ABSTRACT

PETERS, JASON WASHBURN. Oyster Demographic Rates in Sub-Tidal Fished Areas: Recruitment, Growth, Mortality, and Potential Larval Output. (Under the direction of Dr. David B. Eggleston).

Understanding metapopulation dynamics is key to effectively guiding conservation and restoration strategies of focal species. Recent metapopulation modeling efforts suggest that a network of no-take oyster reserves in Pamlico Sound (PS), North Carolina is not self-sustaining, yet oyster densities in 8 of 10 reserves increased sharply during 2006-08, suggesting that these reserves are being supplemented by larvae from neighboring fished oyster reefs (Puckett and Eggleston 2012). To begin to identify larval sources in fished areas that facilitate metapopulation persistence within reserves, we quantified population density, recruitment, growth, survivorship, and potential larval output at two types of sub-tidal fished reef (cultch-planted and natural) in PS. We then made comparisons of these data to demographic data collected at marine reserves during a previous study (Puckett and Eggleston 2012).

A total of 102,634 oysters were counted from 24 harvested reefs in PS during this two year study from May 2012 to October 2013. Mean oyster densities were generally five-times higher in cultch-planted sites than natural reefs, higher along the western shore of Pamlico Sound than the eastern shore, and highest during the start of the study in May 2012 and declined through October 2013. Demographic rates, such as mean growth and survivorship, did not vary significantly between cultch versus natural reefs, region within Pamlico Sound, or over time. There was a striking decrease in oyster densities going from no-take to fished oyster reefs. For example, mean total oyster density was 72.2- and 7.5-times higher in reserves than natural and cultch reefs, respectively. There were also striking differences in size structure between fished and non-fISHED reefs. Natural reefs exhibited low density, little
size structure, and few or no individuals greater than legal size (75 mm). Cultch-planted reefs possessed higher densities and a uni- or bimodal size-structure compared to the truncated size structure of natural reefs. However, cultch-planted and natural reefs both had very few legal sized individuals. Reserves typically had a polymodal size structure, including many large individuals. Mean growth rates during fall at fished reefs (cultch-planted and natural reefs combined, 0.13-0.20 mm d\(^{-1}\)) were considerably less than at reserves (0.2-0.5 mm d\(^{-1}\)).

Highest mean growth rates at both fished reefs and no-take reserves were found in eastern PS. Probability of survivorship varied considerably on small temporal and spatial scales, though highest survivorship occurred in western PS for both fished and un-fished reefs. We estimate that reserves have ~7 to 30 times greater potential larval output per m\(^2\) than cultch and natural reefs, respectively. However, accounting for total sound-wide areal coverage of each reef type, potential larval output of natural reefs was similar to that from reserves due to the ~ two orders of magnitude greater areal footprint of natural reefs. The combined total potential larval output of natural reefs and cultch-planted reefs was 188-501% greater than reserves. These results help define the role that fished areas might play in providing larval subsidies to reserve networks, and also highlight the positive impact that no-take reserves have on demographic rates of focal species.
Oyster Demographic Rates in Sub-Tidal Fished Areas: Recruitment, Growth, Mortality, and Potential Larval Output

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Master of Science

Marine, Earth, and Atmospheric Sciences

Raleigh, North Carolina

2014

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DEDICATION

To my parents, Mark and Jean, for their loving support and encouragement.
BIOGRAPHY

Jason Washburn Peters was born on December 19, 1988 in Washington, D.C. He lived in Great Falls, VA until graduating from Langley High School in 2007. While in high school, Jason participated in a variety of outdoor activities, including backpacking, fishing, camping, hunting, boating, and surfing. Upon completion of his Eagle Scout rank in Boy Scouts, Jason became certain in his desire to pursue a career in environmental sciences. In 2007, he moved to Newport News, VA to attend Christopher Newport University (CNU) in pursuit of a bachelor’s degree in Biology. Between school years, Jason took advantage of his summers to continue his education, by interning for both the North Carolina Coastal Reserve in Kitty Hawk, NC and The Nature Conservancy in Nags Head, NC. Both of those experiences fueled his interest in research ecology and the scientific method. Under the direction of Dr. Jessica Thompson, Jason developed his skills as an ecologist and graduated from CNU in 2011.

Jason moved to Raleigh, NC in the summer of 2011 to begin his graduate studies with the Marine Ecology and Conservation lab at NC State University. Upon completing requisite coursework in Raleigh, he elected to move to the coast where he could conduct his thesis research from the NC State Center for Marine Sciences and Technology (CMAST) in Morehead City, NC. Jason now works for the North Carolina Division of Marine Fisheries, designing and implementing coastal habitat enhancement projects.
ACKNOWLEDGMENTS

Foremost, I would like to thank my advisor, Dr. David Eggleston, for his encouragement, enthusiasm, patience, and incredible knowledge. His mentorship helped improve not only this thesis, but my ability as a scientist. I would also like to extend my gratitude to my committee members, Joel Fodrie and Geoffrey Bell. Thank you for your valuable insight and guidance. My sincere thanks goes to all the field assistants and lab mates who made this research possible, including Ashlee Lillis, Robert Dunn, Brandon Puckett, Lydia Neal, Hunter Eggleston, Becky Gericke, Jordan Byrum, Seth Theuerkauf, Doreen McVeigh, and Amber Perk. Additionally, I would like to acknowledge my funding sources for providing the financial support necessary to carry out this work, including NC Sea Grant (RMRD56/12-HCE-2), NSF (1155609), and the Fisheries Resource Grant Program (11EP06). Distinct recognition goes to my undergraduate advisor, Dr. Jessica Thompson, for inspiring me to pursue my interest in ecology. Lastly, but certainly not least, I would like to offer a special thank you to my awesome friends and family who have provided invaluable support and encouragement throughout my academic pursuits.
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INTRODUCTION

Population dynamics of organisms in marine systems are driven by spatiotemporal variation in demographic rates such as births, deaths, immigration and emigration (Gotelli 2001). In marine systems, births and immigration are typically combined into a single input to a local population, termed “recruitment,” which consists of larval settlement or post settlement immigration (Reyns at al. 2007). Marine populations are also characterized by metapopulations, whereby spatially separated populations are connected via dispersal and subsequent recruitment (Cudney-Bueno et al. 2009). A key process connecting metapopulations is larval dispersal, which is driven by a suite of interacting bio-physical variables such as wind- and tidally-driven currents, and depth-regulating larvae that position themselves within certain currents. Integral to the application of metapopulation dynamics to conservation practices is the concept of source versus sink populations or habitats. Sinks are defined as populations in which local reproduction is less than local mortality; therefore sustaining sink populations are inherently dependent on a supply of recruits from source populations where locally, reproduction is greater than mortality (Pulliam 1988).

Identification of metapopulation dynamics and population sources versus sinks require data on the demographic rates of spatially separated populations, as well as connectivity among populations (Hanski 1998). This information is especially important in citing the location and assessing the performance of no-take marine reserves (Botsford et al. 2001, 2009).

Marine reserves are a powerful management approach for restoration and conservation of marine species and ecosystems. In general, the abundance and size of individuals within reserves is often greater and larger, respectively, than outside reserves,
which can also lead to a “spill-over effect” of larvae and individuals from inside to outside the reserve (Gell and Roberts 2002, Halpern 2003, Sobel and Dahlgren 2004). Because reserves are generally not demographically closed, and metapopulation connectivity is driven primarily by larval dispersal, there is a high potential for interaction and exchange of larvae spawned within no-take reserves and fished sites, yet little information exists on such exchange, particularly “spill-in” of larvae from fished populations to those inside no-take reserves (Botsford et al. 2001). In this study, we present population demographic rates of fished, sub-tidal oyster reefs and estimate their potential larval contribution to no-take, oyster broodstock reserves (see below) in Pamlico Sound, North Carolina, USA.

As an ecosystem engineer and economically important harvested species, oysters serve several valuable ecosystem functions such as water filtration, shoreline stabilization, benthic-pelagic coupling, Essential Fish Habitat, and as food for other aquatic organisms (Coen et al. 2007, Mackenzie 2007, Pierson and Eggleston 2014). Unfortunately, on a global scale, as many as 85% of all oyster reefs have been lost in comparison to historical abundance (Beck et al. 2011). Similarly in the United States, present Crassostrea virginica populations have 64% less spatial extent and 88% less total biomass, relative to historical surveys (zu Ermgassen et al. 2012). Overall decline in oyster habitat and biomass can be attributed largely to habitat destruction from intensive fishing pressure (Gross and Smyth 1946, Rothschild et al. 1994, Kirby 2004).

The status of Eastern Oysters, Crassostrea virginica Gemlin (hereafter ‘oyster’) in North Carolina is no exception to the trend of global decline. The North Carolina Division of Marine Fisheries (NCDMF) recognized the need for restoration and created 10 no-take oyster
broodstock reserves within Pamlico Sound, NC between 1996 and 2008, in an effort to establish a self-sustaining network of reserves, but also to provide spill-over of larvae to harvested areas. Previous research has quantified oyster demographic rates within this network of broodstock reserves, and found that some reserves serve as population sources and others as population sinks in terms of potential larval production, larval connectivity, subsequent recruitment, growth, and survival (Haase et al. 2012, Mroch et al. 2012, Puckett and Eggleston 2012). Previous metapopulation studies of these reserves show that the reserves are not self-sustaining in terms of larval connectivity despite the observation of overall oyster densities in 8 of 10 reserves increasing ~400% between 2006 and 2008 (Puckett and Eggleston 2012). Therefore, the broodstock reserves are likely being sustained by larval dispersal from fished oyster populations.

As part of an oyster restoration effort in Pamlico Sound, the NCDMF created the Shellfish Rehabilitation Program. This program is responsible for creating artificial oyster settlement habitat, suitable for the development of oyster populations and subsequent harvest. Each year, reefs for harvest are created in varying locations throughout Pamlico Sound using a variety of settlement materials, called “cultch.” Cultch material typically consists of oyster shell, concrete, limestone marl (~5 cm diameter), or other shell types. Cultch-planted reefs are constructed by spreading a thin veneer of material within pre-determined coordinates. Vertical relief of cultch-planted reefs is generally low (pers. obs.) and reef size is a function of the amount of cultch material deposited (C. Caroon, NCDMF, pers. comm.). Cultch reefs are monitored for a period of 3 years post-planting, however they are open to harvest as soon as oysters on the reefs have reached harvestable size (75 mm shell height). In general, cultch-
planted reefs typically persist for 5-7 years, but some can last for 20+ years (C. Caroon NCDMF, pers. comm.). Both cultch-planted and naturally occurring oyster reefs exist in Pamlico Sound within a salinity range of about 13 PSU to 29 PSU, and are separated from each other by approximately 1-125 km.

Within North Carolina, naturally occurring oyster reefs are composed primarily of shell or oyster shell hash (Street et al. 2005, this study). Natural oyster reef habitat has been mapped by NCDMF in Pamlico Sound waters less than 5m deep (B. Conrad, NCDMF, unpub data, Fig. 1). Additionally, single reef coordinates have been compiled for natural reefs which have not yet been included in the NCDMF survey (C. Hardy, M. Jordan, M. Marshall, G. Allen NCDMF, unpub. data; Fig. 1). An historical survey of PS natural reefs compares fairly well with more recent NCDMF mapping efforts in terms of distribution and approximate areal extent (Winslow 1886, Fig 1). While these maps provide a reference to natural reef locations and extent, very few studies have been conducted to ground-truth or visually validate their actual existence. In this study, we ground-truthed the putative locations of natural oyster reefs and then refined maps of their distribution and abundance. In addition to quantifying the location and areal extent of natural oyster reefs, we also quantified reef characteristics to help assess the structure and functions of oyster reefs. For example, reef elevation is important for oyster reef water quality. Low profile oyster reefs may experience reduced water flow rate and therefore increased sedimentation, followed by relatively high mortality due to burial or low anoxic stress (Lenihan 1999). Moreover, low profile reefs may experience increased exposure to anoxic or hypoxic bottom water, which reduces survivorship and growth (Lenihan and Peterson 1998, Lenihan 1999). Similarly, habitat
complexity can impact predator-prey interactions, such that low complexity reefs (e.g., lacking clustered oysters) may favor predation on oyster spat (Grabowski 2004). High fouling abundance may also influence population dynamics by inhibiting larval settlement and growth (Ortega and Sutherland 1992, Osman et al. 1989). Recent observations indicate that boring sponge (Cliona spp.) can burrow into and compromise the integrity of the limestone marl used to create certain oyster reefs, and that percent cover by boring sponge increases with salinity (Dunn et al. 2014, in review; N. Lindquist, UNC-CH/IMS, pers. comm.). We have observed high oyster mortality at several high salinity reserves in Pamlico Sound where boring sponge is common (Puckett and Eggleston 2012, N. Lindquist, UNC-CH/IMS, C. Hardy, NC DMF, pers. comm.), however, recent experiments indicate that boring sponge do not negatively impact oyster recruitment and survival over short-time scales (months to year) (Dunn et al. 2014). Nevertheless, while quantifying demographic rates of oysters in fished areas in Pamlico Sound in this study, we also recorded the percent cover of boring sponge to better characterize distribution and abundance patterns of this part of the fouling community.

The main objectives of this study were to: (1) map and characterize the distribution and abundance of two types of fished, sub-tidal oyster populations in Pamlico Sound, North Carolina, (i) natural oyster reefs and (ii) cultch-planted sites, (2) quantify oyster demographic parameters (density, recruitment, growth, and survival) in these fished oyster populations over time, and (3) compare and contrast the oyster demographic rates measured in fished sites in this study, with similar demographic data from non-fished, marine reserve sites within Pamlico Sound from a related study (Puckett and Eggleston 2012).
METHODS

The general approach first involved using GIS and the best available information from NCDMF, fishermen, and scientists to map the locations of fished, sub-tidal oyster populations in Pamlico Sound, followed by field reconnaissance via boat and SCUBA. Once oyster reef locations were mapped, we randomly selected oyster reefs to sample demographic rates via replicate quadrat sampling of oysters over space and time using SCUBA.

Study System

The Croatan-Albemarle-Pamlico Estuarine System (CAPES) in North Carolina is the second largest estuarine system in the United States, with a total area of approximately 6,600 km² (Pietrafesa et al. 1986). It is a bar-built estuary, separated from the Atlantic Ocean by the Outer Banks barrier islands. The CAPES is characterized as a lagoonal estuarine system with three inlets regulating exchange of water with the Atlantic Ocean (Luettich et al. 2002, Lin et al. 2007). Pamlico Sound is the largest water body in the CAPES, covering an area of approximately 4,800 km². The mean depth of this shallow estuarine system is 4.9 m, with maximum depths around 7.3 m (Epperly and Ross 1986). The mean salinity in Pamlico Sound is 15 psu, with salinity ranges affected by fresh water inflow that occurs from the west (Tar-Pamlico and Neuse rivers) and the north (Albemarle Sound and tributaries) (Xie and Eggleston 1999). Salinities in southern Pamlico Sound are generally the highest, averaging about 25 psu. Pamlico Sound is a nursery for numerous recreationally and commercially important fishes (Ross and Epperly 1985, Pierson and Eggleston 2014).

Site Selection and Reef Mapping

Site Selection. – To quantify oyster demographic rates, we randomly selected 24
natural and cultch-planted oyster reefs distributed throughout Pamlico Sound from the NCDMF geospatial database. Pamlico Sound was first divided into two regions: (i) relatively low-salinity sites (10-18 psu) along the western shore and (ii) relatively high-salinity sites (18-26 psu) located along the eastern shore. Within each region, eight cultch-planted sites and four naturally occurring reefs were randomly selected for sampling and numbered 1-26. Two natural reefs (sites 25 and 26) were added to the study in August 2013 to increase replication. This was necessary because field reconnaissance in 2012 demonstrated that certain natural reefs identified via GIS data were non-existent (sites 9 and 17; Fig. 2).

Reef Mapping. – Site reconnaissance was conducted during April-May 2012 by on-site “tonging” to distinguish shell versus soft substrate. At a given site, we initially used a PVC-pipe and, starting from a single reef coordinate, made expanding concentric circles with a boat while probing the sediment for changes in bottom substrate. When the substrate changed from shell to soft, a marker buoy was deployed. This procedure was continued until the reef boundaries were determined on all sides, which was then followed by ground-truthing the corners of a given oyster site via SCUBA. Coordinates of geometric corners were recorded with a hand-held GPS unit and transposed into ArcMAP© geospatial software to calculate the areal footprint of a given reef/site. Estimates of the area of a given site were necessary to (1) allocate sampling effort based on the area of a given type of oyster reef (natural vs. cultch-planted) and location within certain salinity regimes (see below), as well as (2) allow us to scale-up demographic rates, such as potential larval output, from m² to total potential output for a given site or reef type (see below). The total area of natural oyster reefs in Pamlico Sound was estimated using existing NCDMF data on natural oyster reef bottom,
multiplied by a corrective factor of 0.44 to account for missing or nonexistent reefs based on our field reconnaissance (see Results, Ground-Truthing and Reef Mapping). The total area of cultch-planted reefs was estimated by multiplying the average mapped reef size by 137, the total number of existing cultch-planted reefs at the time of our surveys.

Sampling Procedure. – All reefs were sampled during May, August, and October 2012 and June, August, and October 2013. Prior to each sampling time, new replicate coordinates for each reef were randomly generated using ArcMAP© geospatial software. At each site and sampling time, water temperature, salinity, dissolved oxygen, and depth were recorded using a YSI-85 water quality instrument.

Percent Cover of Boring Sponge and Fouling Organisms

Spatiotemporal variability in percent cover of boring sponge (Cliona spp.) and fouling organisms were quantified on live oysters at each site. External surfaces of live oysters only (within each quadrat) were examined for percent cover estimates, considering total surface area of all live oyster shells to be one hundred percent. Two separate 2-way repeated measures ANOVA models were used to test the effects of region (eastern vs. western Pamlico Sound) and reef type (cultch-planted or natural) within time (May ’12, Aug ’12, Oct ’12, June ’13, Aug ‘13, Oct ’13), on percent cover of boring sponge and fouling organisms.

Oyster Demographic Rates in Fished Reefs

Oyster Density. – Each site within a given region and reef type served as the sampling unit. At each site in 2012, oysters were sampled by randomly placing a 0.25 m² quadrat on the reef, with subsequent hand excavation to a depth of 15 cm by divers using SCUBA
(Powers et al. 2009, Puckett and Eggleston 2012). To increase sample size at low density sites, a 1 m² quadrat was used for excavations in 2013. For quadrat samples with abundant oysters, a sub-sample quadrat (0.25 or 0.5 m²) was used within excavated material. All excavated material was placed in mesh bags and brought to the surface for immediate processing. Processing included measuring the left valve length (LVL) of all oysters collected, from the umbo region to the outer edge of the shell. Once excavated, quadrat samples with noticeably high live oyster densities were sub-sampled. The number of excavated oysters were counted and scaled to # oysters/m² to account for sub-sampling. Replicate samples were averaged at each site for each sampling event and then separated into three cohorts, based on left valve length (LVL): (1) recruits (LVL <25mm), (2) sub-legal (25mm ≤ LVL<75mm), and (3) legal oysters (LVL ≥75mm). Mean density of each cohort was calculated in terms of individuals/m².

We tested if a given response variable (oyster total density, legal density, sub-legal density, and recruit density) varied according to reef type (natural or cultch-planted) or region (eastern vs. western Pamlico Sound) with four separate, two-way repeated measures ANOVA models, where the repeated measure was time (May ‘12, August ‘12, October ’12, June ’13, August ’13, or October ‘13). Data were tested for homogeneity of variance using Levene’s Test. For all size classes, transformed data failed to meet the assumption of homoscedasticity, therefore non-transformed data were still analyzed with ANOVA due to their robustness and insensitivity to skewness (Glass et al. 1972). In these instances, examination of residual plots indicated they were close to normal and therefore were used for data analysis (D. Dickey, North Carolina State University Statistics Department, personal
communication). Comparisons among treatments within a factor were conducted with a Tukey-Kramer multiple comparisons test.

**Oyster Growth and Survival.** – Oyster growth and survival were estimated by following the progression of cohort density and mean LVL at each site over time, and applying a modal analysis of oyster length-frequency using 5mm size class intervals. Modes were assigned to length-frequency distributions using FiSAT© NORMSEP, which utilizes a maximum likelihood procedure to separate distributions into multiple normal components. Modes were validated using a two-sample, Kolmogrov-Smirnov goodness-of-fit test. If an assigned mode failed the goodness of fit test (p<0.05), it was not used for growth or survivorship estimates. Those sites where the number of oysters collected was less than 100 were omitted from this analysis (Hoenig et al. 1987). Size modes exhibiting mean LVL progression over time were used for growth and survivorship estimates.

To estimate oyster growth, mean cohort LVL was plotted by site for each sampling event to observe changes in mean oyster length over time. Change in LVL was used as a proxy for growth. To determine rate of growth (G), mean lengths were subtracted and divided by the number of days elapsed between sampling events. We used the following formula to estimate per-day oyster growth for five time periods: (1) May ‘12-Aug’12, (2) Aug’12-Oct. ‘12, (3) Oct’12-June’13, (4) June’13-Aug’13, (5) Aug’13-Oct’13,

\[ G = \frac{\text{LVL}_{tf} - \text{LVL}_t}{d} \]

Where LVL is mean left valve length, (t) is the initial sampling date for a given period, (tf) is the final sampling time for a given period, and (d) is the number of days between (t) and (tf). Growth rates were pooled across cohort and site to generate a mean
growth rate for a particular region (eastern or western PS) and in different reef types (natural or cultch). The effects of time, region, and reef type on growth rates (mm d\(^{-1}\)) were tested using a 3-way ANOVA model with non-transformed data.

Changes in oyster cohort density over time were used to estimate per capita mortality and survivorship. Mortality rates (M) were determined for five time periods (see Growth). Rate of mortality for each site was based on percent loss in cohort density from one time period to the next, and represented by the following formula, where (CD) is the cohort density, (t) is the initial sampling date for a given period, and (tf) is the final sampling date for a given period:

$$M = \frac{OD_t - Od_{tf}}{OD_t}$$

Percent survivorship (S) is simply the inverse of mortality:

$$S = (1 - M) \times 100$$

A three-way ANOVA was used to test the effects of time, location, and reef type on percent oyster survivorship using non-transformed data.

**Oyster Demographic Rates in Fished versus Unfished Populations**

Estimates of oyster demographic rates in this study (density, growth, survival, larval output/m\(^2\), and total potential larval output) were compared with similar data from Pamlico Sound collected in no-take, oyster broodstock reserves during 2006-08 (Puckett and Eggleston 2012). The NCDMF created a network of no-take oyster broodstock reserves in 1996 to enhance the oyster metapopulation in Pamlico Sound, and create oyster reefs that would serve as Essential Fish Habitat. As of 2011, a total of 10 broodstock reserves had been established with footprints ranging in size from 0.16 ha to 12.7 ha. Total footprint area for all
ten reserves is approximately 57.1 ha. Each reserve contains high-relief (2 m) limestone mounds (reefs) that are roughly 20 m apart (Mroch et al. 2012, Puckett and Eggleston 2012, Pierson and Eggleston 2014).

**Potential Larval Output**

Oyster length frequencies from this study were combined with per-capita fecundity for a given oyster size-class to generate mean larval output for each site in terms of eggs/m$^2$ (Mroch et al. 2012). Potential larval output for oysters in Pamlico Sound peaks in May prior to the first spawning event of the season (Mroch et al. 2012). Therefore, we used oyster density and length frequency estimates from May ’12 and June ’13 to estimate potential larval output. Site-specific estimates of larval output/m$^2$ were first pooled to calculate a mean larval output/m$^2$ by reef type. Total potential larval output for each reef type was then computed by multiplying larval outputs/m$^2$ by their respective total areal coverage in Pamlico Sound, and these values were then compared with similar values of potential reproductive output from the no-take reserves (Mroch et al. 2012). A range of total larval output for natural reefs was estimated based on NCDMF-reported natural reef areal extent and an adjusted areal extent from this study (see Results, Ground-Truthing and Reef Mapping).

**RESULTS**

**Ground-Truthing and Reef Mapping**

The majority of fished, sub-tidal oyster reefs in Pamlico Sound appear to be concentrated in the northwestern part of the sound, and near the mouths of the Neuse and Pamlico rivers (Fig. 1). In general, western Pamlico Sound has a much greater number of oyster reefs than eastern PS (NCDMF unpub. data, Fig. 1). We mapped and quantified oyster
demographic parameters in a total of 13.30 ha of fished oyster reefs, including both natural and culch-planted types. In this study, we ground-truthed 18 natural reef coordinates that were randomly selected from the NCDMF’s geospatial database for Pamlico Sound. A total of 10 of 18 reefs (56%) were not present at the reported coordinates. The remaining eight were located and used for this study. The eight natural reefs used in the present study had a total area of 8.66 ha and ranged in size from 0.03 ha to 6.04 ha. Mean areal footprint for natural reefs was 1.08 ha (σM = 0.7 ha). All NCDMF culch-planted reefs were present at the reported coordinates.

A total of 16 culch-planted reefs were mapped, with a total area of 4.64 ha. Culch-planted sites in this study ranged in size from 0.02 ha to 1.1 ha and were, on average, considerably smaller than natural reefs (mean = 0.29 ha, σM = 0.07 ha). Average areal cover of fished reefs in eastern PS was somewhat larger (mean = 0.72 ha, σM = 0.49 ha), yet fewer in quantity than western PS, where reefs were smaller (mean = 0.39 ha, σM = 0.09 ha) and more numerous (Figs. 1 and 3).

According to the NCDMF’s oyster reef mapping database (NCDMF, unpubl. data), there are approximately 5,929 ha of sub-tidal, natural reefs in Pamlico Sound, which is likely an over-estimate. For example, based on our field surveys of potential natural reefs from this NCDMF database, only 44% of reefs were actually present in some form (see above). Therefore, we have also provided an adjustment to the amount of natural reef cover, which was reduced to a total of 2,609 ha. It is important to note, this downward adjustment in area of natural oyster reefs is based on a relatively small sample of natural reefs (n=18) on which we conducted field verification. Thus, within the context of historical reports (4,046 ha;
Winslow 1886), the adjusted area of natural oyster reefs in this study (2,609 ha) may be considered a low-range estimate, whereas the NCDMF estimate (5,929 ha) may be considered a high-range estimate for existing natural reef area in PS.

Cultch-planted reefs created prior to 2005 were considered non-existent due to fishing pressure, sedimentation and shell erosion, therefore only 137 reefs were considered to be in existence by 2011 (C. Caroon, NCDMF, pers. comm.). Based on our mapping of cultch sites, which had an average footprint of 0.29 ha, we estimate the overall areal cover of cultch reefs in Pamlico Sound to be 39.73 ha (137 cultch sites X 0.29 ha/reef). The areal cover of 10 no-take broodstock reserves in Pamlico Sound is 57.18 ha (NCDMF, unpub. data).

We made general qualitative observations on several important reef characteristics, including reef elevation, substrate volume, reef patchiness, sedimentation, and shell morphology, some of which exhibited differences between region (eastern PS vs. western PS) and reef type (natural vs. cultch-planted). With respect to reef elevation, all fished reefs throughout the system were characterized by low vertical relief across the entire reef (<0.25 m height above-bottom). Visual observations suggest western PS reefs may have more settlement substrate (shell or cultch) per square meter than eastern PS, based on the volume of material in excavated quadrat samples (Appendix 1a). Sedimentation and burial of shells was observed more frequently at sites in the western PS than eastern PS. The substrate on natural reefs also varied between eastern and western PS. Reefs in western PS contained numerous small (~1-5 mm) pieces of shell “hash” with scattered live oysters (Appendix 1b), whereas eastern PS reefs contained mainly large, partially eroded shells (Appendix 1c). Morphologically, oyster shells were typically elongate and relatively brittle in eastern PS,
whereas oysters in western PS oysters were more round and smooth, with thick shells (Appendix 1d).

**Percent Cover of Boring Sponge and Fouling Organisms**

Average percent cover of boring sponge (*Cliona* spp.) was significantly higher in eastern PS than western PS (two-way repeated measures ANOVA; location: df = 1, 22 p<0.0001), averaging 45.2% cover on live oysters in the former versus 2.78% cover in the latter. Mean percent cover by fouling organisms on oysters was also significantly greater in eastern PS versus western PS (two-way repeated measures ANOVA; location: df = 1, 22, P<0.01). There were no statistically significant differences in mean percent cover of boring sponge or fouling organisms between natural and cultch-planted reefs or over time, nor any significant interactive effects (2-way repeated measures ANOVA, all p > 0.05).

**Oyster Demographic Rates in Fished Reefs**

A total of 102,634 oysters were counted from twenty-four harvestable reefs in Pamlico Sound during our two year study from May 2012 to October 2013. Mean total density was driven primarily by sub-legal density, which was ~5x higher in western PS than eastern PS (Fig. 5a &5c). Sub-legal density declined by 83.4% between May 2012 and October 2013 (Fig. 4c). Legal and recruit sized oyster densities remained generally low over time and did not vary by region or reef type (Figs. 4, 5, 6; see below for results of statistical tests). Demographic rates such as mean growth and survivorship did not vary significantly between cultch-planted and natural reefs, eastern vs. western Pamlico Sound, or over time (Figs. 7 & 8).
**Oyster Size Class Densities**

**Total Density** – Mean total oyster density varied significantly according region within Pamlico Sound (eastern vs. western shore) and by time (May 2012 to October 2013), but not by reef type (cultch-planted vs. natural reef; 2-way repeated measures ANOVA; region: $df = 1, 21, p < 0.05$; time: $df = 5, 107, P = 0.0215$; reef type: $df = 1, 21, p = 0.0642$; Figs. 4a, 5a, 6a). Mean total oyster density was significantly greater at sampling sites located along the western versus eastern region of Pamlico Sound (Fig. 5a). Total density was higher in 2012 than 2013 (Fig. 6a).

**Legal Density** – The mean density of legal size oysters varied significantly by reef type (Fig. 4b; 2-way repeated measures ANOVA; $df = 1, 21, p=0.0008$) but not by time (Fig. 6b; $df = 5, 44, p=0.58$) or region (Fig. 5b; $df = 1, 21, p=0.0619$). The mean density of oysters on cultch-planted reefs was ~ four times higher than on natural reefs (Fig. 4b).

**Sub-Legal Density** – The mean density of sub-legal oysters varied significantly by time (2-way repeated measures ANOVA; $df = 5, 107, p = 0.0261$) and region ($df = 1, 21, p = 0.0126$). There was a pronounced decline in the mean density of sub-legal oysters over time, with an 83.4% loss from May 2012 to October 2013 (Fig. 6c). Higher densities existed at sites along the western shore of Pamlico Sound compared to the eastern shore (Fig. 5c). Sub-legal oyster density was apparently higher at cultch-planted reefs than at natural reefs, though reef type was marginally non-significant ($df = 1, 21, p = 0.0624$).

**Recruit Density** – Mean density of recruits did not vary significantly by reef type (Fig. 4d; 2-way repeated measures ANOVA; $df = 1, 21, p = 0.0536$), region ($df = 1, 21, p = 0.3331$), or over time ($df = 5, 107, p = 0.3847$). While the mean density of recruits did not
vary significantly over time, there were apparent peaks on recruitment that were detected in May 2012, October 2012, and October 2013 (Fig. 6d). Recruitment also appeared higher on cultch-planted reefs than natural reefs, though the trend was not statistically significant (Fig. 4d).

*Growth and Survivorship*

Mean oyster growth and survivorship was determined for fifteen and fourteen sites, respectively, over five time periods from spring 2012 to fall 2013. For most sites, growth and survivorship were not calculated for all five time periods due to non-normal length-frequency distributions and subsequent inability to perform modal progression analysis (Table 1, Table 2).

*Growth* – Although there was a trend towards relatively high growth in the eastern region of Pamlico Sound than western region, and peak growth during fall 2012, mean growth rate did not vary significantly according to reef type, region, or time, nor were there any significant interactions among the three factors (Fig. 7; 3-way ANOVA; all p > 0.06).

*Survivorship* – Similar to the pattern for growth rates, oyster survivorship did not vary significantly according to reef type, region within Pamlico Sound, time, or any of their interactions (Fig. 8; 3-way ANOVA; all p > 0.06).

*Oyster Demographic Rates in Fished versus Unfished Populations*

*Density* – We compared the mean density of oysters measured on fished reefs in this study with similar data collected recently (June 2006 to July 2008) from six no-take, oyster broodstock reserves in Pamlico Sound (Puckett and Eggleston 2012). There was a striking
decrease in oyster densities going from no-take to fished oyster reefs. For example, mean total oyster density was 72.2- and 7.5-times higher in reserves than natural and cultch-planted reefs, respectively (Fig. 9a). Mean density of legal oysters was 27.3- and 6.0-times higher in reserves than natural and cultch-planted reefs, respectively (Fig. 9b). Similarly, mean density of sub-legal oysters was 52.4- and 4.8-times higher in reserves than natural and cultch-planted reefs, respectively (Fig. 9c). Lastly, the mean density of recruits in reserves was 178.0- and 15.4-times higher than in natural reefs and cultch-planted reefs, respectively (Fig. 9d).

Size Structure – Striking differences in size structure were also observed between fished and non-fished reefs. Natural reefs exhibited low density, truncated size structure, and few or zero individuals greater than legal size (75mm) (Fig. 10a). Cultch reefs possessed higher densities and a generally unimodal or bimodal size-structure compared to natural reefs, however, there were very few legal sized individuals (Fig. 10b). Reserves typically had a polymodal size structure, including many large individuals (Fig. 10c, Puckett and Eggleston, unpubl. data). Relative size class abundance also varied between fished and reserve oyster reefs, whereby recruits composed 62.8% of total individuals within reserves versus only 23.5% at fished reefs. Legal sized oysters represent the smallest fraction of total density at reserves and fished reefs, accounting for 8.0% and 11.9% of the total, respectively. At fished reefs, sub-legal sized oysters were generally most abundant, composing more than half of the total density.

Growth and Survivorship – Oyster growth rates at fished reefs were compared with those reported at the no-take, broodstock reserves by Puckett and Eggleston (2012), who
found fastest growth during fall and little growth over winter. A similar trend was observed in this study on fished reefs, however, growth rate in fall at fished reefs (mean = 0.165 mm d\(^{-1}\), \(\sigma_M = 0.04\)) was less than at reserves (0.2-0.5 mm d\(^{-1}\)), whereas over winter growth rates at fished reefs was similar to rates reported at marine reserves (<0.1 mm d\(^{-1}\), Puckett and Eggleston 2012). Growth was highly variable at small spatial scales (i.e. by site), however, highest mean growth rates at both fished reefs and marine reserves occurred in eastern PS (Table 1, Puckett and Eggleston 2012). Probability of survivorship also varied considerably on small temporal and spatial scales, though highest survivorship occurred in western Pamlico Sound for both fished and un-fished reefs (Table 2, Puckett and Eggleston 2012).

**Potential Larval Output**

Integration of per-capita fecundity for a given oyster size-class from Mroch et al. (2012) with length frequency data from this study and those from Puckett and Eggleston (2012) suggests reserves have ~7 to 30 times greater potential larval output per square meter than cultch and natural reefs, respectively (Fig. 11a). Per square meter potential larval output was 0.23x10\(^6\) ± 0.04x10\(^6\) for natural reefs and 2.69x10\(^6\) ± 0.62x10\(^6\) for cultch-planted reefs, whereas per square meter output of reserves was 1-2 orders in magnitude higher (14.15x10\(^6\) ± 3.7x10\(^6\)). Accounting for total sound-wide areal coverage of each reef type (see Results, *Ground-Truthing and Reef Mapping*), adjusted total potential larval output from natural reefs (20.65x10\(^{12}\) ± 6.55x10\(^{12}\)) was ~2.5 times that from marine reserves (8.08x10\(^{12}\) ± 2.11x10\(^{12}\)) due to the ~two orders of magnitude greater areal footprint of natural reefs. The potential larval output from cultch-planted reefs (1.62x10\(^{12}\) ± 0.42x10\(^{12}\)) was ~13 times less than that from natural reefs (adjusted) and ~5 times less than reserves (Fig. 11b). The combined total
potential larval output of natural reefs (adjusted) and cultch-planted reefs was 188% greater than reserves (Fig. 11c). Unadjusted combined larval output of natural and cultch-planted reefs was 501% greater than reserves.

**DISCUSSION**

Metapopulation connectivity in marine systems is driven primarily by larval dispersal (Botsford et al. 2001, Puckett et al. 2014). No-take reserves likely exchange larvae with fished sites or populations within the overall metapopulation, yet there is little information on the potential contribution of fished sites to the overall larval pool that no-take reserves might draw upon (Botsford et al. 2008). The results from this study suggest that fished, natural oyster reefs in Pamlico Sound potentially contribute, on average, 188-501% more larvae to the Pamlico Sound larval pool than the network of 10 no-take reserves. This higher relative larval contribution is driven by the ~two orders of magnitude greater areal cover of natural reefs compared to reserves, since the density of oysters in natural reefs was ~one order of magnitude lower than that in reserves. Moreover, the size-structure in natural reefs generally lacked large oysters above the legal size limit that would contribute relatively high numbers of eggs (Mroch et al. 2012).

An important finding from this study was the relatively high percentage of natural oyster reefs that did not exist when their reported locations were ground-truthed, and the relatively poor condition of natural reefs when they were found. For example, 10 out of 18 natural reef coordinates visited in the present study did not contain oyster reefs, and the remaining eight were characterized by low density, few to no large oysters, low vertical relief, low shell volume per m², and patchy distribution. The relatively degraded state of
natural reefs in PS is likely to persist over time since these reefs are exposed to harvest through tonging and dredging. Moreover, the low vertical relief of fished reefs pre-disposes them to burial by sediment, as well as exposure to low DO (Lenihan & Peterson 1998, Lenihan 1999).

**Density**

Population densities recorded in the present study contrast with those measured in related studies. For instance, in Chesapeake Bay, oyster densities at fished reefs range from 300-500 oysters/m² (Mann et al. 2009), which compares somewhat favorably with densities found at cultch-planted reefs in this study (mean = 247 oysters/m²), but not densities at natural reefs, which averaged 30 oysters/m².

Results from this study highlight the impact that no-take reserves have on demographic rates of focal species. For example, oyster density in PS no-take reserves averaged 1,936 oysters/m², which is 1-2 orders in magnitude higher than fished reefs in PS and Chesapeake Bay. Moreover, divers’ observations indicate that no-take reserves in PS contained high-relief (2 m) reefs (Puckett and Eggleston 2012), whereas fished reefs had relatively little to no relief. A variety of other studies have also demonstrated the potential for marine reserves to maintain larger organisms and higher habitat complexity than fished areas (Alcala and Russ 1990, Bennett and Attwood 1991, Duran and Castilla 1989, Polunin and Roberts 1993, Roberts 1995). In one study, 59% of all target species expressed greater abundance and biomass within reserves versus adjacent habitat (Polunin and Roberts 1993). In another investigation of fished areas versus reserves, significantly greater density, biomass, and size structure of Bull Kelp (*Durvillaea antarctica*) were observed in areas...
protected from harvest (Castilla and Bustamante 1989).

Recent observations of oyster densities within reserves in PS suggest densities have declined by an average of ~47% since 2008 (NCDMF, unpub. data). While this is a notable decrease, it may be attributable to ecological community development, which occurs over time until steady state has been achieved (Clements 1916, Connell and Slatyer 1977). Restored oyster reefs typically host high oyster densities in early stages of reef development (Luckenbach et al. 2005). As abundance of other fouling organisms and predation increase, post construction, successful recruitment may be limited and therefore oyster density may decline somewhat (Osman 1989, White and Wilson 1996). Most reefs sampled in this study were similar in age (post construction) to reserves sampled in 2006-08, which lends credence to the notion that oyster demographic rates can be reliably compared between fished and unfished reefs.

Oyster recruitment on fished reefs was highly variable, ranging from an average of <1 to 313 oysters/m², which is more variable than estimates from Chesapeake Bay (~45-175 m²; Mann et al. 2009, Southworth et al. 2010). Recruitment was also very episodic during the course of this study. For example, at sites 11 and 12 in northern PS, highest recruit densities were recorded during May 2012, while at site 24 in southern PS, highest recruit density was observed during October 2012. Site 18 in eastern PS, which typically had 0-5 recruits, obtained its highest recruit density in August 2013 (mean = 17 oysters/m²). Although low and episodic recruitment may hinder persistence and growth of oyster reefs in the face of harvesting pressure, it also makes it difficult to track cohorts over time so that one can estimate growth and survivorship. For example, modal analyses of size-frequencies often
require a minimum of 100 individuals per site and time period (Hoenig et al. 1987), which was rarely achieved in the present study.

**Growth and Survival**

At fished reefs in PS, mean growth rate did not vary between salinity regimes (eastern vs. western PS). This contrasts with other studies on oyster growth, which have found higher growth rates in higher salinities (Brown and Hartwick 1998, Ortega and Sutherland 1992). Mean growth at fished reefs was somewhat lower than PS reserves during fall, where daily growth rates were 0.13-0.20 mm d\(^{-1}\) and 0.20-0.50 mm d\(^{-1}\), respectively. Over winter, growth rates at fished reefs and reserves were similar (<0.1 mm d\(^{-1}\), Puckett and Eggleston 2012). Results from this study compare favorably with previous growth estimates in PS fished reefs, which found no difference in growth rate between study sites and an overall mean growth of 0.085 mm d\(^{-1}\) over one year (Ortega and Sutherland 1992).

Oyster survival on PS fished reefs did not vary significantly by time, salinity regime (eastern vs. western PS), or reef type. However, seasonal percent survival of all fished reefs in PS during this study (41%-71%) compares well with fished reefs in Chesapeake Bay, where seasonal percent survival rates of age 1 – 2 oyster ranged from 25%-80% (Mann et al. 2009). Mean percent survival at fished reefs was also comparable to those at marine reserves in PS, where percent survival of age 1 oysters ranged from 36-89% among reserves (Puckett and Eggleston 2012). While no difference in survival was observed between eastern and western PS fished reefs, other studies have noted increased oyster mortality rates at higher salinities, where predation rates and incidence of disease are greater (Wells 1961, Shumway 1996).
One potential factor that may influence oyster survival is the presence of boring sponge (*Cliona* spp.). The relatively high salinity in eastern PS tended to support relatively high percent cover of boring sponge, *Cliona* spp. (this study), which has been implicated in the high mortality of oysters at two of ten broodstock reserves in PS (N. Lindquist, UNC-IMS, pers. comm.). Studies have not established a direct link between oyster mortality and boring sponge infestation, though evidence of stress has been observed through lysosomal assays of oyster tissue from infected individuals (Ringwood et al. 2004).

At relatively high oyster densities, demographic rates may become density-dependent, such that growth rates and survivorship decrease with increasing density (Puckett and Eggleston 2012). In the present study, there were no significant differences in growth and survivorship between natural and cultch-planted reef types, despite significantly different densities. In this study, small sample size for modal analysis (see above) may explain why no differences in growth or survivorship were observed. It is likely that oyster densities in fished sites are too low to detect any differences in growth and survivorship between reef types, or as a function of increasing oyster density (Puckett and Eggleston 2012). It should be noted that growth measured in this study represents two-dimensional increase in shell length. Biomass growth may vary between salinity regimes, however, oyster biomass was not measured in this study.

**Potential Larval Output from Fished Reefs versus Marine Reserves**

Mean reproductive output (eggs/m$^2$) of natural reefs in PS is 220,000 eggs, whereas mean reproductive output from no-take reserves in PS is 14.2 million eggs/m$^2$ (Mroch et al. 2012, this study). This ~2 orders of magnitude reduction in potential reproductive output in
fished areas is attributable to low density and lack of large oysters at natural reefs. The potential for reserves to produce greater larval output per unit area has also been well documented for other species such as Queen Chonch (*Strombus gigas*) and Copper Rockfish (*Sebastes caurinus*), where reserve outputs were 1-2 orders of magnitude higher than nearby fished populations, respectively (Stoner and Ray 1996, Palsson and Pacunski 1995).

Estimation of potential larval output per square meter may be refined by considering other factors that can modify the production of larvae, such as fertilization success of eggs by sperm, and the health of a given oyster (Levitan et al. 1992, Ringwood et al. 2004). For instance, fertilization of broadcast spawning organisms is likely density dependent, thus reducing the probability of larval development at low density reefs (Levitan et al. 1992). Furthermore, oyster health may vary on small spatial scales, impacting reproductive potential (Ringwood et al. 2004, Ringwood, unpubl. data). Reef age may also be an important consideration for estimating larval output based on population density. For example, recent total density estimates at PS reserves suggest a population decline since the reported findings of Puckett and Eggleston (2012). Reduced population density would likely reduce per-square meter larval output and subsequently total output estimates of larval output from reserves.

Another key finding in this study was the apparently large relative contribution of natural reefs to the Pamlico Sound larval pool, despite low oyster density and subsequently low potential larval output per square meter. In a similar study on coral trout (*Plectropomus maculates*) in Great Barrier Reef, degraded fished reefs, which composed 72% of the total habitat area, supplied ~51% of total recruitment to the metapopulation (Harrison et al. 2012). This compares favorably to the results from the present study, in which fished reefs comprise...
~97% of total area and contribute ~68% of all larvae to the larval pool. Both studies emphasize the importance of a large fished habitat footprint area to maintain a sizeable larval contribution to the metapopulation.

In conclusion, this study has two important implications. First, fished areas have great potential to provide a larval supplement to a network of no-take reserves, which may facilitate reserve growth. Thus, it is important to identify if specific fished areas serve as population sources for the reserves, so that these areas might receive enhance protection. Second, this study highlights the efficacy of no-take reserves in preserving population density and size structure of protected species. As a result, larval output/m² can be as high as 2 orders of magnitude greater than fished reefs.
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Winslow F (1886) Report on the waters of North Carolina, with reference to their possibilities for oyster culture; together with the results of the surveys directed by the resolution of the general assembly, ratified March 11, 1885. Raleigh: North Carolina State Printer and Binder. 151 p.

Table 1. Growth rate (mm d\(^{-1}\)) estimates from modal progression analysis of fifteen harvested oyster reefs in Pamlico Sound, NC, USA. In instances where population density was insufficient to perform modal analysis, no growth rate is reported.

<table>
<thead>
<tr>
<th>Site</th>
<th>Spring '12</th>
<th>Fall '12</th>
<th>Winter '12-'13</th>
<th>Spring '13</th>
<th>Fall '13</th>
<th>Mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>1(_{b,2})</td>
<td>0.07</td>
<td>0.2492</td>
<td>0.0737</td>
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<td>X</td>
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<td>3(_{b,2})</td>
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<td>0.163</td>
<td>0.157±0.099</td>
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<td>4(_{b,2})</td>
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<td>X</td>
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<tr>
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<td>0.099</td>
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<td>X</td>
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<td>X</td>
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<td>0.025</td>
<td>0.1765</td>
<td>0.102±0.076</td>
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<td>7(_{b,2})</td>
<td>0.0332</td>
<td>0.0816</td>
<td>0.0168</td>
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<td>0.006</td>
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<td>21(_{b,1})</td>
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<td>26(_{a,2})</td>
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</table>

Mean ± SD: 0.094±0.062 0.214±0.180 0.060±0.032 0.118±0.107 0.079±0.066


33
Table 2. Percent survivorship estimates from modal progression analysis of fourteen oyster reefs in Pamlico Sound, NC, USA. In instances where population density was insufficient to perform modal analysis, no survivorship estimate is reported.

<table>
<thead>
<tr>
<th>Site</th>
<th>Spring '12</th>
<th>Fall '12</th>
<th>Winter '12-'13</th>
<th>Spring '13</th>
<th>Fall '13</th>
<th>Mean ± SD</th>
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<td>0.410±0.315</td>
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<td>X</td>
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<td>0.464±0.270</td>
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<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>0.3585</td>
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Mean ± SD 0.615±0.208 0.651±0.202 0.370±0.268 0.517±0.240 0.483±0.211

Figure 1. Reported locations of natural and cultch-planted oyster reefs in Pamlico Sound, North Carolina, USA. Data compiled from North Carolina Division of Marine Fisheries’ geospatial database for Pamlico Sound oyster habitat, including relic oyster reef, commercial fishing coordinates, and reefs mapped by the NCDMF. Red patches represent NCDMF mapped reefs, though not ground-truthed for exposed material. Black points are representative of unmapped oyster reef coordinates. Blue crosses designate cultch-planted reefs created between 2005 and 2011, or otherwise deemed still present (C. Caroon, NCDMF, pers. comm.). Green patches depict historical natural oyster reefs, as surveyed in Winslow (1886).
Figure 2. Study sites in Pamlico Sound, NC. East/West section designation based on a line drawn from Neuse River, NC, northeastward to Wanchese, NC to denote relatively low (10-18 psu) versus high (18-26 psu) salinity areas in Pamlico Sound (Durham 2009). Cultch-planted oyster reefs are identified by black triangles and natural oyster reefs by black circles. Site numbers are adjacent to the symbol they represent.
Figure 3. Mean oyster reef footprint area by region in Pamlico Sound (Figures 1 & 2). Area is recorded in hectares and error bars represent standard error of the mean.
Figure 4. Mean oyster density (oysters/m²) for a) total (all size-classes), b) legal (LVL≥75mm), c) sub-legal (25mm≤LVL<75mm), and d) recruit (LVL<25mm) size classes for natural versus cultch-planted oyster reefs. Error bars designate standard error. Significant differences are denoted by an asterisk (*). See text for results of statistical rests.
Figure 5. Mean oyster density (oysters/m²) for a) total (all size-classes), b) legal (LVL≥75mm), c) sub-legal (25mm≤LVL<75mm), and d) recruit (LVL<25mm) size classes for oyster reefs in eastern and western PS. Error bars designate standard error. Significant differences are denoted by an asterisk (*). See text for results of statistical tests.
Figure 6. Mean oyster density (oysters/m²) for a) total (all sizes), b) legal (LVL≥75mm), c) sub-legal (25mm≤LVL<75mm), and d) recruit (LVL<25mm) size classes for six sampling times during May, August, and October 2012 and June, August, and October 2013. Error bars designate standard error. Different letters indicate significant differences between months as determined with a Tukey-Kramer multiple comparison test. See text for results of statistical tests.
Figure 7. Mean growth rate (mm d⁻¹) for fifteen sites, pooled across a) time, b) region, and c) reef type. Time comparison is based on five time periods (between six sampling events) of spring 2012, fall 2012, winter 2012-2013, spring 2013, and fall 2013.
Figure 8. Mean percent survivorship for fourteen sites, pooled across a) time, b) region, and c) reef type. Time comparison is based on five time periods (between six sampling events) of spring 2012, fall 2012, winter 2012-2013, spring 2013, and fall 2013.
Figure 9. Mean population density (per square meter) of a.) total, b.) legal, c.) sub-legal, and d.) recruit size class oysters on harvested reefs (natural and cultch-planted, this study) compared to reserve sites (Puckett and Eggleston 2012). Error bars represent standard error.
Figure 10. Example length-frequency distributions of a) natural (site 2), b) cultch-planted (site 11), and c) reserve (Ocracoke, Puckett and Eggleston unpub. data) reef types during May 2012 (natural and cultch-planted) and June 2006 (reserve). Mean frequency is reported in oysters/m² and size class designations are established at 5mm. A vertical dotted line at 75mm represents a cutoff for legally (to the right) and non-legally (to the left) harvested oysters based on local regulation.
Figure 11. Mean contribution (±SE) of oyster larvae to the PS larval pool by natural, cultch-planted, and broodstock reserve reef types in terms of a) maximum potential larval output per square meter, b) total larval output for each reef type within PS, and c) total output of combined fished reefs versus reserves. Adjusted larval output bars in graphs b. and c. represent modified output based on ground-truthed observations of existing natural reef (see Results, Ground Truthing and Reef Mapping). Larval output per square meter is reported as number of eggs in millions and total output is reported as number of eggs in trillions.
c. Total Larval Output of Fished Reefs vs. Marine Reserves

- **Reef Type**
  - Fished
  - Fished (Adjusted)
  - Reserve

- **Larval Output (#eggs x 10^12)**
  - 0
  - 10
  - 20
  - 30
  - 40
  - 50
  - 60
  - 70

- **Mean Potential Larval Output (m^2)**

- **Reef Type**
  - Natural
  - Cultch-Planted
  - Reserve

- **Larval Output (#eggs x 10^6)**
  - 0
  - 2
  - 4
  - 6
  - 8
  - 10
  - 12
  - 14
  - 16
  - 18
  - 20
APPENDIX
APPENDIX 1

All photographs and videos for this study may be seen at

https://sites.google.com/a/ncsu.edu/peters2014/

a. Examples of substrate materials from oyster reef excavations for western (left) and eastern (right) Pamlico Sound oyster reefs. Quadrat samples represent 1m$^2$ and are typical of substrate abundance at reefs in their respective region.
b. **Western Pamlico Sound Natural Reef Composition.** Shell hash, scattered whole shells, and scattered live oysters typical of natural oyster reefs in western Pamlico Sound, North Carolina, USA. Photograph was taken at site 2, near the Neuse River mouth, in May 2012.
c. **Eastern Pamlico Sound Natural Reef Composition.** Example of natural oyster reef in Eastern Pamlico Sound, North Carolina USA, characterized by large, partially eroded oyster shells and scattered live oysters. Photograph was taken at site 13, near the Buxton, North Carolina, in August 2012.
d. Western Pamlico Sound Oyster Morphology. Example of oyster shells from cultch-planted oyster reefs in western Pamlico Sound, North Carolina, USA, and characterized by rounded and smooth oysters with thick, dense composition compared to their eastern Pamlico Sound counterparts.