North Carolina Fishery Resource Grant Project

Final Report for 07-EP-04

Oyster Settlement and Reef Mapping in Pamlico Sound

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Abstract: A large-scale field program mapped the height and spatial extent of oyster mounds within six broodstock sanctuaries in Pamlico Sound, and a large-scale educational outreach program tested settlement hypotheses for oyster in Pamlico Sound. The height of oyster mounds off the seafloor changed in one year from an increase of 0.03 m at Deep Bay to a decrease of 0.37 m at Ocracoke. A significant linear relationship was found between the average bottom depth and the change in mound height, with the greatest reduction in mound height at the deepest depths. Reasons for the decrease in mound height could be sediment accumulation, settling of mound material, and sinking into the bottom. Deeper mounds may be subject to higher sediment accumulation. During storms, it is possible that the tops of some mounds could receive direct wave action which could displace material. In all years sampled, oyster settlement peaked in June and was highest at Hatteras. Water quality parameters were sufficient to support settlement, growth and survival of oysters at al sampling stations, and there was a positive relationship between salinity and settlement. Using 2006 as a baseline, cumulative annual oyster settlement in Pamlico Sound increased 57% in 2007, but decreased 26% (through July) in 2008. Oyster settlement in Pamlico Sound was spatially coherent at small (~10s of km) and large (~100 km) spatial scales, but may be decoupled in the northern and southern portions of Pamlico Sound due to Bluff Shoal. As a result, reserves at intermediate distances from one another may be important for maintaining larval connectivity (i.e., stepping stones) at the metapopulation scale of Pamlico Sound. As expected, settlement in southern and northern Pamlico Sound was most influenced by northeasterly and southwesterly winds, respectively. A longer lag (e.g., 3 wks) between settlement and wind forcing suggests that the wind conditions present when larvae are first spawned (and presumably passive drifters) may determine their final destination. To this end, the prevalent southwesterly wind during peak larval settlement suggests that reserves located in southwestern Pamlico Sound may serve as sources for oyster populations distributed northeastward and, are, thus, important for metapopulation persistence.

Introduction

Populations of eastern oysters (*Crassostrea virginica*) in North Carolina have reached historic lows, and the NC Division of Marine Fisheries (NC DMF) is building new oyster reefs in Pamlico Sound to be used as unfished, broodstock spawning sanctuaries for oyster restoration (Fig. 1). It is not yet known whether the new sanctuaries will support self-sustaining populations, seed other areas in Pamlico Sound, or both. The lack of population data on NC's oyster broodstock sanctuaries is a major limitation to effective implementation of oyster population rebuilding efforts and the Coastal Habitat Protection Plan that was recently passed by the NC legislature. Moreover, the NC DMF is concerned about the degree to which newly created oyster shell and rip-rap mounds may be sinking into the sediment. The overall goal of this study was to provide data that will aid the State of NC in locating oyster broodstock sanctuaries in Pamlico Sound.

Objectives & Hypotheses

The main goals of this study were to: (1) map the height and spatial extent of reefs in six oyster sanctuaries in Pamlico Sound, and (2) quantify spatiotemporal variation in oyster settlement near the six spawning sanctuaries mapped in #1.

We tested two hypotheses associated with Objective #2.
H_1: Mean oyster settlement (# spat mm^{-2}) will vary significantly among sites near sanctuaries and over time, with most of the variation in spatial and temporal variation explained by salinity and water temperature, respectively.

H_2: Spatial coherence in mean oyster settlement between sanctuaries will increase with decreasing distance between sites, with the strongest correlations in settlement at sanctuaries where modeled larval dispersal trajectories overlap (dispersal trajectories generated by Dr. Cynthia Cudaback's research group at NCSU via NC Sea Grant funding).

Methods

1. Oyster Sanctuary Mapping

Using data from project 06-EP-03, oyster mounds originally mapped in 2007 were re-surveyed in 2008. We chose oyster mounds where the north-south and east-west transects conducted in 2007 were laid over the top of the mound. Three to six mounds at each site met this criterion. Surveying was initiated at the Ocracoke sanctuary site on May 14, 2008 and was completed at the West Bay site on June 24, 2008.

Geo-referenced depth profiles of the mounds were created by running ArcPad on an IPAC Pocket PC, outfitted with a differential GPS receiver, concurrently with a Lowrance color depth sounder (0.1 foot accuracy). Boat position data provided to the ArcPad software by the GPS allowed for navigation on an overlay of nautical charts, side-scan sonar images, or other shapefiles imported from ArcView. Digital pictures of the Pocket PC and the depth sounder were taken as the boat progressed along each transect (Fig. 2). These pictures were then imported into ArcView where the position information was transferred to ArcView manually. The depth information was extracted from the depth sounder by digitizing the depth line then a projective transformation and reference points were used to add points to a depth shapefile. ArcView scripts were used to select reference points on the pictures of the sonar screen and using a spreadsheet and plane projective transformation, the digitized depth sounder values were transferred to a sequence of depth points and values of northing and easting in the UTM coordinate system. The UTM values were converted to geographic positions in decimal degrees and the average of the projected depth readings given for northing and easting used for the final corresponding depth value.

The software script HYDRO_FT allowed interactive insertion in GIS ArcView of the intersection points of the 2008 transects with the 2007 transects. A histogram was then created. The next step was to graph these depth values in feet vs. distance traveled along the north-south and east-west transects. Reef height change was calculated by:

1. A *delta* value, which was computed as the difference in the 2008 intersection depth and the average of the depths of the 2007 points nearest to it in the ArcView graph (Fig. 3)

2. Comparing depth readings obtained in 2007 and 2008 on the relatively flat areas off the riprap mounds and calculating a shift factor called *offset*, after selection of 2008 bottom points on the graph.
3. *delta_mean* - *offset_best,* which was the change in measured mound height in feet from 2007 to 2008.

Comparison of the 2007 and 2008 depth histograms with 0.1 foot bin size, in many cases, confirmed the choice of bottom points from the intersection graphs, since sometimes there were few intersection points on flat bottom areas around the mounds. Calculation of the offset in measured depths at the various sites from 2007 to 2008 was done using the method of least squares. *Offset_ best* was usually the histogram value, unless the bottom had roughness around the mound and there was no prominent peak in the histogram. Regression analysis was performed using Graphpad InStat version 3.00 for Windows 95, Graphpad Software, San Diego California USA.

2. Spatiotemporal Variation in Oyster Settlement

Oyster spat settlement was measured during the reproductive season (May through October) at seven locations in 2006 and nine locations in 2007 and 2008. The sites were located near six oyster sanctuaries around the perimeter of Pamlico Sound (PS) (Fig. 1). The sampling sites were: Wanchese at the northern end of PS, Engelhard, Swan Quarter, and Hoboken on the western side of PS, Oriental, Turnagain Bay and Cedar Island to the south, and Ocracoke and Hatteras on the eastern side of PS. Turnagain Bay and Swan Quarter were sampled only in 2007and 2008. The Turnagain Bay shell strings were placed at the sanctuary while the other strings were located off docks near sanctuary sites. Turnagain Bay was sampled by the NC DMF (S. Slade), whereas all other sites were sampled by local high school students.

Shell-strings for collecting oyster spat were constructed by stringing 12 clean oyster shells face down on a wire. At each site, 3 strings were suspended off a dock with the bottom of the shell-string hanging just above the sediment. Shell-strings were allowed to collect spat for one week, after which they were retrieved and replaced with clean shell-strings by students from secondary schools in coastal NC counties. The 10 inner-most shells from each shell-string were removed from the string and placed in containers containing a preservative (ethanol) and then transported to the lab at NC State University's Center for Marine Sciences and Technology in Morehead City where the spat were counted and measured using a stereo microscope. Laboratory processing of shell-strings and data analyses has been completed, as of this report, for the 2006, 2007 data, and May-July 2008. We are completing processing of 2008 and 2009 shell-string samples via funding from NC Sea Grant.

During the weekly retrievals of shell-strings, students measured environmental conditions that may be important for successful oyster settlement and growth. Water temperature and dissolved oxygen were measured with a YSI 55 instrument and salinity with a refractometer. At each site, three replicate 200 ml water samples were each filtered onto a glass fiber filter by low-vacuum filtration for chlorophyll *a* analysis. Chlorophyll *a*, the green pigment in algae and other plants, might be an indicator of food (phytoplankton) availability for larval oysters. Chlorophyll *a* measurements can also be used as an indication of water quality since increased nutrient input will cause phytoplankton blooms. The chlorophyll *a* was extracted from the phytoplankton captured on the filter with 90% acetone. Acetone dissolved the algal cell walls and released the chlorophyll pigment into the liquid. The resulting chlorophyll extract was analyzed by a fluorometer. Blue light beamed through the sample caused the algal pigments in the extract to emit red light of specific wave lengths. These wave lengths were measured by the fluorometer, and the resulting measurement was used to calculate the concentration of chlorophyll *a* (and therefore how much phytoplankton).

The average number of spat per shell per day, which was the primary response variable, was calculated by dividing the total number of spat observed on a shell-string by the number of shells (10) examined and then by the number of days a given shell-string "soaked" in the water (usually 7 days). Preliminary cross correlation analyses using autoregressive moving average (ARIMA) models were conducted to determine the degree of spatial coherence in oyster settlement among the settlement stations. The residuals from ARIMA models were cross correlated with weekly wind components decomposed into principal axes of variation to determine wind forcing effects on settlement. Patterns of spat settlement in space, time and relationships with environmental parameters and the prevailing winds were analyzed using ANOVA and regression models.

Results

1. **Oyster Sanctuary Mapping**

Transects over the selected mounds for 2007 and 2008 are shown for each sanctuary in Appendix I. Table 1 summarizes the data collected for 2007 and 2008. Mound height changed from an increase of 0.03 m at Deep Bay to a decrease of 0.37 m at Ocracoke. A significant linear relationship was found between the average bottom depth and the change in mound height ($p=0.01$, $r^2=0.842$), with the greatest reduction in mound height at the deepest depths (Fig. 4). Sampling effort was higher at some sites than others but there was no correlation between number of data points and change in mound height ($p=0.12$).

2. **Spatiotemporal Variation in Oyster Settlement**

<u>*Education Outreach*</u>

Over the 3 years of this study, a total of 21 students, 8 teachers, and 5 parents from 6 schools participated at the spat settlement study. The students attended a training session at the beginning of each season that instructed them in the background and protocol of the study, as well as the use of water quality instruments such as the YSI 55, refractometer, and chlorophyll *a* filtering apparatus. In addition, two high school students assisted with the data analysis. NCSU staff and graduate students have provided data and information for science projects and have been available to answer questions throughout this study.

Physical Parameters

Dissolved Oxygen

The average dissolved oxygen (DO) concentrations (Fig. 5, Appendix II) were well above the minimum necessary for oyster survival (3mg/l) at all sites.

Chlorophyll a

The chlorophyll *a* data was highly variable both within a season and among years (Fig. 6, Appendix II). There were higher concentrations of chlorophyll *a* in 2007 than 2006 at some western Pamlico Sound sites (Fig. 6) and very low concentrations at all sites during 2008. There was no statistical relationship between chlorophyll *a* concentrations and spatiotemporal variation in spat settlement (linear regression; al $p > 0.10$).

Salinity

The average salinity at the shell-string sites was also within the 10-30ppt tolerances of oysters (Fig. 7, Appendix II). Salinity at most sites was relatively high during 2007 and 2008 because of drought conditions. Sites along the western shore of PS exhibited the greatest variation in salinity, since they were influenced by river input, ranging from 15-18ppt, which is optimum for oyster growth. The Hatteras and Ocracoke sites were nearest to the ocean inlets, therefore salinity remained high within a season and among years. High salinity has been linked to decreased oyster growth and survival because disease and parasites are more prevalent. The relationship between settlement and salinity was positive and significant ($p=0.008$) in 2006, but not during 2007 (Fig. 8). Thus, we accept **H1** for salinity but not for temperature (see below).

Temperature

The average temperature was similar in magnitude and temporal pattern among most of the shell-string sites during the study. Temperatures at the beginning of the study in May were near or just over 20^{o}C. However, at Oriental, the temperature on May 20 was already well over 20^{o}C (Table 2). Temperature peaked in July and August then fell during the late summer and fall, falling below 20^{o}C in late September or early October (Fig. 9, Appendix II). Oysters are known to spawn at temperatures over 20^{o}C, therefore spawning would be expected to begin sometime in early to mid May and the first settlement event would occur 2 to 3 weeks later. Spawning and settlement would be expected to end in October, which was the case (see below).

Spatiotemporal Patterns in Oyster Settlement

In 2006 and 2007, mean weekly oyster settlement varied significantly by site (2006: $p < 0.0001$; 2007: $p < 0.0001$) and over time (2006: $p < 0.0001$; 2007: $p = 0.03$). For example, during 2006, oyster settlement was protracted from 20 May to 4 November, but peaked from mid June through July with smaller secondary peaks in early September (Fig. 10). In all years sampled, settlement peaked in June and was highest at Hatteras (Figs. 11 & 12). Using 2006 as a baseline, cumulative annual oyster settlement in PS increased 57% in 2007, but decreased 26% (through July) in 2008.

Settlement was spatially coherent among nearly all sites sampled ($R > 0.6$) at lags ranging from -2 to 4 weeks, although it should be noted that settlement at the northern-most site (Wanchese) was only spatially coherent with the site most proximal (Engelhard). In general, spatial coherence decreased with increasing distance between sites as hypothesized (**H2**). Settlement at Engelhard and Wanchese (northern-most sites) was most correlated with southwest winds lagged 1 to 3 weeks ($R > 0.5$). In contrast, settlement at Ocracoke and Hobucken was most correlated with northeast winds lagged 0 to 3 weeks ($R > 0.5$). Settlement at Hatteras, Oriental, and Cedar Island were not significantly correlated with winds along the major (NE and SW) axis.

Discussion

1. Oyster Sanctuary Mapping

The estimates of sinking by oyster mounds in this study were consistent with the sinking observed of an artificial reef barge near the Ocracoke Sanctuary, both in terms of depth transects recorded of it in this study and the trend observed by party boats that frequent the area of the barge (G. Ballance, pers. obs.). Reasons for the decrease in mound height could be sediment accumulation, settling of mound material, and sinking into the bottom. Deeper mounds may be subject to higher sediment accumulation. During storms, it is possible that the tops of some mounds could receive direct wave action which could displace material. Some of the mounds may be older than others and it might be expected that new mounds might go through a period of settlement right after deployment, and then stabilize. Deep Bay was the only site that showed an increase in height which may be due to sediment accumulation.

One year of data suggests that some oyster sanctuary mounds could be loosing as much as 0.3m per year. More data is needed to better determine the sinking rate. Deeper mounds appear to be sinking faster than shallower ones. The mechanisms causing loss of mound height are not known. Given the three-dimensional integrity of oyster habitat is essential to maintain a viable oyster population it would be prudent to investigate this further. Annual documentation of mound heights for old and new materials placed in the oyster sanctuaries can be done using the method in this report.

2. Spatiotemporal Variation in Oyster Settlement

Three years of oyster settlement data showed consistent patterns of natural oyster settlement in Pamlico Sound both in time and space. Spat settlement commences in late May to early June. Temperatures over 20^{o}C that trigger spawning occur about 2-3 weeks before the first settlement is observed. Oysters in the Neuse River may spawn sooner than those in Pamlico Sound because temperature rises sooner in the shallow Neuse Estuary. This spatial variation in spawning appears to produce a small early settlement event at areas adjacent to the mouth of the Neuse River and on the western end of Pamlico Sound. This early event comes about 2 weeks before a series of massive sound-wide settlement events occurring during the first 6 weeks of the settlement season (June 1 to July 15$^{th)}$ resulting in over 60% of the total settlement for the year.

Prevailing winds from the SW and NE appear to facilitate larval delivery to sites on the northern and southeast sides of Pamlico Sound, respectively. Tides have minimal impact in Pamlico Sound unlike other large estuaries such as Chesapeake Bay. Water movement coupled with episodic spawning is likely a major factor underlying settlement variation in time and space, however, successful settlement also depends on additional pre-settlement factors such as local hydrodynamics, predation, and competition for space. Any one or combination of these factors might also contribute to the highly episodic nature of the settlement observed in this study.

Although settlement occurred throughout the summer and early fall, spatial settlement patterns were established during the first 6 weeks of sampling. Higher settlement measured at the northern (Wanchese) site and southeastern sites (Cedar Island, Ocracoke and Hatteras) was likely due to relatively high larval delivery facilitated by prevailing northeast and southwest winds. Constraints on site placement, however, may have biased settlement patterns in 2007 and 2008 at Ocracoke, in which the site was re-located to a relatively small cove in these years which may have had restricted hydrodynamic connection with Pamlico Sound. In 2009, we switched the Ocraocoke sampling station back to where it was located in 2006. .

The results from this study oyster settlement in Pamlico Sound is spatially coherent at small (~10s of km) and large (~100 of km) spatial scales, but may be decoupled in the northern and southern portions of Pamlico Sound due to Bluff Shoal (A. Haase, MS thesis, NC State University). As a result, reserves at intermediate distances from one another may be important for maintaining larval connectivity (i.e., stepping stones) at the metapopulation scale of Pamlico Sound. As expected, settlement in southern and northern Pamlico Sound was most influenced by northeasterly and southwesterly winds, respectively. A longer lag (e.g., 3 wks) between settlement and wind forcing suggests that the wind conditions present when larvae are first spawned (and presumably passive drifters) may determine their final destination. To this end, the prevalent southwesterly wind during peak larval settlement suggests that reserves located in southwestern Pamlico Sound may serve as sources for oyster populations distributed northeastward and, are, thus, important for metapopulation persistence.

Tables and Figures:

Table 1. Summary of 2007 and 2008 data on the height of oyster mounds. ***Delta_Mean*** is the mean of the differences in the 2008 intersection depth and the average of the depths of the 2007 points nearest to it for each mound in the ArcView graph for the given sanctuary. ***Offset_ ArcView*** is the offset computed for 2007-2008 ArcView intersection points. ***Offset_Histogram*** is the result of the least square comparisons of depth histograms. ***Offset_Best*** is usually Offset_histogram, unless it had no definitive value. This occurred when the bottom was not flat enough in the vicinity of where transects were taken around the mound. ***Delta_mean - Offset_best*** is the change in measured mound height in feet from 2007 to 2008.

sanctuary	**crabhole**	**deepbay**	**hatteras**	**ocracoke**	**westbay**	**westbluff**
Average 2007 + 2008 total points	778.0	1977.3	1251.2	721.3	2067.8	1024.5
average bottom depth>>>>	-12.8	-7.9	-11.0	-18.9	-7.2	-12.5
delta_mean - offset_best	**-0.4**	**0.1**	**-0.3**	**-1.2**	**-0.1**	**-0.1**
Average 2007 points	247.0	966.0	443.0	243.8	776.5	243.0
Average 2008 points	531.0	1011.3	808.2	477.5	1291.3	781.5
Average delta count	12.7	36.0	24.6	13.0	47.8	16.5
delta_mean	-1.2	-0.8	-0.8	-2.5	-0.2	0.6
offset_arcview	-0.7	-0.9	-0.4	-1.3	-0.2	0.8
offset_histogram	-0.8	-0.9	-0.5	-1.3	-0.3	0.7
offset_best	-0.8	-0.9	-0.5	-1.3	-0.2	0.7

Table 2. Temperature at each site shell-string sampling site on May 20, 2007 & 08. The data illustrate higher temperature at Oriental than at the other sites. No data was available for Swan Quarter in 2007 and for all sites in 2006.

Site	2007	2008
Wanchese	17.4	20.5
Engelhard	19.5	21.8
Swan Quarter	x	21.1
Hobuckan	21.7	22.9
Oriental	**24.9**	**24.9**
Turnagain Bay	21	22.6
Cedar Island	20.6	21.3
Ocracoke	19.1	21.7
Hatteras	20.3	19.9

Table 3. Total settlement density at the sites during 2007.

Site	Total settlement density
Cedar Island and Hatteras	$50000/m^2$
Wanchese	$20000/m^2$
Turnagain Bay	$7400/m^2$
Ocracoke, Engelhard, Swan Quarter, Hobucken	$1000\text{-}3000/m^2$

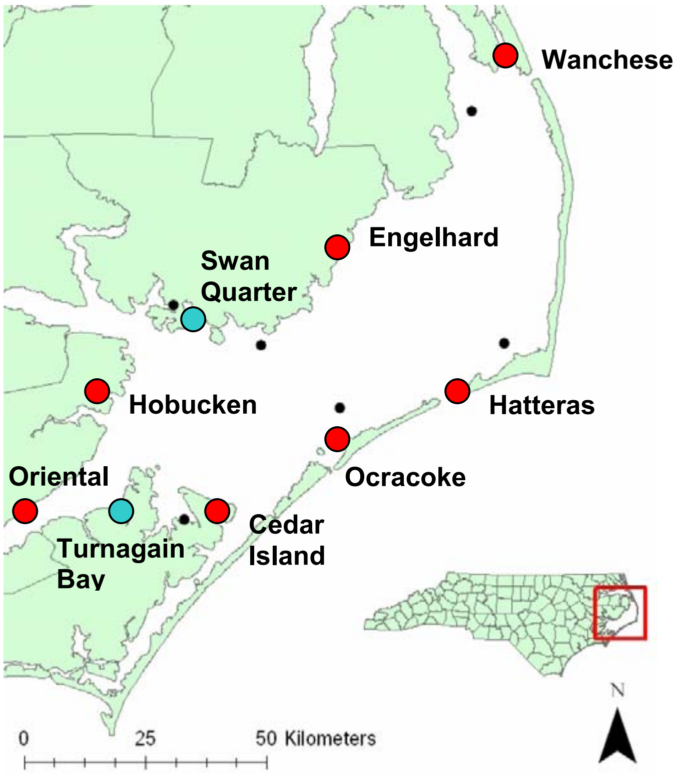


Figure 1. Map of Pamlico Sound showing oyster shell-string sites (large, colored dots) and their proximity to the 6 oyster sanctuaries (small, black dots). 2006 sites (red) were Oriental, Hobucken, Engelhard, Wanchese, Hatteras, Ocracoke, and Cedar Island. Turnagain Bay and Swan Quarter (blue) were added for 2007 & 2008.

Figure 2. Picture taken of the Pocket PC and Lowrance depth sounder output used to provide position and depth information for mapping the height of oyster mounds. Position information that was manually extracted is circled on the Pocket PC screen. Reference points along the digitized depth contour were related to position using an ArcView script.

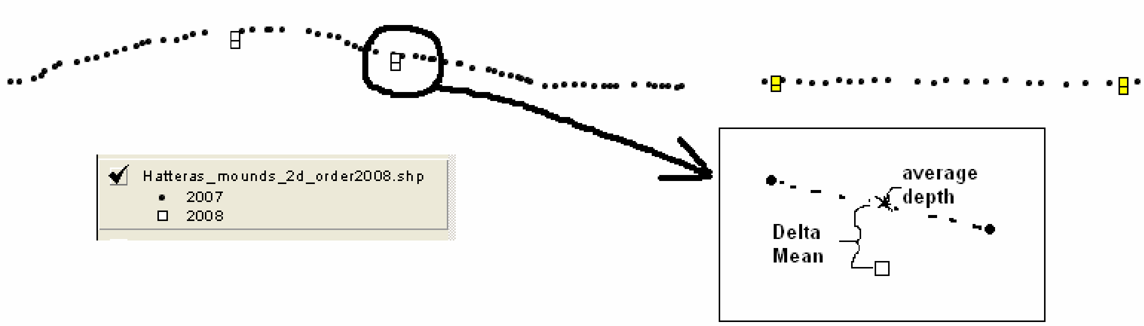


Figure 3. Depth profile of Hatteras mound in 2d. A ***delta*** value is the computed relative (vs. absolute) difference in the 2008 intersection depth and the average of the depths of the 2007 transect alongside it.

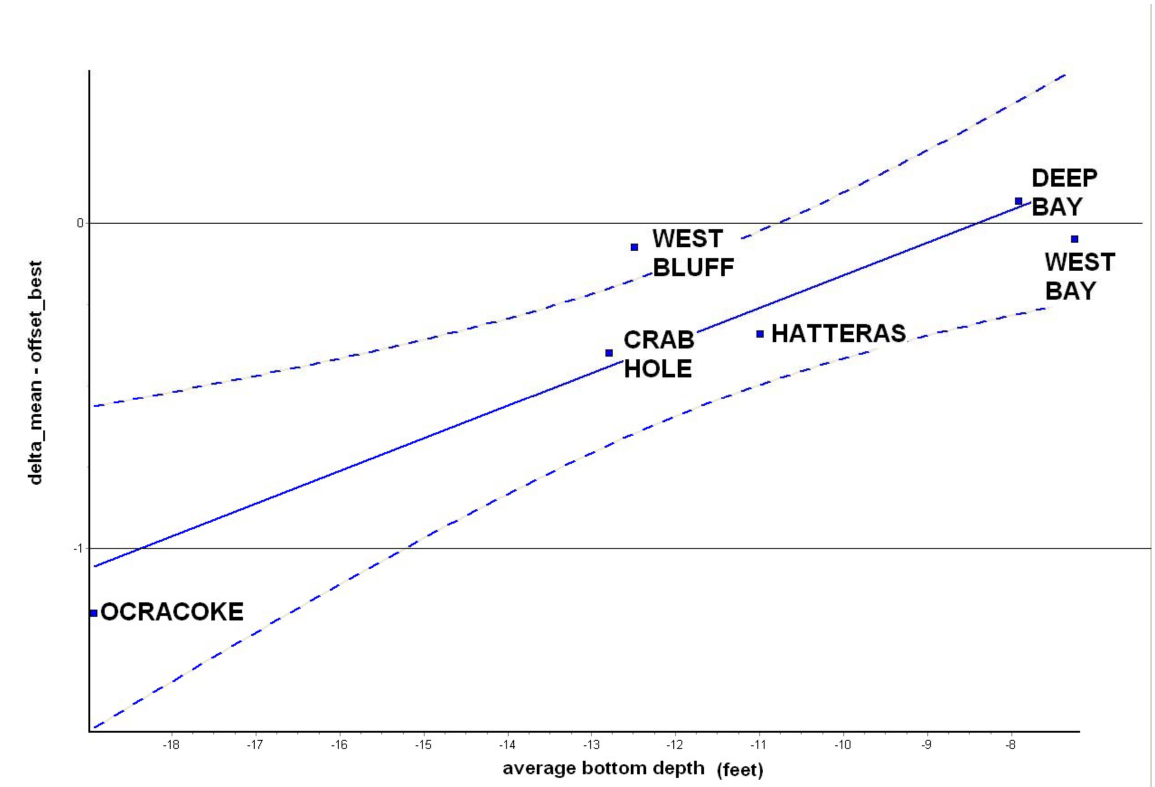


Figure 4: Relationship between average bottom depth and change in mound height at the 6 study oyster sanctuaries. P=0.01, r^2=0.842

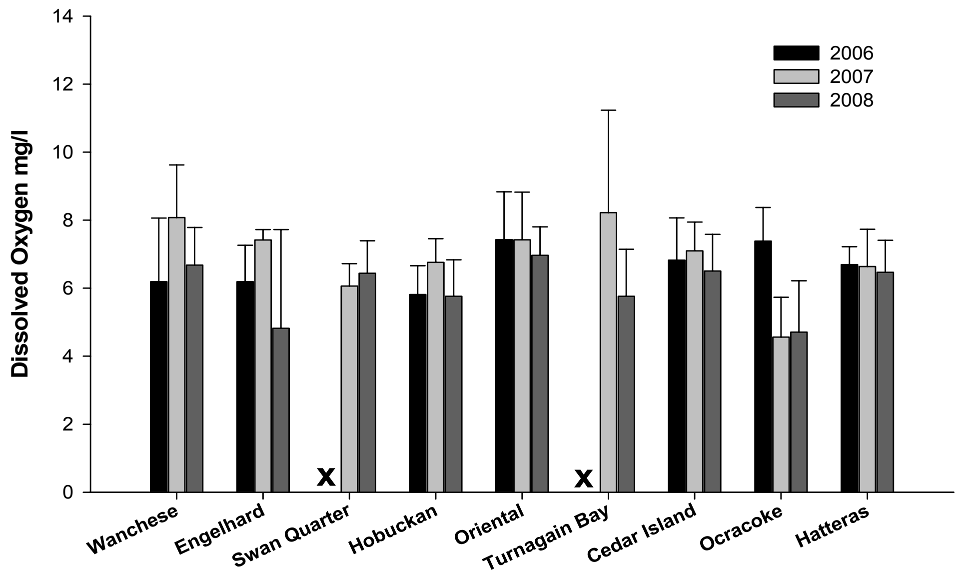


Figure 5. Average dissolved oxygen concentrations at each of the sites during 2006, 2007, and 2008. Error bars are 1 standard deviation and X indicates no data.

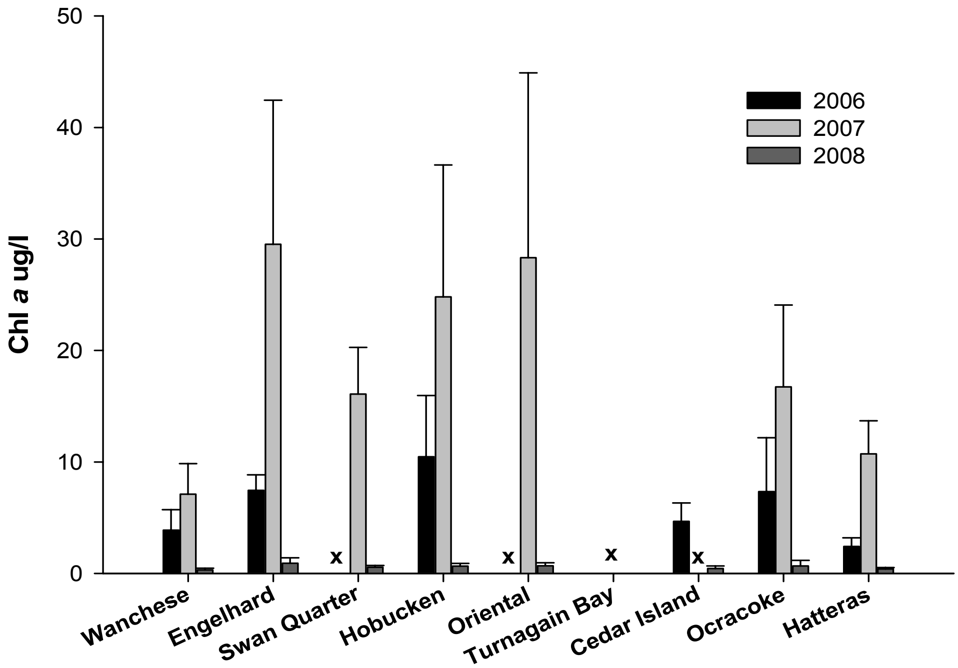


Figure 6. Average chlorophyll *a* concentrations at each of the shell-string sampling sites during 2006, 2007, and 2008. Error bars are 1 standard deviation and X indicates no data available.

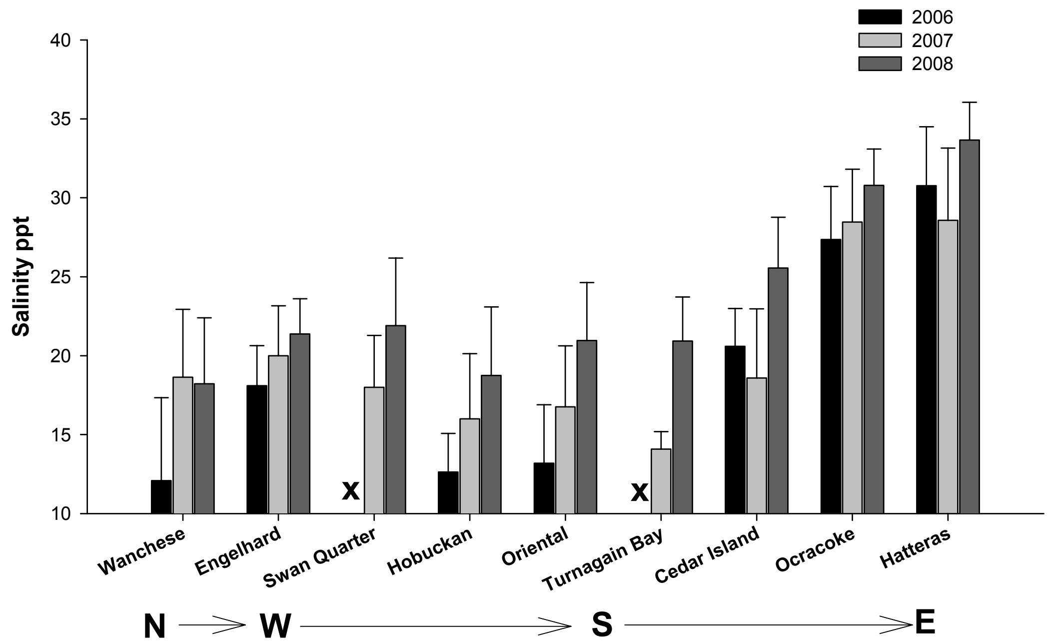


Figure 7. Average salinity at each of the shell-string sampling sites during 2006, 2007, and 2008. Error bars are 1 standard deviation and X indicates no data available. Geographic position of the sites in Pamlico Sound (northern, western, southern, eastern) is indicated under the graph.

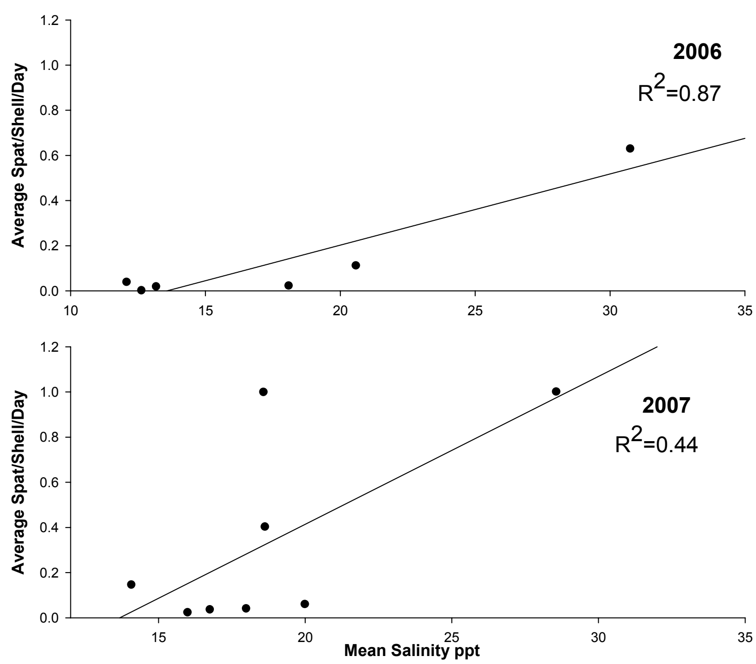


Figure 8. Relationship between salinity and oyster settlement during 2006 (top) and 2007 (bottom). Data from Ocracoke was removed in 2007 because it may not have been comparable with the 2006 because of a change in the location of the shell-string station.

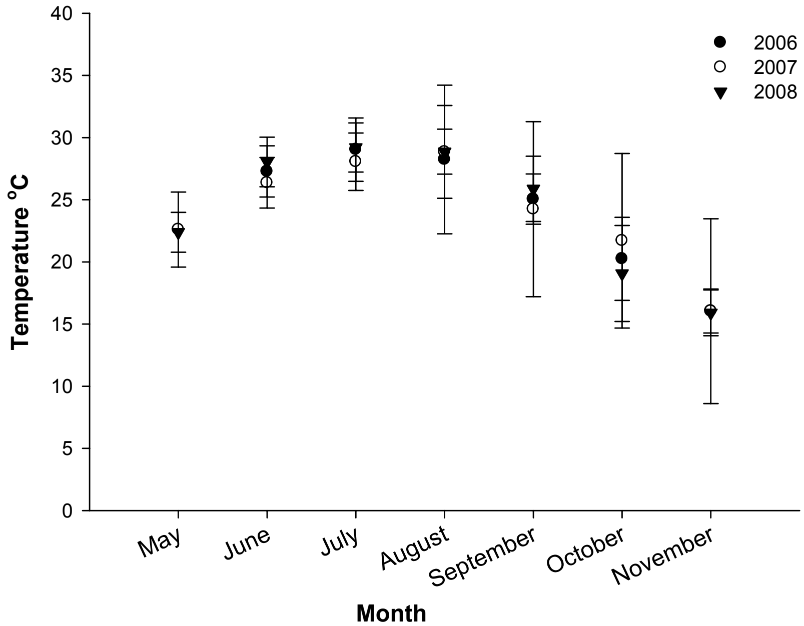
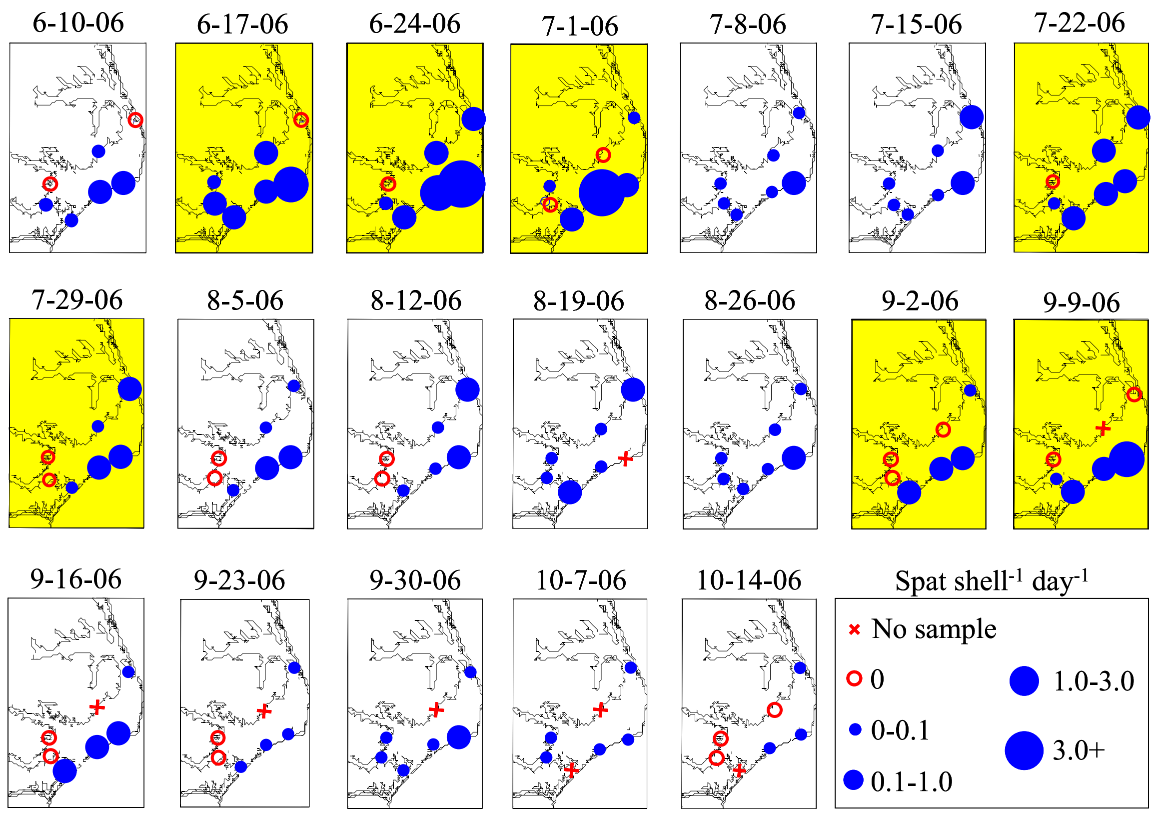


Figure 9. Average monthly temperatures during 2006, 2007, and 2008. Error bars are 1 standard deviation.

Figure 10. Mean weekly settlement (spat shell^{-1} day^{-1}) at 7 sites in Pamlico Sound from June 3 to October 14, 2006. Size of circle is proportional to mean settlement. Temporal peaks in settlement are highlighted.



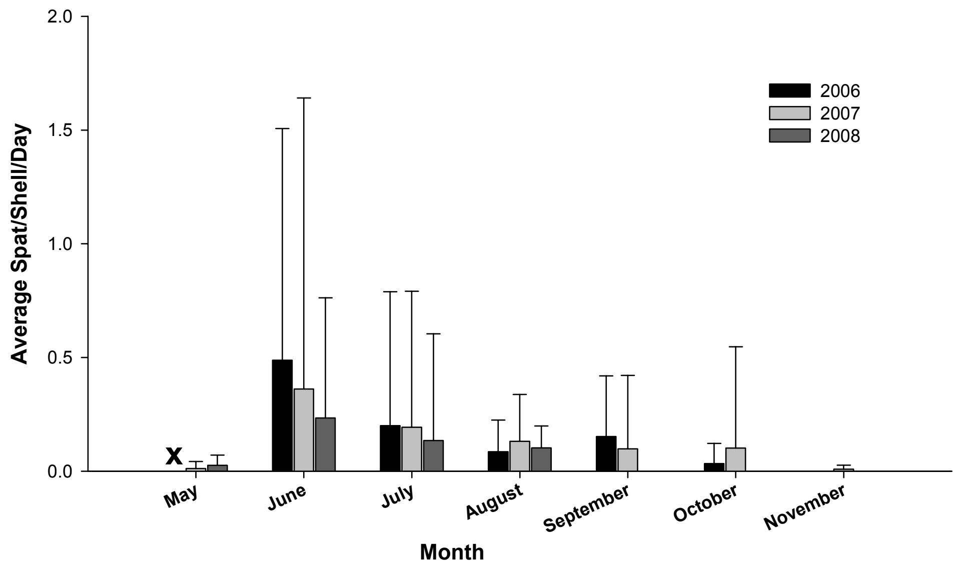


Figure 11. Average monthly settlement during 2006, 2007, and 2008. Error bars are 1 standard deviation and X indicates no data available.

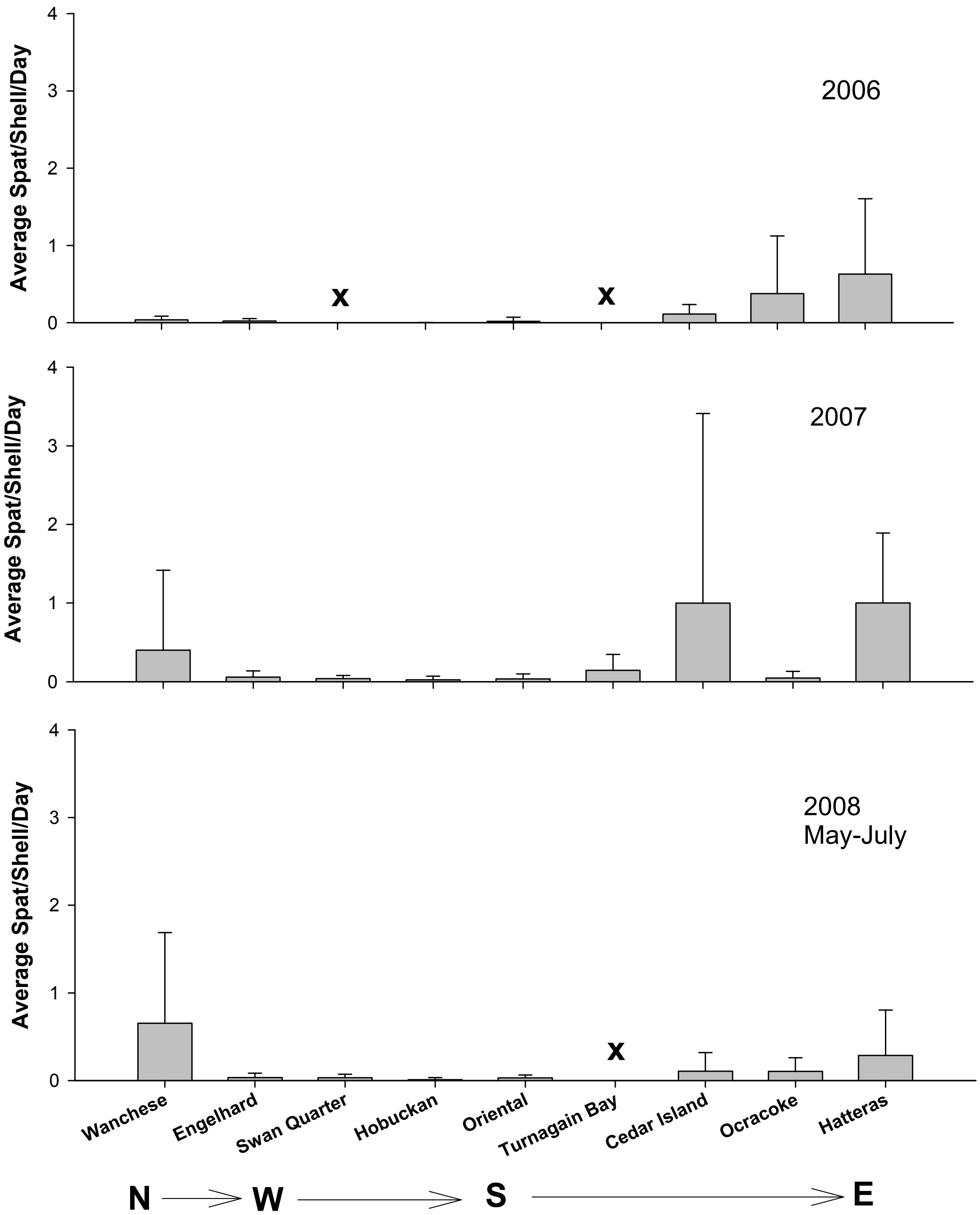


Figure 12. Average settlement at each of the shell-string sampling sites during 2006 (top), 2007 (center), and 2008 (bottom). Error bars are 1 standard deviation and X indicates no data available. Geographical position of the sites in Pamlico Sound (northern, western, southern, eastern) are indicated on the x-axis.