

DISTRIBUTION PATTERNS AND SELECT LIFE HISTORY CHARACTERISTICS OF
OSTREA EQUESTRIS (SAY 1834) IN SOUTHEASTERN NORTH CAROLINA

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ABSTRACT

Ostrea equestris (crested oyster) is a relatively unknown native oyster along the Atlantic coast. Originally considered a subtidal species, recent observations indicated that there may be significant intertidal populations in southeastern North Carolina (the northern extent of its known range). This study evaluated distribution patterns among various habitat types in southeastern North Carolina, as well as select life history characteristics (condition index and reproductive period). Eleven habitat types common to this area were sampled at five sites from Long Bay to Core Sound, NC. All sites and habitats were sampled in 2008 to assess spatial distribution of populations using transects/ quadrats or subtidal dredges. Select habitats were resampled in 2009 to evaluate the temporal stability of previously observed patterns. *Ostrea equestris* was present at all sites and in almost all habitats sampled in 2008. The UNCW Research Lease and Middle Marsh (among the southern-most sites and northern-most sites, respectively) had significantly higher densities than the other sites, suggesting an abundance cline did not exist for this area. Among habitats, floating docks, low intertidal oyster reefs, and low intertidal shell hash had the highest densities, while the subtidal dredges had the lowest. Densities differed significantly among sites for various habitats. *O. equestris*, while initially thought to be uncommon, comprised up to 100% of live oyster seen in some areas. However, coefficient of variance data indicated that all populations were extremely patchy in their distributions. Densities in most cases increased from 2008 to 2009 and populations were less patchy in 2009. This though may be an artifact of non-random site selection in 2009. Condition indices for *Ostrea equestris* were sampled for 3 seasons (summer, winter, and spring) at three sites, and showed significant differences by site and season. Reproductive samples were collected April- November at three sites. There was a peak in reproductive output (as indicated by the presence of brooded larvae) in July, though no significant differences were observed among sites for larval counts. At most only 3% of the oysters were found to be brooding at a site. This study indicates that *Ostrea equestris* is more common than previous literature would indicate, comprising 50-100% of the live oyster

in some habitats. The densities that have been found to occur in this study indicate that not only are there meaningful populations farther north than previously indicated, but in habitats not previously described. As a result, it is probable that these oysters may play a larger ecological role than previously thought because of their relatively high abundances, especially on floating docks and in areas of shell hash.

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INTRODUCTION

Crassostrea virginica (the eastern oyster) is the most conspicuous of North Carolina's native oysters; however the crested (or horse) oyster (*Ostrea equestris*, Say 1834) is found in many of the same environments but is rarely noted. While there is considerable literature on the eastern oyster due to its economic, ecological, and historical importance, not much is known about the smaller, cryptic *O. equestris*.

The reported distribution of *Ostrea equestris* stretches from North Carolina to the Gulf of Mexico and the West Indies (Galtsoff and Merrill 1962; Walker and Power 2001; Abbott 1974). It has been reported as far south as Brazil (Abbott 1974) and Argentina (Harry 1985). There have been reports of *O. equestris* occurring as far north as Delaware (Galtsoff and Merrill 1962), but these were isolated observations. *O. equestris* has been reported in high numbers offshore particularly on shipwrecks, buoys, and drilling platforms in the Gulf of Mexico (Gunter 1951; Wells and Gray 1960; Merrill 1961). It is thought to be a strictly subtidal stenohaline species found in waters with a minimum salinity tolerance of 20-25 ppt (Menzel 1955; Galtsoff and Merrill 1962; Gunter 1951) and found in areas of low turbidity (Menzel 1955). In general *Ostrea* spp. tend to exhibit a patchy distribution and survive better in higher salinity areas (Baker 1995; Menzel 1955; Feeny 1983), their salinity tolerances limiting their intrusion into estuaries (Gunter 1951). Hoese (1960) indicated that salinity tolerance may be a primary factor in excluding this oyster from an area. Population distribution may also be influenced by their feeding ability, as *Ostrea* species are poorly adapted to feeding in turbid areas (Menzel 1955). However, this was only postulated based on one series of experiments and never proven in the field.

Ostrea equestris is a larviparous (brooding) species that may be reproductively active year round in warmer waters (Walker and Power 2001). In cooler areas, the active reproductive

period may be more seasonal. Like other *Ostrea* species, *O. equestris* starts spawning at cooler water temperatures (20° C) compared to *Crassostrea virginica* (25° C) (Menzel 1955; Walker and Power 2001). This leads to a prolonged spawning season within the Gulf of Mexico and Georgia, with larvae potentially present from April to October/ November (Walker and Power 2001; Menzel 1955), and settlement occurring within the same time period (Menzel 1955).

Ostrea equestris may develop gametes within a month of settlement, taking 26 days to produce sperm and 33 days for ova (Menzel 1955). Males can be reproductively active at 5.5 mm and females at 8.5 mm (Menzel 1955). Though initially thought to be protandric (like most other oyster species), the crested oyster is actually a sequential hermaphrodite, changing sex multiple times throughout its life, and potentially within a breeding season as well (Menzel 1955). It is possible that the crested oyster may develop as female in the first phase rather than as a male as would be expected based on other oyster species (Menzel 1955). Both Menzel (1955) and Walker and Power (2001) have shown the majority of the populations are male, with <10% female presence and only 3-15% of total females have been shown to be actively brooding at a given time (Walker and Power 2001; Menzel 1955 respectively). The large number of males in these populations is most likely density dependent, as the closer the oysters occur together the higher the proportion of males (Menzel 1951 and Buroker 1983 in Walker and Power 2001). In some cases those oysters in a transitional state have had both sperm and eggs within histological sections, as well as larvae (Menzel 1955). Despite this transitional state there has been no documentation of self- fertilization.

Larval numbers of *Ostrea* spp. are almost an order of magnitude lower than those of *Crassostrea* spp. Individual specimens of *Ostrea* spp. have been shown to produce 250,000 larvae per spawning event (Peter-Contesse 2005; Baker 1995). To put this in perspective, specimens of *Crassostrea* spp. can produce up to 50,000,000 gametes (Menzel 1955; Kennedy 1996). The eggs of *Ostrea* spp. are much larger than those of *Crassostrea* spp.; most likely reflecting differences in reproductive strategy (brooding vs broadcast spawners) (Menzel 1955).

While very little information is available on the specific reproductive strategy of *O. equestris*, there is information from related species that may provide some insight into the potential larval production and timing of *Ostrea equestris*. Korringa (1941) demonstrated that *Ostrea edulis* retained fertilized eggs in the branchial chamber until the larvae were at an advanced stage of development; anywhere from 6-18 days (Waller 1981) or until they reach 165-200um (Korringa 1941) depending on temperature. This has been shown to be true for *Ostrea lurida*, as well (Peter-Contesse 2005; Baker 1995). Larvae of *Ostrea* spp. have been shown to have a residence time in the water column that ranges from 2 to 8 weeks (Menzel 1955; Baker 1995; Peter-Contesse 2005), depending on water temperature. For *Ostrea edulis* the average has been up to 17 days (Waller 1981; Korringa 1941), and *O. lurida* has been shown on average to take 10-14 days (Peter-Contesse 2005). Oysters will set on both the top of hard surfaces and the underside as well, especially remnant shell of conspecifics (Baker 1995; Merrill 1961; Menzel 1955; Trimble *et al.* 2009). *Ostrea* spp. larvae settle on remnant shell (Trimble *et al.* 2009) and those shells of other oyster species (Polson and Zacherl 2009). The larvae of the crested oyster have been shown to exhibit a negative phototaxic response in the water column (Baker 2003) following a diel vertical migration.

Ostrea spp., especially *Ostrea lurida*, tend to settle close to the parent populations (Baker 1995). This is most likely the result of their brooding life history. Populations are patchy and can sometimes occur hundreds of kilometers apart (Baker 1995). These populations of *Ostrea lurida* are genetically distinct, likely related to the tendency to settle close to parent populations (Stick *et al.* 2009). *Ostrea equestris*, however, seems to exhibit modest levels of genetic structuring with no genetic break between the Gulf Coast and Atlantic populations (Kirkendale *et al.* 2004) and has a potentially global distribution (Shilts *et al.* 2007).

Ostrea equestris overlaps with *Crassostrea virginica* throughout much of its known range, and co-occurs with *Ostrea frons* and *Ostrea permollis* (Galtsoff and Merrill 1962; Gunter 1951) in the Gulf of Mexico. In areas of co-occurrence with the eastern oyster, stratification of

settlement tends to occur. *Ostrea equestris* tends to set lower on pilings and the lower edges of *C. virginica* reefs. In some cases *O. equestris* has been shown to completely replace *C. virginica* assemblages in periods of extreme drought with extremely high salinities (>40ppt) (Hoese 1960; Parker 1960 in Galtsoff and Merrill 1962), though this is a rare phenomena. Hoese (1960) also indicated complete mortality of crested oyster assemblages when the salinity rapidly dropped at the end of the drought.

Preliminary observations (Markwith, Alphin, and Posey, personal observations) over the past five years indicate *Ostrea equestris* can be found intertidally in several environments in North Carolina, with average densities as high as 66.25 m² in certain habitats. Preliminary surveys indicated that this oyster may be more common than once thought, forming small dense aggregations (5-25 oysters per aggregation) in areas where other native oysters are less common and they may constitute a significant portion of the total bivalve counts for some oyster reefs.

O. equestris occurs both individually and in groups on rock, shell, and rubble (Menzel 1955), and has also been found within the interstitial spaces of existing *C. virginica* reefs (Markwith, personal observation). Crested oyster spat will settle in relatively large numbers on live horseshoe crabs, live and dead scallop shells, and hermit crab shells (Gutsell 1926; Gunter 1951). Upon settlement *Ostrea equestris* will reach 2/3 of its approximate adult size within a month (Menzel 1955). On average this is no larger than 25-40mm, dependent upon the location and degree of crowding (Walker and Power 2001; Menzel 1955), although there are reports of larger individuals: 72mm from the Gulf of Mexico (Gunter 1951) and 82mm from the southwestern Atlantic (outside Charleston, SC) (Galtsoff and Merrill 1962; Merrill 1963). *Ostrea equestris* does not form reefs (Galtsoff and Merrill 1962; Walker and Power 2001), and little is known about its ecosystem impacts or interactions with other species.

In recent years, some attention has been focused on *Ostrea equestris* as a potential intermediate host for the novel haplosporidian parasite *Bonamia* sp. In an effort to restore oyster populations to the Chesapeake Bay and in some areas of North Carolina, *Crassostrea ariekensis*

(the Suminoe oyster, a non-native from China) was considered for potential introduction and aquaculture. Experiments with caged *C. ariekensis* in North Carolina showed high mortality (up to 100%), of juveniles (<40mm), due to *Bonamia* sp. at several locations (Carnegie *et al.* 2006, 2008; Bishop *et al.* 2006; Burreson *et al.* 2004; Alphin *et al.* 2006). This was the first known observation of this parasite within North Carolina waters. It was initially proposed that *O. equestris* was a reservoir for *Bonamia* sp., although small *Crassostrea virginica* was found to test positive by PCR for the presence of the parasite (Wilbur *et al.* 2008). However, infections of *C. virginica* by *Bonamia* sp. have not been confirmed histologically. As a result of these findings (and other concerns), *C. ariekensis* is no longer being considered for aquaculture in North Carolina.

The potential for *O. equestris* to be a parasite reservoir emphasizes the lack of information and understanding related to the life history and ecology of *Ostrea equestris*. Prior to the current interest in *O. equestris*, much of the work was published pre-1965. Based on the preliminary population surveys mentioned above it can be suggested that the crested oyster may be much more common than initially thought, raising questions about current populations and abundances. Not only might it be present in the intertidal (though literature suggested it was primarily subtidal), it can also be part of the lower reef complex in high salinity waters (Galtsoff and Merrill 1962; personal observations). *O. equestris* may also constitute a large part of the community found on subtidal shell in areas adjacent to where it has been found intertidally. As a result, there may be direct and indirect interactions, such as discussed by Wootten (1994), between *Ostrea equestris*, *Crassostrea virginica*, and other species (oyster and otherwise) within the ecosystem.

While the majority of literature is from the Gulf Coast of the United States, this study focuses on southeastern North Carolina as it is considered the northern extent of the crested oyster's range. Eleven habitats, common to most of the southern Atlantic and Gulf Coasts, were differentiated (subtidal to 5-10 ft; fouling community on floating docks; shallow subtidal/low

intertidal and mid intertidal oyster reefs; shallow subtidal/low intertidal and mid intertidal shell hash (20-40% and >75% shell cover); shallow subtidal/low intertidal and mid intertidal sand; and seagrass beds, when present). Subtidal habitat was sampled because of the previous literature reports defining *O. equestris* as purely subtidal (Menzel 1955; Galtsoff and Merrill 1962). Fouling community habitats were mentioned in the literature (Merrill 1961 and 1963; Gunter 1951) and *Ostrea equestris* were found on docks in marinas at Wrightsville Beach, North Carolina (Ami Wilbur, pers. communication). Oyster reefs were sampled because the co-occurrence of the two native species is well documented (Menzel 1955; Gunter 1951; Gutsell 1926; Hoese 1960; Wells 1961; Wells and Gray 1960; Galtsoff and Merrill 1962). Various percent cover of shell hash (mostly free of live *Crassostrea virginica*) was sampled to look for possible effects shell cover. *Ostrea lurida* have been shown to recruit to small patches and individual pieces of shell (Baker 1995). *O. lurida* have also been shown to occur on shell hash within seagrass beds (Baker 1995; Archer 2008). This coupled with the presence of *O. equestris* on dead and live scallops (Gutsell 1926; Cassie Martin and Mark Sherman, pers. communication) which utilize seagrass habitat, indicated it should be sampled where present.

With the potential ecosystem importance of *Ostrea equestris*, it is necessary to obtain more information on distribution and relevant life history attributes of this species. This study seeks to fill key gaps in our understanding of *O. equestris*. Distribution among various habitats and the relative density of populations at various locations within southeastern North Carolina was studied. Reproductive period (using brooded larvae as a proxy) and condition index were also determined for a subset of populations to provide initial information on life history for this region.

Given the objectives of the study, and the above general information, it was hypothesized that:

- 1- Population numbers would decline at the more northerly sites
- 2- Population densities should be greater subtidally than intertidally.

- 3- Intertidally, most populations should be found in the low intertidal, as opposed to the mid, due to differences in exposure time due to tides.
- 4- Populations will be relatively patchy based on known life history characteristics.
- 5- Reproduction should be more limited than in GA or TX studies, most likely occurring May through October, with a possible peak in late summer.

METHODS

Field sites

Sites from Long Bay (border of North and South Carolina) through Core Sound (Cape Lookout region) were sampled, as this is the known northern extent of the range for *Ostrea equestris* (except for a few isolated occurrences (Galtsoff and Merrill 1962)). This area was broken down according to the shellfish growing area maps from North Carolina Shellfish Sanitation, which are based on specific areas within large bodies of water. The sites chosen were used as they had been delineated by North Carolina Division of Marine Fisheries based on key coastline characteristics, and many are mapped or are being currently mapped for bottom characteristics. All sites contained most, if not all, of the habitat types sampled (Table 1).

A total of 11 habitat types were sampled: subtidal to 5-10 ft; fouling community on floating docks (FD); shallow subtidal/low intertidal and mid intertidal oyster reefs (LIOR and MIOR); shallow subtidal/low intertidal and mid intertidal shell hash (20-40% and >75% shell cover) (SH40L/M and SH75L/M); shallow subtidal/low intertidal and mid intertidal sand (LIS and MIS); and seagrass beds (SG), when present. For this study, low intertidal was defined as areas exposed only on negative low tides, while mid intertidal was considered areas exposed most low tides. Specific locations included sites where *O. equestris* was preliminarily observed to exist (i.e. UNCW Research Lease (CL), Bogue Sound (BS), New River (NR)), as well as two areas at more extreme ends of the southeastern North Carolina coast (i.e. Lockwood Folly River

(LF), Middle Marsh (Rachel Carson National Estuarine Research Reserve) (MM)) (Figure 1). All sites had salinities of 25 ppt or greater, and similar tidal ranges.

Sampling occurred May 2008 through December 2008/ January 2009 for all parts of the project. A second season of sampling for a subset of habitats/sites (May 2009 through July 2009) was done to evaluate temporal variation in distribution patterns. Of the five sites sampled, three (Lockwood Folly River, UNCW Research Lease, and New River) were chosen for continuous sampling for life history characteristics (condition indices and reproductive period). Life history characteristics were sampled during 2008 and beginning of 2009. Water quality measurements (salinity, dissolved oxygen, temperature) were taken with a handheld YSI at each site each time samples were collected.

Distribution

Subtidal habitats were trawled a total of 10 times per site using a small bottom dredge (46x 7.5 x 25.5cm). Each tow was kept consistent at 900rpms for one minute. Both the start and end latitude/longitude were recorded so that the amount of area covered by the tow could be determined. The length of each tow was determined by converting the latitude and longitude to decimal degrees, converting the decimal degrees to meters, and then multiplying this number by 0.46m (the width of the dredge) to standardize the densities. These numbers were then adjusted to a density per 0.25m² to allow comparison to densities obtained from quadrat sampling. Bottom type and amount of material (qualitative measure based on percent of a 5 gallon (18.9L) bucket filled) pulled were determined from the contents of pull.

Intertidal and shallow subtidal habitats were sampled with modified line transects. Transects were 50m long and established parallel to tidal lines. Every 2m a survey was done for a total of 26 pairs of observations, in each case noting presence or absence of *Ostrea equestris*. Each time *O. equestris* was observed a 0.5m X 0.5m quadrat was used to determine densities and size. Two transects were done for each habitat at a site. However, only a total of 16 quadrats

Table 1: List of habitats by site. Habitats are listed by abbreviations as indicated within the text. Plus and minus symbols indicate presence or absence of a habitat from the particular site.

Habitat	Site				
	Lockwood Folly	UNCW Research Lease	New River	Bogue Sound	Middle Marsh
LIOR	+	+	+	-	+
MIOR	+	+	+	+	+
SS40L	-	+	-	+	+
SS40M	+	-	+	+	-
SS75L	+	+	+	+	+
SS75M	+	-	-	+	-
LIS	+	+	+	+	+
MIS	+	+	+	+	+
FD	+	+	+	+	+
Subtidal	+	+	+	+	+
SG	-	-	+	+	+

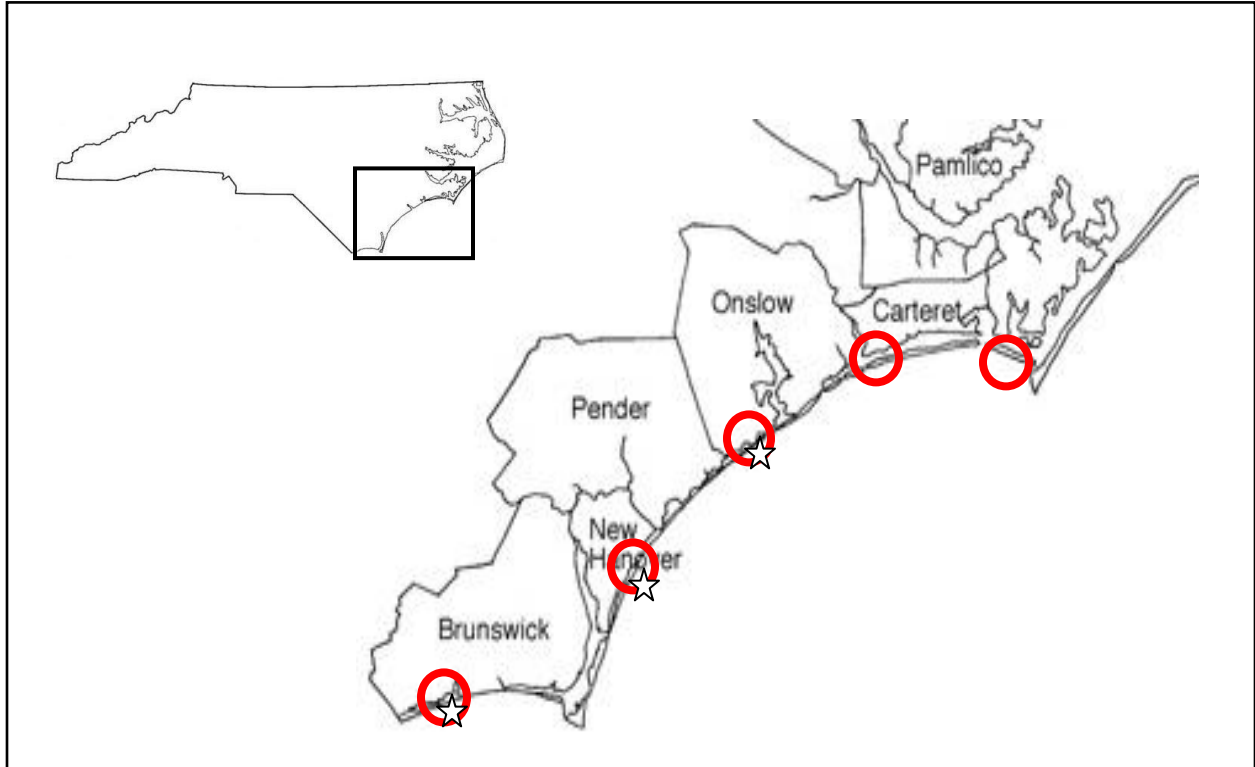


Figure 1: Map of southern and central coastal counties in North Carolina. Circled areas indicate sites sampled, while those starred are recurrent sites (used for life history studies). From south to north the sites are as follows: Lockwood Folly River, UNCW Research Lease, New River/Chadwick Bay, Bogue Sound, and Middle Marsh. (Picture taken from North Carolina Department of Environment and Natural Resources: dcm2.enr.state.nc.us/handbook/sction1.htm).

(8 points per transect: 4 interior and 4 edge.) were taken per site for the oyster reef habitat types. Edge was distinguished from interior because initial observations indicated *O. equestris* seems to occur mainly on the edges of the reefs.

Fouling communities were sampled using modified quadrat sampling (20 X 20 cm). These communities were located on floating docks within several locations per site. A total of 15 random points were sampled if available. At most 5 quadrats were taken from a distinct series of floating docks in order to lower the risk of pseudo-replication (at least 3 or more floating dock complexes were sampled per site). Numbers were standardized to a density of 0.25m² for comparison to the other habitats.

The crested oyster (adult size range 35-55mm) is often hard to distinguish from juvenile *C. virginica*. Based on external characteristics such as crenulations of the shell, cup depth, and in some cases shell color, *O. equestris* was reliably identified nearly 90% of the time in early trials (personal observations). Accuracies of 98% were later achieved during the course of the study. The dentitions on the inner hinge were used as a diagnostic characteristic to verify identifications (Galtsoff and Merrill 1962; Menzel 1955), but results in mortality of the individuals being examined. *Crassostrea virginica* do not have dentitions on their inner hinges. Kirkendale *et al.* (2004) indicated that *Ostrea equestris* multiple morphologies concerning the presence of crenulations, and they can occasionally lack dentitions (Harry 1985), potentially making density estimates conservative from this study.

In the interest of time and accuracy all samples were brought back to the lab for processing, which included identification of scars, articulated boxes, and live *Ostrea equestris* within each quadrat. The number of *C. virginica* was also noted within each quadrat.

Size distribution

The heights (straight line from umbo to furthest point) of all *O. equestris* within each quadrat were measured using digital calipers. This allowed for measurement of size distributions within each quadrat.

Condition index

Oysters were collected from the three recurrent sites (UNCW Research Lease, New River/ Chadwick Bay, Lockwood Folly River) for three seasons (spring (May), summer (August), and winter (January)). Twenty oysters were collected from each habitat where present. Samples were collected from same areas within sites each time. *O. equestris* was separated from the aggregates into individuals, and then measured for shell height and width, weight of the whole oyster including shell, wet and dry weights of soft tissue, and weight of the empty shells. The soft tissue was dried for 24 h at 70°C to obtain dry weight. Condition index was calculated using a version of Hopkins formula (Austin, et al 1993; Lawrence and Scott 1982), which uses the soft tissue dry weights and internal shell volume:

$$CI = [(dry\ weight\ of\ meat) * 100] / [weight\ of\ live\ oyster\ in\ shell - weight\ of\ empty\ shell].$$

This is a commonly used measure of oyster health, and though the original formula uses internal shell volume, the difference in shell weights approximates the volume of the oyster. This method assumes that there are no size, site, or habitat differences in shell thickness.

Reproductive period

Additional oysters were taken from Lockwood Folly River, the UNCW Research lease, and New River on a biweekly basis (April through October) to determine when oysters were reproductively active. Twenty oysters per site were collected and preserved in alcohol for later examination. These were examined for the presence of brooded larvae. Each oyster's gills

(branchial chamber) were agitated and washed using DI water to dislodge larvae. All liquid was sieved through a 32um sieve, and solids were re-suspended in 40 ml of DI water. The presence of brooded larvae was confirmed by microscopic examination, and numbers quantified using a Sedgwick-Rafter gridded (1 x 1mm) cell. For each sample that contained larvae, triplicate 1mL samples were counted and averaged. Larval stage and quantification procedures were based on Carteret Community College Hatchery procedures (Kemp *et al.* 2006). Height and width was taken for each oyster using digital calipers to be able to correlate oyster size with larval presence or absence and numbers.

Statistical analysis

All statistical analysis was done using PC-SAS. The one-way and two-way ANOVAs were calculated to determine significant patterns. When ANOVAs indicated significant overall treatment effects, post-hoc SNK tests were used to determine specific differences.

Distribution

Distribution data was analyzed by shared time period and habitat types: 2008 data, common habitats between years for sites, oyster reefs between years (including edge and interior), and floating docks. Mean densities were calculated by site, habitat, and year (where applicable). Mean densities were also calculated for the edge and interior of the oyster reefs. Means were also calculated for patch densities (densities only where *Ostrea equestris* occurred). For all data categories coefficients of variation were calculated as a means of determining the patchiness of the populations. The higher the coefficient of variation, the greater the patchiness of the population.

Tests for homogeneity of variances (PC-SAS calculates Levene's, Bartlett's, and Brown and Forsyth) indicated that the data needed to be log-transformed before ANOVAs were run. The coefficients of variation were also log-transformed prior to analysis. For the 2008 data a

two-way ANOVA was calculated among sites and habitats. Since the interactive term was significant, two one-way ANOVAs were calculated, among sites and among habitats separately. For the common habitats and oyster reef densities, three-way ANOVAs were calculated with year as the additional variable. If there was a significant interaction, then the appropriate one-way ANOVAs were calculated (i.e. among sites or habitats for year or among years for either site or habitat). For oyster reefs, one-way ANOVAs were calculated among sites by year and intertidal location or interior vs edge. All above analyses were also calculated on the patch density data. Densities were also calculated based on the presence of *Ostrea equestris*, allowing for patch abundances to be observed. An increase in patch size density, relative to the overall density, potentially indicates patchier populations.

Spearman's ranked correlations were calculated. This type of correlation was used due to the differences in sampling methodology for the subtidal and the floating docks. These examined the number of live *O. equestris* to live *C. virginica*, and live *O. equestris* to scars of *O. equestris*.

Size

Mean, maximum, and minimum sizes were calculated by site, habitat, and year (where applicable). A two-way ANOVA was calculated examining site and habitat differences by year. If the interaction was significant (as it was for the 2008 data), a one-way ANOVA was calculated among sites and among habitats independently. One-way ANOVAs were calculated among sites and habitats by year (looking at differences by habitats or sites respectively). One-way ANOVAs were also calculated amongst years by site and habitat.

Condition Index

Mean condition indices were calculated for season, site, and habitat. Three-way and two-way ANOVAs weren't calculated for the condition data due to the unequal N (habitat types weren't consistent across sites). One-way ANOVA's were run among sites by habitat and by

season. One-way ANOVAs were also run among habitats by site and by season, where applicable. A Pearson's correlation was run to look at the relationship between height and condition index values. Pearson's was used instead of a Spearman's ranked correlation because there was no difference in sampling methodology.

Reproductive period

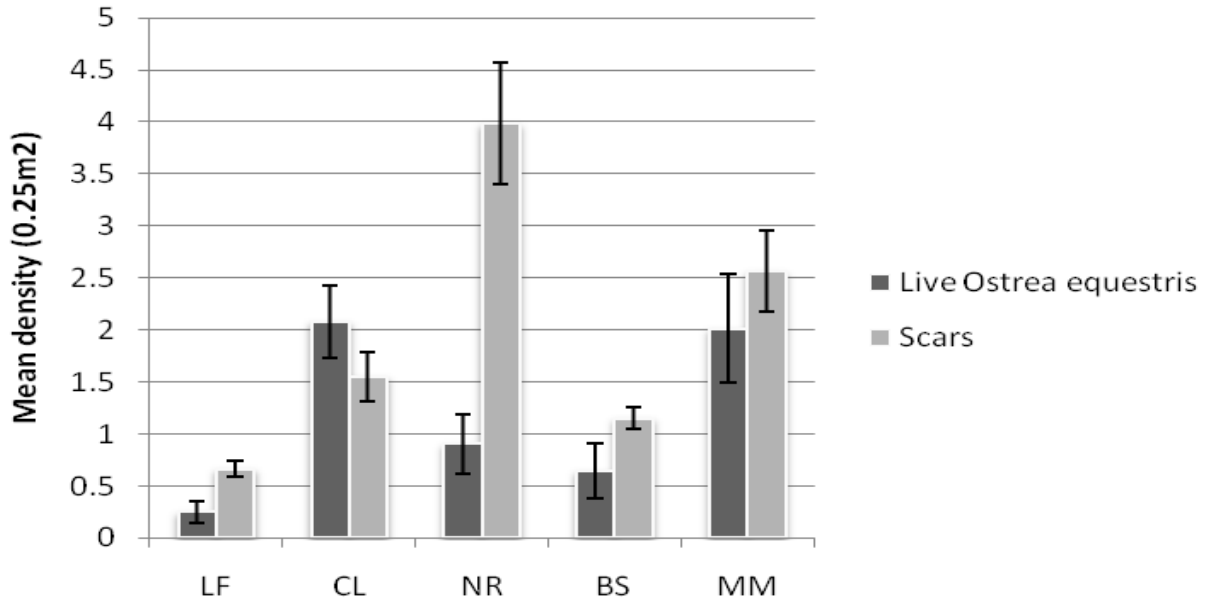
Twelve *Ostrea equestris* (N=4 per site) contained brooded larvae. Due to this small number reproductive condition could not be compared accurately between sites among time periods. A one-way ANOVA was run to compare the average number of larvae between sites over the entire time period. Mean height of the oysters and larval counts were calculated when brooded larvae occurred. Frequencies (number of oysters with larvae divided by total adults collected) by time period and sites were calculated for larvae presence. A Pearson's correlation was run for height and average number of larvae (where present). Pearson's was used instead of a Spearman's ranked correlation there was no difference in sampling methodology.

RESULTS

Density distributions

Though *Ostrea equestris* has been reported to be a relatively rare subtidal species (Menzel 1955; Galtsoff and Merrill 1962; Gunter 1951), site and habitat densities from this study indicate that *O. equestris* is found consistently over a wide spatial scale within the northern extent of its range. Live *Ostrea equestris* was found within every site sampled (Figure 2a), though densities in some locations were relatively low. It was also found within just about every habitat sampled (Figure 2b), the exception being SS40M. In almost all areas sampled, the number of total dead (scars and articulated boxes for *Ostrea equestris*) was greater than the number of live *O. equestris* (Figures 2a and 2b). The majority of dead crested oysters were scars, while very few were represented by boxes. Scars were 73.3% (in Middle Marsh) to 100% (in

a)



b)

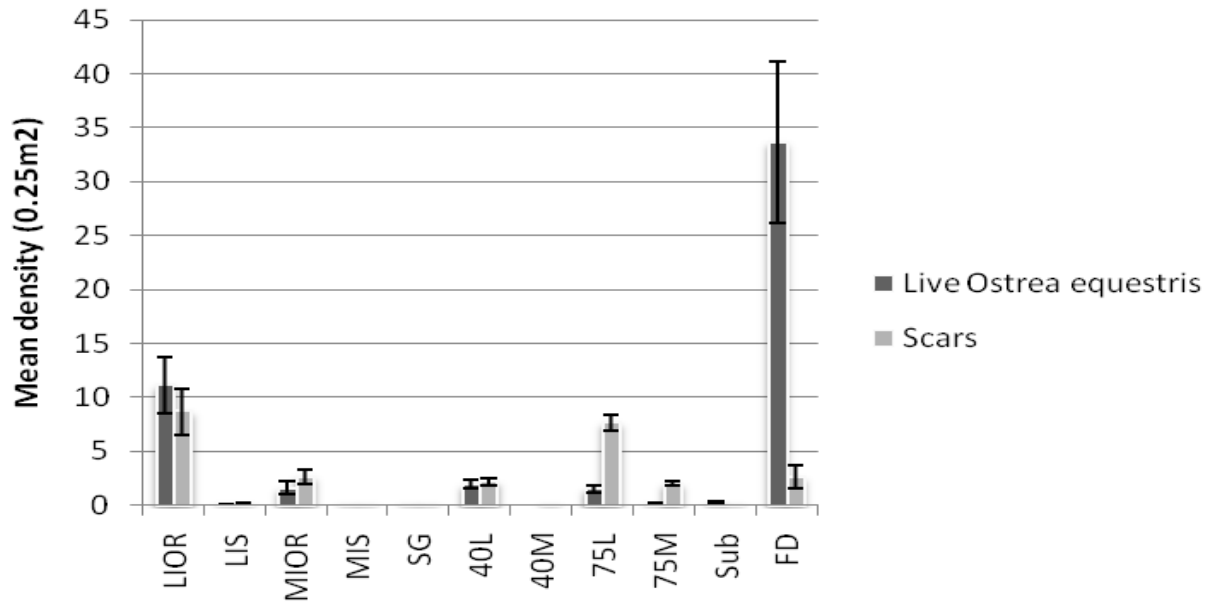


Figure 2: Mean densities for live *Ostrea equestris* and scars by a) site over all habitats for 2008 and b) by habitat over all sites for 2008. Abbreviations for site and habitat indicated in the methods. Error bars are equal to $\pm 1SE$.

Table 2: Percent of total dead (scars and boxes) that scars comprise at sites where dead *Ostrea equestris* were present within sites, habitats, and years.

Site	Habitat	Year	Boxes	Scars	% scars (of total dead)
LF	LIOR	2008	0.000	1.670	100
		2009	2.000	13.000	86.7
	MIOR	2008	0.330	1.000	83.3
	SH40M	2008	0.000	1.360	100
	SH75L	2008	0.383	2.640	92.3
	SH75M	2008	0.279	3.740	92.3
	FD	2008	0.000	10.400	100
	Subtidal	2008	0.125	0.177	60.0
CL	LIOR	2008	2.810	5.180	72.5
		2009	7.400	5.200	42.9
	LIS	2008	0.330	14.000	97.8
	MIOR	2008	0.714	2.570	83.8
		2009	4.400	2.000	23.1
	MIS	2008	0.330	1.670	91.7
	SH40L	2008	0.564	5.560	88.4
		2009	0.435	13.800	97.1
	SH75L	2008	1.310	12.000	93.0
		2009	9.070	7.500	51.1
	FD	2008	0.000	0.000	0
	Subtidal	2008	0.038	0.529	91.4
		2009	0.215	2.520	92.1
	NR	LIOR	2008	2.170	6.170
MIOR			2008	1.000	6.000
MIS		2008	0.500	0.500	50.0
SH75L		2008	0.596	31.800	97.7
		SG	2008	0.000	3.000
2009			0.073	3.850	98.5
FD		2008	0.000	12.500	100
Subtidal		2008	0.003	0.031	88.2

Table 2, continued:

BS	LIS	2008	0.330	1.400	84.4
	MIOR	2008	0.625	5.000	76.0
	MIS	2008	0.000	1.330	100
	SH40L	2008	0.286	4.710	85.2
	SH75L	2008	0.000	5.440	100
	SH75M	2008	0.057	4.670	97.3
	SG	2008	0.000	2.600	100
	FD	2008	8.750	0.000	0
	Subtidal	2008	0.000	0.010	100
		2009	0.000	0.071	100
MM	LIOR	2008	3.930	25.800	83.3
		2009	18.600	29.400	63.9
	MIOR	2008	3.500	9.400	84.3
		2009	0.750	1.500	56.2
	SH40L	2008	4.890	8.050	69.1
		2009	4.740	25.700	85.9
	SH75L	2008	5.870	13.800	69.8
		2009	3.000	25.700	93.9
	SG	2008	0.330	2.330	66.7
	FD	2008	9.370	3.120	50.0
	Subtidal	2008	0.007	0.046	85.7
		2009	1.000	2.670	75.3

Bogue Sound) of remains among sites, and 37.8% (MIOR, though this is an exception as most habitats are >50%) to 98.5% (SG) for habitats. This varied between habitats and years amongst sites (Table 2). For the rest of this discussion scars will be used to represent total dead due to their high percentages. The boxes would indicate relatively recent death, while scars are a potential indicator of longer term mortality. Scars observed were a mix of older and newer (approximately less than a year old, as indicated by the limited degree of fouling and degradation), though the majority were newer especially in Bogue Sound. This possibly indicates that in these areas the oysters are settling but not persisting. A few areas, like Lockwood Folly subtidal, the UNCW Research Lease, and Middle Marsh had a higher proportion of older scars possibly indicating more persistent populations.

2008 densities

Site and habitat comparisons for live *Ostrea equestris* indicated a significant interaction ($F=13.80$, $p<0.0001$), so one way ANOVAs were calculated for site and habitat separately (in general and comparing habitats by site and sites by habitat). Lockwood Folly River had the lowest mean densities for *O. equestris* in 2008, while the research lease had the highest overall mean densities (Figure 2a) ($F=19.51$, $p<0.0001$). It is interesting to note that the two sites with the higher densities are the northern-most site (Middle Marsh) and one of the southern-most sites (UNCW Research Lease) (Figure 2a) indicating the lack of a clear latitudinal cline. However, the coefficient of variation, increased with increasing latitude (Table 3), except for the Lockwood Folly River, indicating patchier populations within the more northern populations.

Among habitats, the FD had the highest mean densities, while lowest were observed in LIS and MIS (Figure 2b) ($F=106.3$, $p<0.0001$). LIOR, MIOR, SH40L, and SH75L had the second highest densities, while subtidal samples had relatively low densities compared to these intertidal habitats (Figure 2b). This may be a reflection of differences in sampling methods, as a dredge can sometimes skip along the bottom not always capturing the full number of individuals

Table 3: Coefficient of variation of density estimates for sites with pooled habitats. Sites are listed in geographical order.

Site	Coefficient of variation
LF	607
CL	359
NR	541
BS	1413
MM	550

Table 4: Coefficient of variation for habitats over all sites.

Habitat	Coefficient of variation
LIOR	206.8
MIOR	329
LIS	1753
MIS	2280
40L	329
40M	N/A
75L	455
75M	536
SG	942
FD	79.7
Sub	358

present. The coefficients of variation for each of the habitats were relatively high (Table 4) indicating that abundances among the habitats, like the sites, were patchy. This is especially true for LIS and MIS, as the total number observed was less than 10. The exception to the high coefficient of variation was FD, indicating overall less patchy population for that habitat.

Mean scar densities for sites and habitats are an order of magnitude greater than live densities (Figure 2a and b). The two exceptions are the UNCW Research Lease and LIOR. This could indicate one of two things for those populations with greater amounts of scars: either these populations are persistent and this is reflective of multiple generations, or they are not persisting and are dying within a year or two of settling. When overall and patch densities are compared relative to each other, patch densities are higher than ambient densities, indicating again the patchiness of the populations. This is not only true among sites (Figure 3) but among habitats as well.

When sites are looked at individually by habitat, there are some patterns that emerge. For the majority of the sites FD had the significantly higher mean densities (Table 5). LIOR, for most of the sites, also had relatively high densities, though in Bogue Sound MIOR had higher. In general, SH40L and SH75L had higher densities than the other habitats after FD, while subtidal and SG tended to be relatively lower (Table 5).

For SH40L and SH75L, the UNCW Research Lease had significantly higher densities ($F=12.45$, $p<0.0001$; $F=14.21$, $p<0.0001$ respectively) than the other sites. New River had significantly higher densities for MIOR ($F=5.64$, $p=0.0006$), while for LIOR Lockwood Folly had significantly lower densities ($F=7.88$, $p=0.0002$). Lockwood Folly did have significantly higher densities for SH75M ($F=9.00$, $p=0.0030$). While there was an overall significant difference among sites for the subtidal habitat ($F=3.14$, $p=0.0236$) specific site differences were not indicated by the SNK test.

Ostrea equestris constituted a maximum of 75.4% of the total live oysters observed at the UNCW Research Lease (Table 6). For habitats, the crested oyster constituted anywhere from 0%

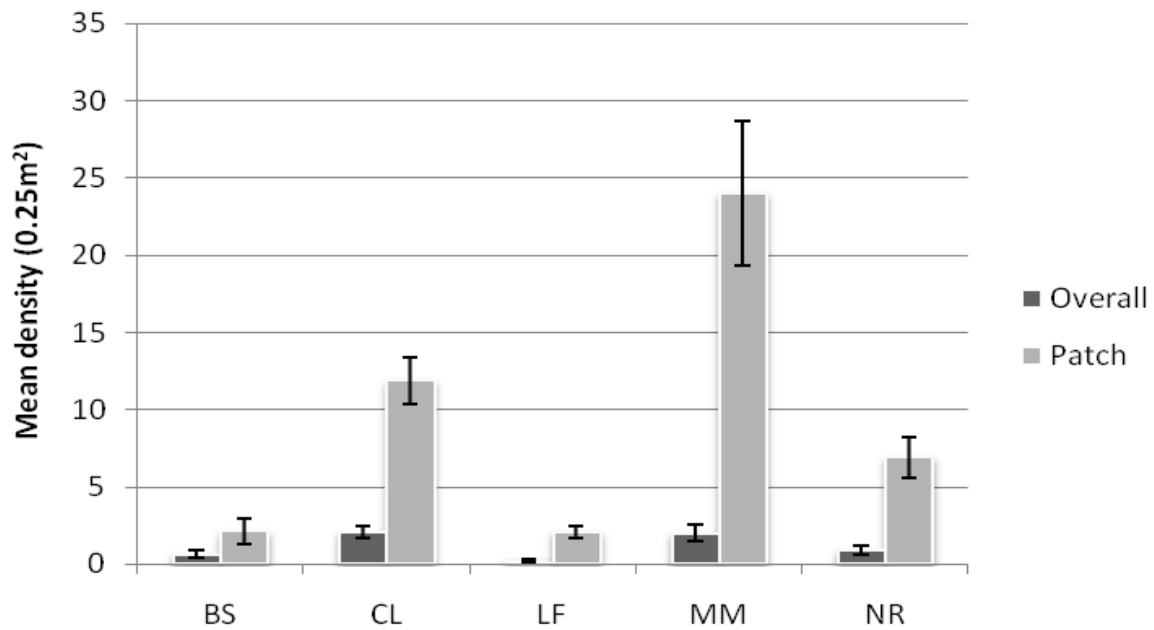


Figure 3: Overall and patch densities for live *Ostrea equestris* for sites over all habitats for 2008. Error bars are equal to $\pm 1SE$. Overall densities include all quadrats, while patches include only those quadrats where crested oysters were present.

Table 5: Statistical significance in density by site among habitats for 2008. Only letters that are different indicate significant difference between habitats (SNK test).

Site	Statistical significance	SNK
BS	F=5.84, p<0.0001	FD ^A , MIOR ^B , SG ^C , SH40L ^C , MIS ^C , LIS ^C , SH40M ^C , SH75L ^C , SH75M ^C , subtidal ^C
CL	F=17.52, P<0.0001	FD ^A , LIOR ^A , SH75L ^B , SH40L ^B , subtidal ^B , LIS ^B , MIS ^B , MIOR ^B
LF	F=12.81, p<0.0001	FD ^A , subtidal ^{BC} , MIOR ^{BC} , SH75M ^{BC} , SH75L ^C , LIOR ^C , LIS ^C , SH40M ^C , MIS ^C
MM	F=59.58, p<0.0001	FD ^A , LIOR ^B , SH40L ^C , MIOR ^C , SG ^C , MIS ^C , LIS ^C , SH75L ^C , subtidal ^C
NR	F=72.35, p<0.0001	FD ^A , LIOR ^B , MIOR ^B , SH75L ^C , SG ^D , subtidal ^D , LIS ^D , SH40M ^D , MIS ^D

Table 6: Percent of live oyster (both *Crassostrea virginica* and *Ostrea equestris*) that *O. equestris* comprises where live oysters are present for sites, habitats, and years. Maximum and minimum percentages are also included.

Site	Habitat	Year	% <i>Ostrea equestris</i>	Maximum	Minimum
LF	LIOR	2008	7.1	100	0
		2009	5.5	5.5	5.5
	LIS	2008	0		
	MIOR	2008	0.3	2.2	0
	MIS	2008	0		
	SH40M	2008	0		
	SH75L	2008	28.6	100	0
	SH75M	2008	21.4	100	0
	FD	2008	75	100	0
	Subtidal	2008	99.5	100	98.3
CL	LIOR	2008	9.1	50	0.4
		2009	0.2	1.4	0
	LIS	2008	100	100	100
	MIOR	2008	0		
		2009	4.2	52.3	0
	MIS	2008	100	100	100
	SH40L	2008	98.8	100	83.3
		2009	100	100	100
	SH75L	2008	98.7	100	82.3
		2009	100	100	100
	FD	2008	100		
	Subtidal	2008	100	100	100
		2009	97.2	97.2	97.2
	NR	LIOR	2008	24.3	57.9
LIS		2008	0		
MIOR		2008	17.9	61.9	0
MIS		2008	0		
SH40M		2008	0		
SH75L		2008	96.7	100	50
SG		2008	100	100	100
		2009	100	100	100
FD	2008	96.3	100	89.9	
Subtidal	2008	100	100	100	

Table 6, continued:

BS	LIS	2008	0		
	MIOR	2008	0.3	1.7	0
	MIS	2008	0		
	SH40L	2008	20	100	0
	SH40M	2008	0		
	SH75L	2008	0		
	SH75M	2008	0		
	SG	2008	60	100	0
	FD	2008	97.7	100	89.3
	Subtidal	2008	0		
		2009	100	100	100
MM	LIOR	2008	33.1	92.8	0
		2009	7.4	65.3	0
	LIS	2008	0		
		2009	0		
	MIOR	2008	4.4	36.9	0
		2009	0	0	0
	MIS	2008	0		
		2009	0		
	SH40L	2008	62.8	100	0
		2009	71.8	100	0
	SH75L	2008	0		
		2009	0		
	SG	2008	100	100	100
	FD	2008	100		
Subtidal	2008	0			
	2009	100	100	100	

(SH40M) to 99.9/100% (subtidal and LIS/ FD respectively). Percentages were 50% or above for MIS, SG, SH40L, and SH75L.

Correlations were run to determine if there was a relationship between *Ostrea equestris* densities and *Crassostrea virginica* densities as well as live and dead *O. equestris*. Correlations among crested oysters and eastern oysters for LIOR and MIOR were negative, though not always significant (Table 7a), while these species were positively correlated in SH40L and SH75L. Correlations were positive for live to dead *Ostrea equestris* for all sites (Table 7b).

Temporal variation

In 2009 various habitats were re-sampled at each of the sites to determine if there was temporal variability in distribution patterns. This sampling was non-random targeted sampling. The following were re-sampled: Lockwood Folly River- LIOR; UNCW Research Lease- LIOR, MIOR, SH40L, SH75L, and subtidal; New River- SG; Bogue Sound- subtidal; Middle Marsh- LIOR, MIOR, SH40L, SH75L, and subtidal. The UNCW Research Lease and Middle Marsh had more extensive re-sampling because of effort was targeted to populations that tended to have higher densities in 2008, so temporal differences may be more readily observed. For the Lockwood Folly River, New River, and Bogue Sound only one habitat was re-sampled because in each case there was anecdotal evidence of higher densities than obtained in 2008.

The UNCW Research Lease had significantly higher densities than Middle Marsh for the subtidal in 2008 ($F=3.14$, $p=0.0236$) and for SH75L both years ($F=14.21$, $p<0.0001$; $F=7.57$, $p=0.0083$). Middle Marsh had significantly higher densities subtidally in 2009 ($F=5.56$, $p=0.0215$). LIOR and MIOR for both years and SH40L for 2008 were not significantly different between sites. However, Middle Marsh did trend towards higher densities.

For MIOR, there was not a significant difference between years for either site. For SH75L, the UNCW Research Lease had significantly higher densities in 2009 than 2008 ($F=4.48$, $p=0.0361$), but Middle Marsh was not significantly different between years for this

Table 7: Correlations for 2008 for a) *Ostrea equestris* to *Crassostrea virginica* and b) live to dead *Ostrea equestris*. Top numbers are r^2 values and bottom numbers are p-values. NS indicates no significance. NC indicates no correlation could be performed.

a)

Region	Habitat										
	FD	LIOR	LIS	MIOR	MIS	SG	SS40L	SS40M	SS75L	SS75M	Subtidal
BS	0.78262 0.0217	N/A	NC	-0.59491 0.0151	NC	NS	NS	NC	NC	NC	NC
CL	NC	-0.5559 0.0254	NC	NC	NC	N/A	0.40209 <.0001	N/A	0.3552 0.0002	N/A	NC
LF	NS	-0.56389 0.0229	NC	NS	NC	N/A	N/A	NC	NS	NS	NS
MM	NC	NS	NC	-0.57421 0.02	NC	NC	NS	N/A	NC	N/A	NC
NR	NS	NS	NC	NS	NC	NC	N/A	NC	0.26362 0.059	N/A	NC

b)

Region	Habitat										
	FD	LIOR	LIS	MIOR	MIS	SG	SS40L	SS40M	SS75L	SS75M	Subtidal
BS	NS	N/A	NC	NS	NC	0.22419 0.0228	NS	NC	NC	NC	NC
CL	NC	0.76157 0.0006	0.8202 <.0001	NC	NS	N/A	0.71829 <.0001	N/A	0.87748 <.0001	N/A	0.91156 0.0002
LF	NS	NS	NC	0.64286 0.0072	NC	N/A	N/A	NC	0.22052 0.0245	0.3419 0.0005	0.91568 0.0002
MM	NS	0.81263 0.0001	NC	0.56965 0.0213	NC	NS	0.34668 0.0196	N/A	NC	N/A	NC
NR	NS	0.88359 0.0036	NC	NS	NC	0.69673 <.0001	N/A	NC	NS	N/A	NS

habitat. For SH40L, the UNCW Research Lease 2008 had higher densities than 2009 ($F=14.66$, $p=0.0002$), while Middle Marsh had higher densities in 2009 ($F=4.07$, $p=0.0471$). Middle Marsh may not have shown a decrease because the SH40L is more shallow subtidal and protected from freezing temperatures in this area. For both these sites, LIOR densities were higher in 2008 than 2009 ($F=48.00$, $p<0.0001$; $F=11.63$, $p=0.0019$ respectively). Decrease among years is most likely due to extreme cold temperatures experienced in the 2008-2009 winter. This habitat is addressed in greater detail in the next section. Coefficients of variation for the sites over all the habitats indicate that these populations are patchy between years (Table 8).

Correlations for 2009 distribution patterns did not show the same patterns as the 2008 data. Most correlations could not be calculated for both the live to dead *Ostrea equestris* and live *O. equestris* to *Crassostrea virginica* (Table 9a and b). For the latter correlation, this is due to the fact almost no *Crassostrea virginica* were found in these habitats in 2009. However, the live to dead crested oysters were significantly positively correlated for the UNCW Research Lease (Table 9b).

In Bogue Sound, the mean subtidal density increased between years, however this increase was not significant. As in 2008, Bogue Sound had significantly lower densities for the subtidal ($F=5.56$, $p=0.0215$). It was significantly different than Middle Marsh and was less than the UNCW Research Lease. In New River, the mean density for SG increased between years (Figure 4) ($F=23.11$, $p<0.0001$). In 2009, the samples were taken from the sound side of the islands in Chadwick Bay not the ICW side, perhaps allowing for more protection. In the Lockwood Folly, the mean density for live *O. equestris* also increased between the two years for LIOR (from 0.406 to 3 oysters/ 0.25m²) (Figure 5) ($F=5.24$, $p=0.0370$). In 2008, Lockwood Folly had significantly lower densities ($F=7.88$, $p=0.0002$) for LIOR than the other sites, but no significant difference was seen between sites in 2009. While the unequal sample sizes and use of different sites may confound the data from the above three habitats, the statistics still fall within the general data trends.

Table 8: Coefficient of variation by year for sites and re-sampled habitats.

Habitat	Year	Site				
		Lockwood Folly	UNCW Research Lease	New River	Bogue Sound	Middle Marsh
LIOR	2008	163.6	53.2	N/A	N/A	77.2
	2009	----	273.2	N/A	N/A	273.8
MIOR	2008	N/A	----	N/A	N/A	206.9
	2009	N/A	245	N/A	N/A	----
SH40L	2008	N/A	193	N/A	N/A	218.4
	2009	N/A	327.3	N/A	N/A	89.8
SH75L	2008	N/A	202.5	N/A	N/A	----
	2009	N/A	95.3	N/A	N/A	----
subtidal	2008	N/A	155.3	N/A	----	----
	2009	N/A	141.4	N/A	316.2	123.7
SG	2008	N/A	N/A	1019	N/A	N/A
	2009	N/A	N/A	202.7	N/A	N/A

Table 9: Correlations for habitats re-sampled in 2009 between sites for a) *Ostrea equestris* to *Crassostrea virginica* and b) live to dead *Ostrea equestris*. Top numbers are r^2 values and bottom numbers are p-values. NS indicates no significance. NC indicates correlation could not be calculated.

a)

Region	Year	Habitat					
		LIOR	MIOR	SH40L	SH75L	subtidal	SG
BS	2008	N/A	N/A	N/A	N/A	NC	N/A
	2009	N/A	N/A	N/A	N/A	NC	N/A
CL	2008	-0.5559 0.0254	NC	0.40209 <0.0001	0.3552 0.0002	NC	N/A
	2009	NS	NS	NS	NS	NC	N/A
LF	2008	-0.56389 0.0229	N/A	N/A	N/A	N/A	N/A
	2009	NC	N/A	N/A	N/A	N/A	N/A
MM	2008	NS	-0.57421 0.02	NS	NC	NC	N/A
	2009	NS	NC	NS	NC	NC	N/A
NR	2008	N/A	N/A	N/A	N/A	N/A	NC
	2009	N/A	N/A	N/A	N/A	N/A	NC

Table 9, continued:

b)

Region	Year	Habitat					SG
		LIOR	MIOR	SH40L	SH75L	subtidal	
BS	2008	NA	NA	NA	NA	NC	NA
	2009	NA	NA	NA	NA	NS	NA
CL	2008	0.57892 0.0118	NC	0.71776 <0.0001	0.87903 <0.0001	0.91156 0.0002	NA
	2009	NS	0.57595 0.0196	NS	0.31743 0.0381	NC	NA
LF	2008	NS	NA	NA	NA	NA	NA
	2009	NC	NA	NA	NA	NA	NA
MM	2008	0.7869 0.0003	0.59749 0.0145	0.38921 0.0082	NC	NC	NA
	2009	0.53767 0.0317	NC	NS	NC	NC	NA
NR	2008	NA	NA	NA	NA	NA	0.69673 <0.0001
	2009	NA	NA	NA	NA	NA	0.40444 0.0029

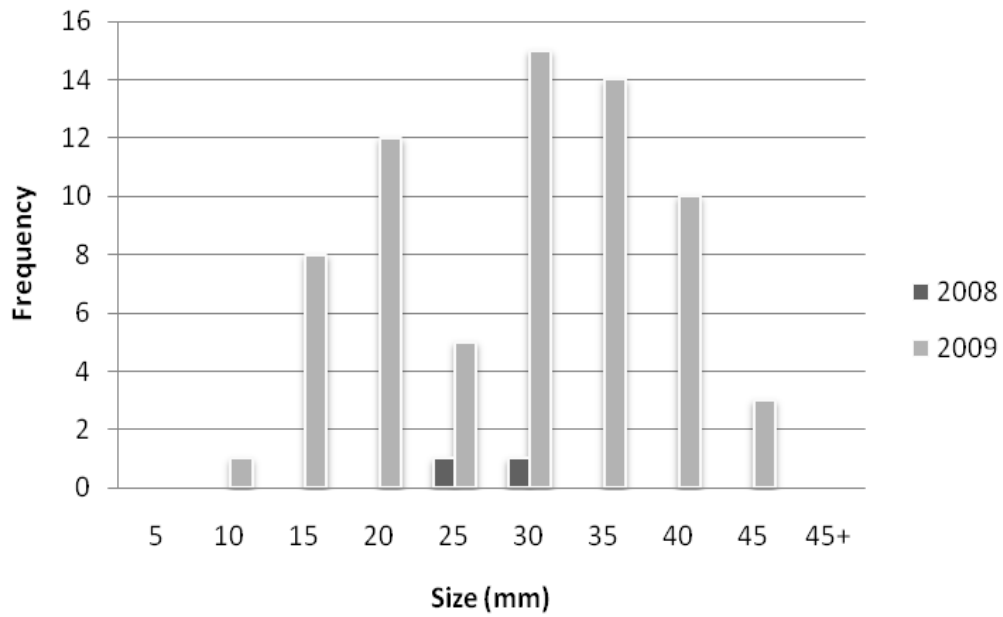


Figure 4: Frequency distribution of size for *Ostrea equestris* for SG in the New River between 2008 and 2009 ($N_{2008}=2$, $N_{2009}=68$).

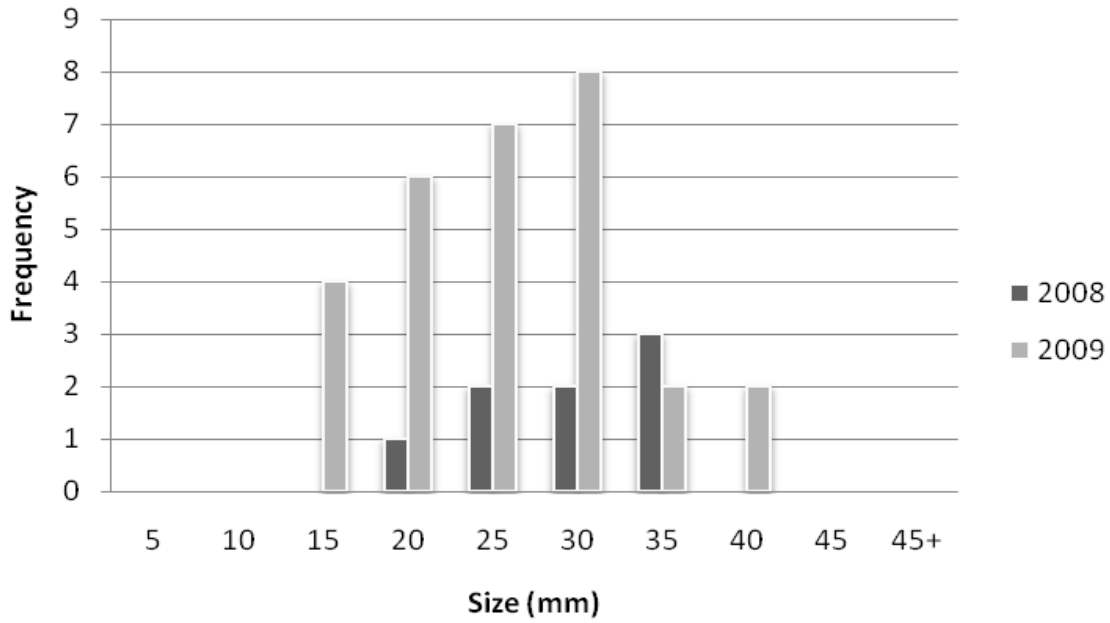


Figure 5: Frequency distribution of size for *Ostrea equestris* for LIOR in the Lockwood Folly River between 2008 and 2009 ($N_{2008}=8$, $N_{2009}=29$).

Oyster Reefs

While oyster reefs are included in the above analyses, it was hypothesized that there may be an edge effect for *Ostrea equestris* related to tidal height and density. Therefore, while all quadrats were pooled for the overall analysis, edge vs interior of the reef was distinguished during collections. Edge of the reefs had higher overall densities of *Ostrea equestris* than the interior (Figure 6) ($F=24.39$, $p<0.0001$), for both LIOR and MIOR ($F=8.70$, $p=0.0039$; $F=5.81$, $p=0.0176$ respectively). LIOR had higher densities than MIOR (Figure 6) ($F=27.33$, $p<0.0001$). In most cases higher densities were observed in 2008 than 2009. Coefficients of variation indicate patchy distribution among all reef habitat types, but are lowest for LIOR-edge (Table 10). The variance increases in most cases between 2008 and 2009.

For those sites that had both mid and low intertidal reefs, the lower intertidal reefs had significantly higher densities than the mid intertidal area (Table 11). For sites where edge and interior could be compared, the edge had significantly higher densities than the interior of the reefs (Table 11). LIOR-edge was the only habitat that was significantly different between sites and between years for sites. In 2008, the Lockwood Folly River had significantly lower densities than Middle Marsh, New River, and the UNCW Research Lease ($F=4.70$, $p=0.0056$), but was not significantly different in 2009. In general 2008 had significantly higher densities than 2009 ($F=17.81$, $p<0.0001$). 2008 had significantly higher densities for Middle Marsh and the UNCW Research Lease than 2009 ($F=11.63$, $p=0.0019$; $F=27.11$, $p<0.0001$ respectively)

Overall, *Ostrea equestris* comprised up to 20% of the live oyster present in the edge of the reefs (especially the low intertidal reefs) and up to 10% of the interior of reefs where present. Data from oyster reef collections for live *Ostrea equestris* to *Crassostrea virginica* and live to dead *O. equestris* showed significant negative correlations for the former and significant positive ones for the later (Table 12a and b) for all oyster habitats but MIOR-edge. These correlations are strongest for the interior of the reefs, further indicating the possibility of an edge effect.

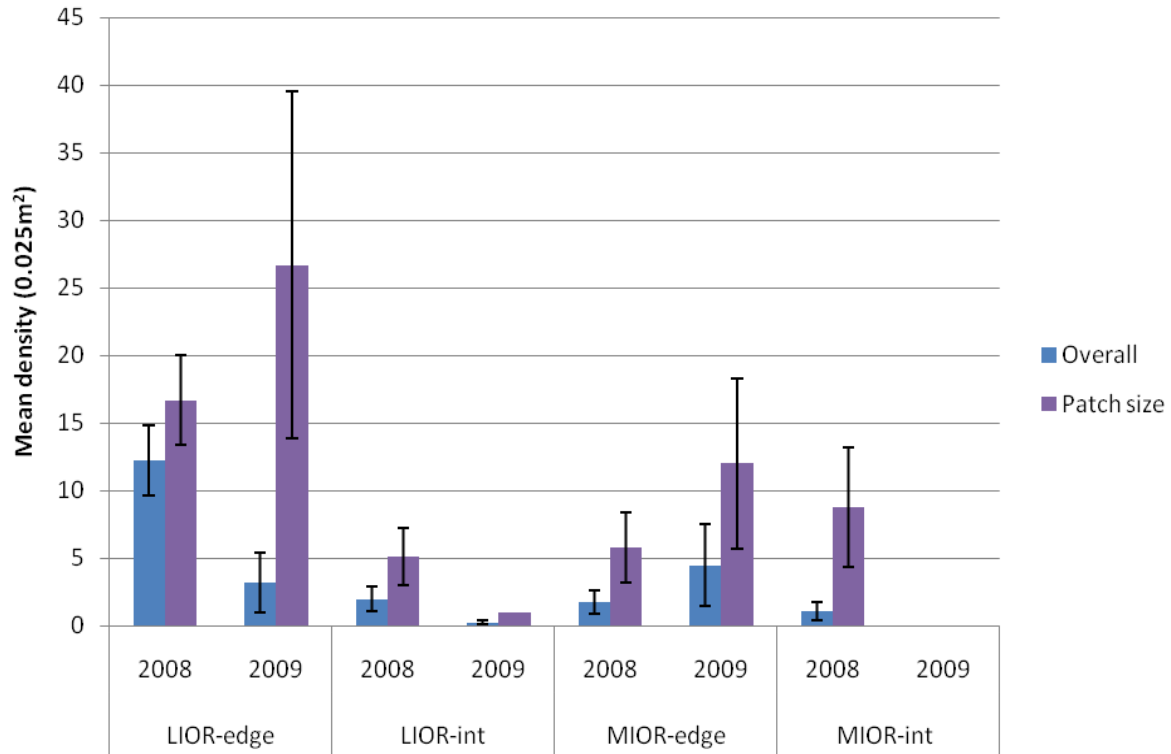


Figure 6: Mean densities for all sites by oyster reef habitats and year for live *Ostrea equestris*, both overall and for only those quadrats where present (patch size). Error bars are $\pm 1SE$.

Table 10: Coefficient of variation for oyster density in variable habitats by year for combined sites.

Habitat	Year	Coefficient of variation
LIOR-edge	2008	84
	2009	297.5
LIOR-interior	2008	159.8
	2009	185.2
MIOR-edge	2008	201.2
	2009	162.6
MIOR-interior	2008	318.4
	2009	0

Table 11: Statistical significance by site and year among oyster reef habitats. NS indicates that there was no significance. Only letters that are different indicate statistical difference between habitats for the SNK test.

Site	Year	Statistical Significance	SNK
BS	2008	F=7.00, p= 0.0192	MIOR-edge ^A , MIOR-interior ^B
CL	2008	F=10.32, p<0.0001	LIOR-edge ^A , LIOR-interior ^B , MIOR-edge ^B , MIOR-interior ^B
	2009	NS	
LF	2008	NS	
MM	2008	F=12.20, p=0.0015	LIOR-edge ^A , MIOR-interior ^B
	2009	NS	
NR	2008	F=7.17, p=0.0119	LIOR-edge ^A , MIOR-edge ^B

Table 12: Correlations for oyster habitats between sites for a) live *Ostrea equestris* to live *Crassostrea virginica* and b) live to dead *Ostrea equestris*. Top numbers are r^2 values and bottom numbers are p-values. NS indicates no significance. NC indicates correlation could not be calculated.

a)			b)		
Habitat	Year		Habitat	Year	
	2008	2009		2008	2009
LIOR-edge	-0.23858 0.0766	NS	LIOR-edge	0.73133 <.0001	0.36184 0.0755
LIOR-interior	-0.76515 <.0001	-0.75593 0.03	LIOR-interior	0.61873 0.0016	NS
MIOR-edge	NS	NS	MIOR-edge	NS	NS
MIOR-interior	-0.49505 0.0012	NC	MIOR-interior	0.52369 0.0005	NC

Floating Docks

While floating docks were addressed briefly with the 2008 data, it was important that they also be addressed separately as fouling communities have been reported to contain a significant number of *Ostrea equestris* (Wells and Gray 1960; Gunter 1951; Menzel 1955). Within southeastern North Carolina floating docks are representative of a subtidal fouling community that is easily accessible and sampled. There was a significant difference between sites ($F=16.59$, $p<0.0001$). The northern populations (MM, NR, BS) had significantly higher densities than the southern sites (CL, LF) (Figure 7).

O. equestris was 93.7% (LF) to 100% (CL, MM) of the total oyster populations on the floating docks. Correlations for live *Ostrea equestris* to live *Crassostrea virginica* were not significant except to at Bogue Sound ($r^2=0.78262$, $p=0.0217$). For live to dead crested oysters all correlations were not significant.

Size distributions

Mean size for *O. equestris* over all sites, habitats, and years ranged from 4.42- 29.7mm (Table 13). The minimum size that could be observed was 1.34mm, while the largest was 48mm. Both these observations were obtained with oysters from the UNCW Research Lease (Table 13). The minimum on the research lease was lower than other sites and habitats due to a large settlement event just prior to collection within the first year (personal observations, indicated by large amount of small spat), which gave the research lease significantly lower mean size in 2008 ($F=346.5$, $p<0.0001$) than the other sites. New River and Middle Marsh had oysters with maximum sizes 44mm and 45.7mm respectively, while those from the Lockwood Folly River and Bogue Sound were 39.1mm and 35.3mm (Table 13). In most cases mean size increased between the 2008 and 2009 sampling seasons (Figure 8), though this may be an artifact of the more targeted sampling in 2009. The size increase was only significant for New River ($F=8.12$, $p=0.0046$) and the research lease ($F=855.3$, $p<0.0001$). The exception to this increase was in

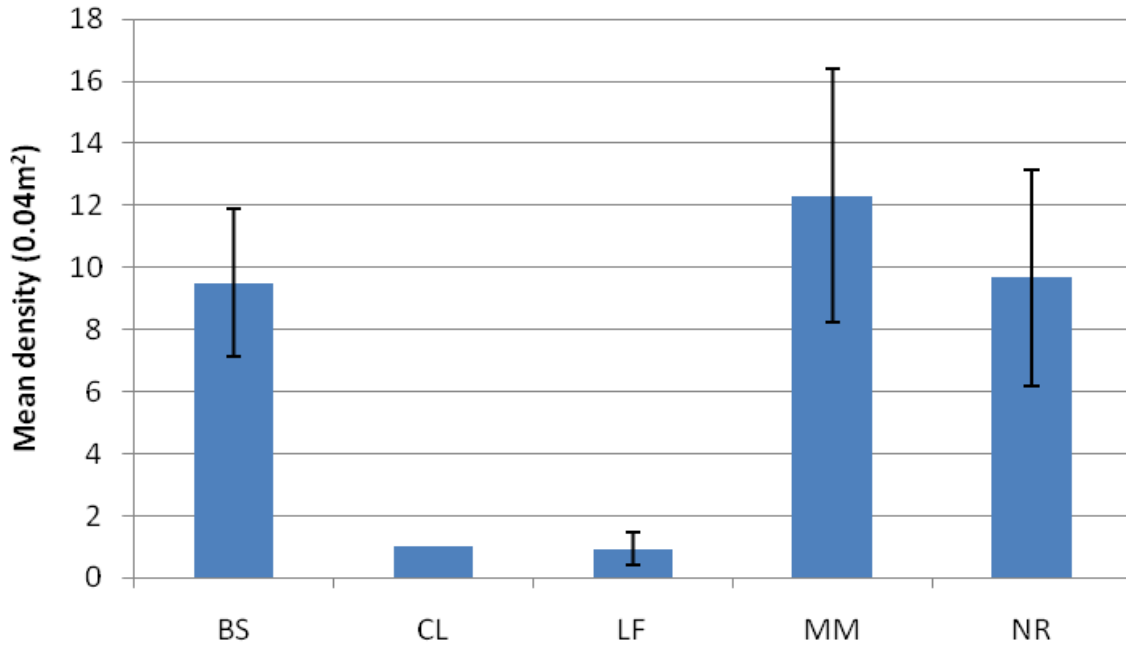


Figure 7: Mean densities of *Ostrea equestris* by site for the fouling communities on floating docks. Error bars are equal to ± 1 SE.

Table 13: Mean, maximum, and minimum sizes (mm) for sites, habitats, and years. Sites are listed in geographical order.

Site	Habitat	Year	Mean	N	Max	Min
LF	LIOR	2008	27.1	8	32.1	18.8
		2009	23.1	29	39.2	10.2
	MIOR	2008	26.9	5	34.3	23.2
	75L	2008	6.98	7	10.8	4.01
	75M	2008	14.7	27	25.1	5.75
	FD	2008	20.7	13	29.9	5.08
	Sub	2008	23.2	343	39.1	9.99
CL	LIOR	2008	25.6	152	46.2	10.8
		2009	26.1	24	48.0	5.55
	LIS	2008	25.2	7	31.9	14.9
	MIOR	2009	21.9	35	36.5	7.37
	MIS	2008	25.9	1		
	40L	2008	13.2	319	34.7	3.88
		2009	23.2	28	42.4	8.84
	75L	2008	15.8	463	44.3	3.31
		2009	21.9	697	47.1	6.14
	FD	2008	31.4	1		
	Sub	2008	7.22	913	30.6	1.34
		2009	16.6	132	31.7	6.24
	NR	LIOR	2008	24.4	242	42.5
MIOR		2008	20.2	8	32.2	10.5
SG		2008	24.3	2	24.3	21.9
		2009	26.5	68	44.4	8.52
75L		2008	19.8	90	40.4	6.54
FD		2008	25.5	29	41.0	8.70
Sub		2008	25.8	6	29.9	16.5
BS	MIOR	2008	29.3	4	35.3	23.6
	SG	2008	14.5	11	34.6	6.3
	40L	2008	17.2	2	19.9	14.5
	FD	2008	25.6	76	43.2	8.5
	Sub	2009	22.9	3	27.7	19.2
MM	LIOR	2008	20.2	420	45.7	3.48
		2009	22.4	159	39.9	5.28
	MIOR	2008	23.7	48	40.2	7.24
	SG	2008	4.42	4	8.91	2.42
	40L	2008	24.3	170	44.8	3.94
		2009	21.9	159	41.7	9.99
	75L	2009	16.5	5	26.1	9.92
	FD	2008	23.3	37	41.8	9.67
	Sub	2009	15.6	1468	80.9	3.41

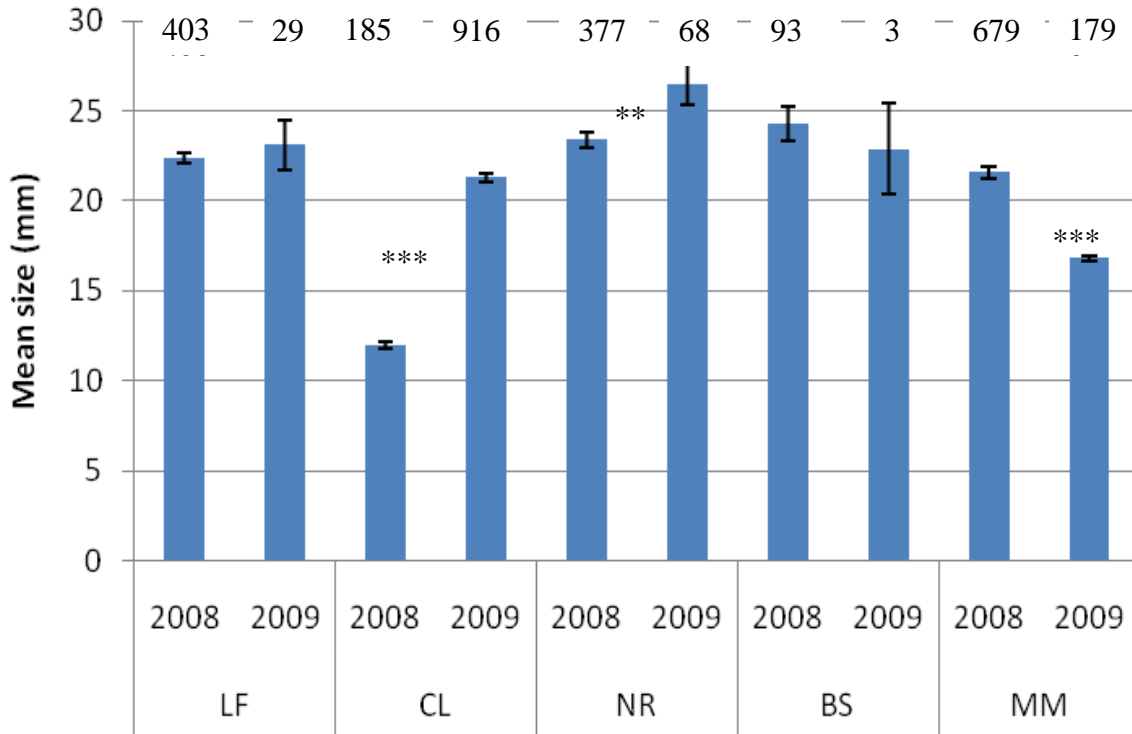


Figure 8: Mean size of *Ostrea equestris* by site between years. Sites are listed in geographic order south to north. Numbers above bars are total oysters measured for that site. Stars on graph indicate significant differences between years for that site (** $p < 0.01$; **** $p < 0.0001$). Error bars are equal to $\pm 1SE$.

Middle Marsh (Figure 8), where oysters were significantly smaller in the second year ($F=214.2$, $p<0.0001$). This is most likely due to a large number of smaller subtidal oysters observed in the second year at Middle Marsh, giving this site significantly lower mean size than the other sites ($F=93.09$, $p<0.0001$) in 2009.

Mean size range varied between habitats, with the smallest averages attributable to *O. equestris* (11.7mm) found in the subtidal dredges and the largest on the floating docks (24.7mm). For both years, dredged subtidal populations were significantly smaller than oysters in the other habitats ($F=114.6$, $p<0.0001$; $F=151.4$, $p<0.0001$, for 2008 and 2009 respectively). While LIS and MIS oysters had larger mean size, the number of individual oysters was less than 8. For those habitats that were re-sampled the second year the mean size of oysters located in MIOR decreased, though the differences were not significant (Figure 9). SH40L, SH75L, subtidal, and seagrass *Ostrea equestris* all had greater sizes between 2008 and 2009 ($F=47.21$, $p<0.0001$; $F=165.0$, $p<0.0001$; $F=210.2$, $p<0.0001$; $F=26.24$, $p<0.0001$ respectively) (Figure 9). LIOR stayed approximately the same, with no significant differences (Figure 9). For seagrass, New River was the only site re-sampled, though the increase in size at site level for this habitat was not significant. The subtidal increase was probably driven by the large number of spat at the UNCW Research Lease the first year not seen in the second. Subtidal size increased significantly between 2008 and 2009 at the UNCW Research Lease ($F=733.5$, $p<0.0001$). Sites, when looked at by habitat and year, didn't exhibit the same patterns for mean size. However, in most cases FD, SH40L and the oyster reefs tended to have the significantly larger sizes, while the subtidal and seagrass had smaller overall means (Table 14). The two best examples of this are the UNCW Research Lease and Middle Marsh (one of the southern-most and the northern-most site) (Figures 10a and b). Habitats compared among sites showed significant differences (Table 15).

Frequency distributions of *Ostrea equestris* sizes indicate three settlement patterns (the only pattern not seen was no recruitment in either 2008 or 2009). The first is a suggestion of

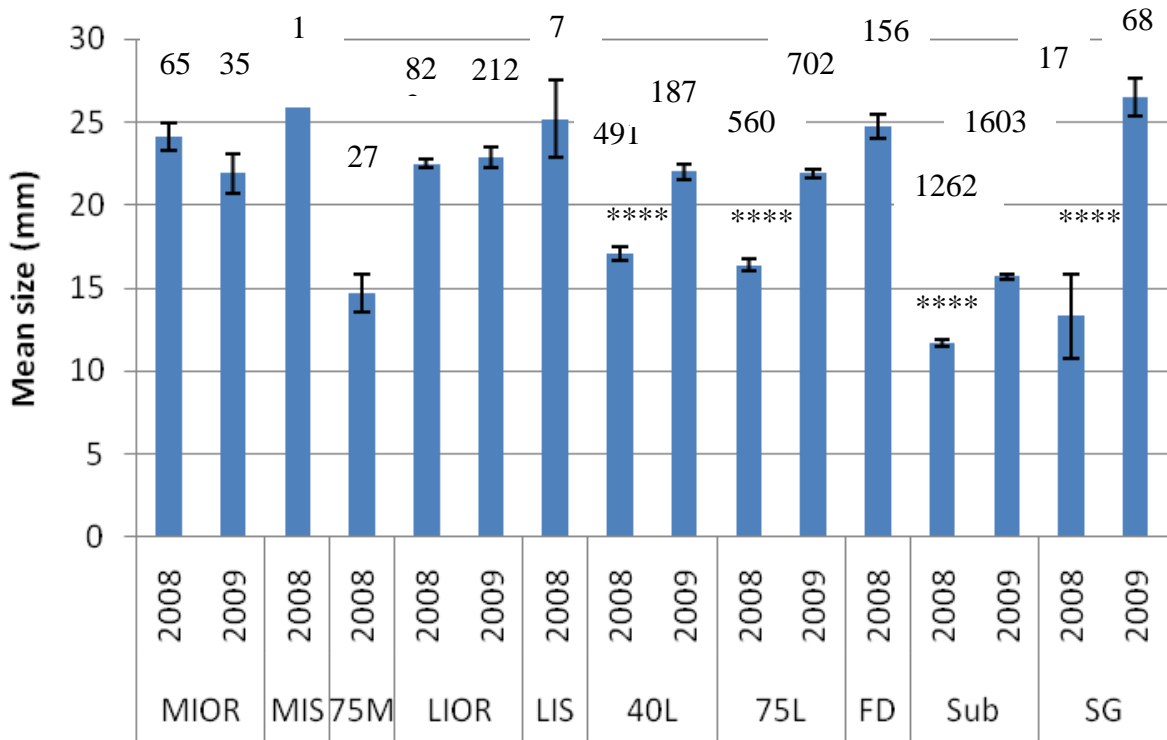


Figure 9: Mean size of *Ostrea equestris* by habitat between years. Numbers above bars are total oysters measured for that habitat. Stars on graph indicate significant difference among years for that habitat (**p<0.01; ****p<0.0001). Error bars are equal to ±1SE.

Table 14: Statistical differences between sites by habitats and years. Letters on table by habitats indicate significant differences, only those letters that are different indicate a significant difference. Letters that are the same indicate no difference.

Site	Year	Statistical significance	SNK differences
BS	2008	F=6.57, p=0.0005	MIOR ^A , FD ^{AB} , SH40L ^{AB} , SG ^B
CL	2008	F=254.3, p<0.0001	FD ^A , MIS ^{AB} , LIOR ^{AB} , LIS ^{AB} , SH75L ^{BC} , SH40L ^{BC} , subtidal ^C
	2009	F=21.51, p<0.0001	LIOR ^A , SH40L ^B , Sh75L ^B , MIOR ^B , subtidal ^C
LF	2008	F=25.15, p<0.0001	LIOR ^A , MIOR ^A , subtidal ^{AB} , FD ^B , SH75M ^C , SH75L ^D
MM	2008	F=13.01, p<0.0001	SH40L ^A , MIOR ^A , FD ^A , LIOR ^A , SG ^B
	2009	F=95.02, p<0.0001	LIOR ^A , SH40L ^A , SH75L ^B , subtidal ^B
NR	2008	F=5.07, p=0.0002	subtidal ^A , FD ^A , LIOR ^A , SG ^A , MIOR ^A , SH75L ^A

Table 15: Statistical differences between habitats by sites and years. Letters on table by habitats indicate significant differences, only those letters that are different indicate a significant difference. Letters that are the same indicate no difference. NS indicates no significance.

Habitat	Year	Statistical significance	SNK
MIOR	2008	NS	
LIOR	2008	F= 24.31, p<0.0001	LF ^A , CL ^A , NR ^A , MM ^B
	2009	NS	
SH40L	2008	F= 119.9, p<0.0001	MM ^A , BS ^{AB} , CL ^B
	2009	NS	
SH75L	2008	F= 12.88, p<0.0001	NR ^A , CL ^A , LF ^B
	2009	NS	
SG	2008	NS	
FD	2008	NS	
subtidal	2008	F= 1961, p<0.0001	NR ^A , LF ^A , CL ^B
	2009	F= 4.44, p=0.0019	BS ^A , CL ^B , MM ^B

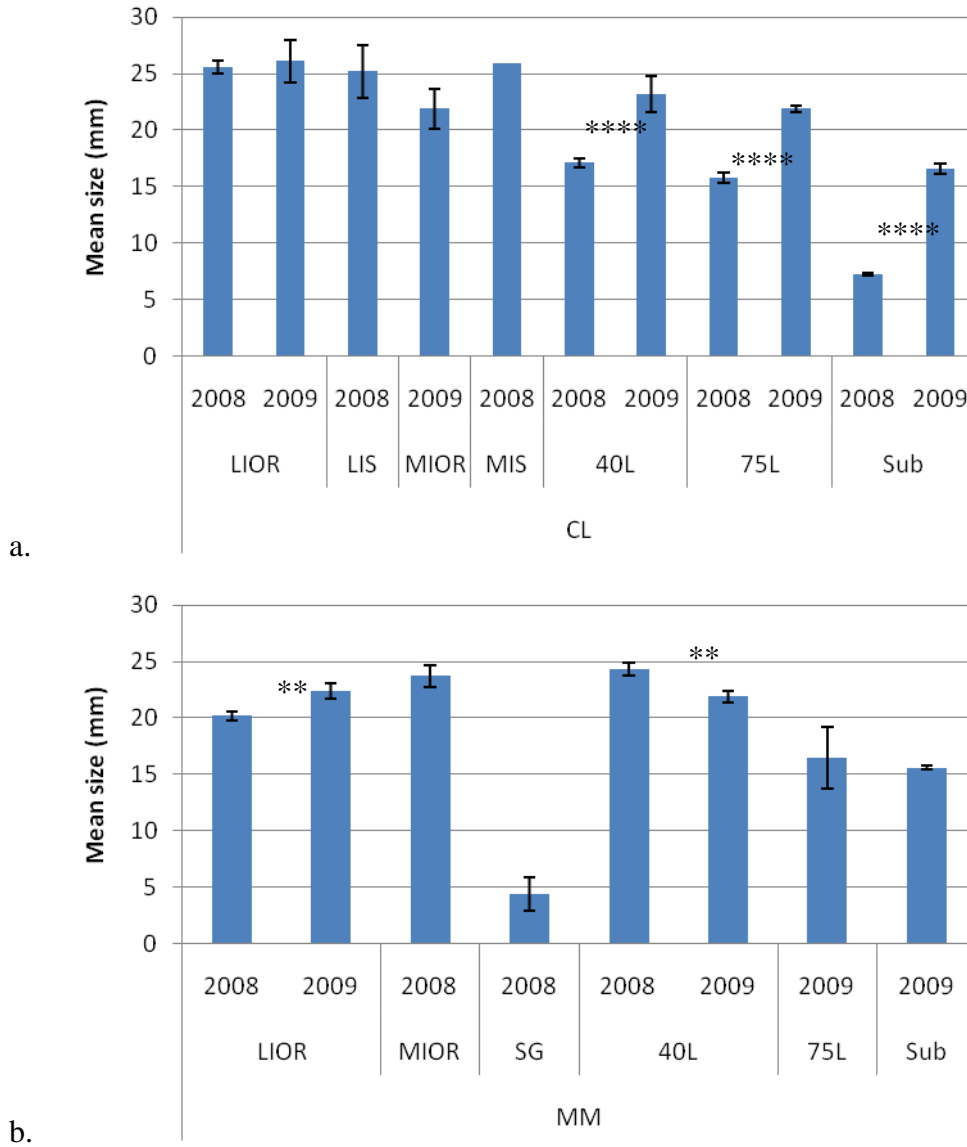


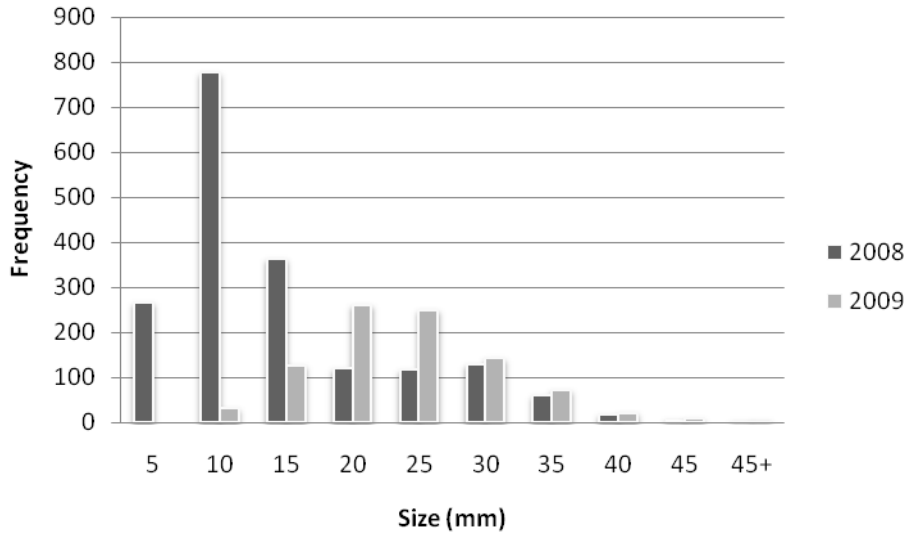
Figure 10: a) Mean size of *Ostrea equestris* by habitat between years for the UNCW Research Lease. b) Mean size of *Ostrea equestris* by habitat between years for Middle Marsh. Stars on graph indicate significant difference (** $p < 0.01$; **** $p < 0.0001$). Numbers of total oysters measured for that habitat at the site are listed in Table 13. Error bars are equal to $\pm 1SE$.

recruitment within the first year. This is based on the large number of smaller oysters in 2008 than 2009 for some sites or habitats. The best overall site example of this is the UNCW Research Lease (Figure 11a) ($F=858.6$, $p<0.0001$). While no other sites really exhibit this pattern, several habitats do: SH40L ($F= 47.21$, $p<0.0001$) (Figure 11b), SG ($F=26.24$, $p<0.0001$), SH75L ($F=165.0$, $p<0.0001$), and subtidal ($F=210.2$, $p<0.0001$). Within the Research Lease, SH40L ($F=45.89$, $p<0.0001$), SH75L ($F=181.9$, $p<0.0001$), and subtidal ($F=733.5$, $p<0.0001$) all represent this pattern. The second pattern indicates recruitment in the second year for different sites and habitats than above, as there is a much larger number of smaller *O. equestris* in 2009 than the year before. The best overall site example of this is Middle Marsh (Figure 12) ($F=200.1$, $p<0.0001$). This is also exhibited by SH40L within Middle Marsh ($F=8.71$, $p=0.0034$). The third pattern reflects a drop in overall densities. Mean size in this case is not significantly different between years. The best site example is the Lockwood Folly River. However, this is misleading as the sample size is severely restricted in the second year for this site. The best overall habitat example is LIOR (Figure 13), though MIOR also exhibits this pattern. Within sites, only LIOR for the Research Lease exhibits this pattern.

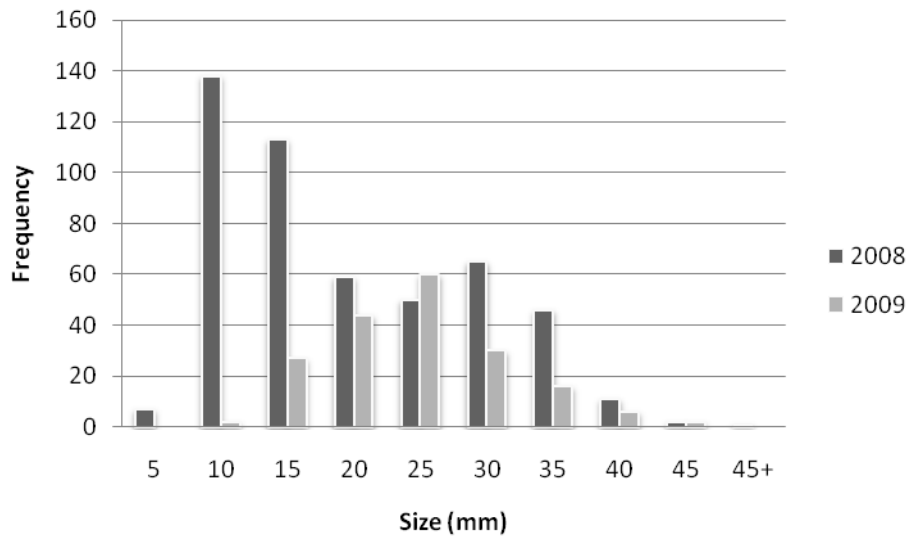
Condition Index

Mean condition indices of oysters ranged from 5.8 (at the UNCW Research Lease LIOR for spring) to 11.8 (at New River SG for summer). In general, most values fell between 8.3 and 9.8. The mean height of the oysters used ranged from 26.2mm to 34mm. There was no correlation between height and condition index, except for the subtidal habitat ($r^2=0.24334$, $p=0.0175$).

Summer and winter condition indices tended to be higher for all sites, while the lowest condition indices tended to be for the spring samples (Figure 14). The only site where this trend was significant was the UNCW Research Lease ($F=5.44$, $p=0.0048$). When the sites are compared by season, only the winter season showed no significant differences between sites.



a.



b.

Figure 11: a) Size distribution of *Ostrea equestris* between years for the UNCW Research Lease. This includes all habitats summed together ($N_{2008}=1856$; $N_{2009}= 916$). b) Size distribution of *Ostrea equestris* between years for SH40L over all sites ($N_{2008}= 492$; $N_{2009}= 187$).

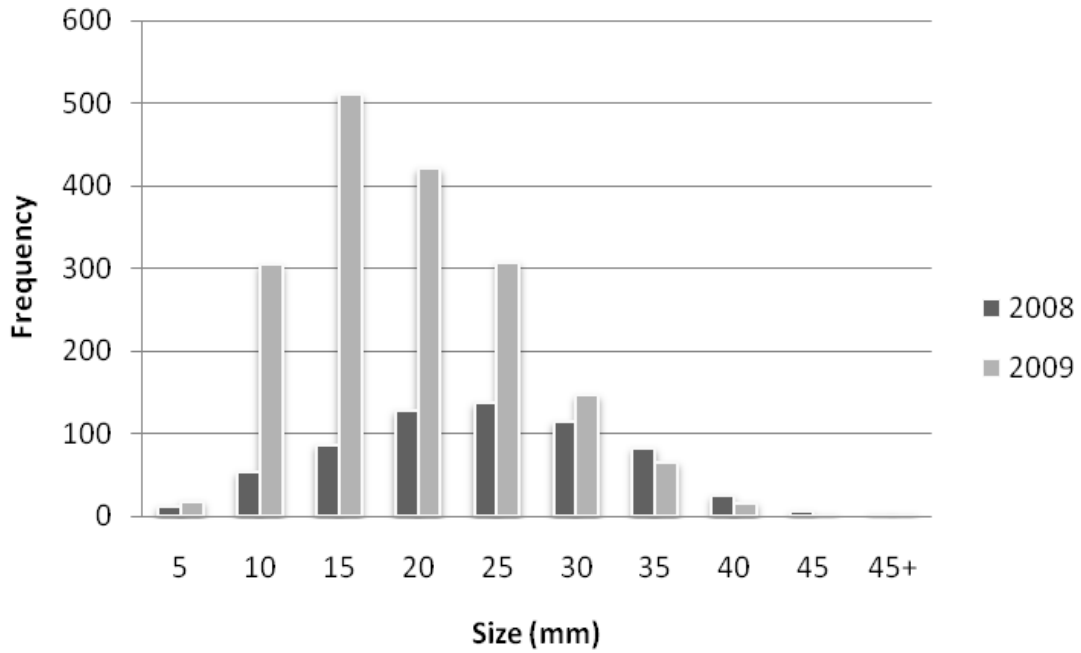


Figure 12: Size distribution of *Ostrea equestris* between years for Middle Marsh, for all habitats ($N_{2008} = 642$; $N_{2009} = 1792$).

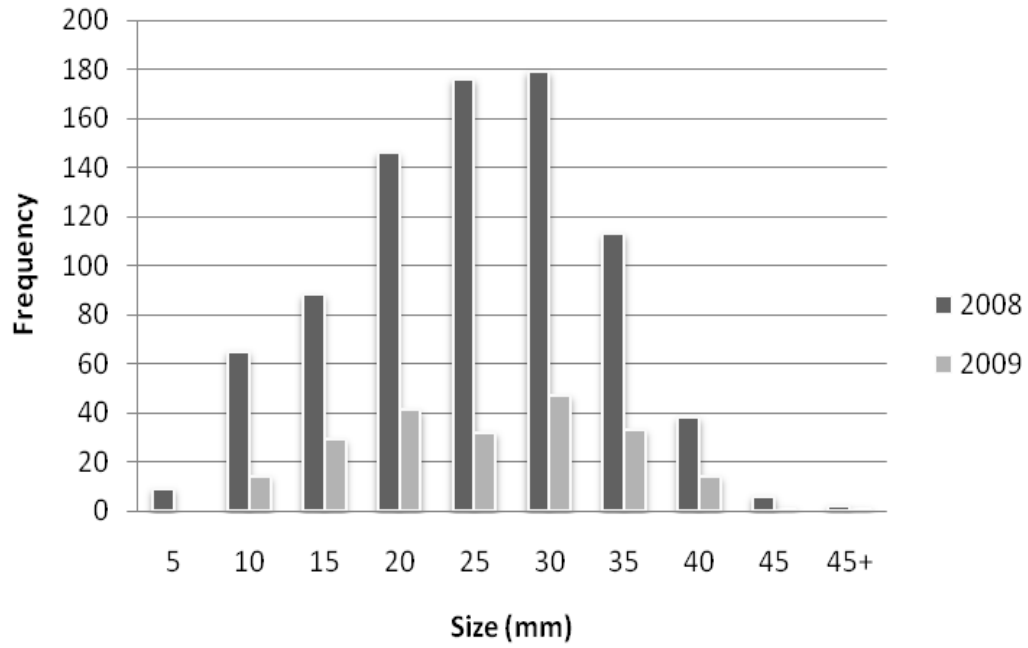


Figure 13: Size distribution of *Ostrea equestris* between years for LIOR, over all sites ($N_{2008}=822$; $N_{2009}=212$).

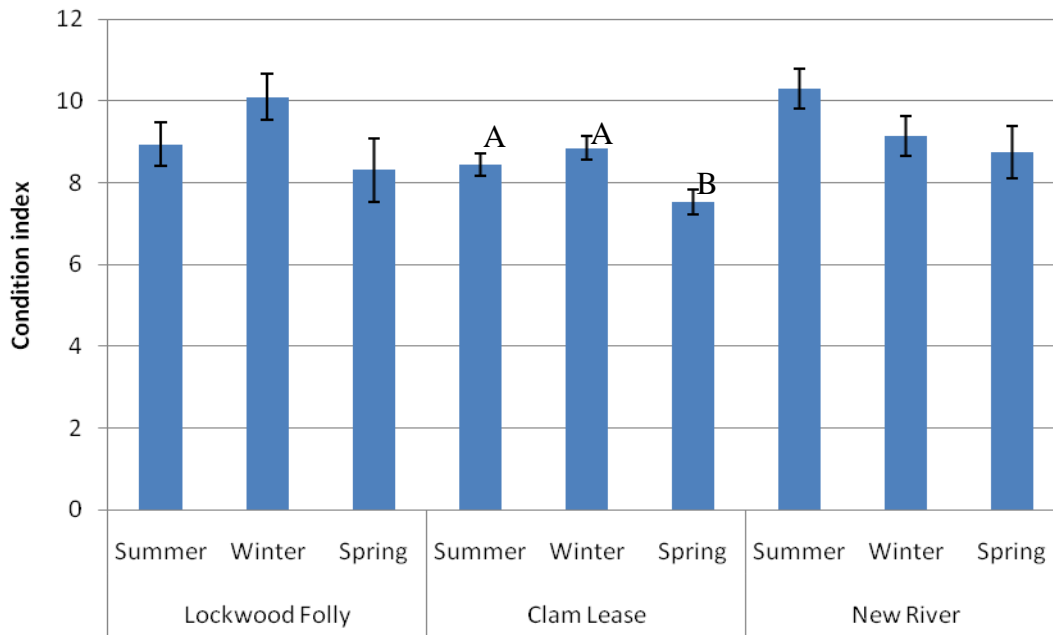


Figure 14: Condition index values for each site separated by season. Sites are in order from south to north. Letters on graph indicate significant differences, only those letters that are different indicate a significant difference. Letters that are the same indicate no difference. No letters present equal no significant for that site. Error bars are equal to $\pm 1SE$.

Both the summer ($F=3.87$, $p=0.0229$) and the spring ($F=14.78$, $p<0.0001$) showed significant differences between sites. Post-hoc SNK testing indicated New River condition indices were significantly higher for the summer than the Lockwood Folly River or UNCW Research Lease. Though the ANOVA picked up differences for the spring, post-hoc testing didn't distinguish between sites. However, spring samples followed the same trend as the summer samples.

Habitats exhibited stronger seasonal signals than the sites themselves (Figure 15), the only two habitats with no significant differences were LIOR and SS75L. Habitats followed the same seasonal trend as sites, wherein spring had the lower condition indices for almost all habitats, the exception being subtidal where spring condition values were not significantly different from either summer or winter. For SS40L ($F=3.71$, $p=0.0306$), MIOR ($F=5.54$, $p=0.0063$), and subtidal ($F=3.98$, $p=0.0220$) habitat winter condition was significantly higher than the other periods. For SG ($F=13.42$, $p<0.0001$) summer conditions were significantly higher.

As Lockwood Folly only had one habitat (subtidal) differences by site and season could not be observed. The UNCW Research Lease and New River both showed habitat differences by season. For the UNCW Research Lease all three seasons showed significant differences within the habitats, though SNK differences changed with season (Table 16). The same is true of New River, though the winter season showed no significant differences between habitats (Table 16).

Only two habitats were similar between sites (LIOR and subtidal), and only two out of the three sites were comparable. In the spring, New River had significantly higher condition index values than the research lease ($F=22.25$, $p<0.0001$) (Figure 16a). Summer and winter values were not significant though they exhibited the same trend (Figure 16a). In the summer, the Lockwood Folly River had significantly higher condition indices for subtidal sites than the UNCW research lease ($F=6.25$, $p=0.0168$) (Figure 16b). No other seasons were significant.

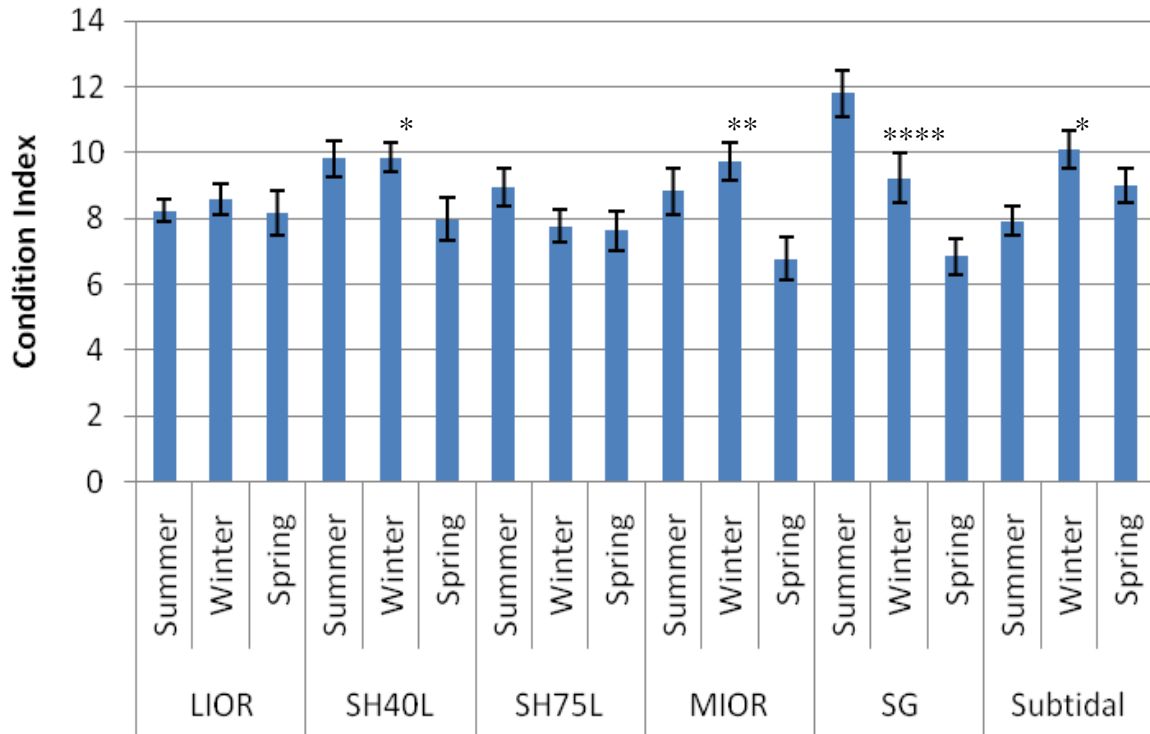


Figure 15: Condition index values for each season separated by habitat. Stars on graph indicate significant difference (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$). Error bars are equal to $\pm 1SE$.

Table 16: Statistical differences between habitats by site and season differences. LF is not included as only the subtidal habitat was sampled. Letters by habitat indicate significant differences if different.

Site	Season	Statistical significance	SNK differences
CL	Summer	F=3.75, p=0.0072	SH40L ^A , SH75L ^{AB} , MIOR ^{AB} , LIOR ^{AB} , subtidal ^B
	Winter	F=4.03, p=0.0103	SH40L ^A , MIOR ^A , LIOR ^{AB} , SH75L ^B
	Spring	F=5.42, p=0.0006	subtidal ^A , SH40L ^B , SH75L ^B , MIOR ^B , LIOR ^B
NR	Summer	F=12.98, p=0.0009	SG ^A , LIOR ^B
	Winter	NS	
	Spring	F=13.42, p=0.0008	LIOR ^A , SG ^B

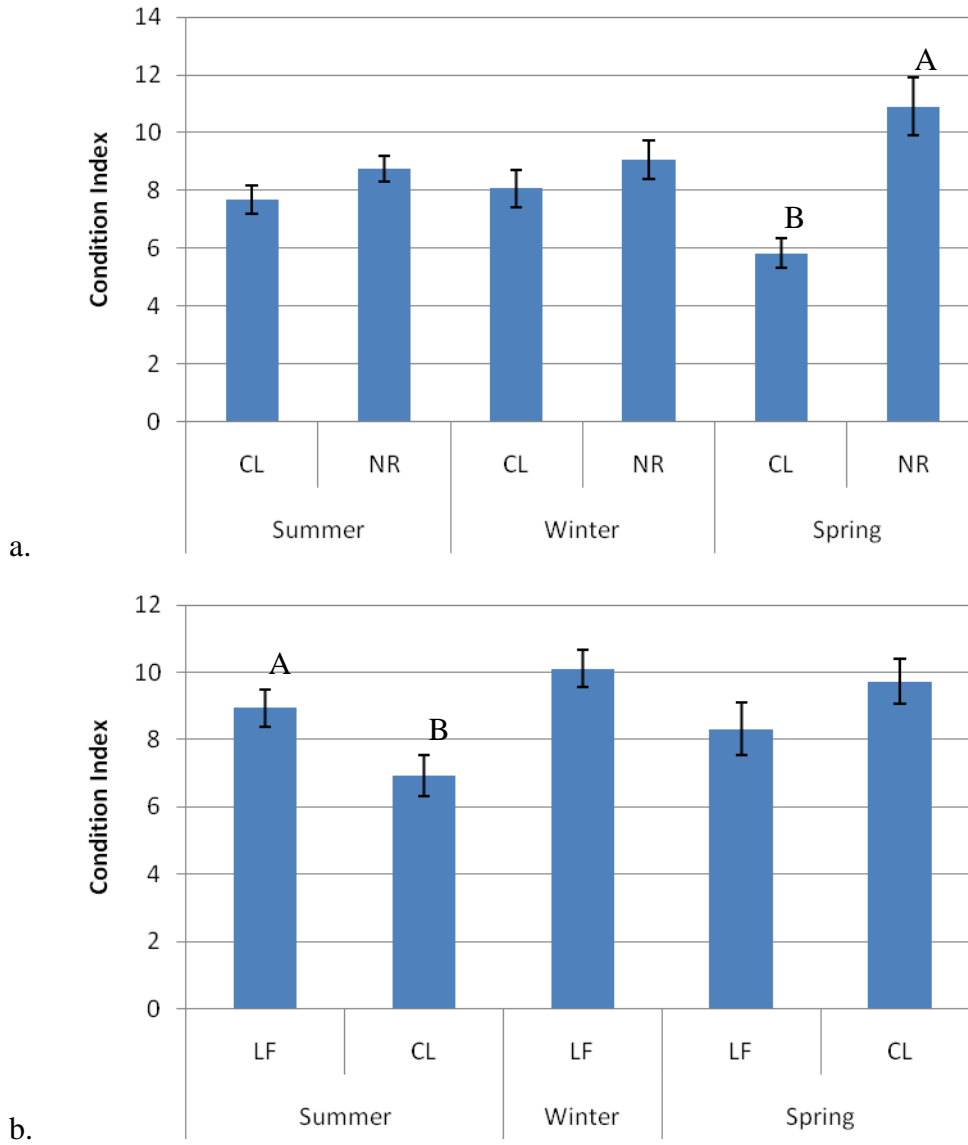


Figure 16: a) Condition index values for Lior by site and season. b) Condition index values for subtidal by site and season. Letters on graph indicate significant differences, only those letters that are different indicate a significant difference. Letters that are the same indicate no difference. Error bars are equal to $\pm 1SE$.

Reproduction

It was found for the overall time period studied <3% of all oysters were brooding (Table 17), which agrees with numbers seen in Georgia (Walker and Power 2001). However, if the data is broken down into individual time periods 5 ~12% of the oysters collected were brooding larvae (Table 18). The largest number of brooders were observed in July (N=6). However, the percentage of brooders was higher in May (10% compared to 5.26%), though this discrepancy may be due to differences in total N collected (115 and 20 for July and May respectively).

The average number of larvae being brooded was 156407 (SD=49377) veligers per oyster that contained larvae. There was no significant difference between sites for the number of larvae being brooded (F=0.17, p=0.8461), though the Lockwood Folly River had noticeably fewer on average (Table 17).

The average size of the *Ostrea equestris* that contained larvae was less than 35mm, with the largest oysters in the UNCW Research Lease and the smallest in the Lockwood Folly River (Table 17). This may be attributed to the fact that those oysters collected from New River and the UNCW Research Lease were low intertidal oyster reefs, while those from the Lockwood Folly River were purely subtidal. Average number of larvae present increased with increased size, though there was no significant correlation for this relationship ($r^2=0.40479$, p=0.1918).

Physical data

There was no significant difference between sites for any of the water quality data collected. Salinity at all sites averaged above 30ppt, and DO was above 5mg/L (Table 19). Temperature was above 20°C for all sites (Table 19), though this is likely due to the fact that most samples were collected in the summer months.

Table 17: Summary of reproductive data by site over the entire collection period.

Site	N-total	N-larvae present	% brooding	avg larval ct	avg height for brooding oyster (mm)
CL	155	4	2.58	174920	31.09
LF	154	4	2.6	112375	27.99
NR	137	4	2.92	181926	29.87

Table 18: Summary of reproductive data by sites for dates when larvae were present

Site	Date	N-total	N-larvae present	% brooding
CL	30-Jun	19	1	5.26
	30-Jul	18	2	11.1
	8-Sep	19	1	5.26
LF	16-Jul	20	1	5.00
	28-Jul	17	2	11.8
	29-Aug	40	1	2.56
NR	28-May	20	2	10.0
	12-Jun	19	1	5.26
	24-Jul	20	1	5.00

Table 19: Means of physical water characteristics for each site over the sampling period.

Site	Salinity (ppt)	DO (mg/L)	Water temperature (°C)
LF	30.46	6.50	24.43
CL	32.85	7.97	25.51
NR	31.57	7.13	23.92
BS	31.18	6.61	25.78
MM	32.63	8.61	21.00

DISCUSSION

Ostrea equestris is thought to be a stenohaline, subtidal species (Gunter 1951; Menzel 1955; Galtsoff and Merrill 1962). However, data from this study indicates that this is not the case in the northern extent of its range. In fact, for all the sites sampled, the highest densities were within the intertidal (either oyster reefs or sandy shell hash) with the exception of the floating docks (subtidal), though this could be influenced by the differences in sampling techniques. This pattern was also consistent between years, though densities did increase in the subtidal dredges from 2008 to 2009. Though densities for the trawl data was significantly lower at all sites and across habitats, these populations can be considered less patchy than the intertidal populations. This may relate to the sampling methods since trawls integrate numbers over a larger area. The other subtidal population that was sampled was fouling communities on floating docks. This habitat had higher densities at the three northern sites, than the two southern sites, a pattern not observed in the other habitats.

Intertidally the highest densities tended to occur in the lower intertidal (especially LIOR, SS40L, and SS75L). This is most likely due to the longer exposure time in the mid intertidal, which is generally exposed for 1-2hrs more than the low intertidal. *Ostrea* species tend not to be tolerant to desiccation (Peter-Contesse 2005; Baker 1995), and the lower intertidal areas allow for *Ostrea equestris* to remain somewhat wet at dead low (exposed at most 1.5hr on a negative tide). Those live *O. equestris* that were found in the mid intertidal were normally found in areas that were lower than the rest of the habitat sampled and did not completely dry out at low tide (i.e. depressions in the habitat). The crested oysters from the mid intertidal were patchier than those in the low intertidal. The fact that *Ostrea lurida* can be found up to 2m past the low tide line (Peter-Contesse 2001; Baker 1995; Polson and Zacherl 2009) indicates that intertidal settlement can be a normal pattern for *Ostrea* species, though survivorship may decrease with exposure (Trimble *et al.* 2009). *Ostrea lurida* can have patches of >30 oysters for mean maximum densities (per 0.25m²) intertidally with moderate coefficients of variation (Polson and

Zacherl 2009). This indicates that these populations are not as patchy within a site as some of *Ostrea equestris* populations are. Patch density (density where *Ostrea equestris* is present) is variable by site and habitat. It can range from less than 5 oysters/ 0.25m² to over 125 oysters/ 0.25m². Relative to the overall densities, patch density is an order of magnitude higher. Patches of *O. equestris* can range from 100s of meters apart at a site or within a habitat to less than a meter apart.

Populations were found across all sites and almost all habitats that were sampled. Even those sites that had low live densities had higher total dead densities. This is important because it indicates that these oysters are settling in these areas, but are not persisting. In fact, antidotal evidence at some of these sites confirms this, particularly at the Bogue Sound site, which has been observed to have a large population occurring on scallops (pers. communication, Tina Moore, NCDMF). This indicates that subtidal and seagrass can have larger densities than those observed at this site in this study. However, the majority of *Ostrea equestris* found in these habitats in Bogue Sound during this study was represented by scars. These mortalities can be caused by variations in weather patterns (particularly extreme cold (Peter-Contesse 2005; Baker 1995) and large scale rain events which dramatically drop the salinity (Hoese 1960). Scar to box ratios at the UNCW Research Lease indicated that there was a mortality event between the two sampling seasons, which was most likely caused by extreme cold. During the winter between the two seasons temperatures reached as low as -15°C on a series of extremely low tides (<-0.5ft). Normal winter lows are between -4° and -1°C. Likewise, freshets that occurred in Cedar Island, North Carolina most likely caused mass mortality of a large *Ostrea equestris* population that existed with an oyster farm (pers. communication, Jay Styron, UNCW). However, the Cedar Island information is antidotal and there is no empirical evidence.

One site pattern that requires further study is that the UNCW Research Lease and Middle Marsh (a southern site and the northern-most site) have very similar densities for the majority of their habitats. The exception being the floating dock communities which had significantly lower

densities at the UNCW Research Lease. The general trend was unexpected as one would expect populations to decline closer to the edge of the range. This may suggest that the northern edge of the range is expanding. However, while densities are similar, Middle Marsh is relatively patchier than the research lease based on the coefficients of variation. The similarity in densities between the UNCW Research Lease and Middle Marsh may have climate change implications. However, the relatively high densities that were observed at the northernmost site sampled, as well as the high densities within the intertidal may indicate a potential range expansion for this species. The potential for *Ostrea equestris* to be an indicator organism for climate change needs to be further evaluated in the future with continued intensive monitoring of some of the populations from this study, but also of potential populations further north.

Physical parameters that were measured did not seem to explain the differences in patterns at sites. However, available cultch type may help to explain these patterns. *Ostrea equestris* have been shown to settle readily on scallop shells (Gutsell 1926), as well as other bivalve species—*Mercenaria* sp. and pen shells (personal observations)—within the intertidal habitats. At Bogue Sound the majority of *O. equestris* were found on scallop shells, and some were found on scallops in New River. While this seems to be an important habitat for *Ostrea equestris* settlement in Bogue Sound, it was observed for the majority of the sites that crested oysters occurred on degraded shell hash. In some cases this was from other bivalve species, but in most cases it was *Cliona* sp. ridden *Crassostrea virginica* shell. Settlement on this shell may allow *Ostrea equestris* to settle on the edges of oyster reefs and in areas where there are degraded beds of shell. *C. virginica* does not seem to settle on this shell, and it has often been shown for this species that clean shell is the preferential cultch (Kennedy 1996). Menzel (1955) in his settlement experiments did indicate that *Ostrea equestris* readily settled on the older shell (though older was never qualified). This preference for older, degraded shell may be limiting to *Ostrea equestris* settlement in areas due to relative lack of substrate. Other *Ostrea* species have

been shown to be limited by lack of preferred shell substrate, particularly in the Pacific Northwest (Baker 1995, Trimble *et al.* 2009).

This relative preference for older, degraded shell can be seen in the habitat preferences of *O. equestris*, the exception being the significantly high populations from the floating docks. Within the floating dock habitat *Ostrea equestris* settled on the untreated floats, and were observed on the pilings for the docks if the wood was untreated. Those intertidal habitats with the highest densities had the highest amount of degraded shell. This is particularly true of LIOR edges, as well as SH75L and SH40L. The UNCW Research Lease was the best site example of this as everywhere there were high densities there was degraded shell. In the areas (Lockwood Folly River and Bogue Sound) where there were very low densities of *Ostrea equestris* the shell hash was clean and not degraded. The Lockwood Folly River probably best illustrates the importance of this cultch, as the persistent population was subtidal where there was a large amount of fouled and degraded shell. Also, the *Ostrea equestris* found within the second year for LIOR occurred on a reef that was older with more degraded shell.

Not only was there variability spatially, both on the small and large scale, but there was temporal variability as well. In many cases the densities between 2008 and 2009 decreased. However, this pattern could be the result of the more target sampling within 2009. Those populations that had increased densities in 2009 (SG at New River and LIOR at the Lockwood Folly River and the subtidal at most sites) were mainly subtidal. This offers a degree of protection from freezing temperatures, as there would be no direct exposure of the oysters in these areas. Also, for New River and the Lockwood Folly, these populations were located within areas that were relatively protected from boat wakes and waves. The populations for the 2009 sampling were less patchy than their 2008 counterparts. This however may be an artifact of a more targeted sampling within the second year.

It is interesting that these same populations, when revisited in 2010 for different sampling schemes, are different from the previous two years (personal observations). Some populations

seem now to be disappearing (Jay Styron, pers. communication) possibly due to freshets or cold winter weather (though there is no data to confirm this). Other populations have been observed in spots that they hadn't before (shell hash areas within the ICW near the Lockwood Folly River) (Markwith, personal observations).

Despite their variability and patchiness, it was observed that *Ostrea equestris* can constitute up to 100% of the live oyster within low intertidal shell hash habitats, and up to 10% on oyster reefs. *O. equestris* can also make up the majority of live oysters for fouling communities, subtidal areas, and seagrass beds. For these areas, especially the subtidal and low intertidal shell hash, large populations may be providing habitat for other organisms. It is unclear if aggregates of *Ostrea equestris* provide similar habitat either on the macro or micro scale, as other oyster populations do. Korringa (1951) showed that *Ostrea edulis* shells could act as habitat to a large number of epiphytic and benthic species. *O. edulis* (Tadorova *et al* 2009) and *O. lurida* (Baker 1995) both form reef-like structures that act as habitat. Preliminary observations (Markwith, personal observations) indicated that *Ostrea equestris* aggregates were not only utilized by epiphytes but goby species as well.

The patchiness and variability between habitats, sites, and years may be a product of their reproductive mode. *Ostrea equestris*, like all *Ostrea* species, are brooders. Based on congenetics it is assumed that they have a relatively short planktonic period (Baker 1995; Peter-Contesse 2005; Waller 1981), settling near parent populations (Baker 1995). This would then explain density differences between sites. Variability between years is probably due to very low brooding activity at a site. Walker and Powers (2001) showed that 3% of the GA populations were actively brooding, though most oysters were at some stage of reproductive activity. This was based on histological cross sections, which were labeled ripe, spent, indeterminate, etc. In North Carolina <3% of the populations are actively brooding veligers at a given site from May to October, indicating a low reproductive output.

Across seasons *Ostrea equestris* had relatively high condition indices values. These high values may be the result of their small size, as size can sometimes confound the condition index calculation. In general condition indices are higher in winter and lower in the summer (as it corresponds with the spawning season) (Shumway 1996). This was found to be true for *Ostrea edulis* in Spain (Cano *et al.* 1997), but not for populations in Croatia (Zrncic *et al.* 2007). *Ostrea equestris* does not seem to follow the expected pattern either, as summer and winter condition indices were highest for all sites, while the lowest were from the spring samples. This may be due to their seemingly low reproductive rates (explaining the high summer indices), impacts from the cold winter (explaining the low spring condition indices), or some environmental factor that is not yet fully understood.

Based on the distribution patterns seen in this study, settlement studies identifying preferred substrates need to be undertaken as well as an extensive population genetics study to see if the patchy distributions have a genetic consequence. Predation studies need to be considered to possibly explain some of the distribution patterns (i.e. SG in New River 2009). It was observed that there was some predation by drills and crabs during collections. However, it has been postulated by Wells and Gray (1960) that drills preferentially predate *Crassostrea virginica* in the subtidal, not *O. equestris*. These possible contradictions need to be examined further. A more intensive reproductive study that includes the northern populations may also explain some of the patterns. Condition index needs to be considered along with other oyster health indicators to find the best possible one for *Ostrea equestris* populations. Finally, one of the biggest unknowns that needs to be addressed based on this study, is the habitat potential for *Ostrea equestris*. Patch density is large compared to overall density. These larger patches could be considered islands providing structure where there is very little other structure.

This study indicates that *Ostrea equestris* is more common than previous literature would lead one to believe, comprising 50-100% of the live oyster in some habitats. As a result, it is probable that these oysters may play a larger ecological role than expected based on their

numbers, especially in areas of shell hash, which could be considered patch-type reefs as with *Ostrea lurida* in the Pacific Northwest (Baker 1995). The densities that have been found to occur in this study indicate not only are there meaningful populations further north than one would expect, but intertidally as well. Both observations are contrary to expectations, but within these areas populations tend to be patchier. Patchiness of populations is fairly variable through time and space though. This is most likely due to the fact these oysters are brooders, and have what seems to be a fairly low reproductive output.

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