Barnegat Bay oyster reefs; biological and cost benefit analyses for scale up efforts

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Project lead
Stockton University
Marine Field Station
30 Wilson Avenue
Port Republic, NJ 08240
609-652-4486

Steve Evert (PI), Pete Straub and Christine Thompson (co-PI's)
Contributing staff and research assistants: D. Ambrose, J. Baez, N. Robinson and E. Zimmermann

Project partners

Parsons Mariculture, Dale Parsons (co-PI)

American Littoral Society, Alek Modjeski (co-PI), Helen Hendersen, and Julie Schumacher

INTRODUCTION

Reef-building oysters, such as the eastern oyster, *Crassostrea virginica*, are important components of estuarine ecosystems. Thriving oyster populations and their associated structural reefs provide many ecological and economic benefits to coastal areas. Ecosystem services include water quality improvement and habitat creation for invertebrates and many managed species of fish (Coen et al. 2007, Grabowski and Peterson 2007). Economic benefits include direct and indirect support of commercial and recreational fishing (finfish and shellfish) and benefits to coastal tourism (Grabowski and Peterson 2007). Many areas on the eastern U.S. have seen marked declines in natural oyster populations due to changes in estuarine hydrodynamics, pollution, disease, and overfishing. In the most extreme cases, as for much of the Eastern U.S., oysters have become functionally extinct, replacing three-dimensional reef habitats with bare bottom (Baggett et al. 2015).

Barnegat Bay is a lagoon-type estuary that runs north to south along the coast of New Jersey, separated from the Atlantic Ocean by barrier islands with two inlets. Oyster beds of Barnegat Bay historically extended from the southern portions of the watershed north in the system to the Forked River (Ford 1997). It is believed that today almost the entire historic oyster habitat has been degraded due to overharvesting, changes in estuarine hydrodynamics, siltation and disease. In 1999, Barnegat Bay was officially classified as highly eutrophic by NOAA's National Estuarine Eutrophication Assessment model. It was determined that eutrophic conditions were extensive and widespread within the Bay, the level of human influence was high, and the associated negative impacts to SAV, shellfish and fish habitats were substantial (Bricker et. al. 1999). The need to restore these estuarine habitats, as well as to identify restoration techniques that can be applied bay-wide are important to the region's ecological, economic and societal needs.

Oyster restoration projects can return some of these services with varying amounts of start-up investment. Projects can range from large efforts to restore hundreds of acres to pilot-level efforts on the scale of an acre or less. Large projects require evidence for potential success to justify steep monetary investments, while pilot projects are best utilized when there is a desire to document an area's potential for supporting a larger investment. This project was developed around the latter approach and represents a proof of concept for oyster restoration in Barnegat Bay. Successful restoration in areas where natural recruitment potential is unknown relies on the remote set method (aquaculture) and/or seed transplant from a brood stock source. The goal of an oyster restoration project is to create a reef that can become self-recruiting as demonstrated by the settlement of natural oyster spat. In some cases, annual investments through remote set and/or wild seed transplant can also have benefits for improved water quality, habitat creation and potential public harvest programs (Brumbaugh and Coen 2009). Habitat creation alone via the placement of shell and its associated encrusting benthic community can even be enough to justify an investment. However, to provide services such as water filtration and denitrification and to achieve restoration goals for oyster biomass, there must be an adult population able to survive typical life spans of 3-5 years for any given cohort.

In the northern part of Barnegat Bay, small scale restoration efforts have been made on the Good Luck Point (GLP) reef prior to this project (Figure 1). Those efforts suffered significant post-Sandy deterioration and had not yet explored the use of local brood stock (Thompson et. al. 2014). In 2014 the Society conducted a small scale *in situ* spat on shell set, and the Barnegat Bay Shellfish Restoration Program and ReClam the Bay seeded an adjacent area with oyster spat on shell. In 2015, 110 cubic yards

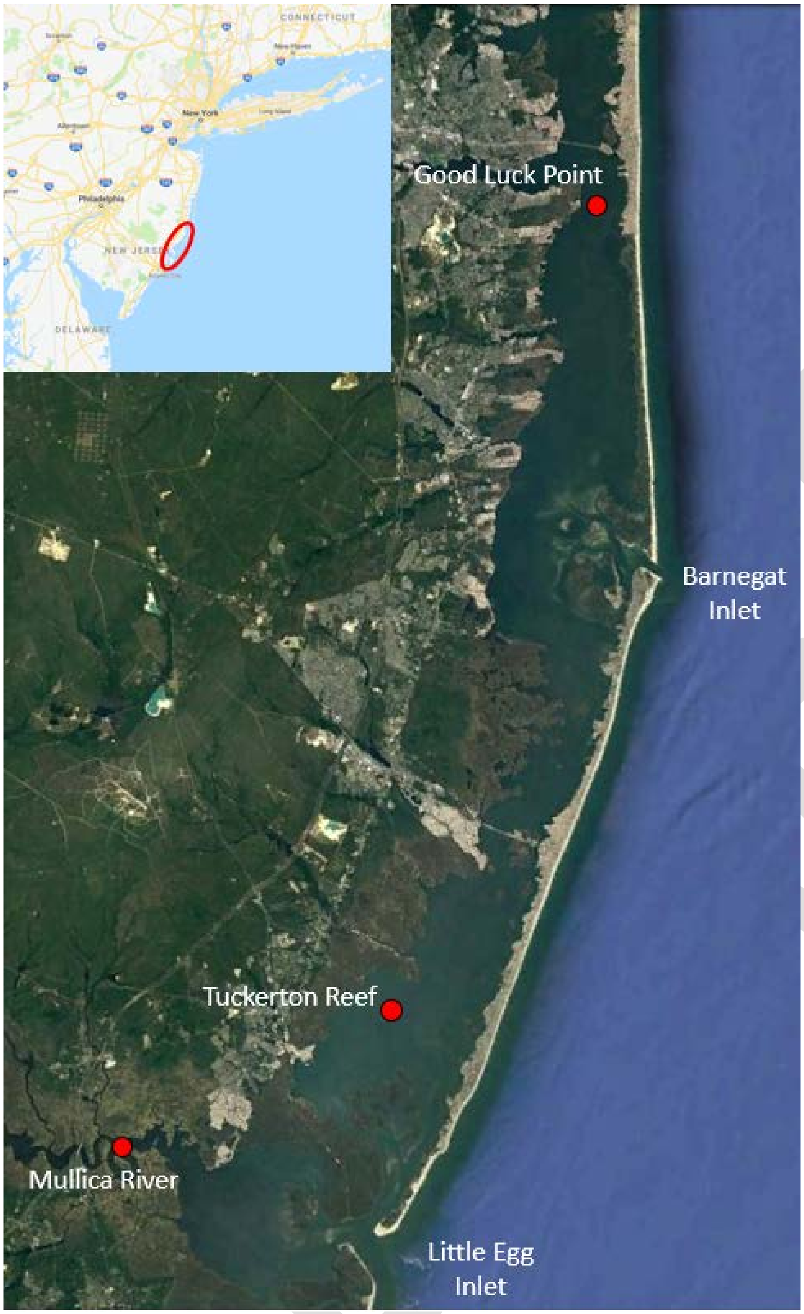


Figure 1. Overall study area showing the northern and southern sites and the Mullica River.

of (bare) whelk shell were placed over a ½ acre area of the reef to increase rugosity of the bed. This project provided a continuation of those efforts, with an added experimental design aimed at quantifying the success metrics and cost benefits between using remotely-set disease-resistant eyed larvae spat on whelk shell (SOWS) and wild-set Mullica River transplanted seed oysters (MRT). As part of this project the GLP site was planted (2016) with 75 bushels of SOWS and 75 bushels of MRT oysters. The planting and monitoring efforts represented 25% of the project's budget.

Areas of the southern Barnegat Bay, specifically Little Egg Harbor bay (LEH), lack on-the-ground restoration activities, yet oyster mariculture on commercially leased beds is on the rise (NJDEP, Normant/leasing records). Multiple oyster farm operations have grow-out leases located in LEH bay. Observational data of wild-set intertidal oysters shows natural recruitment potential for this area (Parsons and Evert, personal observation). Spatfall data collected during the study period further demonstrated this potential. The majority of this project's budget provided the first LEH bay-located oyster reef, the "Tuckerton Reef" (Figure 1). The Tuckerton Reef (TKR) was permitted as a research lease and utilized the same experimental design (as GLP) aimed at quantifying the success metrics and cost benefits of using remotely-set disease-resistant eyed larvae spat on whelk shell (SOWS) and wild-set Mullica River transplanted seed oysters (MRT). As part of this project the Tuckerton Reef was planted (2016) with 150 bushels of SOWS and 150 bushels of MRT oysters. The planting and monitoring efforts represented 75% of the project's budget.

Establishing which method works best for survivorship and ecosystem services is an important step for justifying larger scale restoration in the Barnegat Bay system. Prior to this project oyster restoration in Barnegat Bay was limited in scale, location and assessment. Fisheries and ecosystem managers would find it difficult to develop the Barnegat Bay Partnership's recommended Shellfish Management Plan without this system-specific restoration data. The continuation of existing pilot projects, coupled with new site development in the southern Barnegat Bay region, has helped provide this data. This report includes those biological and economic considerations for scale-up options.

METHODS

Site selection (north)

The Good Luck Point site is a pre-existing one acre state-permitted site for oyster restoration. The American Littoral Society has been monitoring and seeding small amounts of SOWS in this area of oyster habitat since 2013. A separate unplanted ½ acre of this reef was marked for this project.

Site selection (south)

Efforts in Little Egg Harbor bay required a site selection process that was initiated at the proposal stage by consulting area baymen and reviewing areas for potential leasing with the State Bureau of Shellfisheries and the Atlantic Coast Section of the Shellfisheries Council. The research site had to be in approved growing waters and could not conflict with existing leases, SAV or other users in the area. Four sites were considered; West Creek (4), Long Point (3), mid-Bay (2), and Mordecai Island (1) (Figure 2). The Mordecai Island site was dismissed without further investigation due to the nearby dredging activities and the known issue of a migrating channel toward the potential lease area. West Creek and Long Point were dismissed after broad-scale sonar surveys and qualitative bottom grabs indicated soft sediment structure, leaving the final site selection to concentrate on the mid-Bay area.

The mid-Bay area was assessed for sediment type, current flow, water depth at MLLW, proximity to loading points, room to expand, gear/industry conflicts and visibility to law enforcement. A very small salinity gradient exists in the LEH bay south of the Route 72 bridge, leaving tidal flow, bottom firmness and water depth as the most important considerations for site selection. Bottom firmness was important to be sure that bed settlement did not negate shell and live oyster placement, however it was equally important to recognize that sand-dominated bayfloor areas are very dynamic and can cover an established bed during storm events. Moderate to high tidal flow decreases sedimentation and the occurrence of drift algae, both negative influences on oyster reef success. Water depth criteria required the site to be relatively easy to work (i.e. < 4m) but deep enough (>2m) to negate ice scour and navigation concerns.

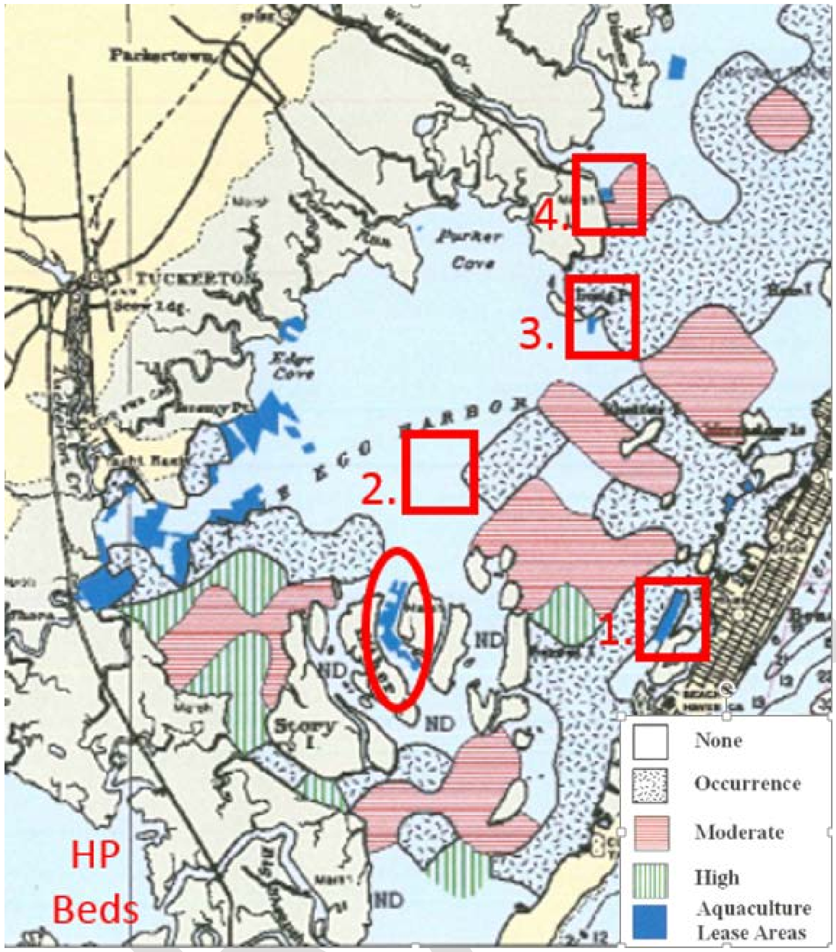


Figure 2. Little Egg Harbor bay sites of consideration.

The final two-acre selection was based on Stockton-led investigations of the proposed areas as well as existing data sets and qualitative ground-truthing. Several sources contributed to the data sets that were reviewed, including direct sonar work (Stockton), direct bottom sampling (Stockton and Rutgers), direct bottom sampling and mapping (USDA NRCS), and direct hydrodynamic modeling efforts to assess potential tidal flow (USGS).

The 2015 USDA (NRCS) soils data set for Barnegat Bay shows an area of the bay to be comprised of Indian River Series soils per the regional classification (Tunstead, 2015). The IRS soils are described as “light gray sand; nonfluid; nonsticky; nonplastic; 3 percent crushed seashell fragments; slightly alkaline; no odor and no peroxide reaction; gradual smooth boundary” from the surface to 61 cm core depth (R. Tunstead communication). Figure 3 shows the sediment classification of “WIr3” standing for “water” “Indian river” “with 3m depth”. Nearby areas are represented by Pasture Point Series (Pp) sediments as well as Tingle Series (Tf) sediments, both of which are described as sediments types containing greenish black silty clay loam; massive; very sticky; very fluid; abrupt boundary (USDA NRCS, Tunstead). These are softer unconsolidated sediment types not conducive for oyster site placement. Stockton University performed qualitative bottom grabs in each of these zones and compared those results to the area’s bathymetry to further narrow the site to a relatively flat two-acre site with an average MLLW depth of ~ 2.4 meters (Figure 4).

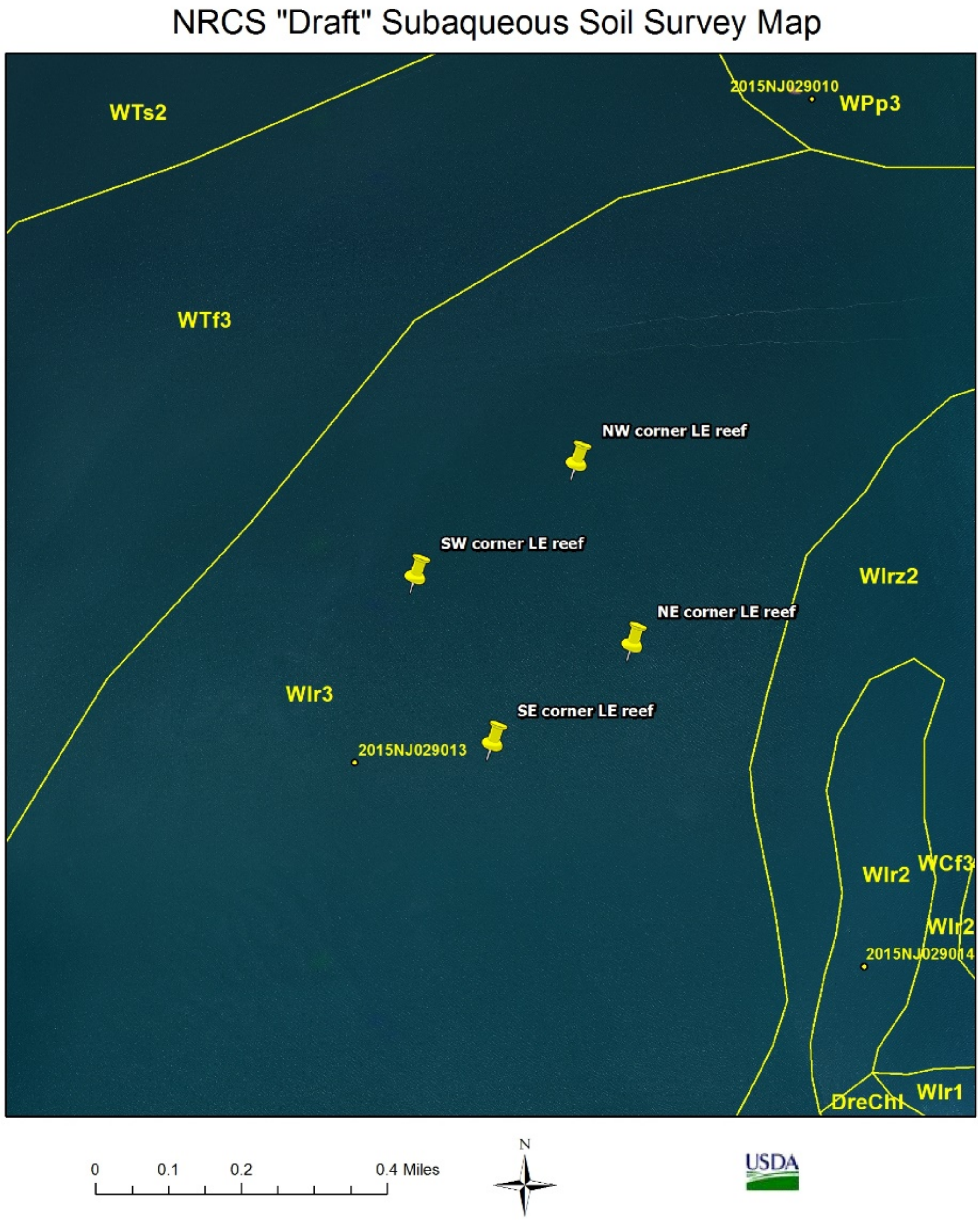


Figure 3. Subaqueous soil classification of the selected site (USDA/NRCS).

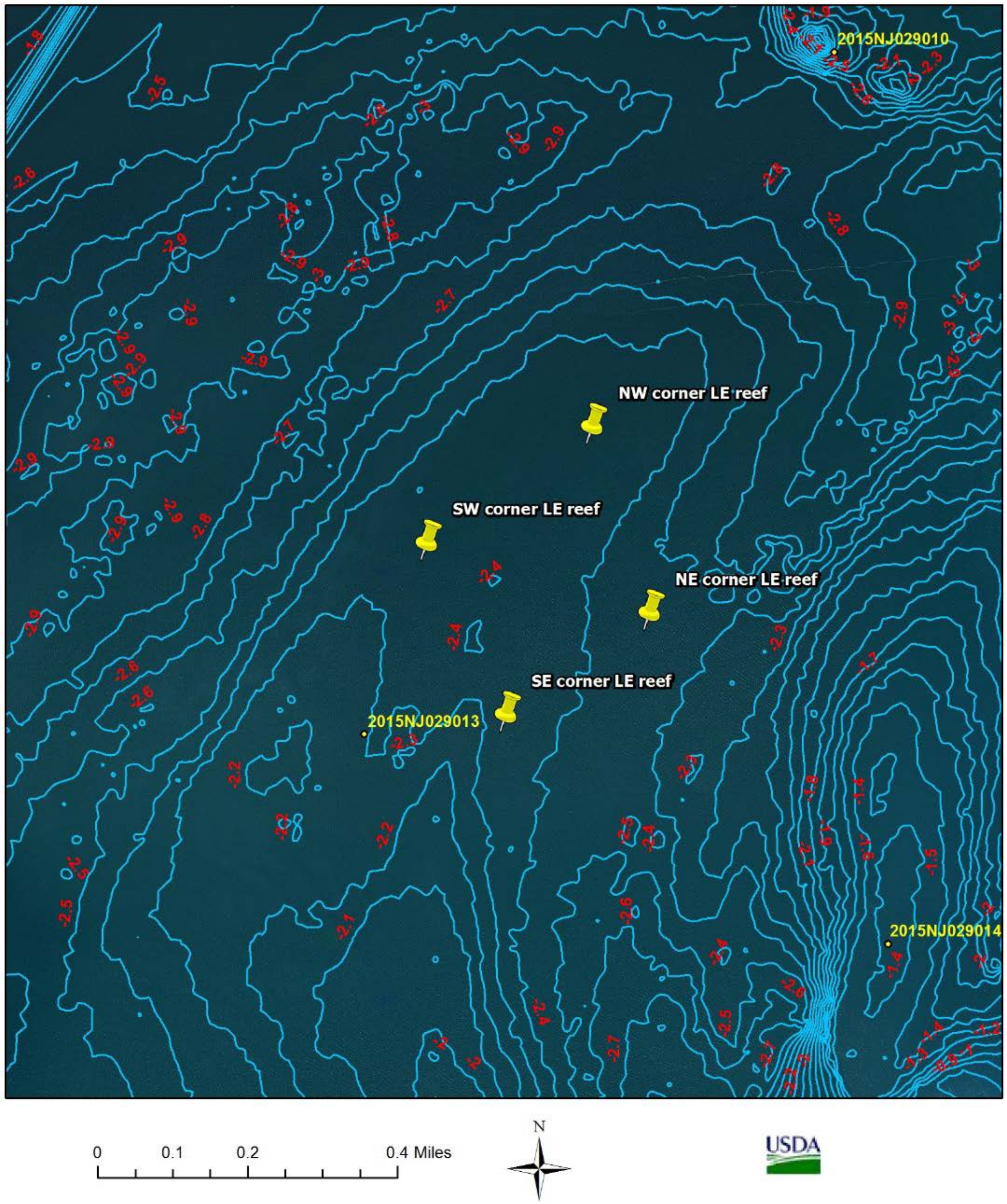


Figure 4. Bathymetry of the selected site (USDA/NRCS).

The defined two-acre area met the criteria for enforcement visibility from Dock Road (West Creek) and the Parkertown boat ramp. These nearby land-based sites also provide areas for loading and staging efforts by vehicle and trailered vessel, an important consideration for future efforts. The two-acre rectangle approximately in the middle of the flat area of preferred sediment type was proposed to the Council and the State for final approval. In January 2016 the Tuckerton Reef research lease was approved, followed by required ACOE permits by May 2016.

Remote setting and planting

Good Luck Point. Three separate remote setting events took place using a 2'x4'x8' setting tank at the public pier in Ocean Gate, NJ. Prior to each setting event, 60 bags of whelk shell were placed in the tank for a total of 180 bags equivalent to 75 bushels. For the first event (June 7th) clean shell bags were in the tank for approximately one week before setting. For the subsequent events, clean shell bags were placed in the tanks the same day after the prior set was removed. Rutgers NEH eyed larvae were obtained the day before each setting event from Rutgers' Aquaculture Innovation Center (AIC). The number of larvae set varied for each setting event, as did the amount of time spent in the tanks versus hanging off the pier before planting day (Table 1).

Table 1. Spat set data from the ALS northern site efforts (GLP).

Date of Set	No. of Larvae Set	Days in Tank	Days off Pier	No. spat/shell	Est. no. of spat set	Est. spat set ratio
6/7/16	750,000	7	28	45.9 +/-3.2	95,880	12.8%
6/14/16	610,000	14	14	9.5 +/-1.4	32,760	5.4%
6/28/16	1,400,000	14	0	17.7 +/-2.1	34,560	2.5%

The NEH eyed larvae were acclimated to lower salinities by placing them in a bucket of mixed water from the hatchery (31 PSU) and the Toms River (17-21 PSU) for about an hour prior to setting. Larvae were set onto the whelk shell by gently pouring them into different areas of the tank. The flow-through pump was shut off for three days to allow larvae to set and an air bubbler was turned on. After three days, the flow was turned on, and the tank was cleaned and inspected weekly.

Three days prior to deployment on the reef, SOWS were subsampled from each of the three sets to assess initial oyster density. Three randomly selected bags of whelk shell were marked from three different regions of the setting tank; top, middle and bottom depths and 3x across the circumference of the tank from each vertical region. Ten shells from each bag (10 x 3 x 3 = 90) were inspected for visible oyster spat and their numbers recorded separately by two workers. Differences in spat counts that exceeded 5% were discarded and re-sampled. Spat counts that were within 5% of each other were averaged to estimate initial SOWS density. On July 15, 2016, under the direction of the ALS team, all remote-set SOWS were planted (via multiple vessels) to a marked area of the GLP reef site (Appendix 3 outreach). The average size of planting ranged from 2.3 – 12.8 mm, representative of the three sets.

Tuckerton Reef site. A single remote set event was conducted at Parsons Mariculture facilities in Little Egg Harbor, NJ. Two 3,000-gallon circular tanks typical of remote setting facilities were loaded with a total of 30 cages and ambient seawater of approximately 25 PSU. Each cage contained approximately 650 whole whelk shells equating to ~ 7 bushels of whelk shell for a total volume of approximately 210 bushels. Shell was caged and washed before remaining in ambient water for 2 days prior to the introduction of the eyed larvae. Haskins NEH eyed larvae were acclimated with 50% solution of hatchery water and local sea water (filtered to 50um). Larvae were held in buckets until the majority were swimming, then evenly dispersed throughout the two setting tanks. Aeration was provided throughout the system and water exchanges were conducted every 2-3 days.

Spat per shell values and set ratios for the mariculture operation were obtained by counting the number spat/shell from six cages. Shells were sampled from three different regions of each cage; top, middle and bottom depths and cages were selected from across the circumference of the two tanks (Table 2). Spat counts were performed the same way as for the GLP reef described above.

Table 2. Spat set data from the Parsons' southern site efforts (TKR).

Date of Set	No. of Larvae Set	No. of whelk shell	No. of *spat/shell*	Est. no. of spat set	Est. spat set ratio
6/23/16	4,000,000	19,500	32.2 +/-7.8	627,900	15.7%

After 28 days in the setting tank the 30 cages of SOWS were emptied onto the *R/V Petrel* for planting. The average size at planting was 5.8mm (+/- 1.7). The process of emptying the cages onto a planting vessel is the first of several sources of expected planting mortality. Unexpected tide restrictions prevented planting for 24 hours and spat on whelk shell were kept viable by remaining wet, shaded and cool aboard the planting vessel. Some (additional) mortality may have occurred with the planting delay but it is expected to be low and was not able to be measured. On July 22nd the full volume was blown off by 3" water hose to the TKR reef site. A known source of planting mortality expected through the planting process itself is caused by water-forced mortality of the small spat (dislodging small spat from their cultch). The final source of planting mortality is via smothering by the random distribution of spat on any given whelk shell (i.e. spat on the underside of a whelk shell once settled to the bottom).

Wild seed transplants

Wild seed transplants to both sites came from the Mullica River beds of Maxwell Shellfish, lease #209, approximately 1 NM downriver of the GSP bridge (Figure 1). Mixed size and age class oysters were mechanically dredged via the *R/V Petrel* on November 16 and 17, 2016. Seed was shoveled and hosed off onto each site; 150 bushels to TKR and 75 bushels to GLP. During transit to the GLP site a total of (4) 0.25-bushel volumes were assessed for # live oysters/0.25 bushel volume and included individual sizes. A total of 1495 live oysters were measured and classified (Figure 5).

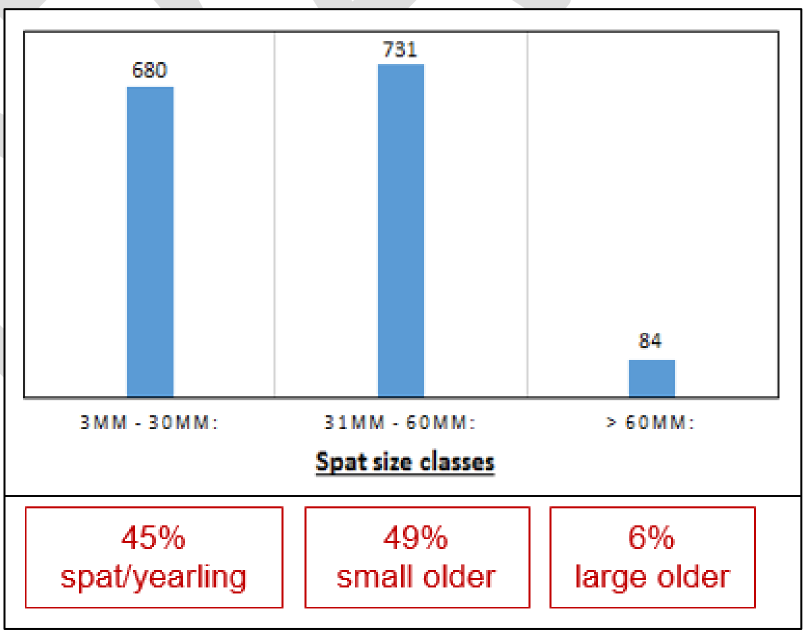


Figure 5. Size distribution of the Mullica River transplant seed.

Reef monitoring.

SOWS. Monitoring SOWS at each site was performed in Fall 2016, Spring 2017, and Fall 2017. Oysters were sampled at 2 randomly selected locations on the GLP site and 4 randomly selected locations at the TKR site (within the SOWS-planted areas). Water quality was taken at the surface and bottom of each site. Whelk shells were brought up in tongs and rinsed in a bucket. Any large fouling organisms (primarily sponges) were removed and quantified by dry volume (liters). Whelk shells from each sampling location were placed into bushel baskets until half full (early sampling events) or full by the October 2017 event to maintain sample size because the larger oysters equated to less whelk/bushel. Comparisons were made by calculating number of oysters per whelk shell, negating volume discrepancies between sampling events. Live oysters on each whelk shell were enumerated and measured. Mortality was estimated as dead oysters with hinge still intact and classified as gaper (tissue present), box (no tissue present), or box with drill (no tissue present, visible oyster drill mark).

To assess habitat enhancement for other species, motile organisms that were rinsed off shells were enumerated by sieving the rinse bucket through a 0.5 mm sieve. Additional motile organisms encountered when processing were added to the totals. Encrusting epifaunal organisms were assessed per whelk shell unit and divided into solitary individuals assessed as number per shell. Colonial organisms were assessed as percent cover of an individual whelk shell.

MRT. Monitoring of MRT oysters was completed in May and October 2017 at both sites. Despite accurate deployment coordinates, sampling the MRT oysters at the GLP site was difficult. Both May and October 2017 sampling event quantities were lower than called for despite several hours of dredging effort. It is suspected that the GLP site experienced bed spreading in major storm events, as documented after superstorm Sandy and perhaps again after winter storm Jonas in January 2016. Using small side scan sonars and available coordinates from the original deployment the sampling teams were still not able to recover sufficient numbers of MRT oysters for proper assessment.

Tuckerton Reef MRT oysters were recovered without difficulty. Four dredge samples were collected using a standard commercial oyster dredge. Oysters were rinsed into a bucket as described above, and separated into subsamples. Live and dead oysters were sampled as described above for SOWS. Motile species were enumerated from rinse buckets. Epifaunal organisms were not recorded.

Fish surveys. Unbaited mesh fish traps were set to capture fish and larger motile crustaceans around the periods of reef monitoring. Traps were 26” x 19” x 9” x ¼” mesh. Three traps were placed at each location: SOWS portion of reef, MRT portion of reef, and a control area off the reef. After a 24-hour deployment period, all species in the trap were measured and enumerated.

Hard clam surveys (TKR site only). Prior to shell planting at the Tuckerton site a hard clam assessment was performed via a snorkeler-deployed modified Peterson grab (Lamotte model 1061, 800 cm^3). A total of 18 grabs were collected; 6 in each of three zones (control; future SOWS area; future MRT area). Samples were wet-sieved in the field to 0.5mm and stained/preserved via rose bengal/10% formaldehyde solution. Species identification and enumeration via microscope was conducted.

The post-reef establishment hard clam surveys were not able to be conducted via snorkeler-deployed bottom grabs. The sampling protocol called for samples to be taken from shelled areas of both oyster types, as well as a control area. Due to the added time needed to locate and move shell to take a bottom grab it was nescessary to perform these follow-up surveys via SCUBA. Diver-collected bottom grabs utilizing the same modified Peterson grab were collected from the same zones (control; planted SOWS area; planted MRT area). Six samples from each zone were wet-sieved in the field to 0.5mm and stained/preserved via rose bengal/10% formaldehyde solution. Species identification and enumeration via microscope was conducted in the laboratory.

Disease. Histopathology testing for MSX and Dermo was conducted by Haskins Shellfish Research Lab (HSRL, Bushek and McGurk). Twenty samples were collected at transplant for the MR seed population (November 2016) to establish baseline prevalence for the wild seed. At the end of the study period 20 larger individuals from each population (TKR site SOWS & MRT; GLP site SOWS & MRT) were collected. This total of 80 samples was taken during the October 2017 sampling events, kept cool and delivered to HSRL.

RESULTS

Water Quality. The Tuckerton site had a 3 PSU higher average salinity than the GLP site (Table 3). There were not large differences in temperature or salinity between surface and bottom waters at either site at each monitoring event (Figure 6). Temperatures represent seasonal trends and weather events, and pH and dissolved oxygen (not shown) was not expected to be limiting. No water quality data was obtained for the spring 2017 sampling event at GLP.

Table 3. Average bottom salinity and pH for both sites averaged over multiple site visits.

	Tuckerton Reef	Good Luck Point Reef
Average Salinity	26.8	23.66
Average pH	7.88	7.93

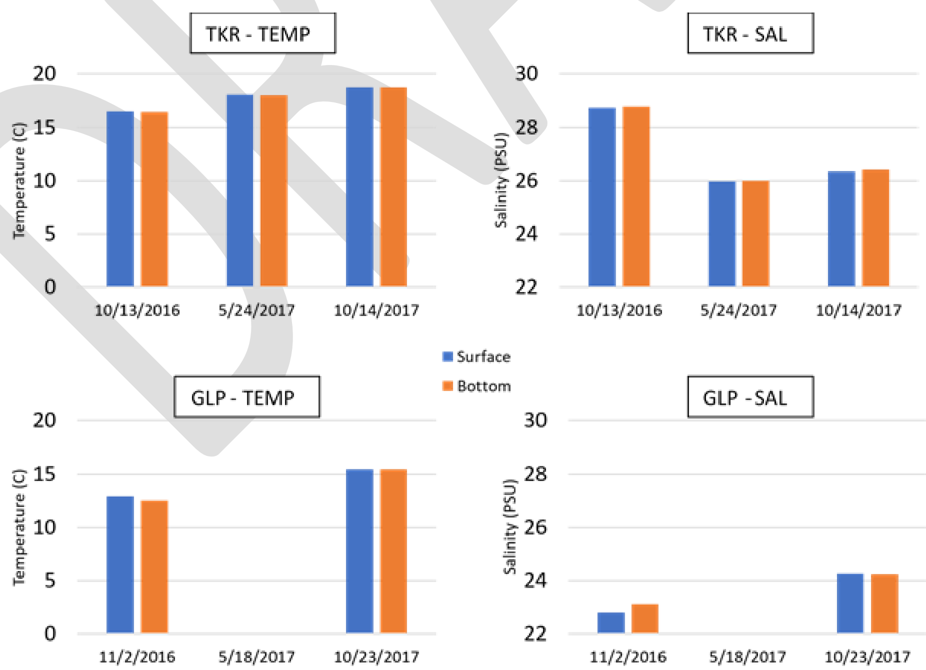


Figure 6. Temperature and salinity at the surface and bottom for Tuckerton (TUK - top) and Good Luck Point (GLP - bottom) reefs during sampling events.

SURVIVORSHIP & GROWTH

SOWS survivorship. Starting densities at both sites were high, with an average of 24.2 spat per shell for GLP, and 32.2 spat per shell for TKR. Survivorship for the remote set oysters at both sites was highest at the end of YR1 (October 2016), with mortality reaching a steadier state between spring and fall 2017 (Figure 7). Percent survivorship was calculated by comparing the average number of live oysters per whelk shell to the initial planting density. The GLP site showed a 25% survivorship rate at the end of YR1 (6.0 oyster per shell vs. 24.4 initial density) and TKR 24% (7.77 oyster/shell vs. 32.2 initial density).

At the end of YR 2, after two full growing seasons on the bottom, the GLP site had 9% survivorship (2.2 oyster per shell vs. 24.4 initial density) and the TKR site showed 18% survivorship (5.7 oyster/shell vs. 32.2 initial density). The increase in survivorship at the TKR site between May 2017 and October 2017 is the result of either 1) sampling variability and/or 2) observation and inclusion of natural set data from the wild-set cohorts observed in 2016 and 2017.

It is important to note that the GLP data (9% survivorship) represents a high estimate due to sampling difficulty and site history of naked shell deployment. Although the shell had been planted on an undisturbed area of the permitted reef, during each sampling event there was evidence that previously added shell (without remote set) to the GLP reef may have migrated to the monitored area. Initially these whelks were used for sampling in order to get to the volume protocols but after closer consideration they were left out of the final live oyster per shell analyses. At the Fall 2017 monitoring event, 64-78% of the whelk shells collected at the GLP site had no oysters or obvious scars and were presumed to be from separate plantings of just whelk shell (no remote set). These were excluded from survivorship estimates presented here. At the TKR site, 8.5% of oysters in the fall 2017 monitoring event did not have obvious scars, but these were not excluded because we know that site was (only) planted with SOWS from this study.

Mortality attributed to oyster drills was highest for the TKR reef, with 9.4-40% of oysters showing drill marks (range of four subsamples). The highest drill mortality was observed in spring 2017 (Table 4). Drill mortality was much lower at the GLP reef, from 0-7.5% of oysters having drill marks (two subsamples). Lower drill mortality at the GLP site is likely the result of salinity-depressed biomass of oyster drills relative to the TKR site (oyster drills prefer higher salinities).

SOWS growth. Oysters grew similarly at both sites in Year 1. Size at planting for the GLP site was 2.3 – 12.8mm as a result of the three setting events. Size at planting for the TKR site was 5.8mm (+/-1.7). Growth over the second summer (Year 2) was higher at the TKR site (Figure 8). Average size of oysters at the TKR site was ~ 10 mm greater than oysters at GLP, with many oysters > 90 mm.

MRT survivorship. Survivorship estimates of Mullica River transplanted oysters was assessed based on the number of live oysters per one half bushel of dredged oysters. In May 2017, 6 months post-transplant, survivorship of MRT at the TKR site oysters was 49%. Survivorship dropped to 19% of initial transplant numbers by October 2017 (Figure 9). It is noted that due to the volumetric approach to sampling and the growth of the transplanted seed oysters that this number under-represents survivorship by some small amount (due to oyster growth = less oysters/bushel). Future efforts should be directed toward standard bed health assessments using a volumetric approach to quantify live oysters/dead hinged oysters/shell hash per bushel (this data was not collected initially).

(A)

(B)

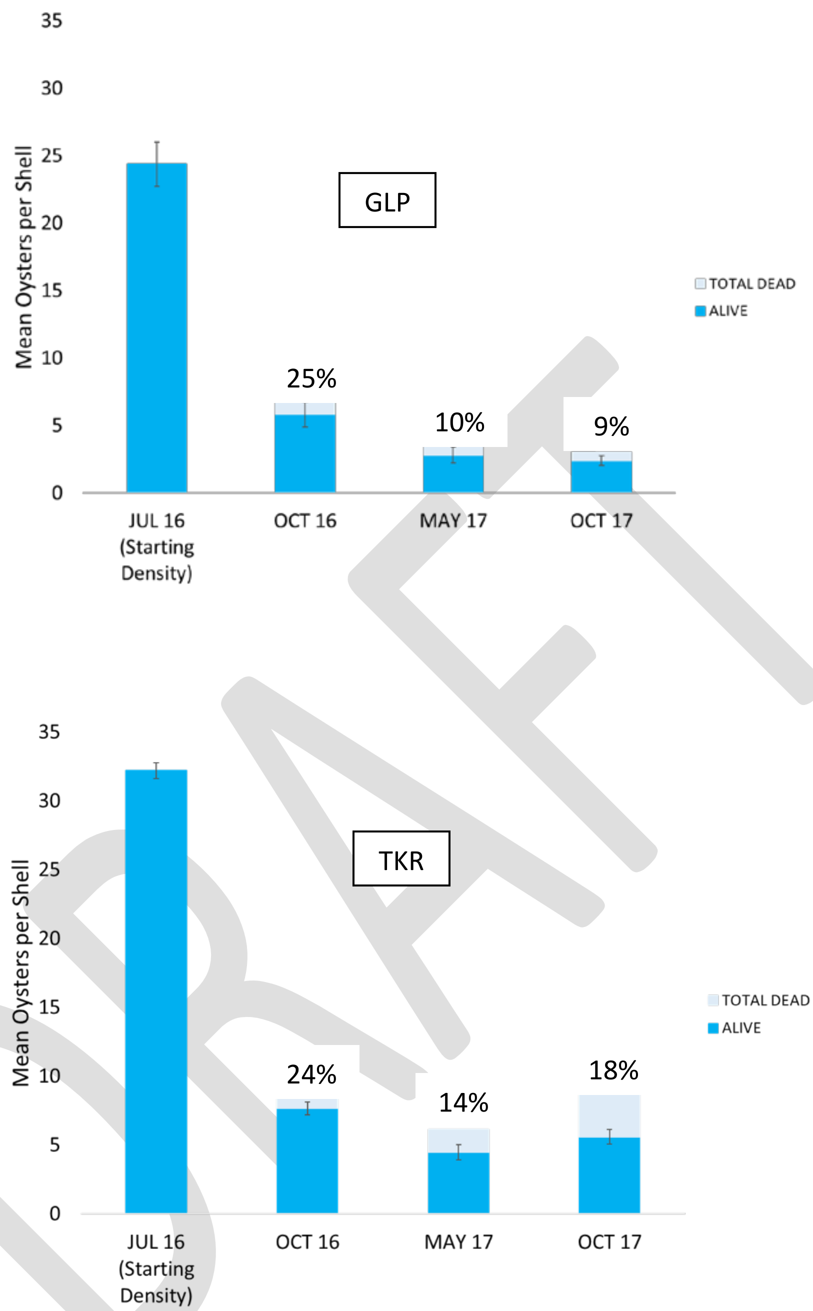


Figure 7. Survivorship of SOWS at (A) Good Luck Point (GLP) reef and (B) Tuckerton (TKR) reef. Average number of live oysters per whelk shell is shown from each monitoring event. Mortality was assessed by the presence of dead oysters with both valves still present.

Table 4. Mortality and percent drill mortality of SOWS oysters at both sites.

	TOTAL DEAD OYSTERS	DRILL SCARS	PERCENT DRILL MORTALITY
Tuckerton Reef			
FALL 2016	317	30	9.4%
SPRING 2017	155	62	40%
FALL 2017	263	36	13.7%
Good Luck Point Reef			
FALL 2016	159	12	7.5%
SPRING 2017	51	1	2%
FALL 2017	18	0	0%

Sampling challenges at the GLP site made recovery of MRT oysters difficult due to presumed movement or burial of the initial plantings. Despite accurate planting coordinates and the use of side scan sonar, only 42 (MRT) oysters were recovered after repeated dredging in spring 2017, and 100 (MRT) oysters were recovered in fall 2017 (Figure 9). Therefore, no survivorship estimates, relative to the initial transplant data, can be adequately determined.

MRT growth. Oysters showed steady growth through each season at both sites, though appeared to level off at the GLP site relative to TKR (Figure 10). Growth of MRT oysters at the GLP site was lower than at the TKR site, similar to trends with SOWS oyster growth and again likely the result of lower salinity and current flow (at the GLP site).

Natural set. Natural set oysters (spatfall) were not observed at any sampling event for the GLP site during this study period. Spatfall was observed in both years at the TKR site, with Year 1 (2016) data showing 52.25 wild-set spat per bushel of whelk shell equating to 1.08 spat per whelk shell. These observations were consistent with qualitative observations of spatfall on hardened structures throughout the bay in 2016 (Evert, personal observation). In 2017 spatfall at the TKR site was much less at 3 wild-set spat per bushel of whelk equating to 0.16 spat per whelk shell.

Disease – both oyster types, both sites. At the time of transplant (November 2016) MRT oysters had a 45% prevalence of Dermo with 10% advanced infections. There was no MSX detected in the MR transplants. At the time of resampling one year later MRT oysters still did not present any MSX detections but had 95% and 90% prevalence of Dermo with large percentages of advanced infections at the TKR and GLP sites (75% and 25% respectively). Additional details are found in Appendix 1.

ECOSYSTEM SERVICES

Appendix 2 shows a list of all species found throughout the monitoring events during this study, grouped by sampling type (Motile, Encrusting, or Fish Trap) and taxonomic level. The species data are summarized and compared below.

Motile species. Motile organisms were assessed for both SOWS and MRT at the Tuckerton reef, but only for the SOWS at the GLP site due to sampling difficulties described elsewhere (Figure 11). Decapod crustaceans (seven species) and gastropods dominated the motile fauna at the TKR site, but GLP had greater species richness overall, and greater abundances of errant polychaete worms, fish, and amphipods (‘other’). Comparing SOWS to MRT at the TKR reef, MRT oysters had greater abundances of polychaete worms and amphipods per half bushel samples.

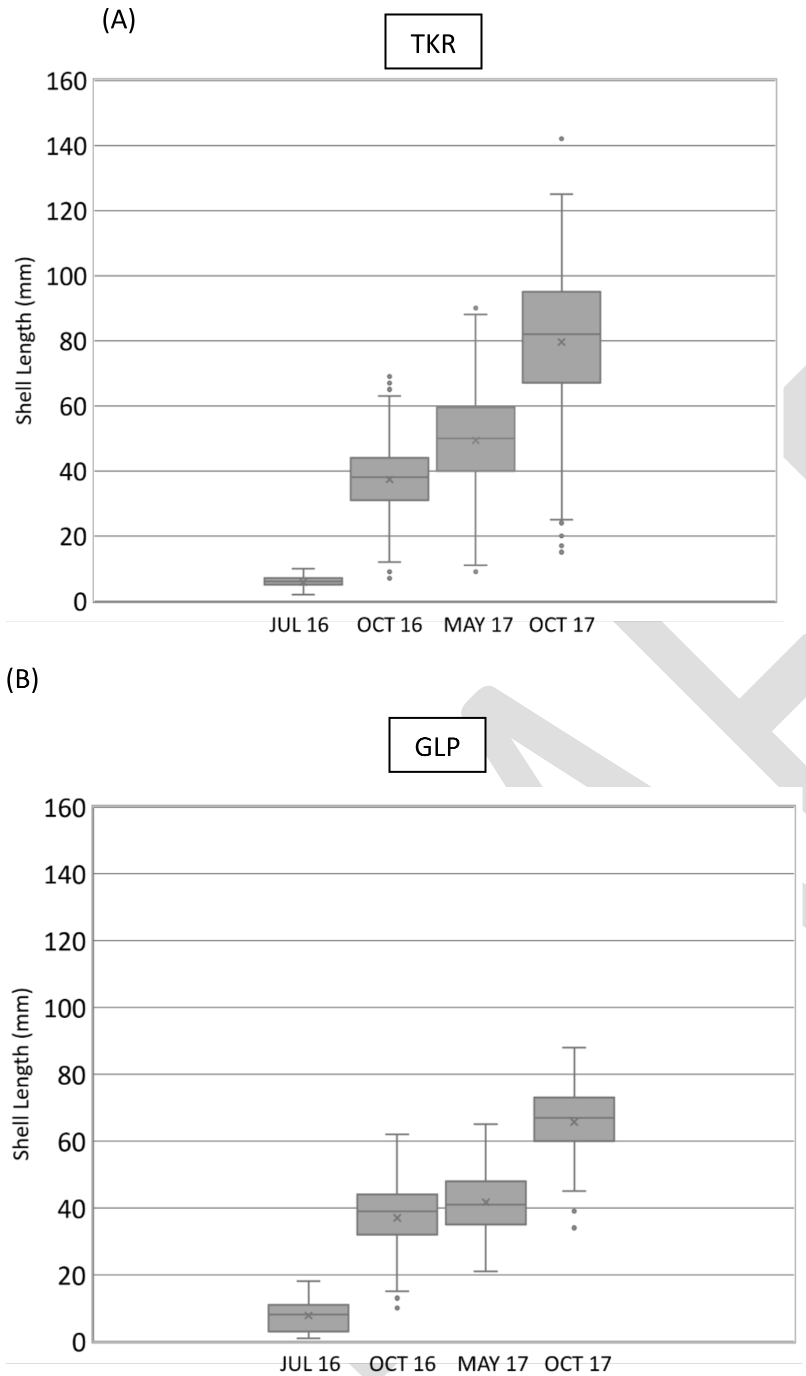
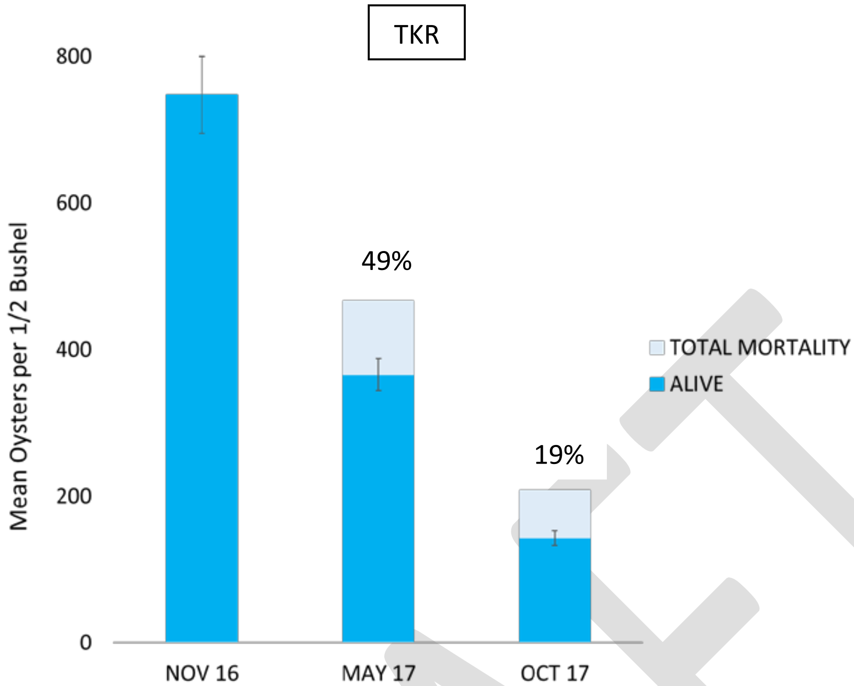


Figure 8. Size frequency of live SOWS oysters for each monitoring period at (A) Tuckerton and (B) Good Luck Point. Middle lines represent the median, ‘x’ marks represent the average size, and outliers are beyond the 95% confidence limits.

(A)



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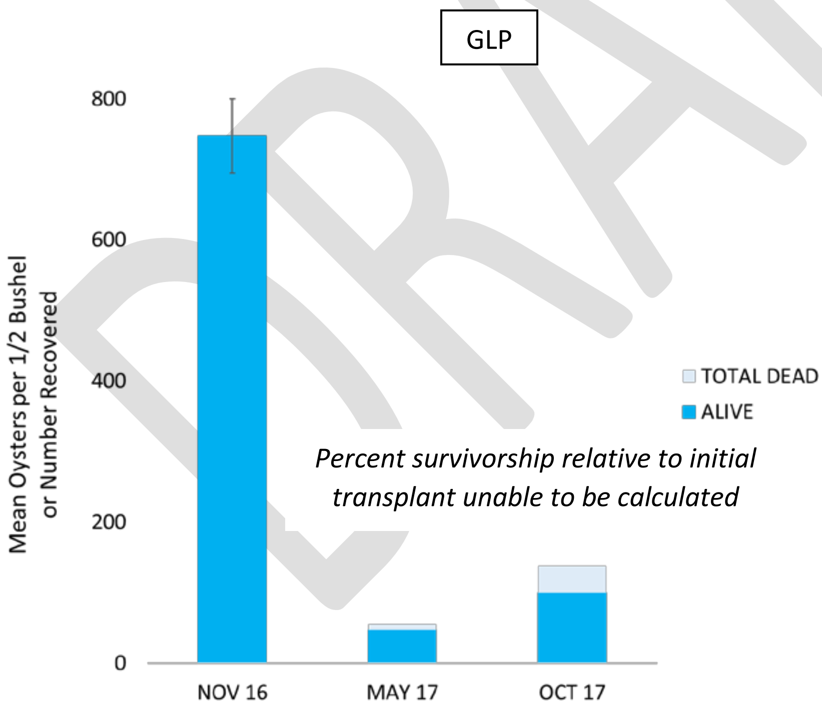

Figure 9. Survivorship of MRT oysters at (A) TKR and (B) GLP sites.

(A)

(B)

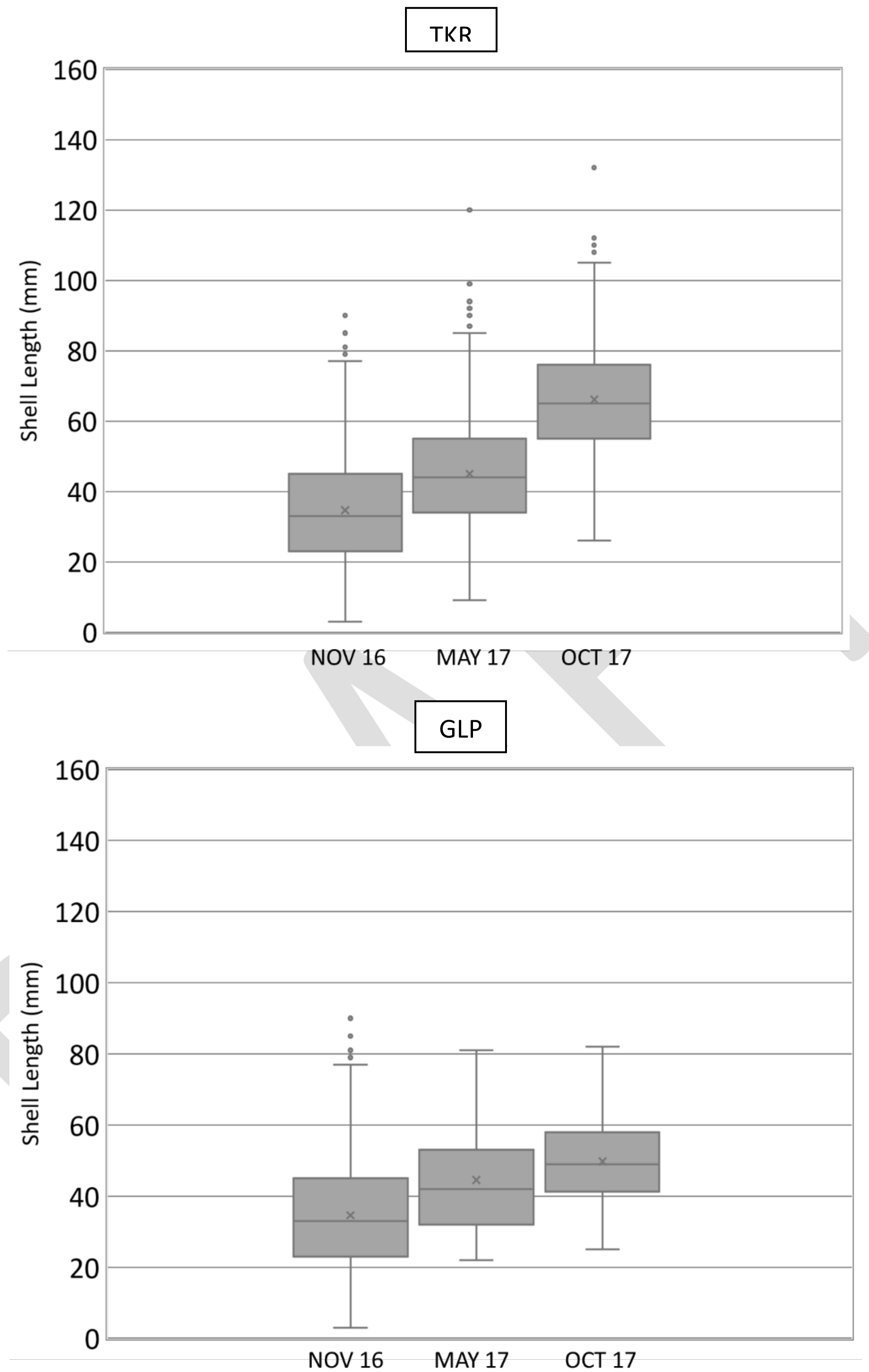

Figure 10. Size frequency of live MRT oysters for each monitoring period at (A) Tuckerton (TUK) and (B) Good Luck Point (GLP). Middle lines represent the median, 'x' marks represent the average size, and outliers beyond the 95% confidence limits.

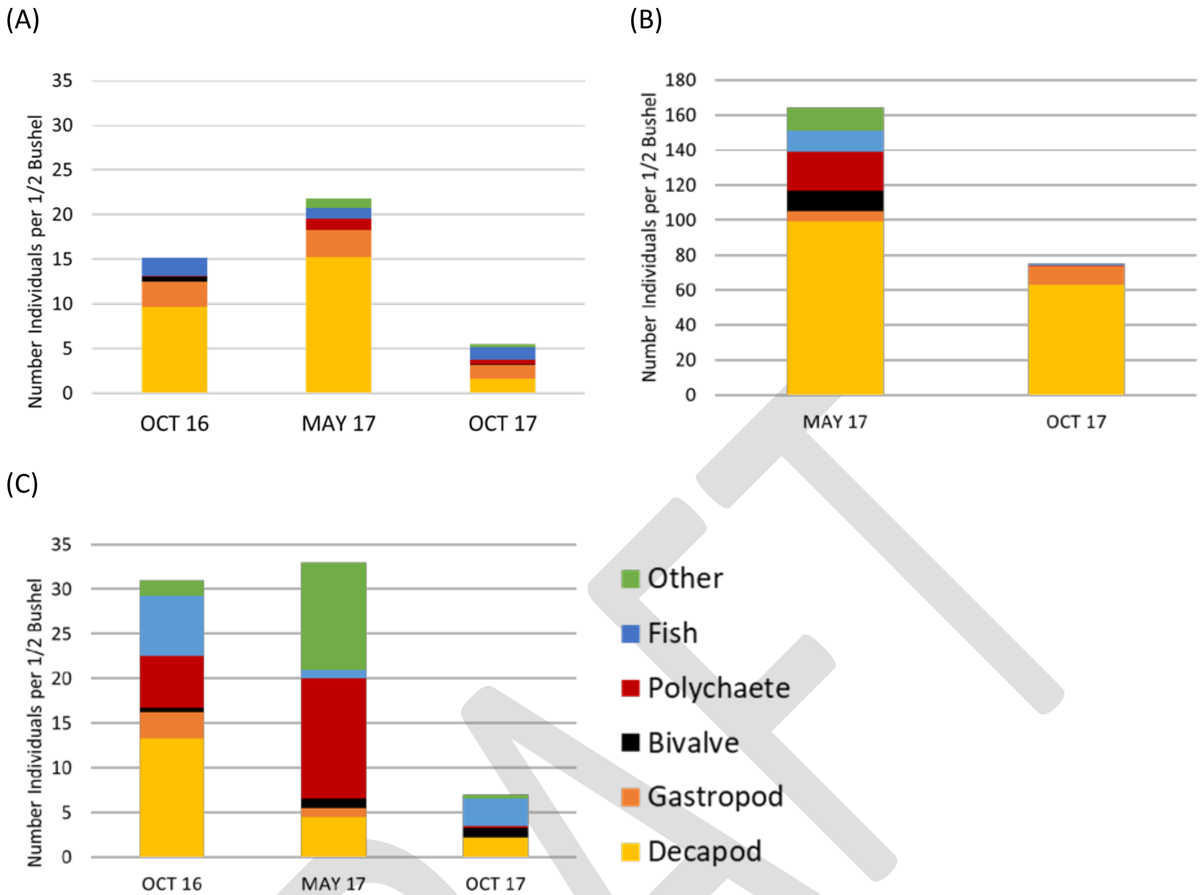

Figure 11. Motile species from (A) TKR SOWS, (B) TKR MRT, and (C) GLP SOWS. Average number of organisms per ½ bushel subsample are plotted for each monitoring event. Fauna is grouped by taxonomic unit for simplicity. 'Other' group represents mostly smaller crustaceans like amphipods.

Encrusting species. Encrusting fauna was assessed on all SOWS samples. Figure 12A and B shows estimates of solitary species (barnacles, limpets, etc) and Figure 12C and 12D shows colonial encrusting species (sponges, tube worms, bryozoan, recently settled barnacles). Differences were noted between encrusting communities at both sites, with jingle shell (*Anomia simplex*) and white limpet (*Crepidula plana*) more abundant at the TKR site, and brown limpets (*Crepidula convexa*) and barnacles (*Balanus sp*) dominating the GLP site. Most shells were extensively covered in Bryozoans. Sponges were more prevalent at the TKR site, in particular the Yellow-boring sponge (*Cliona celata*). Large yellow boring sponge heads have been noted in acoustic and video records of that area.

Fish. Greater numbers of finfish were found on the SOWS or MRT portions of the reef relative to the unplanted controls. Black sea bass, silver perch, and oyster toadfish were found around shell-planted areas versus the control area (Figure 13). The crabs caught in the traps did not show a large difference between reef or control areas. Both spider crab and blue crab seemed similarly abundant on the different areas of the reef (Figure 14). At Good Luck Point vessel problems prevented deployment in Fall 2016, and a severe thunderstorm event prevented recovery of traps in Fall 2017. Only one fish trap sampling event occurred at GLP, making it difficult to compare sites.

Hard clam surveys (TKR site only). There was no evidence of recruitment enhancement of the hard-clam *Mercenaria mercenaria* or other infaunal bivalves after shell planting on the TKR site. *M.*

mercenaria was found in two pre-shelling samples and one in the control sample follow up. Abundances of the stout razor clam *Tagelus pleibeus* declined in pre- and post-sampling events and the small *Macoma spp.* clam remained about the same (Figure 15). Further study and greater sample sizes are required to fully investigate the benefits of shelling and oyster reefs on hard clam recruitment.

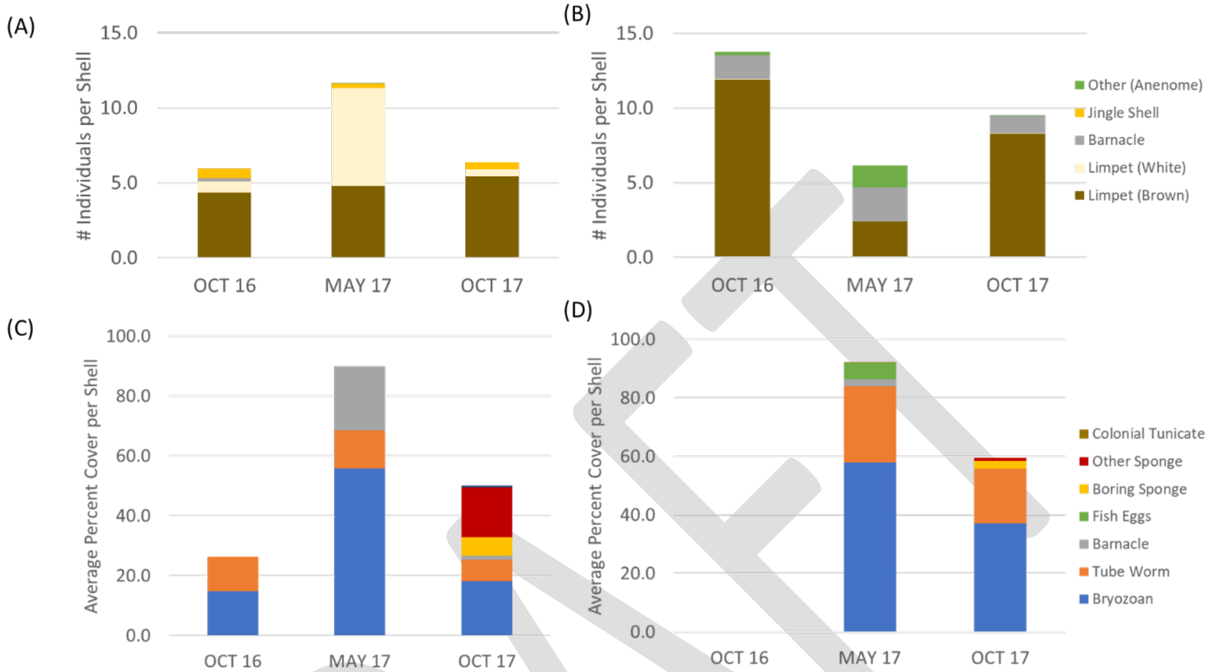

Figure 12. Encrusting species from SOWS at each site. (A) Solitary encrusting species at TKR, (B) Solitary encrusting species at GLP, (B) Colonial encrusting species at TKR, (C) Colonial encrusting species at GLP (no colonial encrusting data was recorded in fall 2016).

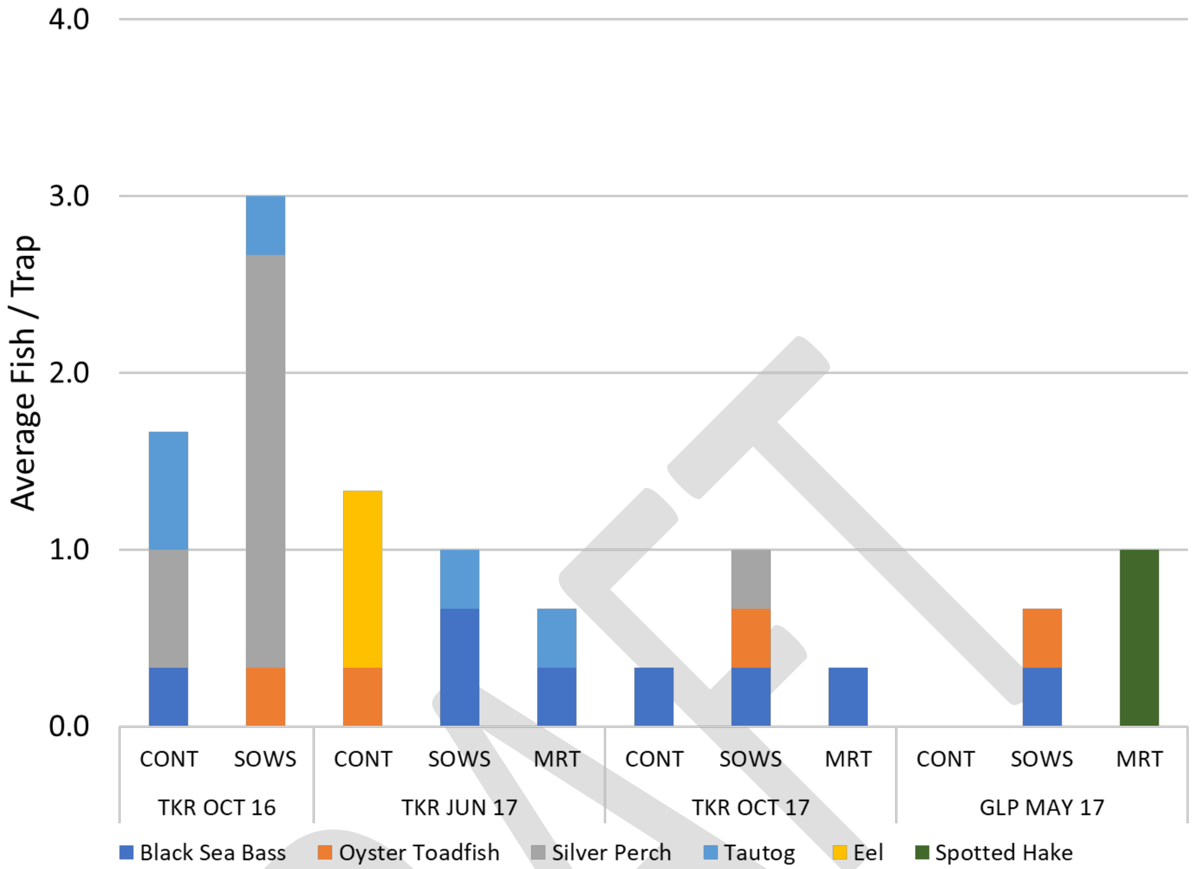

Figure 13. Finish species caught from traps at SOWS and MRT reef locations and a control area. Data shown are from all sampling periods at Tuckerton, but only traps were recovered in May 2017 at Good Luck Point.

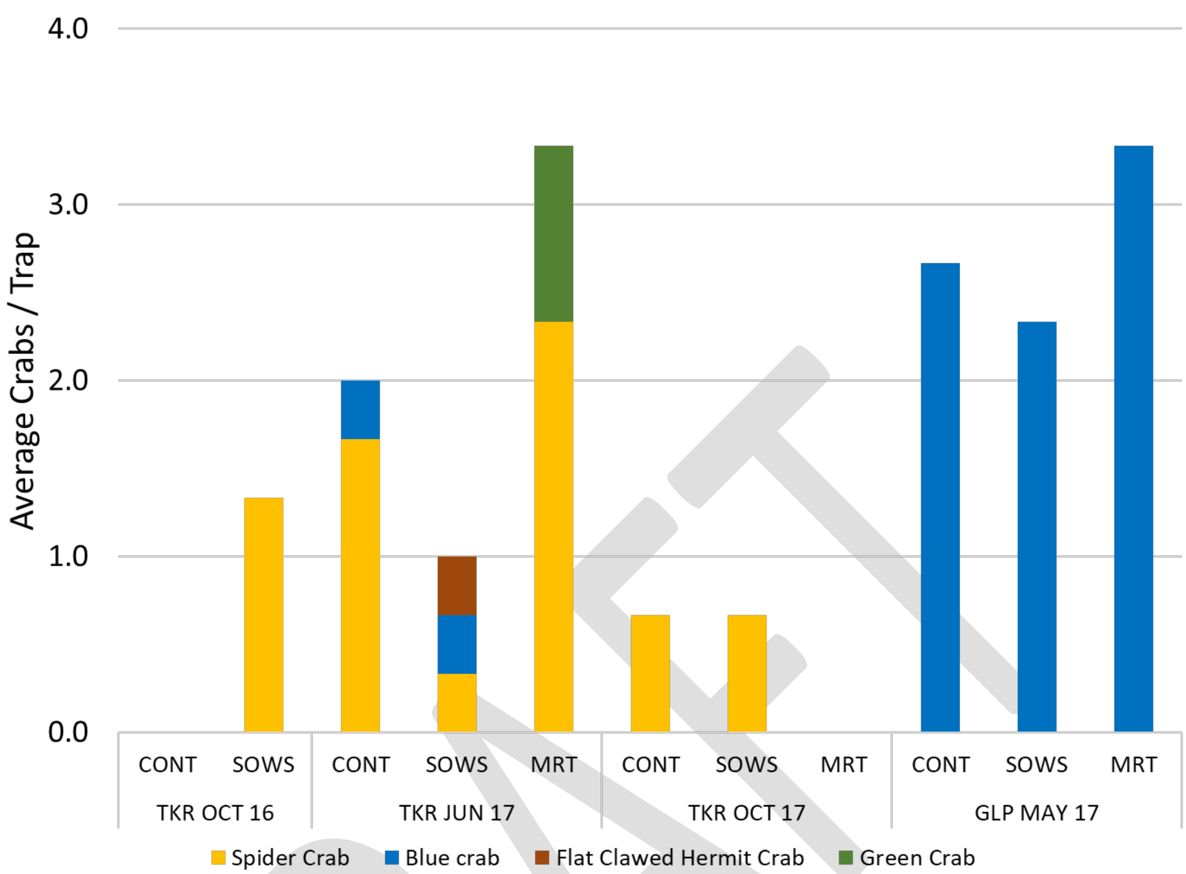

Figure 14. Crab species caught from traps at SOWS and MRT reef locations and a control area. Data shown are from all sampling periods at Tuckerton, but only traps were recovered in May 2017 at Good Luck Point.

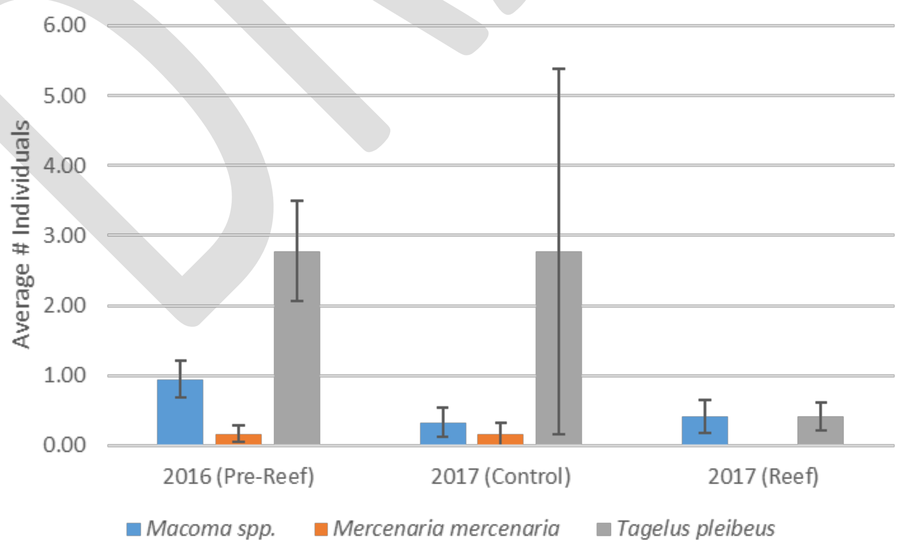

Figure 15. Average number of infaunal bivalves counted from core samples taken at the Tuckerton site prior to reef creation, and after reef creation in a control and a reef area.

DISCUSSION

Comparisons between sites

The physical setting of each site reflects the tidal properties of the bay with the northern site (GLP) having lower salinity/lower tidal exchange than the southern site (TKR), which is closer to the inlet and in an area of the bay that has significantly more tidal range than GLP. Salinity differences between sites can explain the observed difference in oyster growth, but other aspects of the GLP site such as shallow water depth, unconsolidated sediment structure and low tidal exchange may have also contributed to the observed lower growth and survivorship relative to the TKR site.

Both the GLP and TKR sites were planted at high densities (average 24 and 32 spat per whelk shell, respectively). Although there are no published studies available on optimal spat densities when remote planting spat on whelk shell, we anticipated that 15-25 spat per shell would be ideal for survival based on oyster planting methods achieving success in other locations (Paynter et al. 2014). Lower spat survival can sometimes be a result of high-density settlement leading to overcrowding (Andrews 1955, Stanley and Sellers 1986). Initial planting survivorship was 25% and 24% for GLP and TKR efforts, respectively. In the Chesapeake Bay, remote setting survivorship of oyster spat ranged from 12-37% in post-planting surveys, however, there was no evident trend with starting densities (Paynter et al. 2014). Overwintering mortality was not as severe as initial planting mortality, and mortality then leveled off by Year two. This indicates that most of the mortality on the reef was experienced early on and those that survived were able to grow. At the GLP reef, the unconsolidated sediments and lower tidal exchange may have caused additional mortality. The shallow waters that allow for storm-induced energy to reach the bottom also contributed to our sampling difficulties at this site. At the GLP site there was evidence that shell from previous efforts had moved and mixed with SOWS from this effort. As such, the survival estimates presented for the GLP are the higher of possible estimates because we did not include whelk shell that did not show signs of spat set from this project's directed 2016 SOWS planting.

The lower growth rates observed at the Good Luck Point site are likely a result of salinity and tidal flow, as oysters generally grow more slowly at lower salinities (Kraeuter et al. 2007). A potential benefit of the lower salinity regime of this site does lie in its lower disease prevalence profile (Appendix 1). Mortality with the transplanted oysters due to disease may be lower at the GLP site, suggesting it may allow longer survival for transplanted wild seed and could be used as a transplant-to-harvest site (i.e. allow harvest within 18 months of transplant). Predation by the oyster drill *Urosalpinx cinerea* was more common at the TKR reef, however we do not find this to be the most significant source of mortality overall for either site. It is likely that post-planting mortality and spatial competition led to much of the observed mortalities at both reefs, with mortality at the GLP reef further enhanced by shell burial, transport and low tidal flow.

There was no spatfall observed at the GLP site during this study period. Spatfall was observed in both years at the TKR site, with Year 1 (2016) data showing 52.25 wild-set spat per bushel of whelk shell equating to 1.08 spat per whelk shell. These observations were consistent with qualitative observations of spatfall on hardened structures throughout the bay in 2016 (Evert, personal observation). In 2017 spatfall at the TKR site was much less at 3 wild-set spat per bushel of whelk equating to 0.16 spat per whelk shell. It is possible that encrusting organisms and growth limited the amount of available shell for natural set to occur in year 2. It is also important to note the high inter-annual variability of spatfall. Spatfall indices (avg. spat/shell over the season) for the Mullica River beds has ranged from 0.37 – 4.59 over the past 4 seasons (Evert, unpublished data). Monitoring spatfall moving forward will be important and should include clean spatfall bags in addition to the bed health assessment techniques (observing spatfall on previously-planted shell).

The observed ~75% mortality rate at both sites in Year 1 is comparable with other studies and is a function of starting densities, size at planting, planting mortality by physical forces, loss due to sedimentation (how the shell settles to the bottom), and early predation before shell thickness begins to protect the small oysters (Paynter, 2014). Each of these factors should be considered for future efforts. When reviewing this data it is important to recognize that the remote set process mirrors the oyster's natural high fecundity survival approach where actual cohort recruitment (alive going into winter one) often falls well short of initial spatfall numbers. Mortality by sedimentation was not recorded for this study but could be added for future plantings by using the black staining as a proxy for sediment mortality. Mortality by crabs and flatworms certainly occurs but cannot be identified. Mortality by disease is not likely in YR1 for the SOWS due to the lower filtering capacities of the small oysters but is possible and even likely for the mixed age/size oysters from the MRT. See the Histopathology results and discussion.

There were different reef communities observed at each site, possibly attributed to the salinity differences. Some encrusting species, such as the white limpet *Crepidula plana* and jingle shell *Anomia simplex* were more often observed at the higher salinity TKR reef. Anenomes and barnacles were found more often on shells at the GLP site. It is likely that salinity tolerances are driving these differences. Species differences may reflect seasonal recruitment patterns as well as competition for space. As shells became more encrusted with bryozoan, tubeworms, or sponges, this leaves less available substrate for limpet or barnacle settlement. Barnacles were most abundant in the spring. Although sponges were found at both sites, there were higher densities of encrusting sponges at the TKR site. This may have contributed to the reduced natural set observed in year 2, although there are other factors, such as those that influence larval supply, that would also contribute to this. Encrusting species may be a concern going forward, particularly for those that cause damage to oysters such as the boring sponge (*Cliona spp.*).

Mobile species showed seasonal patterns as well as potential habitat preference between the GLP and TKR reefs. Species of crab had greater abundance and richness at the Tuckerton reef, with more fish, worms and amphipods ('other') found at the GLP site. Some of the data (worms and amphipods) may show seasonal patterns being more abundant in spring sampling (Grabowski et al. 2005). Reef associated fauna, such as oyster toadfish, blennies, gobies, and mud crabs were readily observed on the planted areas of both sites, thus achieving the habitat enhancement goal for these species (Lukenbach et al. 2005, Cohen et al. 2007). The extra relief provided by the whelk increases habitat heterogeneity for many of these species. Silver perch and tautog were found in higher numbers for reef samples versus the off-reef controls. Higher frequency sampling and sampling during migratory periods is suggested to get at the question of reef enhancement for juvenile and larger fish. Similarly, the small size of the restored reefs in this effort may reduce the ability to detect many mobile fish species (Grabowski et al. 2005).

Among the anticipated ecological benefits was the potential for the TKR site to increase hard clam (*Mercenaria mercenaria*) recruitment. Bricelj et. al. (2012) reports a major decrease in the landing of hard clams in the Barnegat Bay, with significant drops in the 1980's and 1990's. In 2003, Kraeuter et. al. published an 11-year report on the benefits of shelling toward increasing hard clam recruitment. The Tuckerton Reef site is located adjacent to naturally occurring areas of hard clams (Fig. 2). Data from this study was inconclusive, and it is suggested that further study and greater sample sizes are required to fully investigate the benefits of shelling and oyster reefs on hard clam recruitment.

Comparisons between planting methods

Comparisons between methods are to some degree qualitative but presented as constructive nonetheless in terms of understanding what options exist for Barnegat Bay oyster restoration. Each planting method had specific qualities that makes a direct comparison difficult. Remote-set oysters are represented by a single cohort of disease-resistant larvae and can be set on individual shells that allow directed per-shell sampling. Wild seed transplants represent multiple cohorts and grow in clusters of varying size, requiring volume estimates to measure mortality and survivorship. Transplanted oysters also contained a background level of disease prevalence as well as may have also transplanted epifaunal species, such as the bright-orange lemondrop or limpet nudibranch *Doriopsilla obscura,* which was not found with any of the SOWS shells. Because of this, epifaunal data was not recorded for MRT oysters. We will also only be making this comparison between planting methods at the Tuckerton site, since sampling difficulties limited the recovery of MRT oysters at Good Luck Point. For sampling and estimating mortality, it is noted that the whelk shell provided a discrete sampling unit to measure mortality over time.

Despite different deployment and sampling methods, we can still make comparisons between the two planting methods that result in both future biomass predictions and ecosystem services rendered to the system as a whole. MRT oysters experienced less post-deployment mortality compared to SOWS at the Tuckerton reef (50% relative to 25%). This is expected due to the larger size and shell thickness of the wild seed. The high mortality observed between May and October 2017 could be attributed to increasing disease prevalence, as our results indicated 95% of the largest oysters sampled were infected with Dermo, and of those, 75% were in an advanced stage at the TKR site. The resistance of the SOWS to Dermo was evident in our sampling, however, further sampling may reveal if older oysters become susceptible. Year 3 and 4 sampling of the original cohort is critical to documenting the disease-resistance of the NEH strain and funds should be sought to monitor multiple cohorts for disease. The SOWS at Tuckerton grew to a larger average size than the MRT by the October 2017 sampling period. It is possible that MRT mortality caused by disease is more prevalent in larger oysters causing a downward shift in max average size even at the end of only one growing season. In a similar practice in the Delaware Bay estuary, mortality attributed to Dermo exceeded 50% for larger oysters in early transplants (Kraeuter et al. 2003).

Seed oysters from virtually all waters of the east coast have some level of MSX and Dermo prevalence, and it was important to establish that level prior to the seed transplant to higher salinity. In contrast, remote-set oysters are introduced into their planting waters with no former exposure to disease pressure. Monitoring disease prevalence of the remote-set oysters allows hatcheries and restoration scientists to assess the effectiveness of these selective breeding programs for the target waters. Disease is a major cause of oyster mortality, in particular MSX and Dermo. Other common mortality causes to either oyster type include planting mortality, predation by Oyster drill (*Urosalpinx cinerea*), flat worms, crabs and finfish (including cow nose ray). It is assumed that predation by small crabs, flatworms and even drills is more likely on the SOWS than seed transplants due to their initial smaller size and thinner shell. It is possible that the small clusters of MRT oysters may be more susceptible to cow nose ray predation compared to SOWS, but this is difficult to assess and no reference is made here.

In terms of habitat enhancement for other species, mobile species were more abundant in the MRT section of the Tuckerton reef. Numbers were an order of magnitude higher for crabs, errant polychaete worms, non-oyster bivalves (like the ribbed mussel *Geukenisa demissa*), and fish. This could be due to the increased volume of shell habitat per bushel for seed oysters compared to whelk shell which leave more space between shells. Based on biomass enhancement alone, transplanted oysters may provide a greater abundance and richness of prey items for higher tropic levels, although this was not evident based on fish trap data. Other factors such as oyster density and substrate relief may also play a role in ecosystem services of each approach.

Cost analysis

An important component of the project was to assess the costs of the different approaches to oyster restoration in this area, and to include in that discussion the logistical challenges and potential outcomes of varied approaches. In stark contrast to the Chesapeake Bay and southern New England regions, New Jersey has a very limited regional support system for oyster restoration. In particular, only one operation is available to provide eyed larvae (Rutgers Aquaculture Innovation Center, Cape May) and only one commercial remote-set operation is available (Parsons Mariculture, Tuckerton). Eyed larvae can be obtained from remote hatchery locations via overnight shipping, but it is logistically difficult and/or cost-prohibitive to utilize remote setting facilities that are any significant distance away from a restoration site. Likewise, it is logistically difficult and/or cost-prohibitive to secure wild seed from any significant distance away – restoration generally requires regional availability of wild seed or remote-set services. New Jersey is seemingly in its infant stages relative to neighboring coastal states in terms of remote-set and hatchery infrastructure. Locally-driven cost estimates are critical for the consideration of future restoration efforts in Barnegat Bay.

Remote-set costs. Remote-set activities that are within 25 NM of Tuckerton, New Jersey can be serviced by Parsons Mariculture. Currently, Parsons is capable of producing 400/bushels per setting event between April – October of the calendar year, with plans for expansion of those facilities. Up to two setting events per month are feasible. The current cost is $50 - 55/bushel and includes cultch material, setting and planting. Desired cultch material (whelk, clam, oyster shell, etc.), planting site distances, volume and changing costs in eyed larvae contribute to the range in costs per bushel.

Wild seed transplant costs. Transplant efforts that are within 25 NM of Port Republic, New Jersey can be serviced by any vessel of opportunity that can dredge and transport seed to a desired site (and is permitted by the lease holder and the State to do so). Areas in extreme southern New Jersey may be able to consider seed transplants from the Delaware Bay but for the purposes of this report these estimates are based on the only available seed source along New Jersey's coastal bays – the Mullica River system. Purchasing seed from a private lease holder in the MR system currently goes for $40-50/bushel and includes the transplant cost. Planting site distances and desired volume contribute to the reported range in cost. Additionally, it is becoming more common-place for grow-out operations to purchase seed from MR lease holders and so additional changes in cost per bushel can be affected by open market demand. There also exists with the MR system a series of State-owned and managed seed beds that have been used for restoration activities (Fitney Bit project, 2005). The resource-based decision to allow transplant of this State-owned seed rests on the Bureau of Shellfisheries and the Atlantic Coast Section of the Shellfish Council. If a State-approved seed transplant from these areas without negative effects to the brood stock biomass was permitted, costs could be as low as $10-20 per bushel.

Benefit analysis

Beyond the cost considerations of the available restoration methods for Barnegat Bay lies the ecological and economic benefits to the region. Analysis of this is qualitative to some degree but warrants discussion and a broad understanding of what approach might work to accomplish different restoration goals. Examining the survivorship of each method is likely the greatest contributing factor to selecting an appropriate approach. Our data suggests MR seed with background levels of Dermo at 45% or higher (as was the case for our study) are not likely to survive beyond two growing seasons. In contrast, the two-year (on-the-bottom) data set for the SOWS effort suggests a leveling off of survivorship and anticipated survivorship into Year 3 and possibly 4-5, with shell length equal to that of the MRT by the end of Year 2 (Figure 8).

Identifying the restoration goal is the first step toward selecting a method. For instance, investments in restoration to achieve water quality improvement goals such as denitrification would be best served with multiple-year plantings of disease-resistant spat on shell and its associated multi-year survivorship and its increased biomass benefits relative to filtering capacity. The same remote set approach would be best applied to meet restoration goals aimed at increasing spawning stock biomass in the Barnegat Bay system. In contrast to the purely biomass-driven benefits of remote set restoration, investment in restoration to achieve finfish habitat goals may be realized by a large single planting of remote-set oysters (on whelk especially), recognizing that decrease in biomass in years 4-5 could still provide many of the habitat benefits of the shell placement and its associated epifaunal growth, and will have contributed to spawning biomass for at least several years.

Figure 16 shows the anticipated Yr. 3 biomass of a 1000-bushel planting of SOWS versus a 1000-bushel transplant of wild seed based on the survivorship values observed in our 150-bushel study. Initial set, transplant and survivorship input values for this theoretical biomass example are seen in Table 5. The Year 3 biomass of a disease-resistant remote-set effort is 4.8x greater than a wild seed transplant with these survivorship assumptions. This analysis has great implications for suggesting the biomass-driven benefits of restoration efforts in Barnegat Bay (denitrification, water clarity, and increased spawning stock biomass).

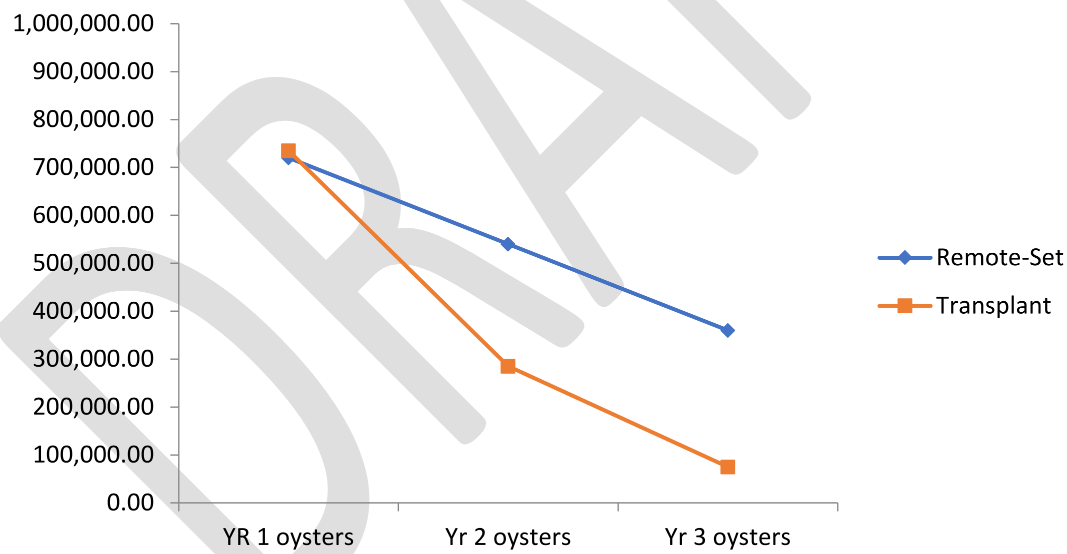

Figure 16. Predicted biomass and survivorship based on a 1000-bushel planting of each oyster type at the TKR reef site using survivorship values from this study (Table 5).

Table 5. theoretical biomass analysis out to 3 years using data values from the project.

Theoretical Live Oyster Yield (#) Over Time													
Method	Larvae Set	Set Ratio	Spatfall	Bushels	oyster/bushel	bushels planted	oysters planted	YR 1	YR 1 oysters	Y2 2	Yr 2 oysters	Yr 3	Yr 3 oysters
Remote-Set	20,000,000	0.15	3,000,000	1,000	3,000.00	1,000.00	3,000,000	24.00%	720,000.00	18.00%	540,000.00	12.00%	360,000.00
Transplant				1000	1,500.00	1,000.00	1,500,000	49.00%	735,000.00	19.00%	285,000.00	5.00%	75,000.00

It is important to note that there are ways to improve the remote set input by increasing set ratios through increasing eyed larvae input (but which could have overcrowding drawbacks) and possibly decreasing the initial planting mortality through better handling methods. In contrast, when conducting wild seed transplants there are no handling methods documented to mitigate the mortality associated with the physical nature of dredging or to increase the number of live oysters per bushel. Furthermore, it is thought that disease prevalence is the main cause of mortality once transplanted which cannot be mitigated by adjusted methods.

Despite these comments and analyses, not only the remote-set method is appropriate for efforts in the Barnegat Bay. MR seed transplants could provide short-term water quality improvement goals, contribute to spawning biomass for at least one season, and may offer an excellent opportunity for community harvest programs at a significantly reduced cost if made available by the State. These types of transplant projects could provide the aforementioned benefits and increase public awareness if harvest could be allowed and the Public could further connect to the projects.

In addition to the finish and motile crustacean ecological services that both sites provided, there is the potential for the LEH site to locally increase hard clam recruitment given its relatively higher salinity and close proximity to naturally occurring populations of hard clams (Fig. 2). Oyster reefs have been shown to have the potential to increase hard clam (*Mercenaria mercenaria*) recruitment, providing additional shellfishery benefits (Kraeuter et al. 2003). However, results of this study were inconclusive and it is suggested that a more robust sampling program to include diver-collected cores in the developing reef area and nearby control areas be implemented as funding allows.

Considerations for scale-up of current restoration in Barnegat Bay

The results of this study indicate that the Little Egg Harbor bay region will yield greater oyster restoration benefits than northern parts of the Barnegat Bay system. The Tuckerton Reef site demonstrated increased survivorship, increased growth and observation of natural set relative to the northern Good Luck Point site. The Good Luck Point reef has a long history of restoration efforts and should not be ignored, especially from a community-outreach perspective (Appendix 3). Natural hydrodynamics of the bay suggest a South to North transport (J. Goodwin, unpub. data), so a healthy, spawning population at the Tuckerton reef could contribute larvae to the northern reef.

There are many benefits to be seen from scaling up oyster restoration in Barnegat Bay. Oyster beds can reduce the impacts of eutrophication by controlling phytoplankton blooms, seston filtration, and removal of material to the benthos (Newell et al. 2007, Coen et al. 2007). Although some of these services, such as water filtration and denitrification, may be difficult to quantify given the limited scale and breath of this effort, other benefits such as increased feeding habitat and protection for mobile species and hard substrate habitat for sessile species (Coen et al. 2007), can be quantified via monitoring. Monitoring programs that consider the impacts on the benthic communities have shown that even without dense populations of oysters, shelled areas increase motile crustacean diversity and occurrence (Hadley et al. 2010), and finfish are also often shown to have higher abundance and species richness in areas of structured oyster habitat (Coen et al. 2007). Whelk shell, which provides greater relief than oyster or clam shell (Figure 17), may also encourage natural recruitment of oysters and hard clams by providing a better hydrodynamic environment for larval settlement (Whitman and Reidenbach 2012). In addition, the outreach component of any of these projects will increase public education of bay water-quality conditions and increase stewardship.

From a habitat value perspective, there is benefit and potential cost savings to transplanting oysters, however, due to disease pressure, survivorship beyond year one post-transplant will be very low.

There may be additional concerns to using significant numbers of wild seed transplant in terms of increasing disease in the restoration waters. A literature search and consultation with restoration managers from other regions is suggested. If wild seed transplants are considered for this area, they may be best suited toward transplant to harvest programs that could allow limited public harvest within one year of transplanting, possibly as a spatially separated site (e.g. transplant 1000 bushels of wild seed in April, allow 500 bushels to be harvest in December of the same year).

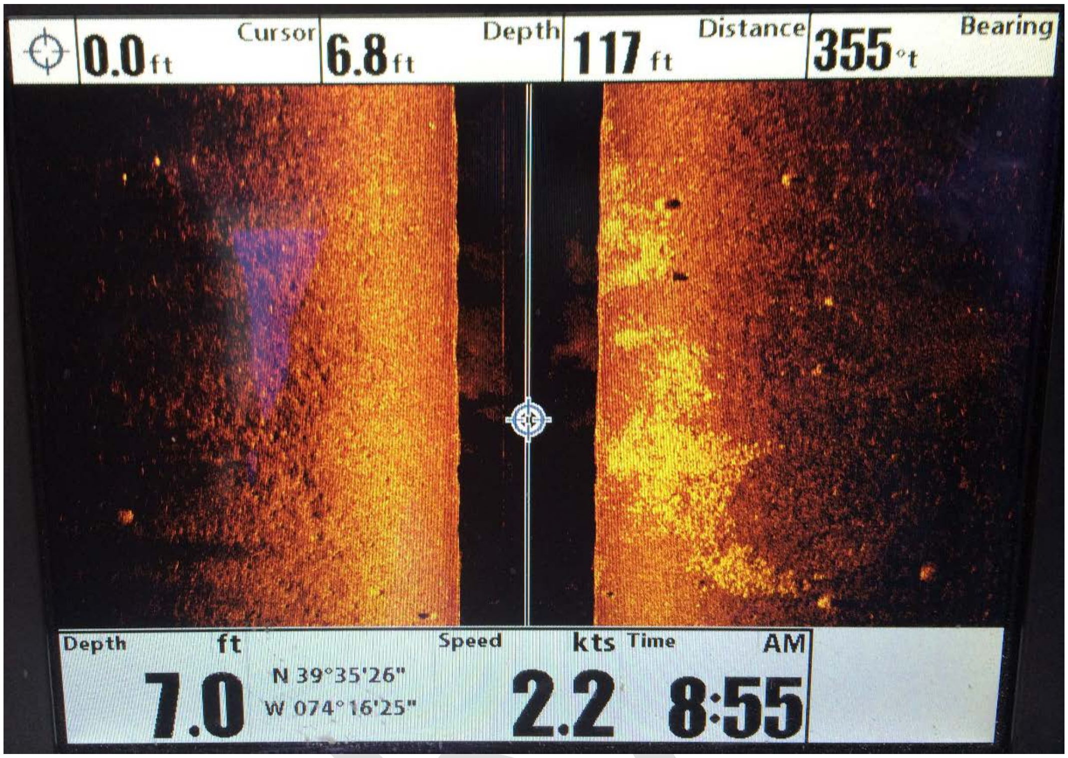

Figure 17. Side scan sonar image showing the clear relief provided by the use of whelk shell as the cultch material.

It was determined that a large portion of the post-deployment mortality was due to handling of oysters during the remote set transplant. Efforts to minimize damage to spat on shells could increase survivorship. There would still be a large portion that would die off depending on how the shell falls onto the substrate, but this would also be expected for transplanted wild seed oysters. Setting fewer larvae may result in less spatial competition and may reduce year one mortality of SOWS. Future SOWS investments at these sites should consider sampling methodology to capture this. There are many factors that can go into optimizing planting of remote sets and mixing in (spat on) oyster shell with whelk shell may be advantageous in terms of habitat heterogeneity.

Sampling methods did not allow calculation of an accurate spatial density of live oysters. This has been listed as an important metric to quantifying restoration reef success (Baggett et al. 2014). Both hand-tongs and dredges, although used widely in fisheries surveys, are not capable of providing accurate estimates of oyster abundance per area due to capture efficiency and unknown sample area (Chai et al. 1992). Moving forward, spatial data would allow us to better quantify the footprint of the reef, as well as the relationship between oyster density and a "successful reef." Current restoration reef monitoring protocols emphasize the importance of oyster density as a fundamental parameter for achieving restoration goals (Baggett et al. 2015) with a target density of 15 oysters/m^2 with an average biomass of

15 g dry weight/m^2 to achieve a successful reef (Allen et al. 2011). Replacing hand tongs with patent-tongs, or using diver surveys, would enable researchers to calculate oyster density in the reef. Moving forward, we suggest that restoration site monitoring in Barnegat Bay follow the Universal Metrics for oyster reef and water quality as suggested by the Oyster Restoration Monitoring and Assessment Working Group (Baggett et al. 2014, Baggett et al. 2015).

There are other aspects of ecosystem services or enhancement that were not done in this study. Estimates of denitrification could indicate potential for water quality improvements, especially if scaling up these efforts is considered. A longer-term fish-usage study would also better indicate any habitat enhancement, especially for managed species. Continued annual investment of live oysters via either method will provide longer term data while increasing the many benefits of a developing reef to these areas. Continued monitoring of the 2016, 17 and 18 cohorts at the TKR site is recommended and should include survivorship, growth, disease prevalence, natural set and ecosystem services.

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Thank You

This effort was made possible with various support of the shellfish industry and science community. Foremost appreciation goes to the Barnegat Bay Partnership for funding this project through their 2015 Shellfish Research program.

Rutgers Aquaculture Innovation Center and Haskins Shellfish Research Laboratory provided technical guidance, eyed larvae and other support, including matching contributions on larvae and histopathology testing. The NJ Bureau of Shellfisheries and the Atlantic Coast Section of the Shellfish council provided general support and permitting. Maxwell Shellfish provided matching contributions to the wild seed purchase and guidance on planting methods and locally observed trends form over 50 years in the industry. Monmouth University and the Town of Ocean Gate assisted ALS with field work and dockside needs. The Shady Rest and Old Causeway restaurants helped get recycling efforts off the ground and hosted events.

In closing, the Public is getting behind oyster restoration in Barnegat Bay through a collision of many interests and projects, including but not limited to increases in coastal aquaculture practices, the Oyster Farmers documentary and the Oyster Recycling Program (https://jettylife.com/pages/jetty-oyster-recycling-program). The encouraging results have led to further financial investments by the Jetty Rock Foundation and Long Beach Township, significantly leveraging this initial BBP investment and providing us an opportunity to continue long-term data collection to demonstrate the ecological and economic benefits of scaling up restoration in these waters. In 2018 another 300 bushels of spat on shell will go to the Tuckerton Reef site through the support of these partners. We thank everyone behind these goals for Barnegat Bay for their continued support of this project.

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Appendix 1
Histopathology Reports

RUTGERS
School of Environmental and Biological Sciences

Haskin Shellfish Research Laboratory
Department of Marine and Coastal Sciences
New Jersey Agricultural Experiment Station
Rutgers, The State University of New Jersey
6959 Miller Avenue
Port Norris, NJ 08349-3167
hsrl.rutgers.edu
856-785-0074
Fax: 856-785-1544

Histopathology Report

Date: January 27, 2017
Location: Mullica River, NJ
Broodstock: native oysters (*Crassostrea virginica*)

Sample	Sample number	Shell length (mm)	Number examined	Date collected	Date fixed / cultured
Lease 209 (209)	2016-101	70.22 ± 7.93	20	12/7/16	12/9/16

Diagnostic Procedures: All oysters were measured and shucked. A portion of the mantle and the rectum from each oyster were incubated in Ray's Fluid Thioglycollate Medium (RFTM) for 5 days at which time the tissues were stained and examined microscopically for evidence of *Perkinsus* species. A section of the remaining tissues containing visceral mass, mantle, and gill was fixed in Davidson's fixative and processed into a tissue slide for each individual. The slides were stained and examined microscopically for evidence of *Haplosporidian nelsoni* (MSX) infection and other parasites or pathological conditions.

Tissue-section examination results:

Nematopsis sp., *Sphenophrya* sp., and *Bucephalus* were observed at 100%, 25%, and 5% prevalence, respectively. Ciliates (*Nematopsis* sp. and *Sphenophrya* sp.) are commonly found in bivalves and are generally not considered harmful to the host. *Bucephalus* is a trematode that produces sporocysts in oyster tissues, ultimately castrating the individual. MSX was not detected in this sample.

RFTM examination results:

	Perkinsus infection levels[1]							Prevalence			Avg intensity of
Site	0	0.5	1	2	3	4	5	Total	Advanced	Weighted[3]	infected indiv.[2]
209	11	4	2	1	1	1	0	45.0%	10.0%	0.65	1.44

Perkinsus spp. infections were in 45% of the sample, with 10% advanced infections.

Emily S. McGurk

1/27/17

Emily S. McGurk
Histopathology Laboratory Technician

Date

[1]*Perkinsus* infection levels are scored on the Mackin scale, 0-5.

0.5 = Less than 20 *Perkinsus* cells present

1 = 21 - 100 *Perkinsus* cells present

2 = *Perkinsus* cells make up 25% of tissue examined

3 = *Perkinsus* cells make up 50% of tissue examined

4 = *Perkinsus* cells make up 75% of tissue examined

5 = *Perkinsus* cells make up 100% of tissue examined

[2]The calculated average of the intensity of infections in only those individuals with *Perkinsus* infections detected.

RUTGERS
School of Environmental
and Biological Sciences

Haskin Shellfish Research Laboratory hsrl.rutgers.edu
Department of Marine and Coastal Sciences
New Jersey Agricultural Experiment Station
Rutgers, The State University of New Jersey
6959 Miller Avenue 856-785-0074
Port Norris, NJ 08349-3167 Fax: 856-785-1544

Histopathology Report

Date: November 28, 2017
Client: Steven Evert, Stockton
Location: Barnegat Bay
Broodstock: Eastern oyster, *Crassostrea virginica* - natives transplanted from Mullica River and NEH remote set

Sample	Sample number	Site	Source	Shell length range (mm)	Number examined	Date collected	Date fixed / cultured
TuckMR	2017-94	Tuckerton	Mullica R transplant	83.49 ± 9.26	20	10/14/17	10/19/17
TuckNEH	2017-95	Tuckerton	NEH remote set	86.27 ± 13.79	20	10/14/17	10/19/17
GLMR	2017-102	Good Luck Pt	Mullica R transplant	60.95 ± 9.14	20	10/23/17	10/26/17
GLNEH	2017-103	Good Luck Pt	NEH remote set	68.04 ± 5.91	20	10/23/17	10/26/17

Diagnostic Procedures: All oysters were shucked. A portion of the mantle and the rectum from each oyster were incubated in Ray's Fluid Thioglycollate Medium (RFTM) for 7 days at which time the tissues were stained and examined microscopically for evidence of *Perkinsus* species. A section of the remaining tissues containing visceral mass, mantle, and gill was fixed in Davidson's fixative and processed into a tissue slide for each individual. The slides were stained and examined microscopically for evidence of *Haplosporidian nelsoni* (MSX) infection and other parasites or pathological conditions.

Tissue-section examination results:

Sample	Inf. intensity range[1]	MSX infection rating[2]					Prevalence			
		0	1	2	3	4	Total	Systemi	Advanced	Weighted[3]
TuckMR	none	20	0	0	0	0	0.0%	0.0%	0.0%	0.00
TuckNEH	none - v light	17	2	1	0	0	15.0%	0.0%	0.0%	0.20
GLMR	none	20	0	0	0	0	0.0%	0.0%	0.0%	0.00
GLNEH	none	20	0	0	0	0	0.0%	0.0%	0.0%	0.00

MSX was detected in the NEH oysters from the Tuckerton site. No systemic or advanced infections were observed.

Sample	Prevalence			
	gill ciliates	*Nematopsis*	*Bucephalus*	RLO/CLO
TuckMR	0.15	0.75	0	0
TuckNEH	0.45	0	0	0
GLMR	0.40	1.00	0.05	0.10
GLNEH	0.55	0	0	0

Sphenophrya-like gill ciliates were observed in all groups. *Nematopsis* sp. were observed in the Mullica River transplants at both sites. *Bucephalus* and *Rickettsia/Chlamydia*-like organisms (RLO/CLO) were observed in the Mullica River transplants at Good Luck Point. Gill ciliates, *Nematopsis* sp., and RLO/CLO are commonly observed in oysters and not generally associated with disease or mortality. Sporocysts of the trematode *Bucephalus* will castrate an individual oyster, and are not uncommon. No other parasites or pathological conditions were observed.

RFTM examination results:

Sample	*Perkinsus* infection levels[4] 0	0.5	1	2	3	4	5	Prevalence Total	Advanced	Weighted[3]	Avg intensity of infected indiv.[5]
TuckMR	1	0	0	4	3	1	11	95.0%	75.0%	0.15	4.00
TuckNEH	19	0	1	0	0	0	0	5.0%	0.0%	0.00	1.00
GLMR	2	1	3	9	4	1	0	90.0%	25.0%	0.22	2.08
GLNEH	18	1	1	0	0	0	0	10.0%	0.0%	0.02	0.75

Perkinsus spp. infections were detected at low prevalence in the NEH samples and at high prevalence in the transplanted samples from both sites. Advanced infections were only observed in the Mullica River transplant samples.

Emily S. McGurk

11/28/17

Emily S. McGurk
Histopathology Laboratory Technician

Date

[1]Infection intensity range describes the number of pathogen cells present.
Rare = less than 10 MSX plasmodia
Very light = 10-100 plasmodia
Moderate = 1-5 plasmodia per field of 100X view
Heavy = more than 5 plasmodia per field of 100X view

[2]MSX infection rating describes the disease progression.
1 = Rare infection
2 = Epithelial infection
3 = Systemic infection
4 = Advanced infection

[3]The weighted prevalence is essentially an average infection intensity of the entire sample, including those with no detectable parasites or pathogens.

[4]*Perkinsus* infection levels are scored on the Mackin scale, 0-5.
0.5 = Less than 20 *Perkinsus* cells present
1 = 21 - 100 *Perkinsus* cells present
2 = *Perkinsus* cells make up 25% of tissue examined
3 = *Perkinsus* cells make up 50% of tissue examined
4 = *Perkinsus* cells make up 75% of tissue examined
5 = *Perkinsus* cells make up 100% of tissue examined

[5]The calculated average of the intensity of infections in only those individuals with *Perkinsus* infections detected.

Appendix 2
Species List

Appendix 2. Table 1. Species list for organisms found during all monitoring events. Organisms are grouped based on sampling category (Motile, Encrusting, Fish Traps) and by Taxonomic category. An ‘x’ is present in a column if that organism was found at either Tuckerton or Good Luck Point and in SOWS or MRT samples.

Motile Species					
		Tuckerton	*Good Luck Point*	*SOWS*	*MRT*
Ctenophora					
Comb jelly	*Mnemiopsis leidyi*		*x*	*x*	
Nemertea					
Ribbon worm	*Cerebratulus lacteus*	*x*		*x*	
Mollusca - Bivalvia					
Ribbed mussel	*Geukensia demissa*		*x*	*x*	*x*
Blue mussel	*Mytilus edulis*	*x*		*x*	*x*
Hard clam	*Mercenaria mercenaria*		*x*	*x*	
Macoma clam	*Macoma spp.*	*x*		*x*	
Dwarf surf clam	*Mulinia lateralis*		*x*	*x*	
Stout razor clam	*Tagelus plebeius*	*x*	*x*	*x*	
Blood arc	*Anadara ovalis*	*x*			*x*
Mollusca - Gastropoda					
Eastern mudsnail	*Ilyanassa obsoleta*	*x*	*x*	*x*	*x*
Threeline mudsnail	*Ilyanassa trivittata*	*x*		*x*	
Oyster drill	*Urosalpinx cinerea*	*x*		*x*	*x*
Knobbed whelk	*Busycon carica*	*x*			*x*
Channeled whelk	*Busycotypus canaliculatus*	*x*		*x*	*x*
Lemon drop nudibranch	*Doriopsilla pharpa*	*x*			*x*
Annelida - Errant polychaetes					
Red line worm	*Nephtys spp.*		*x*	*x*	*x*
Clam worm	*Nereis spp.*	*x*	*x*	*x*	*x*
Scale worm	*Lepidonotus spp.*	*x*			*x*
Blood worm	*Glycera spp.*	*x*	*x*	*x*	*x*
Arthropoda - Chelicerata					
Horseshoe crab	*Limulus polyphemus*	*x*		*x*	
Arthropoda - Decapod crustaceans					
Blue crab	*Callinectes sapidus*	*x*	*x*	*x*	*x*

Rock crab	*Cancer irroratus*	*x*		*x*	*x*
Spider crab	*Libinia emarginata*	*x*		*x*	*x*
Long clawed hermit crab	*Pagurus longicarpus*	*x*		*x*	*x*
Black fingered mud crab	*Panopeus herbstii*	*x*	*x*	*x*	*x*
White fingered mud crab	*Rhithropanopeus harrisii*				
Shore shrimp	*Palaemonetes spp*	*x*		*x*	*x*
Arthropoda - Amphipoda					
Gammarid amphipod	*Gammarus*	*x*	*x*	*x*	*x*
Arthropoda - Isopoda					
Isopod		*x*			*x*
Chordata - Urochordata					
Sea grape	*Molgula spp.*	*x*	*x*		*x*
Chordata - Vertebrata					
Skillet Fish	*Gobiosox strumosa*	*x*	*x*	*x*	
Seaboard goby	*Gobiosoma ginsburgi*		*x*	*x*	
Naked goby	*Gobiosoma bosci*	*x*	*x*	*x*	*x*
Oyster toadfish	*Opsanus tau*	*x*	*x*	*x*	
Striped blenny	*Chasmodus bosquianus*	*x*	*x*	*x*	
Tautog	*Taugoa onitis*		*x*	*x*	
Encrusting					
Porifera					
Red Beard Sponge	*Microciona prolifera*	*x*	*x*		
Boring sponge	*Cliona spp.*	*x*	*x*		
Breadcrumb sponge	*Halichondria spp.*	*x*			
Finger sponge	*Haliclona spp.*	*x*			
Cnidaria - Anthozoa					
Striped anemone	*Haliplanella luciae*		*x*		
Ghost anemone	*Diadumene leucolena*		*x*		
Bryozoa					
Lacy bryozoan	*Membranipora spp.*	*x*	*x*		
Brushy bryozoan	*Bugula spp.*	*x*	*x*		

Mollusca - Bivaliva					
Jingle clam	*Anomia simplex*	*x*			
Mollusca - Gastropoda					
Brown slipper limpet	*Crepidula convexa*	*x*	*x*		
White limpet	*Crepidula plana*	*x*	*x*		
Annelida - Sedentary Polychaetes					
Hard tube worm	*Hydroides dianthus*	*x*	*x*		
Arthropoda					
Acron barnacle	*Semibalanus balanoides*	*x*	*x*		
Chordata - Urochordata					
Star tunicate	*Botryllus schlosseri*		*x*		
Orange tunicate	*Aplidium spp.*		*x*		
White crust tunicate	*Didemnum candidum*	*x*			
Fish Traps					
Arthropoda - Decapod crustaceans					
Blue crab	*Callinectes sapidus*	*x*	*x*	*x*	*x*
Green crab	*Carcinus maenas*	*x*			*x*
Spider crab	*Libinia emarginata*	*x*		*x*	*x*
Flat clawed hermit crab	*Pagurus pollicaris*	*x*			
Long clawed hermit crab	*Pagurus longicarpus*				
Shore shrimp	*Palaemonetes spp*		*x*		
Chordata - Vertebrata					
Tautog	*Taugoa onitis*	*x*		*x*	*x*
Silver perch	*Bairdiella chrysoura*	*x*		*x*	
Oyster toadfish	*Opsanus tau*	*x*	*x*	*x*	
Black Sea Bass	*Centropristis striata*	*x*	*x*	*x*	*x*
American eel	*Anguilla rostrata*	*x*			
Spotted hake	*Urophycis regia*		*x*		

Appendix 3
Outreach Activities

Outreach Summary

The American Littoral Society (ALS) was subcontracted to provide the outreach efforts related to the overall project, as well as to oversee the planting and sampling activities at the northern Good Luck Point site.   ALS was successful at planning and implementing several outreach events, develop a unique partnership with Jenkinson's Aquarium and installing a large exhibit summarizing previous restoration work and work being conducted for this grant. ALS Habitat Restoration Scientist Al Modjeski presented on current grant work at various venues throughout the study period.

For outreach events, the American Littoral Society conducted two (2) coinciding Spat Tank events on July 10 and 12, 2017. The July 10th event was held at Wildwood Avenue Pier in Ocean Gate, New Jersey where we released several hundred thousand larvae (paid for by Jenkinson's Aquarium) into our existing spat tank. Approximately 20 to 30 people attended and were able to see first-hand oyster larvae under a microscope, learn about the work we are doing funded under this grant, tour the R/V Seahawk, and help add larvae to the tank. During this event, a volunteer from the Society was at the Jenkinson's Aquarium "Operation Oyster" exhibit we designed engaging visitors and answering question about the project.

The July 12th event was held at 1400 at Jenkinson's Aquarium. Over 100 people attended the event where we were able to prepare an aquarium tank with shell and have children in the audience help add larvae to the tank. The tank was one component of the exhibit and showed visitors what the restored reefs we have been monitoring look like. Entitled "Operation Oyster: Barnegat Bay", the exhibit also contained a large 8-foot table with shell, a fish trap we use for monitoring, and a number of brochures describing the importance of the project and the ecosystem services oysters can provide. Additionally, 3 heavily branded, large informational posters were attached to the wall that summarized the project and data collected to date. Presenters at the event included Stockton University, the American Littoral Society, and Jenkinson's Aquarium staff. To date, we have had to restock educational literature twice. The exhibit will be ongoing beyond this grant. We were also able to add small native fish species collected during our monitoring to show the diversity and various fish assemblages that may inhabit an oyster reef in Barnegat Bay.

The aquarium also funded printing of educational materials and provided brochures with a "buy one get one free admission" incentive on the day of the event and through October 31st if the word "spat" was said at the admission desk. Brochures were handed out prior at the Ocean Gate Beach and at the spat tank event as well as to visitors at the Society's historic office building. It is estimated that 10s of thousands of visitors have seen the exhibit to date. The exhibit is located on the 2nd floor of the aquarium across from the seal tank so it gets quite a bit of exposure.

On July 27th, we held a "Parade of Boats" at Wildwood Avenue Pier in Ocean Gate as part of our continued outreach for this grant where we took the spat on shell from our spat tank to the Good Luck Point Reef site. The event was attended by over 12 boats and an estimated 85 people. We were able to have the Ocean Gate Yacht Club youth sailing camp attend as well (approximately 40 youth). The young mariners arrived in zodiacs and small motor craft with their counselors, toured the R/V Seahawk, learned about the importance of oysters and of Barnegat Bay, and then helped load spat on shell that was then taken and placed on the reef site. We have been in contact with principle counselor and the camp is eager to attend the event next year.

We were able to present this project at the Restore America's Estuaries National Summit in December 2016. Travel and lodging costs were provided under other grants. Two presentations were completed, one describing the restoration work at Good Luck Point in general and the other summarizing data and results to date.

On April 4, 2017 as part of a larger presentation, ALS presented the Barnegat Bay project to the NJDEP Ecological Project Committee. We presented to the Rutgers Cooperative and ReClam the Bay in Toms River on the 6th of June and we were able to reengage the restaurants with shell recycling outreach materials and pickups throughout the summer. We were unable to schedule a lunch and learn but were able to reach more people through the innovative partnership with Jenkinson's Aquarium.

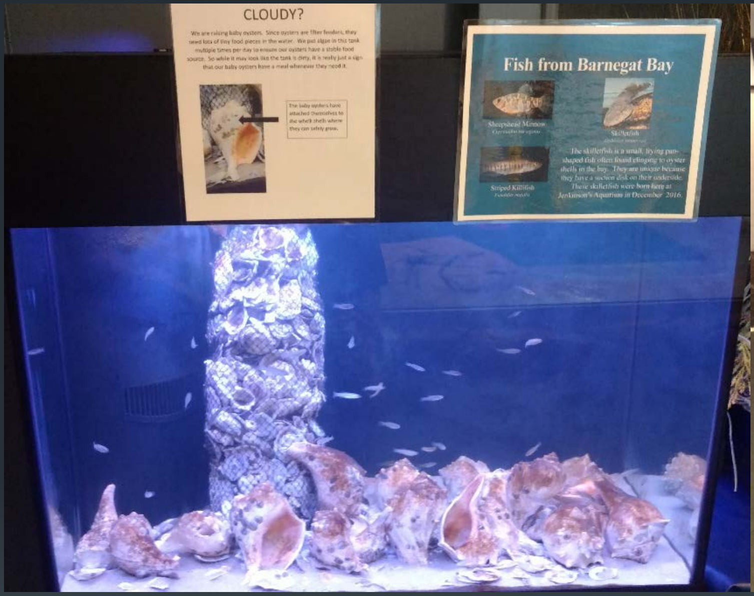
Outreach

Ocean Gate spat set and shell planting outreach event – July 2016 and 2017 with BBP, OGYC, Stockton, ReClam, and Jenkinson's Aquarium

Photo credit S. Am. Lit. Soc.

Outreach

Jenkinson's "Operation Oyster" Exhibit and Spat Set – July 2017 to Current

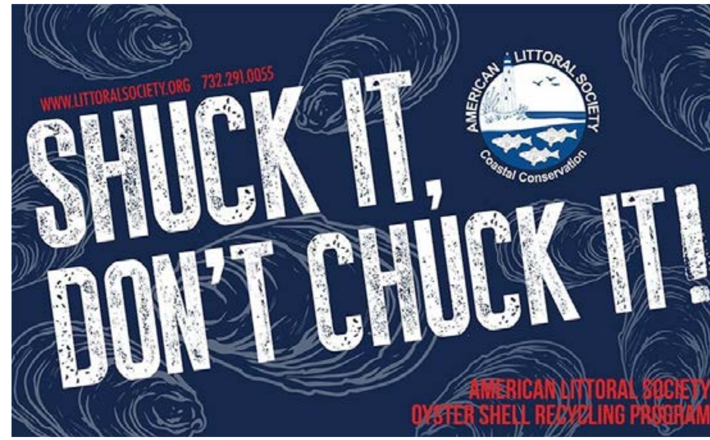


10s of thousands of people reached at Aquarium – "Just say Spat!"

Photo credit S. Am. Lit. Soc.

Outreach - Recycling


Photo credit S. Evert



- ❖ Table-top information available to diners
- ❖ Project posters and materials at the “Rose Garden”, Shady Rest Restaurant, Anchor Inn in Ocean Gate, and Jenkinson’s Aquarium
- ❖ Shell collected used as part of “Operation Oyster” (Separate funding)

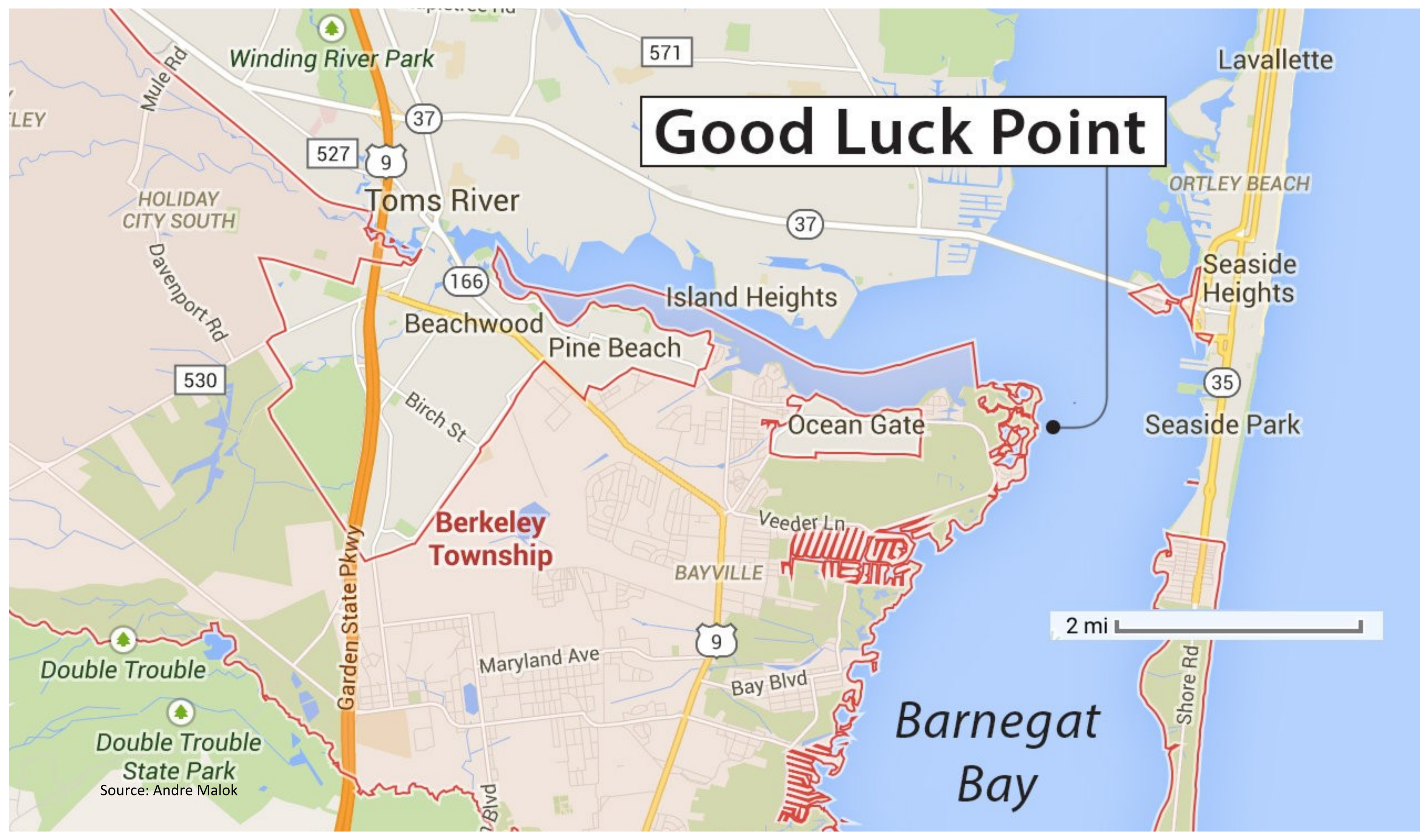
Photo credit S. Ever and ALS

Operation Oyster: Barnegat Bay Reef Restoration Project

Barnegat Bay once held 12,000 acres of oyster reefs, which helped keep the bay's waters clean and clear.

Due to over-harvesting, disease, pollution, natural predators and over-development, most of the bay's oyster reefs have disappeared.

Besides cleaning water (one oyster can filter up to 50 gallons of water a day), oyster reefs protect shorelines from storms and provide habitat for other aquatic creatures.



What's Been Done So Far?

In 2015, the American Littoral Society, along with several partners, installed a one-acre reef off Good Luck Point, as part of an effort to demonstrate that the bay can still support oyster reefs.

Each year the Littoral Society adds more shell to the reef. The shell has been seeded with oyster larvae to help quickly increase the number of oysters growing on the reef.

Shell seeding is done in a spat tank in Ocean Gate, NJ. The tank is filled with whelk shell. The shell provides a hard surface to which baby oysters can attach. Oyster larvae that attach to shell are called spat.

In addition to restoring the ecology of the Bay, the Society and its partners are working to help the surrounding community better understand the bay through education, oyster shell recycling, and public activities such as the spat tank event and Parade of Boats. During the Parade of Boats, seeded shell is carried to its new permanent home on the reef.

You too can play a role in Operation Oyster, in Barnegat Bay, the Delaware Bay or New Jersey's Two Rivers area. Contact the American Littoral Society to learn how.

American Littoral Society - 18 Hartshorne Drive, Highlands, NJ 07732
www.LittoralSociety.org

What’s in the BIG BLUE BOX?

Hint: We are making babies!

Monday, July 10, 11:00am

Join us and partners at Wildwood Avenue Pier to find out more!

Wednesday, July 12, 2:30pm

Jenkinson’s Aquarium in Point Pleasant

Visit our exhibition on the 2nd floor!

Say **SPAT** when you arrive and receive 1 FREE admission with every ticket you purchase.

Bring Your Friends. Bring Your Family.
Bring Anyone Who Loves Barnegat Bay.

But Most of All
BRING YOUR BOAT

Please join the American Littoral Society

Thursday, July 27 at 11 a.m.
for a Parade of Boats.

Gather at the Wildwood Avenue Pier in Ocean Gate, NJ

Help escort our Oyster Babies to their permanent home off Good Luck Point
Carry some of the precious cargo -- we have many bags of shells seeded with oyster spat. Landlubbers and supportive onlookers also welcome

To Join the Parade e-mail Capt. Al Modjeski at
alek@littoralsociety.org

American Littoral Society Coastal Conservation · Barnegat Bay Partnership Research · Educate · Restore · Stockton University · Jenkinson's Aquarium · MU Monmouth University · Ocean Gate N.J. 1918 · ReClam the Bay RCTB Barnegat Bay, NJ