or low for the area based on parameters around Barrell Island will be discarded. Both chl *a* and turbidity measurements are to spot-check for overall trends, but patchiness in these variables is expected. A more accurate measure of TSS (total suspended solids) will be performed from three replicate water samples will be collected from an area outside the reef and an area above the oyster reef (using a submersible pump or Van Dorn sampler). From these samples, a representative sample of 100 mL will be vacuum filtered through a 1 um glass fiber, filter, dried, and weighed as per EPA method 160.2 for TSS. Any TSS replicate that are greater >10% of each other from the same area will be thrown out and resampled if possible.

The water quality meters used for discrete measurements during all site visits will be handled according to the standards of Stockton University's laboratory (Appendix D). Stockton's Marine Field Station maintains YSI water monitoring instruments for numerous research projects, including previously funded EPA/BBP grants and instruments for the Jacques Cousteau National Estuarine Research Reserve, a NOAA monitoring program.

8.1e Other biological sampling. Biological sampling (non-oyster species, motile and encrusting epifauna) will be collected in various ways. During reef surveys, biodiversity measurements will only be measured with tong samples to avoid variation of organism capture rate between tong and dredge methods. Tong sampling will provide data on other fauna (non-oysters) via entrapment of encrusting organisms, motile crustaceans and occasionally finish. These will be enumerated during sample processing. Directed finfish and motile crustacean sampling will be conducted by standard fish traps and substrate basket surveys. See Appendix B, Data Sheets 3, and 5-7.

Precision will be maintained via standardized volume measurements of the tongs and soak times of the fish traps and substrate baskets. During processing, organisms that will be measured (finfish and selected commercially important decapods such as blue crabs) will be measured only once to minimize handling stress but will be measured by the same technician during each sampling event and overseen by the project manager. Identifications will be made using standard field guides for the North Atlantic coastal region and unknown species compared to reference collections maintained by C. Thompson or brought back on ice and frozen until later analysis and taxonomic identification. Precision of balances for any biomass estimates will be maintained as in section 8.1b.

8.2 Bias. There is an inherent bias built into this project via the site selection. The Tuckerton reef site was selected based on a number of variables, including but not limited to bottom hardness, tidal flow, water depth, distance to transplant beds, proximity to existing (successful) oyster grow-out areas, and its ability to be permitted for both a research lease and shell planting under existing State permits. It was not randomly selected but rather targeted by previous or current researchers and State agencies. Because oyster populations in Barnegat Bay are currently low, targeting areas more likely to sustain transplanted oysters and/or experience what limited natural recruitment may be occurring is in the project's best interest.

8.2a Pre-deployment oyster bias. There can only be one age and one source of oyster spat set on remote set whelk shells. Cages of different shell types will be placed randomly in and around tanks to reduce any bias of shell type or individual tank properties. Any personal observation bias is overcome by having a second person subsample the data recorders' initial evaluations and if within 5% of the initial density calculation the data is accepted.

8.2b Reef monitoring and post-deployment oyster bias. The use of half-shell oysters or whelk shell as the remote set cultch decreases the extent of bias when examining any mixed subsamples during post-deployment tong assessments. However, recent surveys on the reef have shown that many oysters have fallen off whelk clusters or migrated to other areas of the reef. Surveys will be assessed with a density metric (# oysters/m^2) and avoid enumerating any whelk or oyster bias, although it is expected that the different areas of the reef are likely to contain more oysters of the cohort that was placed there compared to other migrants. For the surveys to assess planting success of the 2019 cohort, size data will be carefully recorded and any oyster or cluster that appears much larger than the average size, or has a greater amount of fouling, will be discarded and assumed to be part of the older reef areas. Student observers and/or undergraduate research assistants are trained to ask for a second opinion anytime they are uncertain as to a determination of oyster size (measured by length) or survivorship (box, gaper or other metric).

Any natural set that is present on the current oyster reef will be assessed with the oysters in the tong surveys. It will be noted if an oyster is set on top of another oyster, but it will be considered as part of the living reef. For the post-deployment surveys of the new reef in fall of 2019 and 2020, there is the

potential for natural set to co-exist with the remotely set oysters. Collection of size data will reduce this bias, and we will assume any oysters that are smaller than the size of oysters when deployed, or are on top of another oyster, will be counted separately as natural set oysters.

Because we intend to measure oyster density on the reef, we will use side-scan sonar to ensure we are on top of the reef when sampling, but exact area sampled will be haphazardly determined by placement of the boat and tongs. Moving the boat around between samples will allow for coverage of different areas of the reef and not biased towards areas of high or lower density.

8.2c Histopathology bias. Potential bias in histopathology is addressed in Appendix C. We will be choosing the largest oysters for this analysis as those are the ones more likely to show infection. Any potential bias in collections for the individual oysters to be tested in Fall 2019 will be reduced by only choosing oysters that are still on a whelk shell for those cohorts and were pulled up from the given area.

8.2d Water quality measurements bias. Bias in water quality measurements by all meters will be quantified through pre deployment calibrations according to YSI specifications. The QC checks should not require more than slight adjustments to bring the instrument into agreement. Failed calibration checks will initiate a thorough inspection of the unit for obvious sign of malfunction (e.g., loose connections, damaged probes, power source, fouling on DO membrane, etc.). After any maintenance to correct problems, the unit will be re-calibrated with documentation on the appropriate field data form. If the unit will calibrate within the guidelines, water column measurements can be continued. If one or more parameters remain suspect, the nature of the problem will be fully documented on the field form, and the situation will be reported to the Project QA Manager for resolution. If this situation occurs a backup instrument will be made available. Erroneous measurements and/or poor diagnostic values will warrant further scrutiny of the data collected and data outside of the accepted range for each probe will be flagged and removed prior to data analysis. For more information see Appendix D.

Bias in HOBO loggers will be quantified by pre- and post-deployment checks against a calibrated YSI in aerated tanks of freshwater and seawater at 100% oxygen saturation according to specifications in HOBO Dissolved Oxygen and Conductivity-Temperature logger manuals. This method will be used to determine and correct for any sensor offsets and check for instrument drift. HOBO loggers will be periodically recovered and cleaned to prevent biofouling every 3 weeks, or more frequently if the data exhibit signs of drift within three weeks. Fieldwork schedules may be adjusted if sensors must be monitored more frequently.

Bias for TSS sample collections and Chl profiles will be based on accessible locations on the reef and a random control area away from the reef. We will make sure that water samples are over an area of oysters due to side-scan imagery and position the bottom sample approximately 0.5 m above the bottom to avoid resuspending sediment with the pump intake and biasing the data collection this way.

8.2e Other biological sampling bias. There is bias inherent in the methods used for biological sampling. The tong method also allows for some escapement of organisms as the tongs are removed from the water – this can be minimized with proper technique. All mobile and encrusting organisms present on shells will be identified, but this is limited to organisms that can be seen with the naked eye.

For substrate baskets and fish traps, there is bias based on structure-oriented organisms. Also, because the same shell will be replaced each time with fauna removed, some encrusting organisms may remain and be re-sampled the following deployment. Since the baskets will have shell both on and off

the reef, control baskets will be placed in an area at least ½ NM away from the reef to avoid organisms from the reef migrating to the control baskets. Finfish and motile crustacean sampling has bias for those fish that may be more likely to swim into the traps (i.e. structure-oriented fish such as black sea bass, northern puffer and oyster toadfish). The ¼" mesh size of the traps also may allow smaller individuals escape. Traps will be unbaited to reduce any prey bias and also deployed for 24 hours or less to reduce predation among species caught in traps.

8.3 Representativeness Site selection is important to the representativeness of this project. The Tuckerton reef site was selected based on a number of variables, including but not limited to bottom hardness, tidal flow, water depth, distance to transplant beds, proximity to existing (successful) oyster grow-out areas, and its ability to be permitted for both a research lease and shell planting under existing State permits.

8.3a Pre-deployment oyster. Samples will be taken from multiple areas of the tank (three vertical and three horizontal dimensions), at least three cages per tank, and from all tanks to represent the spat-set ratios and planting density of oysters. Sample size will be estimated to represent 5% of the shells in each cage.

8.3b Reef monitoring and post-deployment oyster. Monitoring samples aimed at 50 live individuals for each separate site sampled (old vs new reef, and each cohort on the old reef) will effectively characterize the biological metrics (growth, survival, and biomass) of the reef population via sampling methods described in 8.1b. The May 2019 survey will evaluate the appropriate sample size and representativeness, and if dredge surveys need to be implemented to get up to desired number of 50 live oysters. Twenty oysters from each cohort randomly selected for biomass surveys are assumed to be sufficient to represent the population (see 8.3c).

8.3c Histopathology. The HSRL laboratory, regional experts in histopathology testing of oysters, have indicated that a subsample of 20 oysters will accurately reflect the population of each particular reef. Twenty oysters is the standard "per bed" sample taken from the Mullica River each year during histopathology testing on state beds.

8.3d Water quality measurements. Including continuous water quality sampling instruments will result in a full representation of the temperature, salinity, and DO characteristics on the reef during the oyster growing season. Although we will not be monitoring during the winter season, this data won't be relevant for the oyster filtration model since it will include a time period when oysters do not actively filter water.

Monthly sampling of Chlorophyll and TSS will be less representative of the conditions experienced on the reef. These samples are expected to vary in space and time based on tidal period, weather events, and other random factors not accounted for between sampling periods. These data can be compared to nearby monitoring buoys to better understand the trends in these variables outside of the collection periods. Three replicate samples of each will be collected during each survey to represent spatial variability in these parameters around the reef. Prior to TSS analysis, 1L samples will be vigorously agitated to allow for a 100 mL representative subsample from the collection bottle.

8.3e Other biological sampling. By sampling at least three sites from different areas of each reef type, the samples will be representative of the entire reef area and not clustered due to random chance. The mobile and encrusting species sampled on the reef should be representative of the community

there at the time of sampling. Smaller organisms may be excluded (as noted in previous section). The time of year sampled (May and October) may affect the organisms that are present based on life cycles, community succession, and timing of recruitment for benthic invertebrates. For the traps, there may not be full representation of all demersal fish and invertebrates due to species differences in trap avoidance and potential predation on species in the traps (i.e. not all species utilizing the reef would be expected to swim into the traps).

8.4 Comparability. Project methods are designed to be comparable to other oyster reef restoration monitoring projects by following the methods outlined in the *Oyster habitat restoration monitoring and assessment handbook* (Baggett et al. 2014). This handbook has suggested protocols and metrics for each of the monitoring components in this study and will enable us to directly compare to literature values.

8.4a Pre-deployment oyster. Methods for spat-set counts will be based on methods employed by other hatcheries (Horn Point Laboratory, Billion Oyster Project). This will allow us to assess our set-ratios compared to other efforts in the mid-Atlantic. However, different substrate and settlement processes can affect overall set ratios.

8.4b Reef monitoring and post-deployment oyster. We will perform monitoring surveys to assess universal metrics (Baggett et al. 2014) such as density (#/m^2) and size-frequency distribution to make our data comparable to other restoration projects. Use of whelk shell as substrate, disease-resistant oysters, and subtidal reef placement on bare bottom will affect oyster survivorship relative to other methods. Condition Index is a standard protocol used to assess oyster biological condition in response to environmental stressors.

8.4c Histopathology. We will use 20-30 oysters from each cohort or reef area to send to Rutgers for disease testing. This is comparable to the sample size used by the NJ Bureau of Marine Shellfisheries and suggested in Baggett et al. 2014.

8.4d Water quality measurements. Continuous water quality data will be collected through moorings of data loggers set as close to the reef as possible and recorded in standard units (Celsius for temperature, PSU for salinity, and mg/L for dissolved oxygen). Recordings will be set between 15-60 min as suggested by Baggett et al. 2014 and deployed during the growing season (May-October at minimum).

The water quality measurements during all site visits will be handled according to the standards of Stockton University's laboratory (Appendix D). Stockton's Marine Field Station maintains YSI water monitoring instruments for numerous research projects, including previously funded EPA/BBP grants and instruments for the Jacques Cousteau National Estuarine Research Reserve, a NOAA monitoring program. The data will be directly comparable to numerous projects in the region.

Using standard EPA protocol and 1 µm glass-fiber filters will ensure that the TSS methods are comparable to other studies estimating this variable.

8.4e Other biological sampling. Methods for biodiversity assessments will be consistent with the methodology that has been used on this site since 2016. Reporting for patent tong surveys will be in #/m^2 format allowing for density comparisons. Use of fish traps and substrate baskets will enable testing of reef enhancement for mobile species. Data will be expressed in standard metrics such as richness,

taxonomic groupings, and biodiversity to allow for comparisons to similar studies of biodiversity on oyster reefs.

8.5 Completeness.

8.5a Pre-deployment oyster. Pre-deployment sampling of tank sets will not have weather or other time restrictions. 100% completeness is required based on sampling methodology described in 8.1a.

8.5b Reef monitoring and post-deployment oyster. Post-deployment sampling of both oyster types could be subject to time restrictions based on field conditions. 80% completeness is required based on sampling methodology.

8.5c Histopathology. Histopathology sampling will be based on a minimum of 20 oysters of each type from each reef site (total 60 oysters). Due to the difficulty and expense of re-sampling, should the HSRL laboratories have an unforeseen issue with processing the full sample set an acceptable level of completeness is set at 50% of each oyster type from each site (minimum 10 each type/each site).

8.5d Water quality measurements. Continuous water quality measurements can be affected by instrumentation failures, weather conditions, fouling or disturbance. Monthly checks will be performed on these systems after deployment at a minimum. Water quality measurements could be subject to time restrictions based on field conditions. 80% completeness is required based on sampling methodology.

8.5e Other biological sampling. All biological sampling (biodiversity, oyster growth/survival/finfish trapping) could be subject to time restrictions based on field conditions. In addition, traps set overnight or longer could be lost due to unforeseen circumstance. 80% completeness is required based on sampling methodology.

8.6 Sensitivity

8.6a Pre-deployment oyster. Not applicable.

8.6b Reef monitoring and post-deployment oyster. Not applicable.

8.6c Histopathology. See Appendix C

8.6d Water quality measurements. The minimum instrument detection limit for temperature (-5 C), DO (0 mg/l and 0%), pH (0 units), and conductivity (0 mS/cm) are all below the expected values to be found at our sampling sites. See unit and probe specifications, Appendix D

8.5e Other biological sampling. Biodiversity sampling will be limited to organisms visible to the naked eye – no microscope work will be conducted unless required for ID purposes. Finfish sampling will be limited to organisms that can be entrapped by the ¼" mesh size.

9.0 Non-Direct Measurement. Not applicable. Data collected from previous projects will be used for comparative purposes, but not analyzed here.

10.0 Field Monitoring Requirements.

10.1 Monitoring areas. A map of the general project area is attached in Appendix A.

10.2 Monitoring methods. Monitoring methods for all sample types will follow standard estuarine sampling protocols and equipment use. A timeline of project sampling (*and deployment**)* events follows:

All data sheets are found in Appendix B.

Monitoring Methods Timeline				
Sample Type/Event 2019	**Month**	**Method**	**Quantity**	**Units**
Water quality monitoring	May - Nov	Sensors and YSI, TSS samples	Continuous (HOBO) Profiles (1x Monthly)	Celsius, mg/L (DO, TSS), PSU (Salinity), ug/L (Chl)
Spring oyster monitoring	May	Patent tong	TBD	#/m^2, biomass / m^2, size (mm)
Mobile species habitat enhancement	June (deploy) Aug (recover) Oct (final)	Substrate baskets	5 on reef, 5 off reef	# indiv./area, weight (g)/area, # species/area
Finfish habitat enhancement	June, Aug, Oct	Mesh traps	5 on reef, 5 off	# ind. / trap, # species / trap
SOWS placement	July (2 deployments)	Vessel transplant	800 bushels	N/A
Fall oyster monitoring	October or November	Patent tong	TBD, including new reef	#/m^2, biomass / m^2, size (mm)
Sample Type/Event 2020	**Month**	**Method**	**Quantity**	**Units**
Water quality monitoring	May - Nov	Sensors and YSI, TSS samples	Continuous (HOBO) Profiles (1x Monthly)	Celsius, mg/L (DO, TSS), PSU (Salinity), ug/L (Chl)
Spring oyster monitoring	May	Patent tong	TBD, old and new reef	#/m^2, biomass / m^2, size (mm)
Mobile species habitat enhancement	June (deploy) Aug (recover) Oct (final)	Substrate baskets	5 on reef, 5 off reef	# indiv./area, weight (g)/area, # species/area
Finfish habitat enhancement	June, Aug, Oct	Mesh traps	5 on reef, 5 off	# ind. / trap, # species / trap

Fall oyster monitoring	October or November	Patent tong	TBD, old and new reef	#/m^2, biomass / m^2, size (mm)

10.2a Growth and survivorship. Oyster density will be monitored at the beginning and end of each growing season for each cohort on the reef (May and October). In year 1, all cohorts (2016 – 2 shell types, 2017, and 2019 - Fall) will be assessed separately. Patent tong surveys (3-5 replicates) will be performed at each site. All living oysters will be enumerated and shell length measured in mm. Dead articulated oysters (box, gaper, and drill hole present) will be quantified and shell hash will be estimated by volume displacement. Any visible spat will be assessed in October surveys. Density will be recorded as the mean number of oysters per m_2 averaged for each subsample. Survivorship and mortality will be estimated both as through change in density over time as well as number per whelk (or oyster) shell over time (Evert and Thompson 2018). In year 2, estimates will include the new reef as well as the previous cohorts on the reef. See Appendix B, Data Sheet 2.

For each cohort, 20 oysters will be randomly chosen across the subsamples for biomass estimates. These estimates will be used to obtain filtration and denitrification estimates as well as assess oyster condition, or how they are responding to environmental conditions. Each oyster will be weighed to obtain a whole wet weight (WWW), shell wet weight with tissue removed (SWW), and tissue dry weight (TDW) after drying at 60° for 48 hours. TDW will be used to assess filtration and denitrification abilities (see below) and condition index will be calculated using the equation CI = TDW x 100 / (WWW-SWW) (Baggett et al. 2014). See Appendix B, Data Sheet 3.

10.2b Habitat monitoring. We will assess habitat enhancement for reef resident and transient species in three categories: epifaunal/sessile species, small mobile fish and invertebrates, and larger fish. Epifaunal species will be recorded during oyster surveys and counted on each shell. Individual species (e.g.barnacles, limpets) will be recorded as # ind/m^2, colonial species (e.g. sponges, tunicates) will be estimated as % cover. Large masses of encrusting fauna oyster shells, if present, (e.g. sponges, algae, bryozoans) will be removed from shell if attached and estimated by volume displacement and noted on the back of each data sheet. Mobile species will be assessed in oyster surveys after rinsing and sieving shell material through a 5 mm mesh sieve (See Appendix B, Data Sheet 3).

To better assess reef enhancement of mobile fish and invertebrates, substrate baskets and fish traps will be used. Substrate baskets (~0.1 m^3) lined with 4 mm mesh and filled with clean oyster shell will be deployed at random locations within the reef areas and on a control area away from the reef. In 2020, a third set of baskets will be placed in the new reef area. After approximately 2 months of deployment, baskets will be brought up and shell rinsed and sieved through a 5 mm mesh screen. All organisms will be identified, enumerated, and weighed for biomass estimates. Any fish caught will be measured. Un-baited fish traps to catch larger fish and crustaceans around the reef will deployed in the same areas as substrate baskets around the same periods of substrate basket deployment/collection. Traps will be set for 48 hours and all species identified, enumerated, and measured on site. See Appendix B, Data Sheets 5-7.

10.2c. Water quality monitoring. We will place continuous water quality data loggers in a central location of the reef to log temperature, salinity and dissolved oxygen (HOBO data logger, Onset Corporation) prior to shell deployment in year 1. These parameters will be averaged for each month and used to set up the filtration and nutrient removal estimates. A continuous water quality data logger at

Barrel Island (maintained by Stockton Marine Field Station) will serve as an off reef comparison to the HOBO data collected on the reef.

Discrete samples will be performed monthly on the reef during the oyster growing season (May-October) and during each data collection event (Appendix B, Data Sheet 8). YSI profiles (using YSI 600 or 600 series instrument) will be taken in the middle of the reef and in an area outside the reef to measure chl *a* and turbidity (NTU) along with other parameters (Temp, Salinity, DO, etc). In year 2, profiles will be taken from the old reef (acre 1 seeded in 2016-18) and the new reef established from this study. For TSS, three replicate samples of 1 L will be pumped from 0.5 m above the reef and from a control area outside the reef and vacuum filtered through a 1 um glass fiber filter, dried, and weighed as per EPA method 160.2 for TSS (see Appendix F). The Barrell Island site will serve as an off-reef comparison for longer term trends of chlorophyll a and turbidity as well.

10.3 Field Quality Control. Each member of the field crew will be supervised by a Project Manager (likely C. Thompson) that will be present for all field sampling activities. Checklists and SOP's for each sampling type will be disseminated and checked by a project coordinator. All equipment will be checked for damage each sampling event and repaired as necessary. Materials to repair any equipment issues will be brought to each sampling event.

11.0 Analytical Requirements. The methods for TSS (EPA method 160.2, Appendix F), condition index, and histopathology will require transfer to a laboratory for analysis. Standard methods and protocols will be followed for these methods. See Appendix C for a previously approved QAPP for HSRL that details the analytical methodology.

12.0 Sample Handling and Custody Requirements. All samples being transferred for biomass, condition index, and histopathology will be stored on ice until processed. For biomass and condition indices, if they cannot be assessed immediately after returning, they will be frozen until later analysis. Samples for histopathology testing will be handled per the procedures set forth in Appendix C.

13.0 Testing, Inspection, Maintenance and Calibration Requirements

13.1 Instrument/Equipment Testing, Inspection, and Maintenance. All sampling gear and laboratory instrumentation will be maintained in good repair as per manufacturer's recommendations or common sense to ensure proper function. The YSI handheld water quality meters undergo bi-weekly inspection and are maintained per established procedures (see Appendix D). The HOBO loggers are maintained by removing biofouling using vinegar if necessary, cleaning sensors with soap and water, and replacing the oxygen sensor cap every 7 months.

13.2 Instrument/Equipment Calibration and Frequency. See Appendix D for water quality sensor calibration protocol. All discrete sampling equipment (YSI) will be calibrated prior to each sampling event.

For continuous monitoring instruments, we will conduct a field calibration check every 2-3 weeks with a lab-calibrated YSI. The procedure will be to sample before instrument recovery, check in 100% saturated air on the boat before and after cleaning the sensor. Target calibration precision is oxygen within 0.5 mg/L, temperature within 0.2 deg C and conductivity within 40 uS/cm

13.3 Inspection/Acceptance of Supplies and Consumable. Not applicable.

14.0 Data Management. The project will require that each data generating activity be thoroughly documented. These efforts include pre-plant SOWS counts, size and age structure counts, water quality surveys, and all oyster and biological monitoring. Data will be recorded in a variety of paper and digital formats. Data will be collected under the supervision of the Project Manager (Christine Thompson) who will be present at each survey. If the project manager is unable to attend, co-PI Steve Evert and logistics coordinator will take on the role of project manager. Data will be entered into a Microsoft Excel spreadsheet that will be saved on the Stockton server and backed up daily to a cloud server and campus server. Raw data will be scanned and backed on Stockton's data server. A folder on C. Thompson's office computer will maintain all files for this project (data sheets, QAPP, SOPs, scanned data).

Data from the field will be recorded aboard the sampling vessel on waterproof data sheets. The Project Manager supervising the collection activities will be responsible for transfer of raw data sheets to be copied, scanned, and stored. Hard copies of the raw data sheets will be organized in a 3 ring binder. From the 3 ring binder system staff or undergraduate researchers will enter data into Excel spreadsheets for further analysis. Data entry will be spot checked by a Project Manager and checked thoroughly by separate undergraduate students who did not enter the original data. Checklists will be made and maintained to ensure efficient data entry and quality control for each sampling event.

15.0 Assessments/Oversight. As PI of the project and Project Manager, Christine Thompson will be present at all, if not the majority, of sampling events for this project to ensure consistency and oversight at all data collection event. She will be primarily responsible for recruiting and training of all undergraduate student researchers. Project Logistic Manager and QA officer will be present at the first biological survey and water quality survey event. Water quality project manager A. Pfeiffer-Herbert will be in charge of all water quality monitoring and training of student workers, with help from the QA officer. Any issues such as slight deviation from approved procedures, misidentification of species or oyster ages, measurement techniques, data reporting, etc. will be noted and investigated by the Project Manager or Quality Control Officer until resolved. If issues cannot be resolved, this will be noted and the Project Manager and Quality Control Officer will make a determination as to how the data can be used.

16.0 Data Review, Verification, Validation, Usability.

16.1 Data Review, Verification, Validation. The data generated during the project will be systematically reviewed with varying levels of scrutiny at several junctures along the path from time of collection to final reporting; from quick, on-board screening to in-depth evaluation against established criteria or standards. For much of the field collected data, the first level of validation, a cursory screening, will occur as data are recorded; persons conducting and documenting real-time observations should be aware of the range that constitutes realistic values for a specific measure. Certainly a water temperature of 40 °C should jump out as an obvious outlier and trigger an immediate response to find the source of the error. With other types of data, the initial validation may not occur in such an immediate time frame. Nonetheless, most data are amenable to some form of quick screening soon after being generated and the responsibility for this first- cut validation falls on the personnel performing the measurement. During the data entry process, the field data will be screened for missing or errant information. Checks will be done for all data entered for transcription errors. If any data checked is found to have been entered into a database erroneously all data entered that day will be thoroughly checked. If greater than 5% of entered data is ever found to be erroneous the QAO and the OPM will meet with the individual responsible for data entry to discuss the importance of careful data entry. Any

second occurrence of mis-entered data exceeding the 5% error threshold will result in dismissal from those duties.

Species identifications will be kept as a running list indexed with field guides and photo identifications. If a misidentification appears to have been made, that data will be rechecked and cross-validated with photo records. Grouping data into larger taxonomic units (e.g. gastropod, decapod) will prevent errors at the species level and still allow for abundance estimates to be accurately compared.

16.2 Reconciliation with User Requirements. All data collected as part of this project must meet the QA/QC standards defined by this QAPP. Data analysis will be undertaken in Excel, R, or MATLAB. The software package used will be dependent on the specific spatial, temporal question and the input data type. All output will be saved in an electronic format on the designated folder on a Stockton University network computer, which is in turn backed up on Stockton's main campus system daily and also sorted on Cloud software.

17.0 Reporting, Documents and Records. All electronic project documents will be saved on a designated folder on the Stockton University network. A clean copy of all data will be maintained and re-saved once it is checked; before any data analysis occurs, data will be saved under a different name. All physical project documents will be kept in a project folder at the Stockton offices for the duration of the project and until three years after the performance period ending December 2020 (raw data kept until December 2018). A final report will be written by December 31st 2020 and submitted to the Barnegat Bay Partnership.

18.0 References

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