ABSTRACT

MROCH, RAYMOND MILLARD. Spatiotemporal Variation in Broodstock Reserve Fecundity. (Under the direction of David B. Eggleston).

A major expectation of marine no-take reserves is that organisms within a reserve, over time, will export eggs and larvae to help sustain populations within and outside of reserves. Because fecundity and reproductive output can vary in space and time, the success of broodstock reserves depends on selecting the habitat or location that maximizes reproductive output to the target population. The goals of this study were to quantify spatial and temporal variation in (1) per capita fecundity (# eggs/individual) of female eastern oysters (*Crassostrea virginica*) within a network of marine broodstock reserves in Pamlico Sound, North Carolina; (2) fecundity per square meter (eggs/m^2); (3) reserve fecundity (# eggs/reserve); and (4) the effects of endogenous and exogenous factors on spatiotemporal variation in per capita fecundity.

A total of 2681 oysters were collected by scuba divers from six broodstock reserves over five sampling periods during 2006-2008 and processed in the laboratory for fecundity. Per capita fecundity ranged from 0 to 340,500 eggs, and mean per capita fecundity increased exponentially with oyster size (LVL mm) peaking in May of all years. In general, there were distinct spatial differences in fecundity depending upon the response variable, with per capita fecundity highest at more inshore reserves of moderate salinity, and fecundity per m^2 and reserve fecundity highest at more seaward reserves with relatively high salinity. Ranking of broodstock reserves for management purposes will likely depend upon the specific management goal. For example, if the goal is to expand broodstock reserves at locations that

maximize reproductive potential per square meter of habitat created or improved, then reserves such as Ocracoke and Hatteras, that combine the joint effects of relatively high oyster density, size and per capita fecundity, would be ranked highest for expansion. If the management goal is to rank conservation of current broodstock sanctuaries, then relatively high salinity reserves such as Hatteras, that combine the joint effects of relatively high m^2 fecundity and large substrate surface area, provide the greatest reproductive potential in terms of reserve fecundity. Lastly, if the management goal is to augment oyster densities via stocking with oyster spat at sites with high reproductive potential at the individual level, then more inland reserves such as Bluff Point and West Bay would be ranked highest. These results highlight the need to consider time and space when measuring reproductive potential of marine reserves, as well as the need to consider a comprehensive suite of response variables that best inform managers.

Spatiotemporal Variation in Broodstock Reserve Fecundity

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

May, 2009

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BIOGRAPHY

Raymond Millard Mroch III was born to in Libertyville, IL on July 20, 1978. He spent his youth in the suburbs of Chicago, with frequent trips to northern Wisconsin. There, Ray would mostly be found swimming, rowing, reading, hiking and generally being a nuisance. Ray would often be found doing experiments, discovering important lessons, such as that it is not possible to become undizzy by spinning in the opposite direction. Despite his inquisitive nature, he was an extremely poor student throughout his entire youth. Due to his extremely limited options he joined the Marine Corps immediately after graduating (late) from high school. There, he was given the opportunity to dig holes on a majority of the world's continents. Immediately upon returning to the mainland, he married and enrolled in school. It was in college that Ray discovered that he was a nerd, and that's when things started to click. He started doing well in school, earning a high GPA and several scholarships and fellowships. After graduating and a brief stint testing animal heads for rabies, he enrolled in graduate school. In graduate school, Ray has worked hard in conducting his research and found his work challenging and enjoyable.

ACKNOWLEDGMENTS

This Master's thesis has been a long process through which I am eternally grateful for the encouragement, support, resources and advice that I received along the way. First, I would like to thank my wife and son for their everlasting patience and support through this entire process. Without their understanding and encouragement, I would have never reached this point. I would also like to thank my advisor, Dr. Dave Eggleston for his advice, his insight that helped me become a better scientist, and more importantly that helped me learn more about myself so I can continue to improve and grow. Thank you to my committee. Thank you, Dr. Kamykowski, for providing me with unlimited use of the FlowCam. Without that tool, I would still be counting eggs right now. Dr. Pollock, thank you for your knowledge and guidance with my analysis. Brandon Puckett, I would like to thank you for your help, even when you were busy with your own project. I would also like to thank my labmates for listening me when I needed you and for going on the research trips me to schlep, count and measure oysters, in no particular order: Brandon Puckett, Geoff Bell, Ryan Rindone, Erika Millstein, Mary Radlinski, Christina Durham, Amy Haase, Gayle Plaia, Darren Parsons, Ashton Drew, Jenny Vander Pluym, Michelle Moorman and Grant Parkins. Thanks also to several people that you should consider hiring: my two undergraduate volunteers, Sarah Watts and Kira Lopez, and my paid lab assistant, Jamie Eggleston. Also, thanks to all of the people not associated with NCSU who provided help, most especially, Melissa Southworth at VIMS and Stopher Slade at NCDMF. And finally, thanks to the wonderful, colorful people of North Carolina, in particular, Jack and Jack Jr., pound of bacon, Hollywood Concrete, The owner of the inconvenience store, and the ferry operators, who demonstrated professionally that it is possible to fit 10 pounds of manure in a 5 pound bag. I would also like to express my appreciation for the funding provided through North Carolina Sea Grant.

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Introduction:

Marine populations often exhibit substantial spatial and temporal variability in abundance and demographic rates driven by environmental stochasticity operating on the larval dispersal phase, density-dependent post-settlement processes, and fisheries-induced reductions in spawning stock biomass (Roughgarden et al. 1988, Rumrill 1990, Menge 2000, Levin 2006, Broitman et al. 2008, Rilov et al. 2008). Since the mid-1980s, considerable attention has focused on the role of larval supply on spatiotemporal dynamics of populations by examining the relationship between larval settlement and subsequent juvenile or adult populations, or the influence of stochasticity in larval dispersal, mortality and settlement success (Rumrill 1990, Pawlik 1992, Eggleston and Armstrong 1995, Hughes et al. 2000, MacKenzie 2000, Epifanio and Garvine 2001, Anger 2006, Levin 2006, Jessopp and McAllen 2007). Comprehensive understanding of recruitment dynamics in marine organisms should not only consider supply-side ecology and the role of post-settlement processes (e.g., growth and survival), but also the role of spatiotemporal variation in reproductive processes on subsequent juvenile and adult population dynamics. Quantifying spatiotemporal variation in fecundity and larval output, and the processes underlying this variation, is necessary to understand the effects of both pre- and post-settlement processes on population dynamics of marine organisms.

Despite the relative popularity of supply-side ecology as a research topic, studies that quantify spatiotemporal variation in larval output and associated mechanisms are rare. In one example, spatiotemporal variation in barnacle (*Balanus glandula*) fecundity on the west coast

of the United States was driven by the level of primary productivity in adjacent waters (Leslie et al. 2005). In an example from the Great Barrier Reef, 72% of the spatial variation in recruitment of acroporid corals was due to variation in fecundity of spawners (Hughes et al. 2000). Few studies, however, have examined fecundity directly, instead choosing surrogates that impact or mitigate fecundity. For example, Levitan et al. (1992) demonstrated that fertilization success of the sea urchin (*Strongylocentrotus franciscanus*) decreased with decreasing density and increasing dispersion of spawning urchins.

Fecundity is implicitly linked to stock-recruitment relationships. The premise behind stock-recruitment relationships is that as the spawning stock decreases, the probability of poor recruitment increases due to reduced larval output and subsequent recruitment. Numerous examples of spawning stock-recruitment relationships in fish (Zhao et al. 2003, Arregui et al. 2006, Hanson et al. 2006, Pope et al. 2006) and invertebrates, especially crustaceans (Caputi et al. 1995, Garcia 1996, Hannah 1999, Ye 2000, Wahle 2003), demonstrate the positive relationship between adult populations and subsequent recruitment levels despite the various intervening bio-physical factors between spawning and recruitment. In a management context, spawning-stock recruitment relationships are important because harvest (and/or mortality) thresholds and targets are set to protect sufficient spawning stock to preclude recruitment overfishing, whereby the population of spawners is reduced to such a low level that birth rates cannot overcome death rates in the population (Hilborn and Walters 1992). Traditional management approaches, such as size and catch limits, have tried and often failed to adequately protect spawning-stock biomass.

Accordingly, fisheries managers have moved from more traditional management approaches to establishing no-take marine reserves wherein organisms are protected from harvest, thus protecting the spawning stock and improving the likelihood of recruitment to the fishery (Rowley 1994, Lambert et al. 2006). Reserves with the primary goal of increasing larval supply by protecting the broodstock are therefore called broodstock reserves.

Marine reserves

Broodstock reserves, and no-take marine reserves in general, are expected to positively impact a population by directly protecting the organisms within a reserve from fishing. Such protection increases the density of adults within the reserve, as well as the average size and age of organisms (Halpern and Warner 2002, Halpern 2003). Increased numbers of larger individuals are expected to boost reproductive output since larger individuals invest their energy in reproductive output rather than somatic growth. In cases where multiple reserves are created, the goal is often a series of 'source' populations that seed each reserve, as well as the surrounding fished areas (Pulliam 1988, Eggleston et al. 1999). Because fecundity and reproductive output can vary in space and time, the functional success of broodstock reserves depends on selecting the habitat or location that maximizes reproductive output to the target population.

The spatial scale at which fecundity is considered can also have important management implications. For example, estimating fecundity at the per capita level provides information on how individual fecundity might vary over time and space, but does account

for differences in oyster density or the overall area of a given reserve, both of which can affect how reserve networks might be ranked in terms of reproductive output. For example, high per capita fecundity suggests that an area might be a good candidate for seeding of oysters to augment local densities, whereas reserves with high densities of oysters, when combined with oysters of relatively high fecundity, be ranked highly in terms of overall reproductive output and warrant expansion of reef materials. Lastly, reserves that have high overall reproductive output due to a combination of density, size and area would be ranked highly for conservation without additional reef materials or augmentation via seed oysters.

Studies of the efficacy of marine reserves typically report response variables such as biomass and density of a target population, or overall biodiversity (Guenette et al. 1998, Halpern and Warner 2002, Halpern 2003). For example, in St. Lucia in the Caribbean, reef fish biomass increased in marine reserves and spilled-over into fished areas after reserves were established (Roberts et al. 2001). While increased fish biomass would likely increase fecundity within the reserve, fecundity was not quantified in their study. Similarly, marine reserves in the Florida Keys that were established to protect male spiny lobsters (*Panulirus argus*) were expected to positively impact fecundity since female lobsters preferentially mate with large males, and female clutch size is linked with male size (Cox and Hunt 2005); however, individual or reserve-level fecundity were not quantified. Lastly, a temporal closure to harvest of the blue crab (*Callinectes sapidus*) near the mouth of the Chesapeake Bay reduced fishing mortality of migrating gravid females (Lambert et al. 2006), increasing the

likelihood of these females successfully reaching their spawning grounds, however, fecundity was not quantified.

Objectives

The goals of this study were to quantify spatial and temporal variation in (1) per capita fecundity (# eggs/individual) of female eastern oysters (*Crassostrea virginica*) within six marine reserves in Pamlico Sound, North Carolina; (2) fecundity per meter^2 (the eggs created by all oysters contained in a random square meter of hard substrate (eggs/m^2)); (3) reserve fecundity (the eggs created by all oysters within an entire reserve (eggs/reserve)); and (4) the effects of endogenous and exogenous factors on spatiotemporal variation in per capita fecundity. A sessile species such as the eastern oyster is an ideal study organism in this case because spatiotemporal variation in fecundity is due to the immediate environment rather than being confounded with movement among habitats of differing quality.

Focal species: eastern oyster

The eastern oyster (referred to as oyster hereafter), once highly abundant in the estuaries of the middle Atlantic states of the U.S., declined in abundance sharply during the 20th Century (Chestnut 1951, 1955, Bahr and Lanier 1981, Hargis and Haven 1988, Rothschild et al. 1994, Kennedy et al. 1996, Lenihan and Peterson 1998, Mann and Evans 1998, Breitburg et al. 2000, Jackson et al. 2001, Kirby and Miller 2005). This decline was due to a combination of factors including eutrophication, disease, and destructive fishing

practices (Jackson et al. 2001, Kirby and Miller 2005, Luckenbach et al. 2005). Destructive harvest methods, such as dredging, have resulted in severe degradation of the oyster reef matrix (Dealteris 1988, Hargis and Haven 1988, Lenihan and Peterson 1998), which dramatically reduced reef area and elevation (Rothschild et al. 1994, Lenihan and Peterson 1998). Despite reductions in abundance, oysters remain economically important. In North Carolina, the harvest of oyster meats has averaged more than $1.3 million annually for the decade ending in 2007 (NMFS 2009).

Oysters have long been recognized as a keystone species that supports hundreds of species in estuarine ecosystems (Wells 1961, Zimmerman et al. 1989, Tolley and Volety 2005), providing habitat for diverse fouling organisms, as well as shelter for mobile organisms (Wells 1961, Bahr and Lanier 1981, Lenihan and Peterson 1998, Eggleston et al. 1999, Lehnert and Allen 2002). With time and continual settlement of new spat, oysters can transform flat substrate into a complex area of high relief that can provide an elevated refuge from benthic hypoxia for oysters and associated inhabitants (Lenihan and Peterson 1998, Breitburg et al. 2000). Oysters provide necessary substrate for some fish species, such as the striped blenny (*Chasmodes bosquianus*) and naked goby (*Gobiosoma bosc*) to lay their eggs (Harding and Mann 2000), as well as provide food for estuarine predators such as blue crabs (*Callinectes sapidus*) (Eggleston 1990) and cownose rays (*Rhinoptera bonasus*) (Smith and Merriner 1985). Oyster reefs also provide food indirectly by providing habitat for such prey organisms as annelids, amphipods and peracarid shrimp (Zimmerman et al. 1989). The time period for oyster reef creation can be rapid--within two years of creation an oyster reef can

harbor similar amounts of species and individuals as older, naturally created reefs (Meyer and Townsend 2000).

Oysters also provide water quality ecosystem services. Average filtration rates for *C. virginica* range from 1.5 L/hr to 8 L/hr (Langefoss 1975, Palmer 1980, Pietros and Rice 2003). Field studies show that this filtration rate can limit phytoplankton populations (Lewitus et al. 1998), which may reduce the incidence of hypoxia (Jackson et al. 2001). By being incorporated into the body of the oyster, plankton is made available as food to higher trophic levels (Bahr and Lanier 1981). The precipitous decline in oyster abundance has drastically reduced the filtration benefits of oysters to estuarine water quality. Historically, native oysters were able to filter a volume of water equal to the entire volume of Chesapeake Bay every three days, making the water so clear that a cannon mistakenly dropped from a ship was visible from the deck 10 meters below the surface (Jackson et al. 2001). Today, it is estimated to take the extant oyster population over a year to filter a similar volume (Jackson et al. 2001).

Oyster reproduction

Oysters are protandrous, sequential hermaphrodites, functioning as males initially before transitioning to females (Kennedy et al. 1996), although they can metamorphose back to female (Galtsoff 1964). This reproductive strategy allows it to take advantage of its large size later in life to create and store relatively large numbers of eggs—an evolutionary adaptation to offset the high mortality of oyster larvae (Kennedy et al. 1996). The size at

which an oyster metamorphoses from male to female is complex, and driven by a number of factors that are poorly understood. Oysters in the James River, Virginia were primarily (90%) male when below 35mm in left valve length (Andrews 1979), but larger oysters in this and other areas can vary from having almost 4 times as many females as males (Burkenroad 1931), to an almost even ratio (Smith 1949). Although oysters have been observed to metamorphose from female to male (Galtsoff 1964), the mechanisms behind this transformation are unknown. One potential explanation for the unusual shift from female back to male is increased stress from predation, or decreased food availability, which initiates a shift to sperm production to conserve resources (Davis and Hillman 1971). Another potential explanation for the shift from female back to male involves proximity to other oysters. Oysters in close proximity were found to have a lower proportion of females than oysters growing separately (Burkenroad 1931, Smith 1949). In the laboratory, oysters exhibited a change in sex when placed in trays containing only a single sex, but did not in trays with equal numbers of both sexes (Kennedy 1983).

Oysters sacrifice somatic growth during the summer months for gametic growth (Choi et al. 1994), devoting as much as 48% of their total energy to gamete production during this time (Dame 1976). Oysters initially spawn as females at a size of ~ 30 mm shell length (Kennedy et al. 1996), and the quantity of eggs released per spawn increases exponentially with size (Mann and Evans 1998). Throughout their range on the east and gulf coasts of the United States, *C. virginica* differs in its spawning frequency. Oysters in Texas spawn between 3-10 times a season (Choi et al. 1994), whereas oysters in the James River,

Virginia spawn between 3-4 times a season (Mann and Evans 1998). Oysters in New England may spawn once a year, or not at all if temperatures do not rise sufficiently (Kennedy et al. 1996). Oysters in Pamlico Sound, NC are expected to fall somewhere in between the two extremes, spawning either 2-3 times a spawning season (May-October) (Chestnut 1951, 1955). The number of spawns in a season varies depending on such factors as stress, food availability, salinity and temperature (Kennedy 1996). Temperature is a primary factor affecting gametogenesis (Galtsoff 1964). Based on calculations of gonad maturation time from an experimental study (25 days), and estimation of recovery time of natural populations (100 days) (Loosanoff and Davis 1952, Choi et al 1994), oysters in Pamlico Sound were estimated to be prepared to spawn approximately 60 days after the initial, synchronous spawning event (Eggleston and Puckett, unpublished data).

Oyster settlement patterns in NC exhibit a high degree of spatiotemporal variation. Spatially, settlement is concentrated along the Outer Banks near Hatteras and Ocracoke Inlets with a temporal peak in mid-summer and a secondary peak in early fall (Eggleston and Puckett, unpublished data). This spatiotemporal variation in oyster settlement likely reflects the combined effects of spatiotemporal variation in oyster fecundity and spawning success, as well as meteorologically-forced estuarine circulation patterns. There is little information on spatiotemporal variation in fecundity of oysters in any region (but see Cox and Mann 1992).

Oysters are capable of spawning large numbers of eggs at each spawning event, ranging from 5,200,000 to 115,000,000 eggs (Brooks 1891, Churchill 1928, Nelson 1928,

Galtsoff 1930, Davis and Chanley 1956). For example, Cox and Mann (1992) reported that oysters in the James River, Virginia were at the low end of the range, spawning about 5,200,000 eggs per oyster in one spawning event. This amount of variation makes it difficult to choose a single value for the average fecundity of a mature female oyster. Spatial and temporal variation in oyster fecundity could be driven by a combination of factors including primary production, fluctuations in exogenous conditions such as temperature and salinity (Kennedy et al. 1996), or predation and disease (Villalba et al. 2004).

Study system: Broodstock reserves in Pamlico Sound

Given the economic and ecological benefits of oysters, much effort has been expended to restore oyster populations to historic levels (NCDMF 2006). In North Carolina, nine oyster broodstock reserves have been created in Pamlico Sound (Figure 1) in the hopes that these reserves will be self-sustaining, as well as supply larvae to the rest of Pamlico Sound that is still open to harvest (NCDMF 2006). Like the broodstock reserves created for restoration (Figure 1), the natural oyster reefs in Pamlico Sound primarily fringe the sound in relatively shallow waters near the mainland or barrier islands (Figure 2).

Pamlico Sound makes up a large part of the Croatan-Albemarle-Pamlico Sound Estuarine System (CAPES), the largest lagoonal system and the second largest estuarine system in the United States (Pietrafesa et al. 1986). Pamlico Sound is a wind-driven, shallow, lagoonal estuary that is separated from the Atlantic Ocean by a string of barrier islands (Lin et al. 2007). In a large estuarine system such as Pamlico Sound, it is expected that oyster

fecundity will vary due to variance in habitat quality throughout the sound. Salinity is a key factor that drives much of the variation in oyster demographic rates, and can range from a high of approximately 30 PSU close to the oceanic inlets to ~ 15 PSU near the westernmost part of the sound, with chlorophyll *α* concentrations inversely related to salinity (Lin et al. 2007). This combination of factors is likely to set up conditions favorable for oyster growth and fecundity in locations farthest from oceanic inlets. However, neither the relative magnitude of these factors, nor how they may interact to affect oyster fecundity is known.

The nine oyster broodstock reserves in Pamlico Sound consist of varying numbers of mounds constructed of piles of limestone rip-rap (Figure 3). These reserves are constructed using large (approximately 0.5m diameter) boulders piled on the sound bottom to act as settlement substrate for oysters. These mounds range in height from one meter to approximately five meters depending on depth (Figure 3). The size of broodstock reserves and date created varies (Table 1). The six broodstock reserves studied included: Bluff Point, Crab Hole, Deep Bay, Hatteras, Ocracoke and West Bay (Figure 1). These reserves were selected because they are the target of very active oyster restoration efforts by the NC DMF and span the length/width axes of Pamlico Sound.

Hypotheses:

We hypothesized that (H_1) per capita fecundity, (H_2) fecundity per meter^2, and (H_3) overall reserve fecundity within a given broodstock reserve would vary both spatially and temporally across the six broodstock reserves selected for study in Pamlico Sound. We also

hypothesized that (H_4) average fecundity would vary in accordance with different endogenous (e.g., left valve length (LVL), left valve width (LVW), meat volume) and exogenous (e.g., temperature, salinity, dissolved oxygen and chlorophyll α concentrations) factors. The endogenous factors that were expected to positively influence fecundity (LVL, LVW and meat volume) are all closely related to an oyster's size. Larger oysters are expected to divert more resources to reproduction, due to their ability to capture more food and the decreased need for somatic growth (Kennedy et al. 1996). We expected that average fecundity would be positively correlated with temperature, dissolved oxygen and chlorophyll α concentrations, and negatively correlated with salinity. Increased temperature should be favorable to fecundity because it will increase oyster metabolism, thereby increasing feeding rate. Chlorophyll α concentrations are expected to act as a proxy for food availability and indicate potential energy available to oysters. Increased dissolved oxygen concentrations were expected to increase fecundity by reducing hypoxic stress. Fecundity was expected to be negatively correlated with salinity for two reasons. First, the frequency of parasites should increase with salinity, thereby reducing energy available to oysters in high salinity areas (Villalba et al. 2004). Second, oyster predators are more common in more saline waters (Wells 1961, Villalba et al. 2004), resulting in greater mortality due to predation. While the relative magnitude of the effects of water quality parameters on oyster fecundity are currently unknown, we expected that broodstock reserves at Deep Bay and Bluff Point, located in the mesohaline zones of Pamlico Sound, would have relatively high fecundity since phytoplankton in Pamlico Sound exhibit a negative correlation with salinity (Peierls et al.

2003). Conversely, we expected that fecundity of oysters at high salinity reserves near Ocracoke and Hatteras inlets would be relatively low due to increasing prevalence of oyster predators and disease with salinity (Wells 1961, Villalba et al. 2004), while also being more nutrient-poor than less saline sites (Buzzelli et al. 2003, Lin et al. 2007).

Material and Methods:

Objective 1: Spatiotemporal variation in per capita fecundity

Oysters were collected from each of six broodstock reserves (Table 1) by SCUBA divers using a 0.25m^2 quadrat. To minimize variation in fecundity due to age of an oyster reef, we collected oysters from mounds created during or prior to 2003 whenever possible. For two of the reserves (Ocracoke and Bluff Point) this was not possible, so only mounds that were created in the first year of the reserves' existence (2005 and 2006, respectively) were used. From this subset, mounds were chosen randomly and sampled by placing one quadrat haphazardly on each randomly selected mound. To normalize sampling effort by reserve size, the number of quadrats sampled was standardized by the number of mounds and aerial footprint of each reserve, with a minimum of three mounds sampled at the smallest reserve and five mounds sampled at the largest reserve (Table 1). Samples were collected in June and August 2006, May and August 2007, and May 2008. These sampling dates were selected in an attempt to collect the oysters when they would be at their most ripe and prepared to spawn.

At the surface, all oysters were removed from the rip-rap and spread evenly inside a $0.25m^2$ quadrat; a quarter of this quadrat was randomly sub-sampled. Left valve length and LVW were recorded for individuals to the nearest 0.1 mm. As oyster size measurements were recorded, oysters were separated into six size classes of 15 mm each: 0-15 mm, 15.1-30 mm, 30.1-45 mm, 45.1-60 mm, 60.1-75 mm, and 75+ mm. After all quadrats were sub-sampled for a given reserve, ten oysters from the two smallest size classes and twenty oysters from the remaining size classes were randomly selected from each size class, the meat removed from the shell, and then fixed in a 5% formalin solution (in 2006) or a 30% ethanol solution (in 2007 and 2008) for laboratory processing. An experiment was conducted to test concerns that formalin fixation may cause a stiffening of the oyster tissue, thereby retaining eggs within the discarded oyster leading to egg counts that were biased low. To test for the potential effects of preservation method on estimated fecundity, 20 oysters were assigned to each of three processing methods: fresh without preservation, or stored in 30% ethanol or 5% formalin for a minimum of one week. After this time, oysters were processed identically to the method they were processed in this study. This experiment showed no difference in mean egg counts among the three treatments (ANOVA: df = 29, p=0.27).

In the laboratory, each oyster was removed from its container, soaked for a minimum of five minutes in 25 mL of 1 μm filtered seawater, rinsed, strained and blotted under a fume hood to remove formalin and placed in a graduated cylinder to record its meat volume by displacement volume. Next, oysters from each size class were processed individually to determine the total egg contents (per capita fecundity). The gonad and visceral mass were

sliced front and back with a scalpel and rinsed with one μm filtered seawater until the entire gonad was stripped of its contents. The egg suspension was then washed in a 90 μm mesh-sieve to allow the eggs to pass through, yet retain larger particles such as pieces of shell and oyster meat. The egg suspension was retained in a two liter beaker and its volume recorded. A one mL sample of the stirred and homogenously mixed suspension was taken with an Eppendorf pipette. The eggs in the sample were counted using a Flowcam Flow Cytometer. The flow cytometer captured images of the egg suspension as it passed through a 100 μm cuvet. The eggs from each digital picture were then counted. Three 1 mL samples were counted for each oyster, and the average per capita fecundity (F_{ind}, eggs/oyster) of three subsamples of oysters was estimated as:

$$F_{ind} = E_{mean} \times V_{rinse},$$

Where E_{mean} is the mean egg count from three subsamples, and V_{rinse} is the total rinse volume from the 2 L beaker.

A two-way ANCOVA model (α=0.5) was used to test if mean per capita fecundity of female oysters varied significantly according to month and reserve and, with oyster size (LVL in mm) as the covariate and month and reserve as factors (**H_1**). Initially, variances were heterogeneous (Hartley's F-max test; $p < 0.01$, for the null hypothesis of equal variances) among the treatment levels, which was corrected with a log(x+1) transformation of the response variable (eggs/oyster) (reserve: p=0.57, month: p=0.37). Linear least-squares regression models were fit to the relationship between oyster size and log(eggs/oyster) (binomial z-test, α=0.05). Multiple comparisons between treatment levels were conducted

with a Student Newman-Keuls test to test for significantly different groupings of reserve or month.

Objective 2: Spatiotemporal variation in oyster fecundity per meter^2

The results of Objective 1 were used to determine the mean and standard deviation per capita fecundity of oysters within each size class for each reserve and date of sampling. To incorporate the variability in per capita fecundity in estimates of fecundity per m^2, fecundity per m^2 (F_{area}) was calculated for each quadrat replicate at each reserve during each month as:

$$F_{area} = \sum_{1}^{sc} \sum_{1}^{n} (F_{sc}) + (SD_{sc} \times R),$$

where *sc* is the number of size classes (6), *n* is the number of oysters per m^2 in a size class obtained from quadrat replicates at a reserve during a sampling month, F_{sc} is mean per capita fecundity of oysters in a size class at a reserve during a sampling month (Appendix 1), SD_{sc} is the standard deviation of mean per capita fecundity of oysters in a size class at a reserve during a sampling month (Appendix 1), and *R* is a random number between -1 and 1 generated from a uniform distribution. If the deviation term was sufficiently negative to result in a negative fecundity for individual *n*, fecundity for that individual was set equal to 0 (i.e., fecundity cannot be negative).

Fecundity per m^2 estimates obtained from each quadrat were treated as replicates for a two-way ANOVA model (α=0.5) used to test if mean fecundity per m^2 varied significantly according to month and reserve (**H_2**). As above, variances were heterogeneous for both

reserve ($P<0.001$) and month ($p<0.013$) effects. Data were log(x+1) transformed to satisfy assumptions of normality and homogeneity of variance (reserve $p=0.11$; month $p=0.079$).

Objective 3: Spatiotemporal variation in reserve fecundity

To estimate total fecundity within a given broodstock reserve, estimates of fecundity per m^2 were multiplied by the total surface area of hard substrate within each reserve, determined via side-scan sonar (Eggleston and Ballance 2007). A two-way ANOVA model was used to determine if overall reserve fecundity varied by reserve location and month (**H3**). Once again, variances were heterogeneous for both the reserve factor ($p<0.01$) and month factor ($p<0.01$), but were corrected via a log(x+1) transformation of the response variable (reserve: $p=0.13$, month: $p=0.10$).

Objective 4: Factors influencing per capita fecundity among broodstock reserves

A multiple regression analysis was conducted to examine the relationship between mean per capita fecundity and oyster LVL, LVW, meat volume, chlorophyll α concentration, temperature, salinity, and dissolved oxygen (**H_4**). The mean temperature and chlorophyll α concentrations for each broodstock reserve in Pamlico Sound for the time period 60 days prior to field collections of oysters for fecundity analyses (the minimum time between sampling events) were obtained from the *Ocean Biology Processing Group's* MODIS interface satellite (NASA 2009). In addition, temperature, salinity and dissolved oxygen concentrations were collected concurrent with field sampling events using a YSI-6600 water

quality instrument. Chlorophyll α concentrations were also collected by filtering 200mL of water through a glass fiber filter with a particle retention rating of 98% for particles 1.2μm and greater during sampling events. The retention rate of the filters used was similar to that of oysters, which are poor at retaining particles smaller than 1μm, while having a high retention rate for particles larger than 1μm (Langdon and Newell 1990). The relationship between mean per capita fecundity for a given reserve and various endogenous (oyster size, meat volume) and exogenous factors (temperature, salinity, dissolved oxygen, Chlorophyll α concentration) (**H4**) was tested using a backwards elimination multiple regression model (α=0.05).

Results:

Objective 1: Spatiotemporal variation in per capita fecundity

A total of 2681 oysters were collected and processed for fecundity from the six broodstock reserves over the five sampling periods (Appendix 1). Per capita fecundity ranged from 0 to 340,500 eggs. Oysters containing exactly 0 eggs were assumed to be male, leaving 1768 female oysters (those containing eggs) for use in the per capita fecundity analyses. While female oysters were present at all size classes, the proportion of female oysters approached 1 for oysters over 100mm LVL (Figure 4). Mean per capita fecundity increased exponentially with oyster size (LVL mm) in May (2007, 2008), which is prior to likely spawning in early June (Figure 5). Conversely, there was much less variability and a relatively gradual increase in fecundity with oyster size in June (2006) and August (2006,

2007), which was apparently after the main spawning event in early June (Figure 5). In terms of spatial variation, there was generally a positive, exponential relationship between per capita fecundity and oyster size at all reserves; however, per capita fecundity was two to three-fold higher at West Bay and Bluff Point than at Ocracoke (Figure 5). Average per capita fecundity of female oysters varied significantly by month (Figure 7a), reserve location (Figure 7b) and oyster size; however, a significant three-way interaction precluded contrasts across the main effects (Table 2). This three-way interaction is shown via the different slopes in the regression lines among months and reserve sites on Figures 3 and 4. For example, although the inland sites (Bluff Point, West Bay, and Deep Bay) were all grouped together and ranked the highest in per capita fecundity, the rank order of all reserves was, from highest to lowest: Bluff Point, West Bay, Deep Bay, Hatteras, Crab Hole and Ocracoke (Figure 7b).

Objective 2: Spatiotemporal variability in fecundity per m^2.

When per capita fecundity at each reserve was scaled up and averaged over 1 m^2, fecundity varied significantly by reserve and month (Table 3). When reserves were pooled, fecundity per m^2 was highest in May 2008 and 2007 and lowest in June 2006 (Figure 8a), similar to the pattern observed in per capita fecundity. When sampling months were pooled, however, unlike per capita fecundity there was a positive relationship between fecundity per m^2 and salinity (Figure 8b). The rank order of fecundity per m^2 by reserve location, from

highest to lowest, was: Ocracoke, Hatteras, Bluff Point, Crab Hole, West Bay and Deep Bay (Figure 8b).

A significant reserve by month interaction precluded contrasts across the main effects (Table 3). The significant two-way interaction is evident in the changes in the rank order of reserve-specific fecundity per m^2 during each sampling period (Figure 9) and monthly fecundity per m^2 at each reserve (Figure 10). For example, West Bay was ranked in the highest group for May 2007, 2008 and June 2006, but on the lower end for August 2006 and 2007 (Figure 9). Thus, in contrast to the results for per capita fecundity, Hatteras consistently had the highest fecundity per m^2 of all reserves (ranked 4 of 6 in per capita fecundity), whereas Deep Bay (ranked 3 of 6 in per capita fecundity) ranked lowest in per square meter fecundity (Figures 3b, 6b and 7).

Differences in reserve rank order in per capita and per square meter fecundity were due to differences in oyster density and left valve length (Figure 11). Reserves with both relatively high oyster density and left valve length ranked high in fecundity per m^2, whereas reserves with either high oyster density and small left valve length (e.g., Crab Hole) or larger left valve length and lower oyster density (e.g., Deep Bay) ranked lowest in fecundity per m^2 (Figures 6 and 9).

Objective 3: Spatiotemporal variation in reserve fecundity

When fecundity per m^2 was scaled to the overall size of a given broodstock reserve based the amount of hard substrate estimated from side-scan-sonar mapping (Eggleston and

Ballance 2007), mean reserve fecundity varied significantly by month and reserve location (Table 4). Mean reserve fecundity exhibited the same temporal pattern (peak fecundity in May 2007, 2008) as observed for per capita fecundity and fecundity per m^2 (Figure 12a). Spatially, the reserves were split into two groups based on relatively high or low fecundity; the high grouping consisted of every reserve except Bluff Point and the low group consisted of the three most inland reserves (Bluff Point, Deep Bay and West Bay) (Figure 12b). This spatial trend reflects a positive relationship between fecundity and salinity, similar to fecundity per m^2, with the exception of Bluff Point and Crab Hole (Compare Figures 6b and 10b). A significant reserve by month interaction effect on reserve fecundity precluded comparisons across main effects (Table 4). Although the general trend of peak fecundity in May of 2007 and 2008 was true for most reserve locations, there were some exceptions. For example, reserve fecundity increased steadily over time for Crab Hole (Figure 14e). The spatial trends in reserve fecundity were similar to those for mean fecundity per m^2. As an example, Hatteras consistently was in the highest reserve fecundity grouping, however, no reserve was consistently ranked in the lowest group throughout the course of the study (Figures 7 and 11).

Objective 4: Factors influencing per capita fecundity among broodstock reserves

In general, oyster per capita fecundity pooled across all sampling dates increased exponentially with oyster LVL and meat volume (Table 5; Figure 15). There was a marginally non-significant relationship ($p = 0.08$) between fecundity and LVW (Table 5). In

terms of exogenous variables, there was a positive and statistically significant linear relationship between fecundity and dissolved oxygen concentration (Fig 14d), and statistically significant negative relationships with salinity (Fig 14a), temperature (Fig 14b) and chlorophyll α (Table 5; Fig 14c). When per capita fecundity was pooled across only the two months with the highest per capita fecundity (May 2007 and 2008), there was a statistically significant positive exponential relationship between fecundity and all endogenous variables (Table 6; Figure 17). Conversely, all exogenous variables (except chl α, $p = 0.61$) had a statistically significant negative relationship with fecundity (Table 6, Figure 18). Therefore, when oysters most likely to be ripe are considered, all exogenous variables are negatively related to fecundity while endogenous ones, when they have an effect, are positive.

Discussion:

This study provides a comprehensive suite of three indices of oyster fecundity: (i) per capita fecundity, (ii) fecundity per m^2, and (iii) reserve fecundity, that can be used to rank broodstock reserve locations in Pamlico Sound depending upon the specific management goal. For example, if the goal is to expand broodstock reserves at locations that maximize reproductive potential per square meter of habitat created or improved, then reserves such as Ocracoke and Hatteras, that combine the joint effects of relatively high oyster density, size and per capita fecundity, would be ranked highest for expansion. If the management goal is to rank conservation of current broodstock sanctuaries, then relatively high salinity reserves

such as Hatteras, that combine the joint effects of relatively high fecundity per m^2 and large area of hard substrate, provide the greatest reproductive potential in terms of reserve fecundity. Lastly, if the management goal is to augment oyster densities via stocking with oyster spat at sites with high reproductive potential at the individual level, then more inland reserves such as Bluff Point and West Bay would be ranked highest. These results illustrate the power of using a comprehensive suite of fecundity metrics to guide comprehensive oyster restoration and management, and this approach is not only applicable to networks of oyster broodstock reserves elsewhere, but to any reserve network aimed at enhancing reproductive output.

Temporal variation in fecundity

All three metrics of fecundity (per capita fecundity, fecundity per m^2, and reserve fecundity) were highest in May (this study), and oyster settlement in Pamlico Sound generally peaks in early June (Eggleston and Puckett, unpublished data). Because the duration of oyster planktonic larvae can range from 20 to 60 days, depending on temperature and food availability (Dekshenieks et al. 1996, Kennedy et al. 1996), back-calculating from peak settlement in mid-June suggests a likely spawning time of late May. Thus, the high fecundity measured in this study in May 2007 and 2008 are likely the result of mass spawning just prior to oyster collections in June. These results suggest that oysters build reproductive products during the year prior to a first massive spawning event in mid-May (Galtsoff 1930, Choi et al. 1994, Kennedy et al. 1996). Subsequent oyster spawning events

during the summer and early fall are not likely to be as synchronized as the May spawning due to individual variation in resource allocation, food availability, filtration efficiency, and growth rates (Kennedy et al. 1996). Our data support this assumption of reduced spawning output during the summer and early fall since fecundity and oyster settlement was generally lowest in June and August (this study, Eggleston and Puckett, unpbl. data).

Spatial variation in fecundity

Per capita fecundity

When all reserves were pooled, per capita fecundity varied from 0 eggs to a maximum of 340,502, with an average estimate of 16,513 eggs per oyster. This estimate is much lower than those in the literature, which range from millions to tens of millions of eggs per oyster (Brooks 1891, Churchill 1928, Nelson 1928, Galtsoff 1930, Davis and Chanley 1956, Cox and Mann 1992, Choi et al 1994). There are several reasons why our estimates of oyster fecundity are relatively low. First, the methods used in previous studies varied greatly; they either estimated counts of eggs from a known volume of gametic tissue (Brooks 1891), counted aliquots of egg suspensions by eye (Churchill 1928, Nelson 1928, Galtsoff 1930, Davis and Chanley 1956, Cox and Mann 1992), or conducted immuno-precipitation assays (Choi et al 1994). All of these methods differ in their precision. Our method of using a flow-cytometer to count eggs was probably very precise compared to previous estimates by eye. For example, application of flow-cytometry to fecundity studies in fish increased precision by reducing within- and among-reader variation to 1% of the total count (Friedland et al

2005). Another explanation for the relatively low concentrations of eggs observed in this study versus previous studies is that we selected the entire size range of oysters (5.9mm to 197mm LVL), rather than just the largest. When only oysters from the largest size class (larger than 75mm LVL) were included in our estimates of average fecundity, the mean rose from 16,513 eggs per oyster to 39,878 eggs per oyster. When we selected the 100 most fecund oysters, average fecundity increased to 85,299 eggs per oyster. Therefore, size selectivity is probably not the key reason why our estimates are of fecundity are 1-2 orders of magnitude lower than previous studies. Because many of the rip-rap mounds in Pamlico Sound broodstock reserves were created recently, it is unlikely that the age structure of these reserves would be as old as oyster used in previous studies of fecundity. For example, Bluff Point was the most newly created reserve. Therefore, oysters collected there could not have been more than 18 months old when collected in 2006. Although oysters in Bluff Point grew to legal size in that time, it is unlikely that they would have had as much energy to devote to gamete production as a large oyster that did not need to expend as much energy in recent growth. Finally, the proportion of oysters infected with diseases remains to be studied in Pamlico Sound. Oyster infected with diseases such as MSX and dermo exhibit reduced fecundity (Villalba et al. 2004), and although disease may be inhibiting fecundity in portions of Pamlico Sound, it is doubtful that it could account for a 1-2 order of magnitude difference in fecundity since fecundity of oysters reported by Cox and Mann (1992) and Choi et al. (1994) were also collected from areas where disease was present. We suggest that the main reason why our estimates of oyster fecundity are 1-2 orders of magnitude lower than previous

studies is due to the increased precision of egg counts via the use of a flow-cytometer and digital imaging software. Moreover, regardless of differences in the mean and range of fecundity between this and previous studies, a main goal of this study was to compare fecundity within a network of broodstock reserves in Pamlico Sound over time and space using standardized methods, which is still valid.

In terms of per capita fecundity in this study, with the exception of Crab Hole, broodstock reserves with the highest fecundity were those with low salinity, such as Bluff Point and West Bay. This was surprising because higher salinity reserves typically had larger oysters in this study. Because per capita fecundity increased with oyster size (both LVL and LVW), we expected that oysters from reserves with larger oysters would have higher fecundity, which was not always the case. For example, although oysters from Deep Bay were large and had a high fecundity, oysters from Hatteras and Ocracoke were large and had a low fecundity. Conversely, Bluff Point had the second smallest oysters (due to being the most recently created reserve), but high per capita fecundity. The increase in per capita fecundity with LVL was steeper at Bluff Point, which was a relatively young reserve of moderate salinity, than Ocracoke, which was a relatively old reserve with high salinity. Thus, fecundity does not necessarily increase with reserve age or large sizes in high salinity areas.

Several other factors could explain the relatively low rate of increase in per capita fecundity with LVL observed at Ocracoke and Hatteras. One of these factors could be parasitic infections. Pea crabs (*Zaops ostreum*) were present inside oysters almost exclusively at the Ocracoke and Hatteras, while being completely absent from other reserves. Pea crabs

consume food that would otherwise be consumed by the oyster, or consume the oyster tissue itself, thus reducing an oyster's resources available for growth and reproduction (Kennedy et al 1996). In addition to pea crabs, plasmodium (MSX) and protozoan (Dermo) parasites can extract resources from oysters. These parasites are most common in warm, high salinity waters (Villalba et al. 2004), and may have been somewhat responsible for decreased per capita fecundity of oysters in more saline reserves in this study, although this remains to be tested. Further studies determining the infection rates by pea crabs, and relative impacts of parasites on oyster health and fecundity in Pamlico Sound are needed for a more rigorous assessment of the impacts of salinity-related factors on per capita oyster fecundity.

A second factor that may explain variation in the rate of increase in fecundity with LVL is possible competition for food. For example, Crab Hole is a reserve of low salinity and like Bluff Point, would be expected to have high per capita fecundity, despite having female oysters of relatively small size, but this was not the case. The rate of increase in per capita fecundity with LVL at Crab Hole, a relatively low salinity reserve, was the lowest of all reserves in this study. The Crab Hole reserve was unique in that it had extremely high densities of Atlantic ribbed mussels (*Geukensia demissa*), which are also sessile suspension-feeders. Thus, it is possible that competition for space and food reduced the ability of oysters at Crab Hole to acquire the energy necessary to increase their size and fecundity in accord with other reserves in Pamlico Sound. Although inter-specific competition for substrate and food has impacted oyster growth and survival for oysters in other studies (Lohse 2002, Boudreaux et al. 2009), it remains to be tested in broodstock reserves in Pamlico Sound.

The age of the reserve must also be considered in terms of oyster fecundity. Because broodstock reserves in Pamlico Sound were constructed over the course of a decade, the demographics within the reserves can be highly variable. For example, older reserves, such as Hatteras, West Bay and Deep Bay, are much more likely to have a population of oysters that is older than more newly-created reserves such as Bluff Point and Crab Hole. Older oysters should be relatively large and highly fecund. Thus, the rank order of per capita fecundity may change with time as the population and individual size of oysters within younger reserves (Crab Hole, Bluff Point) increases.

Per square meter fecundity

Broodstock reserves with the highest fecundity per m^2 were those with the highest densities of large oysters, such as Hatteras and Ocracoke. In general, both oyster density and size increased with salinity. Oyster density likely differed among and within reserves during this study due to variation in settlement and mortality. For example, during the primary spawning period in mid-May, winds blew predominantly from the southwest and oyster settlement was highest near Hatteras and Ocracoke along an axis aligned southwest by northeast in Pamlico Sound (Eggleston and Puckett, unpublished data). During late summer and early fall, predominant wind direction alternates between southwesterly and northeasterly, with corresponding oyster settlement patterns that were more uniform spatially across Pamlico Sound than in early summer (Eggleston and Puckett, unpublished data). Thus,

peak settlement in early summer appears to drive high recruitment and subsequently high densities of oysters at relatively high salinity reserves at Hatteras and Ocracoke.

We expected that relatively high oyster settlement at Hatteras and Ocracoke would be off-set by increased predation-induced mortality at these more saline reserves (Wells 1961). Although spatial variation in predation-induced mortality was not quantified in this study, there did not appear to be an appreciable loss of oysters due to predation-induced mortality since both Hatteras and Ocracoke had relatively high oyster densities.

Reserve fecundity

At the reserve level, much of the spatial variation in fecundity was lost due to two factors. First, there was a high degree of variance with square meter fecundity that would have only been exacerbated by multiplying by the differing substrate areas of the reserve. Second, some of the reserves that had a high fecundity per meter^2 measurement were also smaller reserves. For example, Crab Hole had the largest amount of hard substrate for settlement, but was ranked low in terms of fecundity per m^2. These differences in reserve size would reduce any spatial variation in fecundity when scaled up to reserve size. The three most inland reserves (Bluff Point, Deep Bay and West Bay) were ranked lowest in terms of reserve fecundity, and ranked low due to a combination of small reserve size and low fecundity per m^2.

In conclusion, the results from this study highlight the need to consider time and space when measuring reproductive potential of marine reserves, as well as the need to

consider a comprehensive suite of response variables that best inform managers. Moreover, data on spatiotemporal variation in fecundity should be used in conjunction with information on larval connectivity among reserves, and other demographic rates such as growth and mortality within and among reserves, to rank restoration and conservation within a network of reserves. Fecundity is an important demographic metric to rank reserves in terms of health and reproductive potential, and can provide a simple cost/benefit assessment in terms of egg output as a function of dollars spent on restoration and conservation. Lastly, the spatiotemporal patterns in oyster fecundity measured in this study may change as reefs age, new reefs are built, and the biotic and abiotic environment is altered by climate change.

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Table 1: Quadrat sampling effort of broodstock reserves in Pamlico Sound, NC standardized by number of mounds and areal footprint of broodstock reserve.

Oyster Broodstock Reserve	Mounds	Quadrats Collected	Hard Substrate (m^2)	Area Within Reserve Boundaries (m^2)	Reef Construction Year
West Bay	14	3	2,725	8,094	1996
Ocracoke	20	4	4,383	23,876	2004
Hatteras	15	3	5,783	13,759	1996
Crab Hole	20	4	13,648	55,441	2003
Deep Bay	14	3	6,278	20,234	1996
Bluff Point	26	5	5,324	21,853	2005

Table 2: Results of analysis of covariance for the effects of reserve (Bluff Point, Crab Hole, Deep Bay, Hatteras, Ocracoke and West Bay), and month (June 2006, August 2006, May 2007, August 2007 and May 2008), and the covariate (left valve length, LVL) on mean per capita fecundity (log(eggs/oyster + 1) transformed) among oyster broodstock reserves in Pamlico Sound, NC.

Variation Source	SS(DF)	Mean Square	F	P
Reserve	37.70 (5)	7.54	4.54	0.0004
Month	142.27 (4)	35.57	21.41	<.0001
LVL	1259.12 (1)	1259.12	757.78	<.0001
Reserve*Month	98.26 (20)	4.91	2.96	<.0001
LVL*Reserve	43.07 (5)	8.61	5.18	<.0001
LVL*Month	70.48 (4)	17.62	10.6	<.0001
LVL*Reserve*Month	132.25 (20)	6.61	3.98	<.0001

Table 3: Results of analysis of variance for the effects of reserve (Bluff Point, Crab Hole, Deep Bay, Hatteras, Ocracoke and West Bay) and month (June 2006, August 2006, May 2007, August 2007 and May 2008) on fecundity per m^2 (log(eggs/m^2 + 1) transformed).

Variation Source	SS(DF)	Mean Square	F	P
Reserve	30.66 (5)	6.13	10.29	<.0001
Month	538.42 (4)	134.60	225.90	<.0001
Reserve*Month	78.53 (20)	3.93	6.59	<.0001

Table 4: Results of analysis of variance for the effects of reserve (Bluff Point, Crab Hole, Deep Bay, Hatteras, Ocracoke and West Bay) and month (June 2006, August 2006, May 2007, August 2007 and May 2008) on reserve fecundity (log(eggs/reserve+1)).

Variation Source	SS(DF)	Mean Square	F	P
Reserve	39.30(5)	10.42	9.97	<.0001
Month	604.35(4)	151.09	144.67	<.0001
Reserve*Month	89.51(20)	4.48	4.29	<.0001

Table 5: Results of multiple regression analysis of the relationship between per capita fecundity and various endogenous and exogenous factors (n = 1768). The multiple regression analysis was conducted with per capita fecundity pooled across all sampling dates (June 2006, August 2006, May 2007, August 2007 and May 2008). Parameters collected via remote sensing from the Giovanni ocean color time-series online visualization and analysis tool are noted '(Satellite)'.

Variable	Estimate	Standard Error	Type II SS	F	P
Intercept	4.89	0.34	108.10	201.52	<0.0001
Left Valve Length	0.013	0.0013	55.88	104.19	<0.0001
Left Valve Width	0.0038	0.0022	1.62	3.03	0.0819
Meat Volume	0.031	0.0077	8.81	16.43	<0.0001
Temperature (Satellite)	-0.074	0.0051	112.38	209.51	<0.0001
Salinity	-0.079	0.0087	44.36	82.69	<0.0001
Dissolved Oxygen	0.048	0.024	2.09	3.90	0.0483
Clorophyll α (Satellite)	-0.024	0.0051	11.82	22.04	<0.0001

Table 6: Results of multiple regression analysis of the relationship between per capita fecundity and various endogenous and exogenous factors (n = 824). The multiple regression analysis was conducted with per capita fecundity pooled across the spring sampling dates (May 2007 and May 2008). Parameters collected via remote sensing from the Giovanni ocean color time-series online visualization and analysis tool are noted '(Satellite)'.

Variable	**Estimate**	**Standard Error**	**Type II SS**	**F**	**P**
Intercept	7.06	0.60	62.02	136.17	<0.0001
Left Valve Length	0.0070	0.0017	7.55	16.58	<0.0001
Left Valve Width	0.019	0.0032	15.95	35.01	<0.0001
Meat Volume	0.045	0.010	8.55	18.78	<0.0001
Temperature (Satellite)	-0.077	0.011	23.83	52.32	<0.0001
Salinity	-0.099	0.013	25.08	55.07	<0.0001
Dissolved Oxygen	-0.22	0.042	12.93	28.38	<0.0001
Clorophyll α (Satellite)	-0.040	0.0078	0.12	0.26	0.61

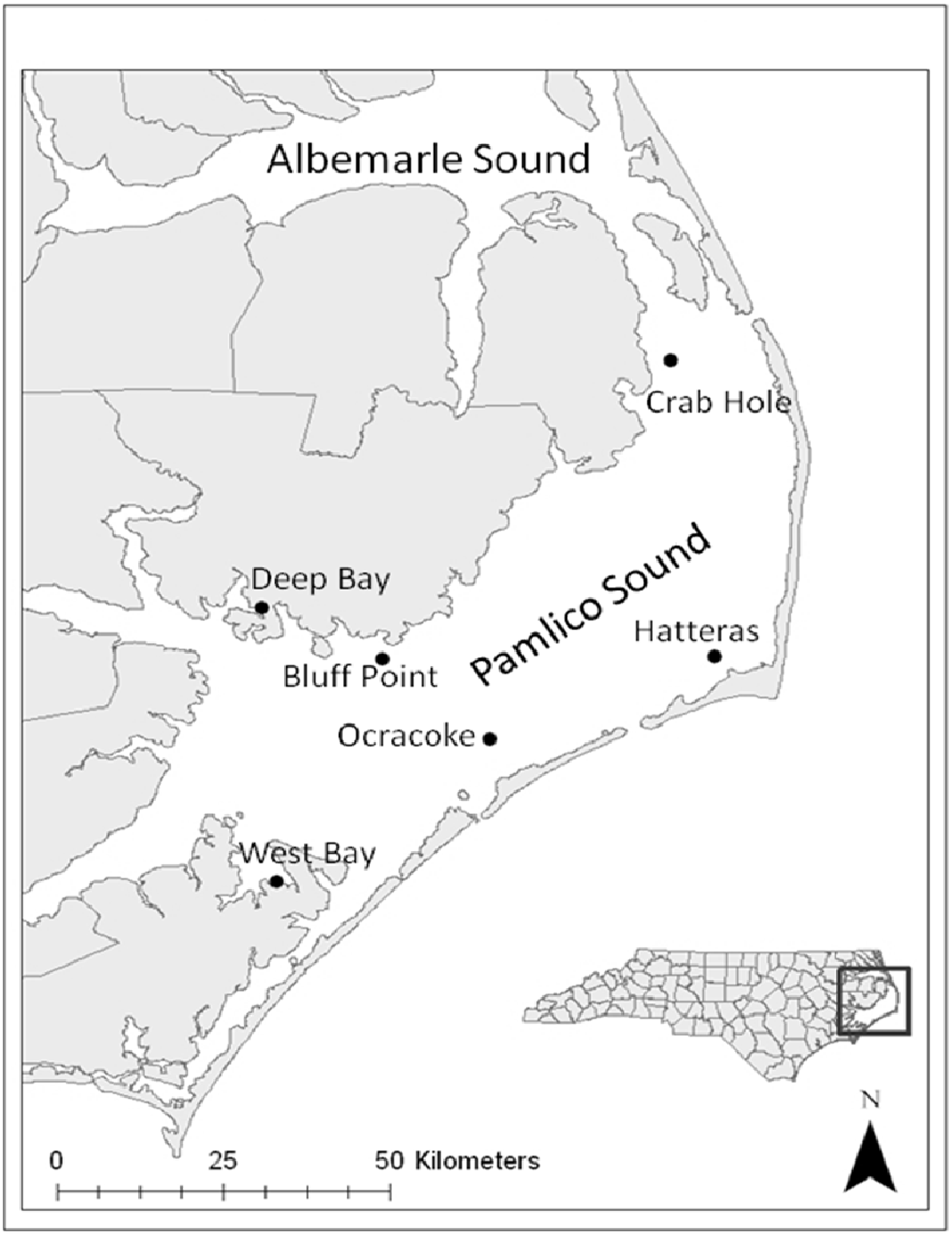

Figure 1: Oyster broodstock reserves selected for fecundity sampling in Pamlico Sound, NC, USA.

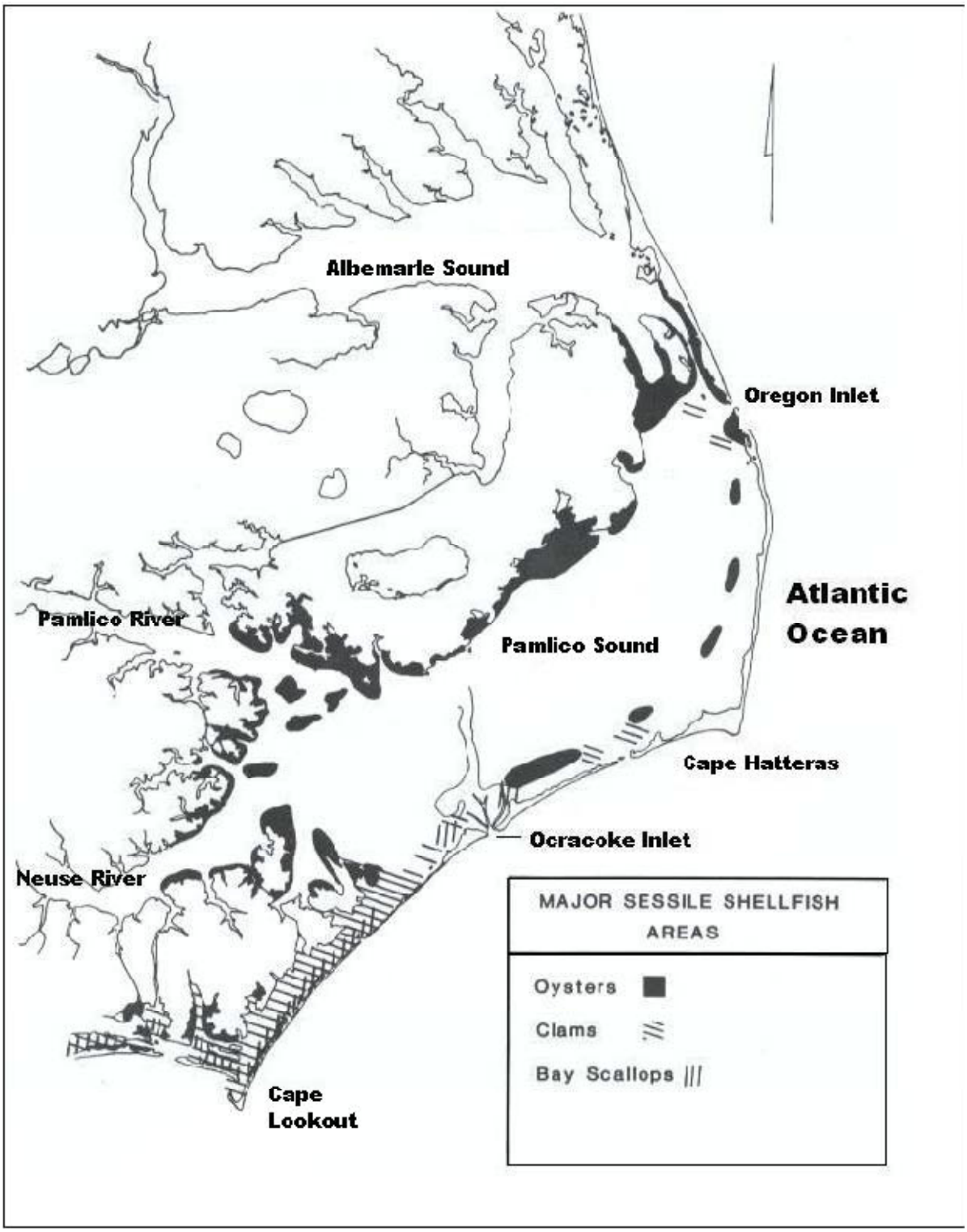

Figure 2: Natural shellfish areas in Pamlico Sound, NC, USA.

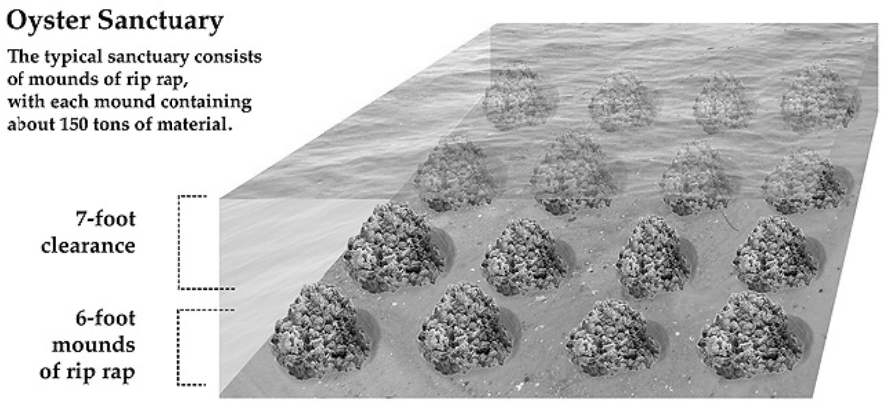

Figure 3: Schematic of broodstock reserves constructed in Pamlico Sound, NC by the NC Division of Marine Fisheries. Mounds with reserves were constructed of piles of limestone rip-rap; each piece of rip-rap was approximately 50cm in diameter and provided a clean settlement site for oyster larvae. Figure 8rom NC DMF website at:http://www.ncdmf.net/shellfish/sanctuary1.htm

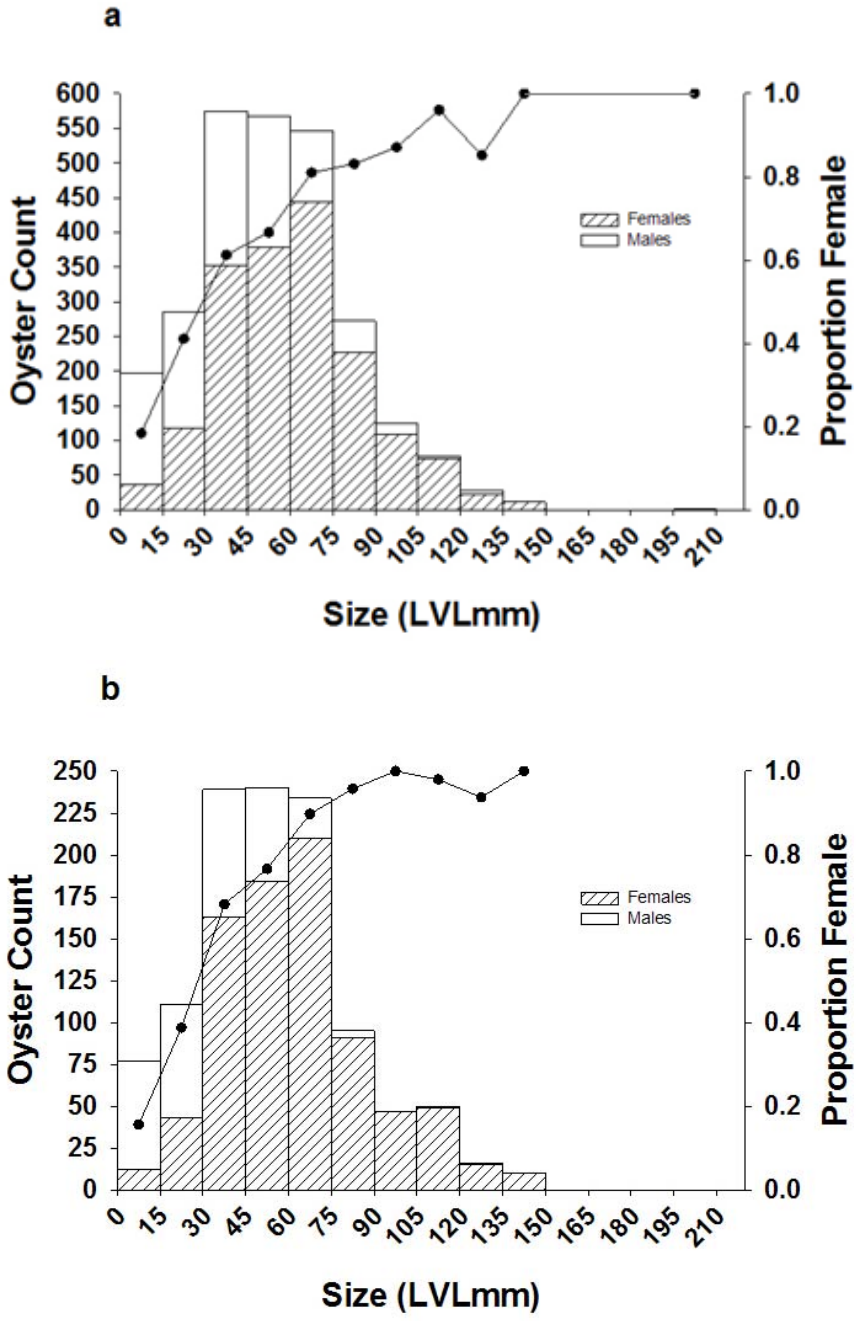

Figure 4: Counts of male and female oysters from a) all sampling dates (June 2006, August 2006, May 2007, August 2007, May 2008) and b) May 2007 and 2008 only, pooled among all reserves. The line represents the proportion of female oysters in that size range. A total of 2681 oysters were present for all sampling dates, and 1119 total pooled across the May 2007 and 2008 sampling dates.

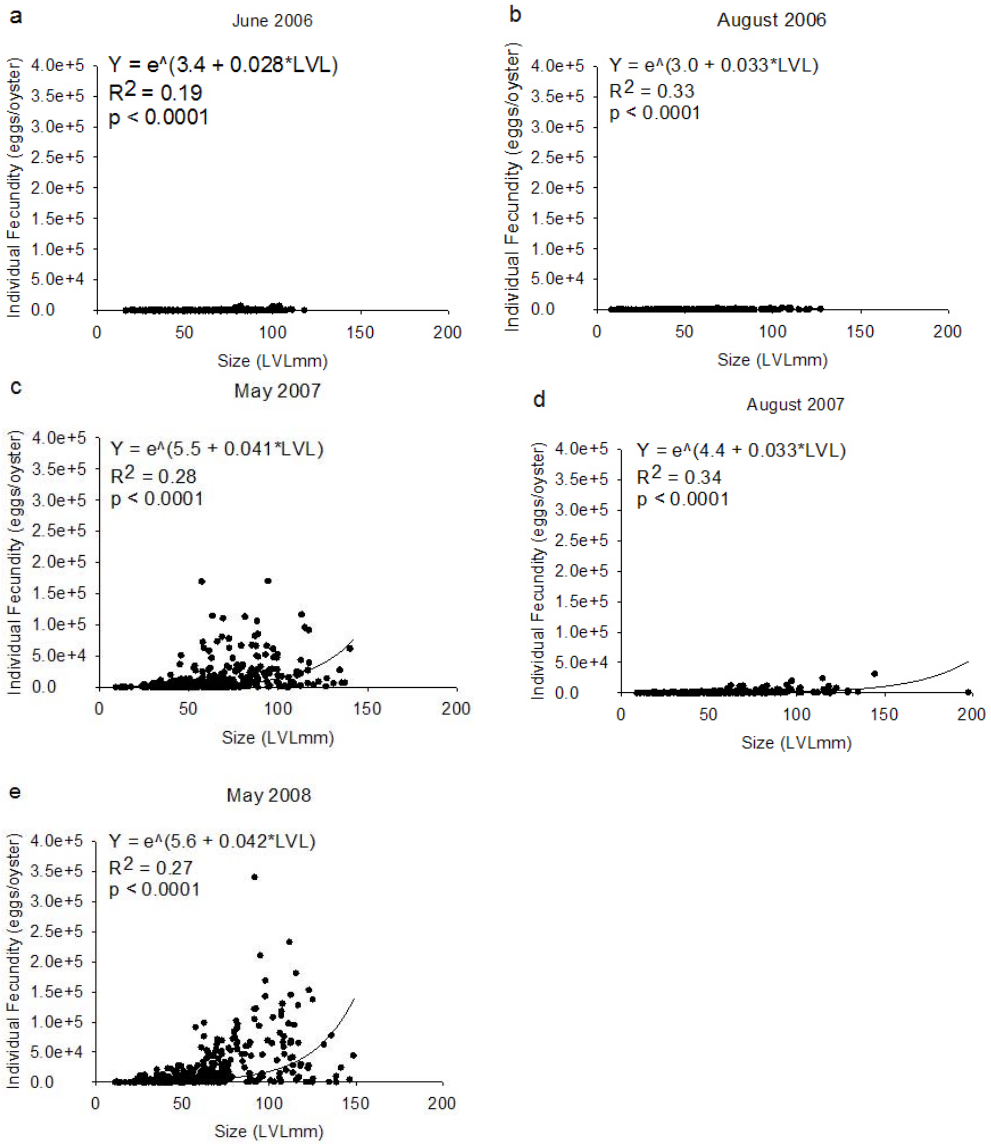

Figure 5: Mean per capita fecundity (eggs/oyster) pooled across all six broodstock reserves in Pamlico Sound for a) June 2006, b) August 2006, c) May 2007, d) August 2007, and e) May 2008. A maximum of 10 oysters were selected from each of the smaller two size classes (0-15.0, 15.1-30.0 mm) and a maximum of 20 from the remaining size classes (30.1-45.0, 45.1-60.0, 60.1-75.0, and ≥75.0 mm), oysters found to contain eggs were included for a total of 1768 female oysters, see Appendix 1 for numbers of oysters collected and sampled. Regression equations were back-transformed to eggs/oyster to facilitate reader comparison with the results of other studies.

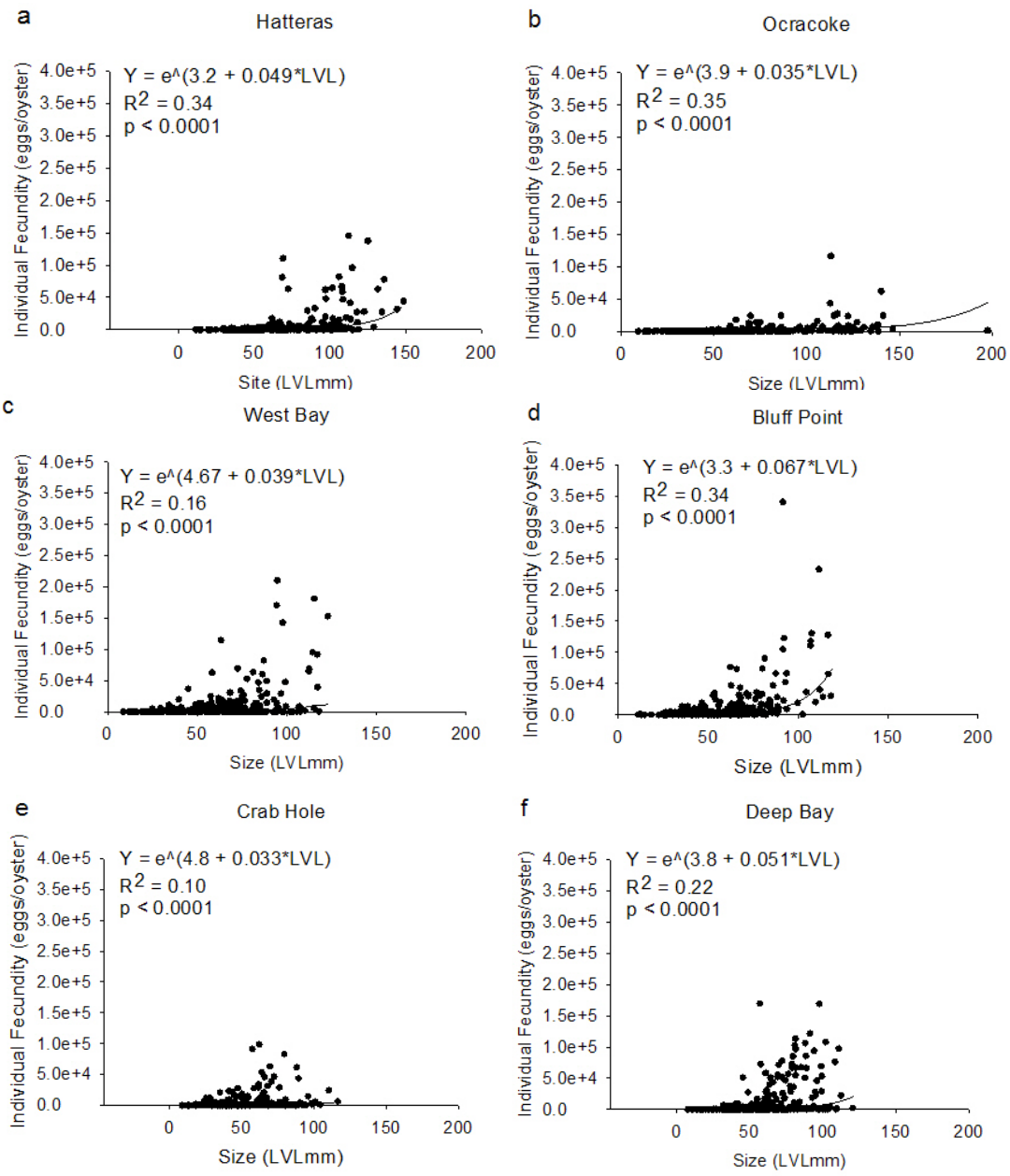

Figure 6: Mean per capita fecundity (eggs/oyster) pooled across all sampling months for a) Hatteras, b) Ocracoke, c) West Bay, d) Bluff Point, e) Crab Hole, d) Deep Bay, ranked by salinity, highest to lowest. A maximum of 10 oysters were selected from each of the smaller two size classes (0-15.0, 15.1-30.0 mm) and a maximum of 20 from the remaining size classes (30.1-45.0, 45.1-60.0, 60.1-75.0, and ≥75.0 mm) for a total of 1768 female oysters, see Appendix 1 for numbers of oysters collected and sampled. Regression equation were back-transformed to eggs/oyster to facilitate reader comparison with the results of other studies.

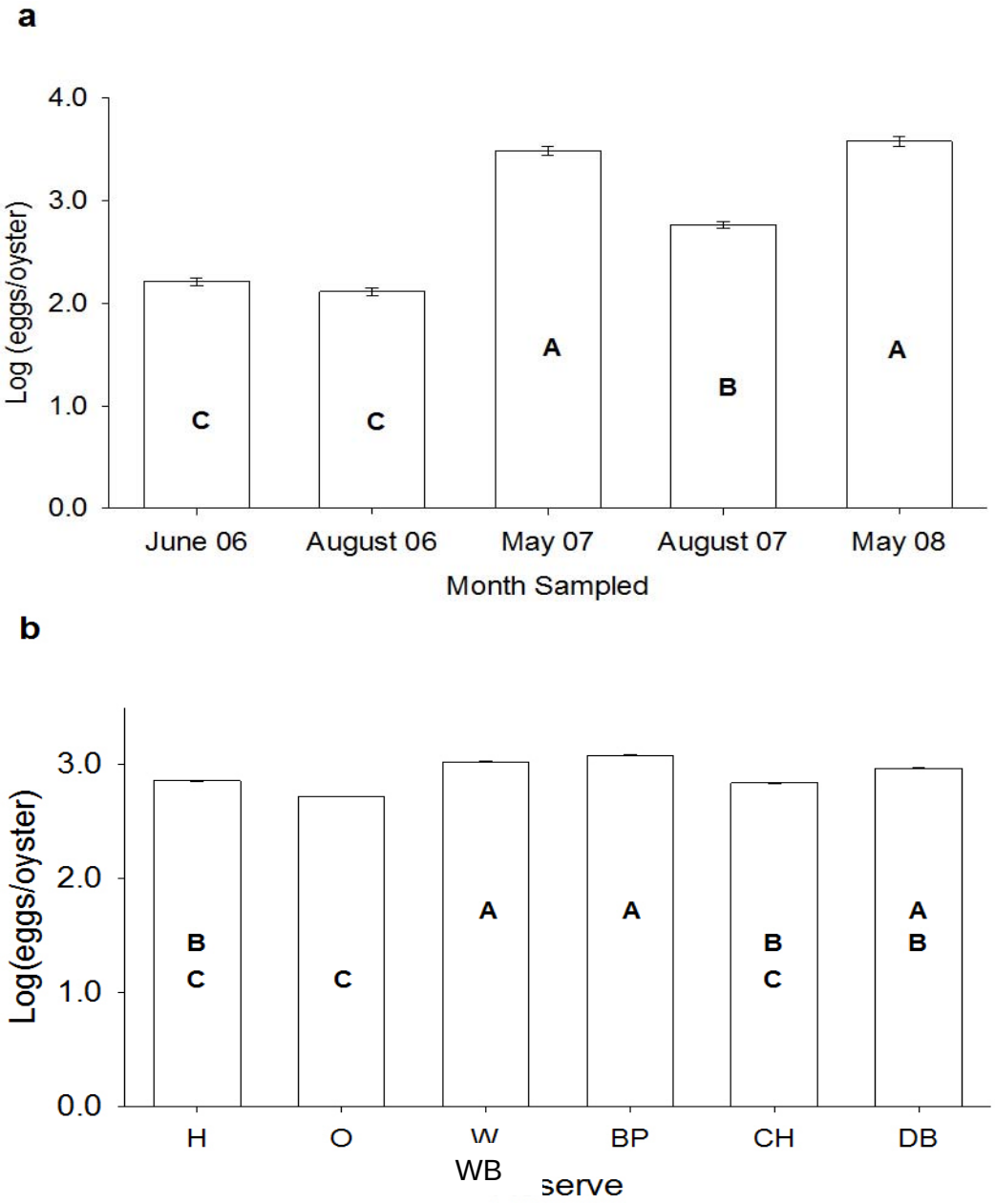

Figure 7: Temporal (a) and spatial (b) variation in per capita fecundity with either reserves (Hatteras (H), Ocracoke (O), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB)) (a) or sampling months (b) pooled. Fecundity in reserves or months with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ±1SE and n= 1768 oysters.

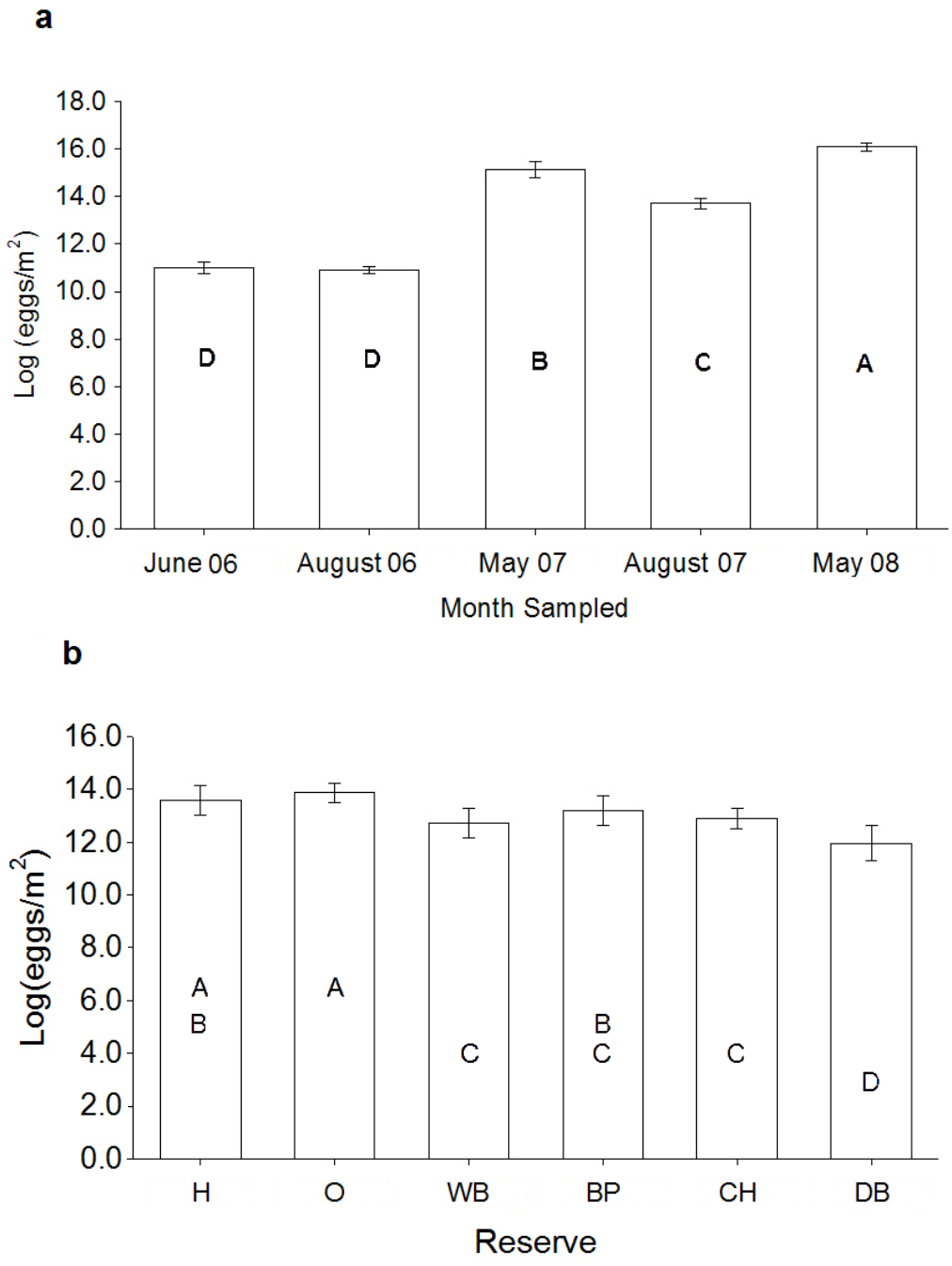

Figure 8: Temporal (a) and spatial (b) variation in fecundity per m^2 ($log(eggs/m^2)$) with either reserves (Hatteras (H), Ocracoke (O), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB)) (a) or sampling dates (b) pooled. Fecundity in reserves or months with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ±1SE and n= 124 quadrats.

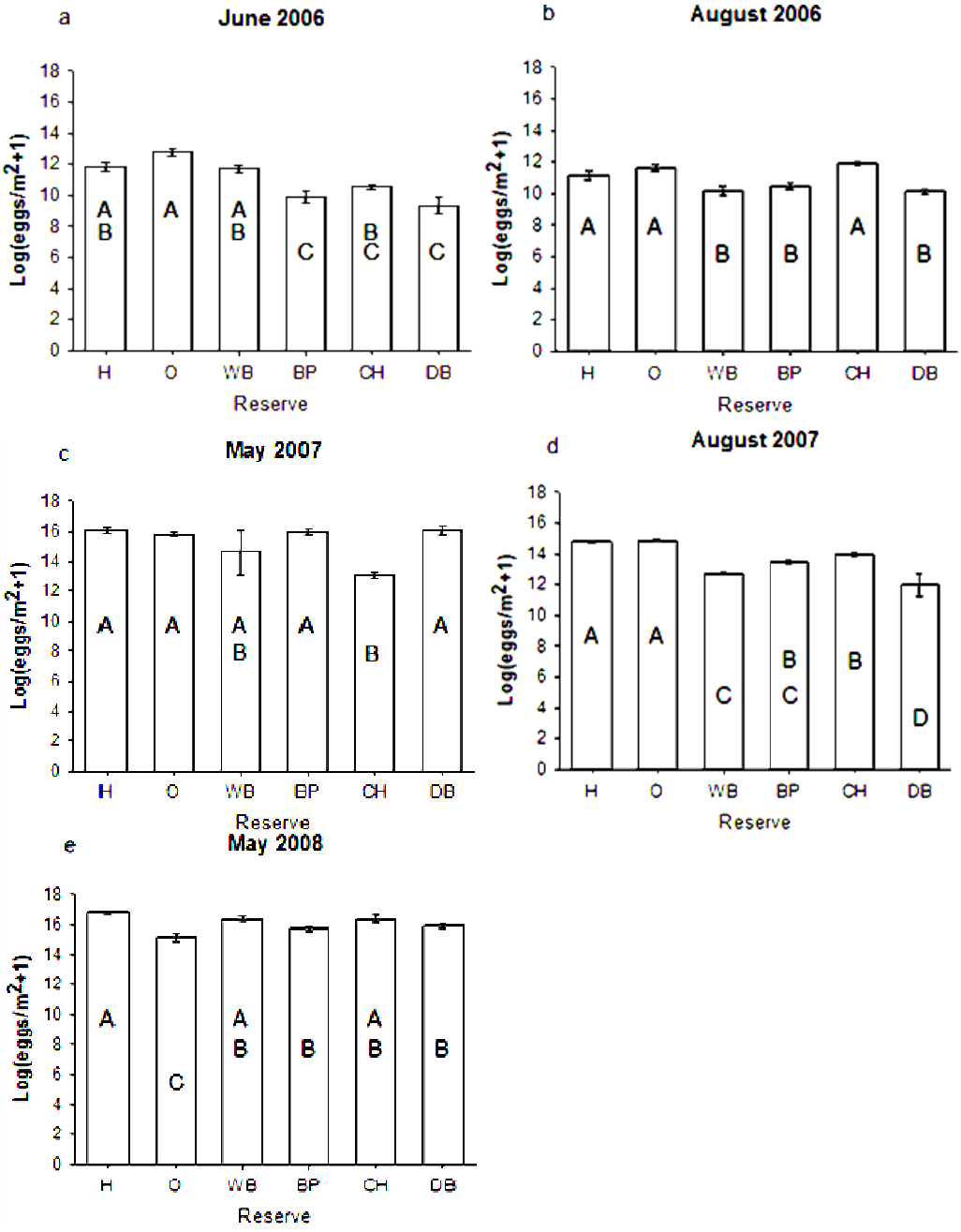

Figure 9: Mean fecundity per m^2 (log(eggs/m^2) transformed) for each reserve, Hatteras (H), Ocracoke (O), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB), during: a) June 2006, b) August 2006, c) May 2007, d) August 2007, and e) May 2008. Fecundity in reserves with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ±1SE and n = 22 quadrats for each sampling date.

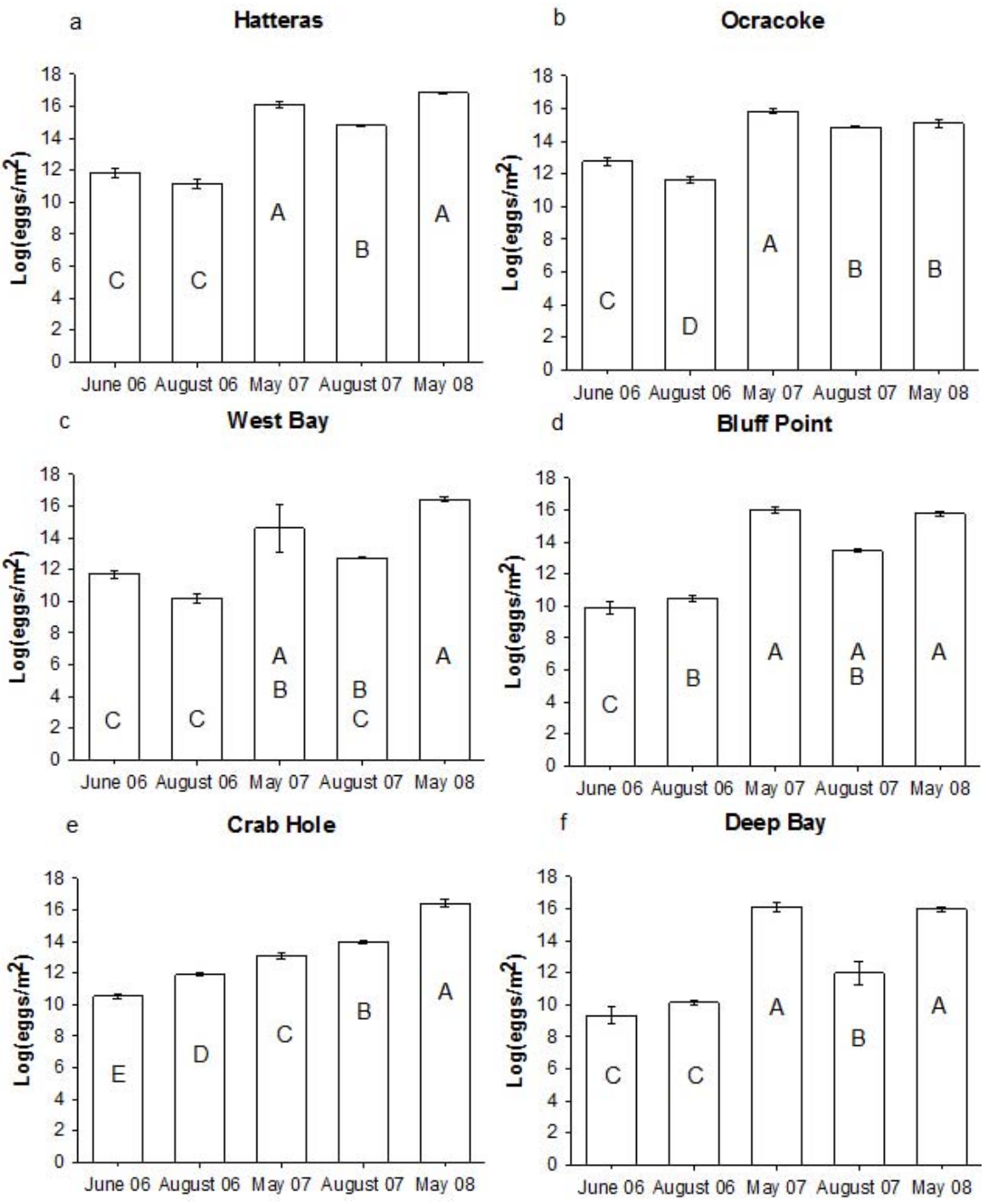

Figure 10: Mean fecundity per m^2 (log(eggs/m^2) transformed) for each sampling date. Fecundity for dates with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ±1SE and n = 22 quadrats for each sampling date.

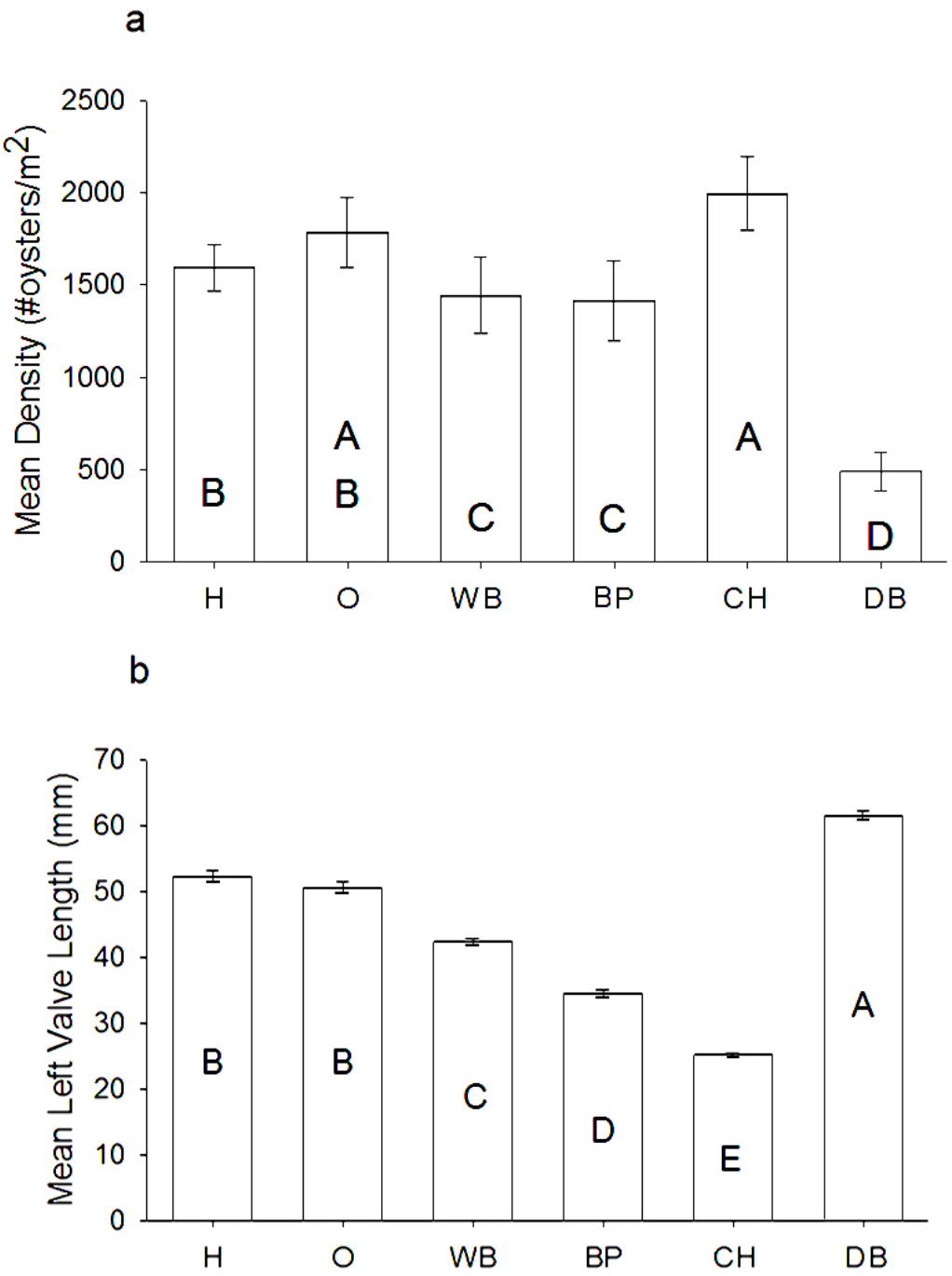

Figure 11: Spatial variation in mean reserve (Hatteras (H), Ocracoke (O), West Bay (WB), Bluff Point (BP), Crab Hole (CH) and Deep Bay (DB)) oyster density (a) and size (b). Density and left valve length in reserves with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ± 1 SE and n= 126.

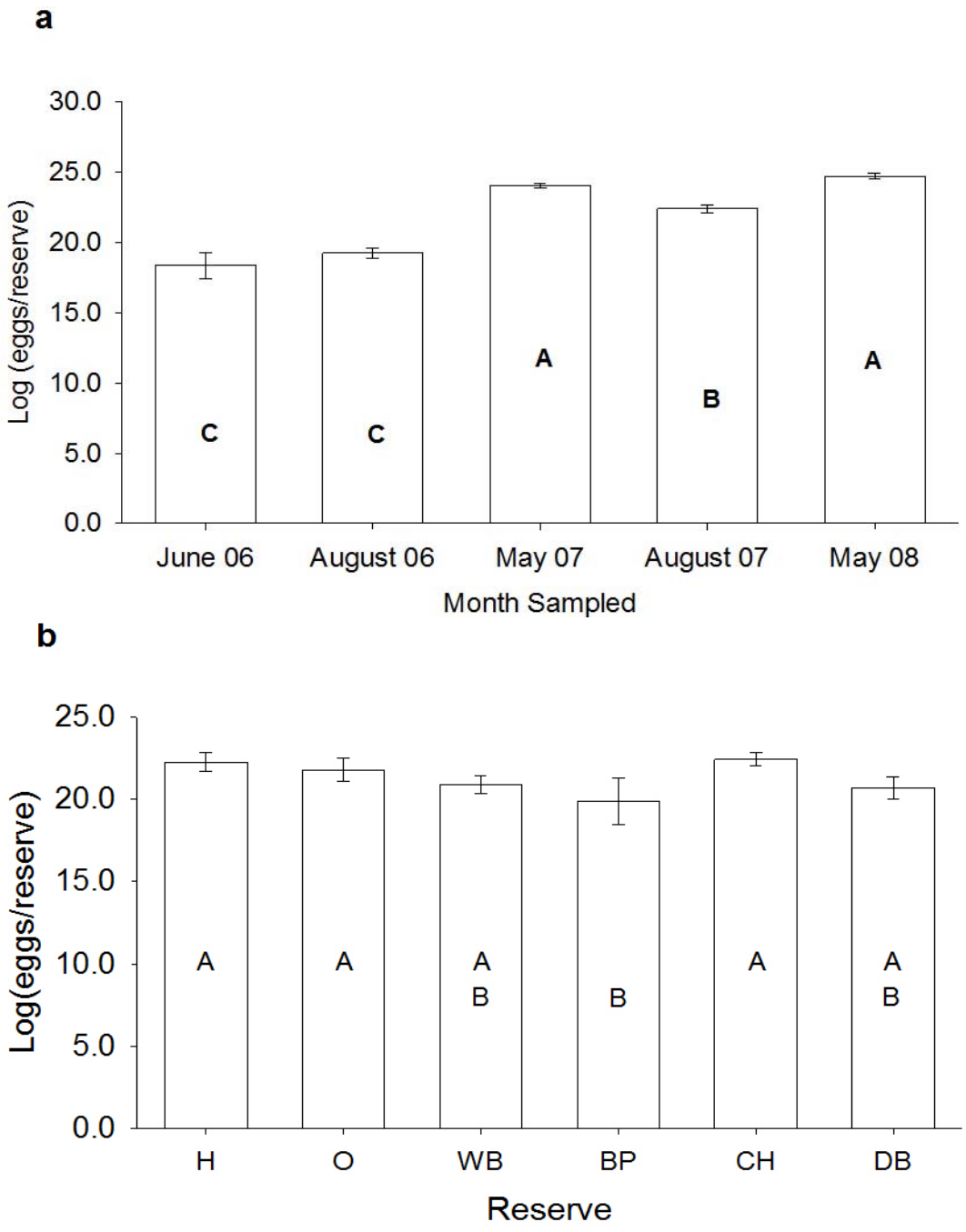

Figure 12: (a) Temporal and (b) spatial variation in mean reserve fecundity (log(eggs/reserve)) with reserve (Hatteras (H), Ocracoke (O), West Bay (WB), Bluff Point (BP), Crab Hole (CH) and Deep Bay (DB)) or sampling date pooled. Reserve fecundity in reserves or sampling dates with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ±1SE and n= 124 quadrats.

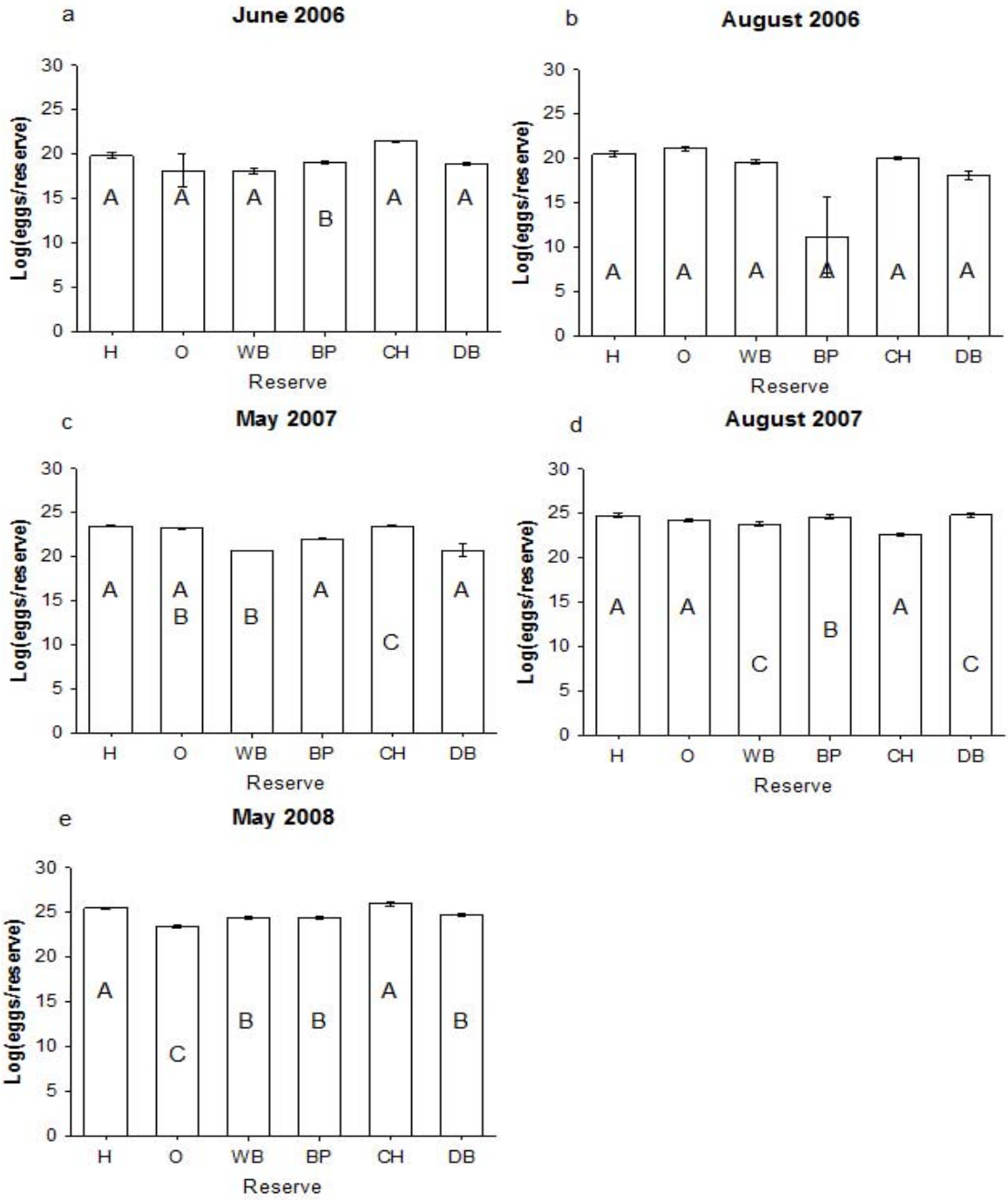

Figure 13: Mean reserve fecundity (log(eggs/reserve) transformed) for each reserve, Hatteras (H), Ocracoke (O), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB), for each sampling event. Fecundity in reserves with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ±1SE and n = 22 quadrats for each sampling date.

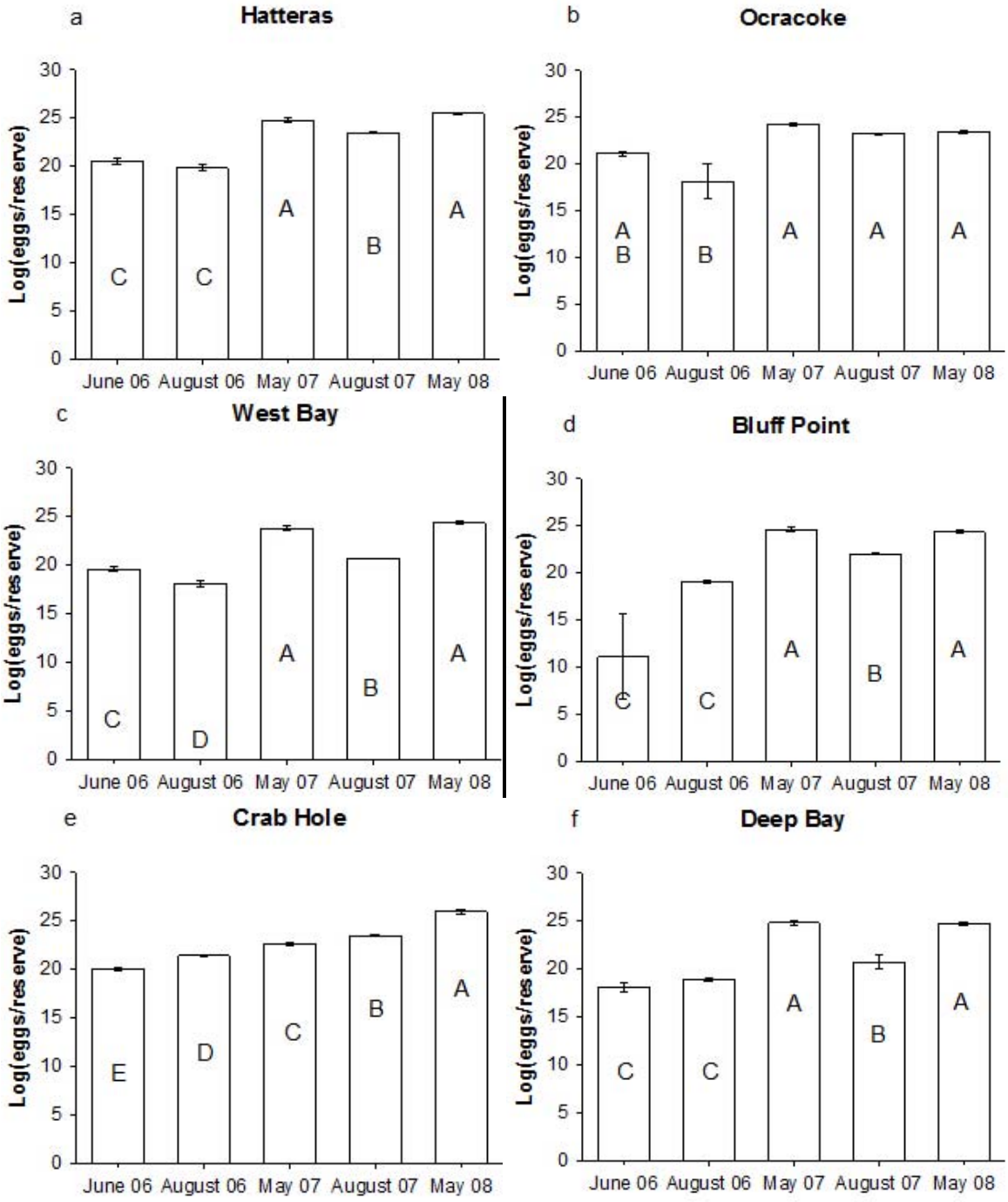

Figure 14: Mean reserve fecundity (log(eggs/reserve) transformed) for each sampling date. Fecundity for dates with the same letters were statistically similar as revealed with a Student Newman-Keuls multiple-comparisons test. Error bars are ±1SE and n = 22 quadrats for each sampling date.

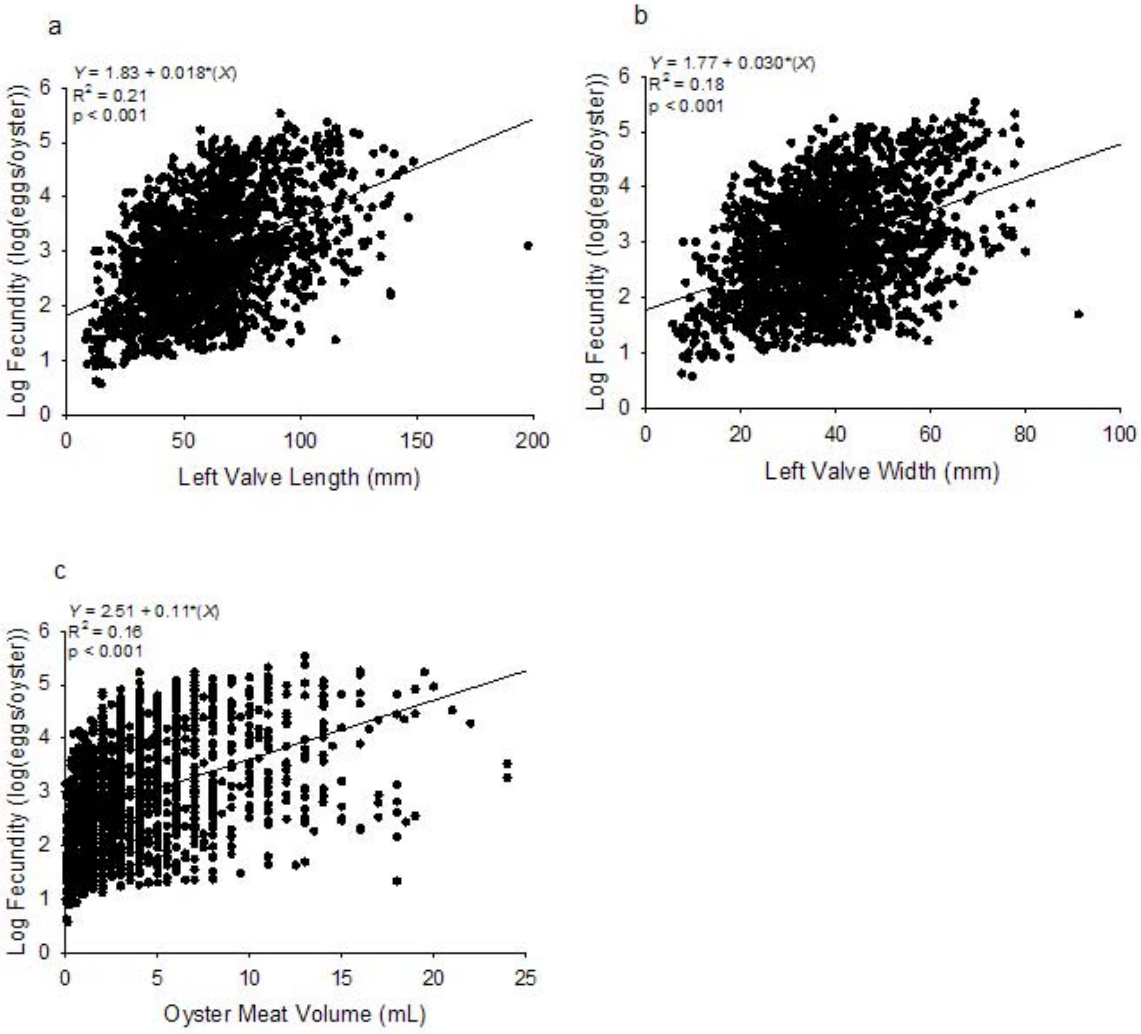

Figure 15: Relationship between a) left valve length, b) left valve width, and c) meat volume and per capita fecundity (log(eggs/oyster)) pooled across all months sampled (June 2006, August 2006, May 2007, August 2007 and May 2008). Mean per capita fecundity (n = 1768) measurements were log(eggs/oyster +1) transformed to satisfy the assumptions of a linear model.

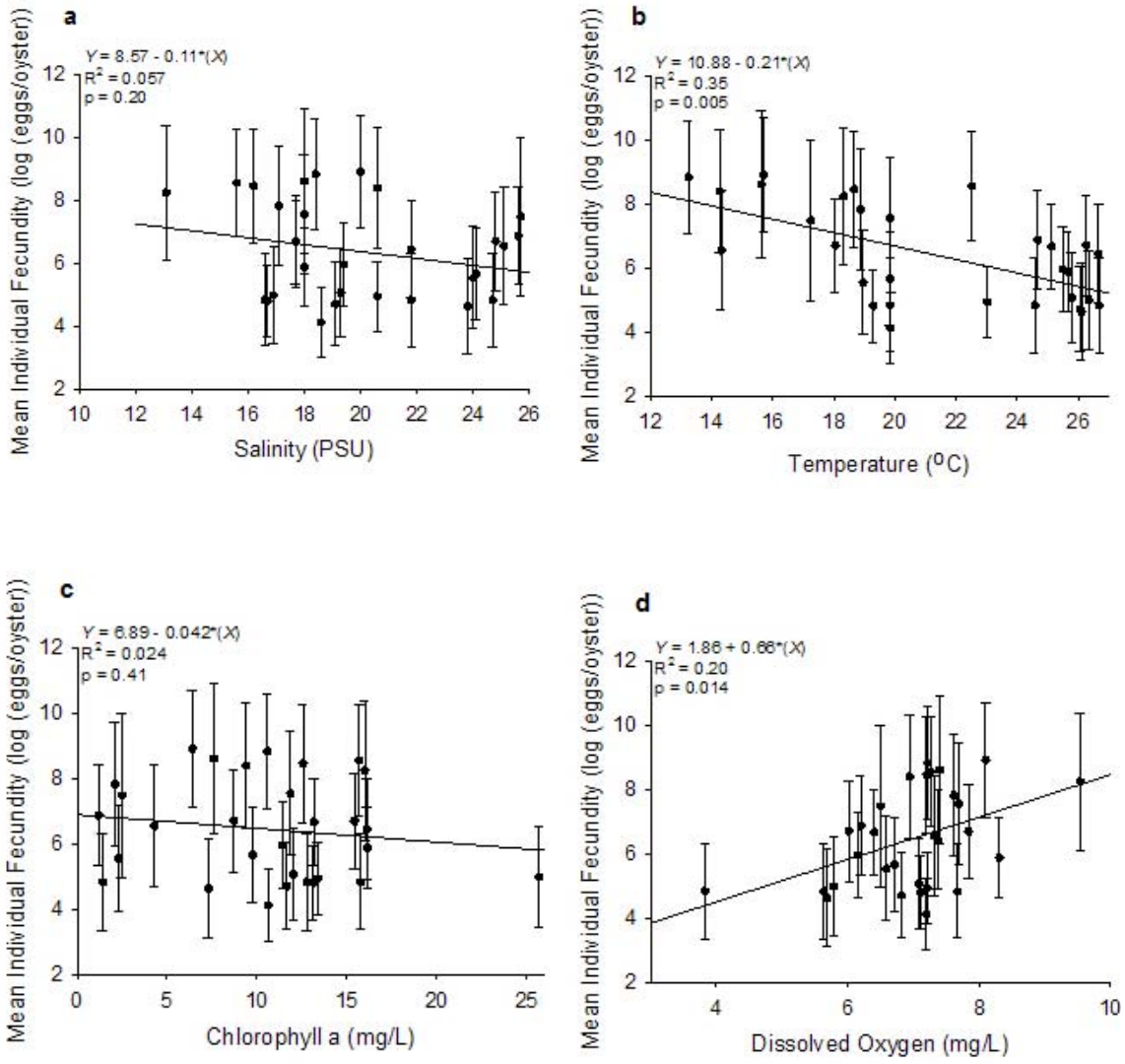

Figure 16: Relationship between a) salinity, b) temperature, c) chlorophyll α, and d) dissolved oxygen and mean per capita fecundity (log(eggs/oyster)) pooled across all months sampled (June 2006, August 2006, May 2007, August 2007 and May 2008). Mean per capita fecundity (n = 1768) measurements were log (x+1) transformed to satisfy the assumptions of a linear model. Error bars are ± 1 SE.

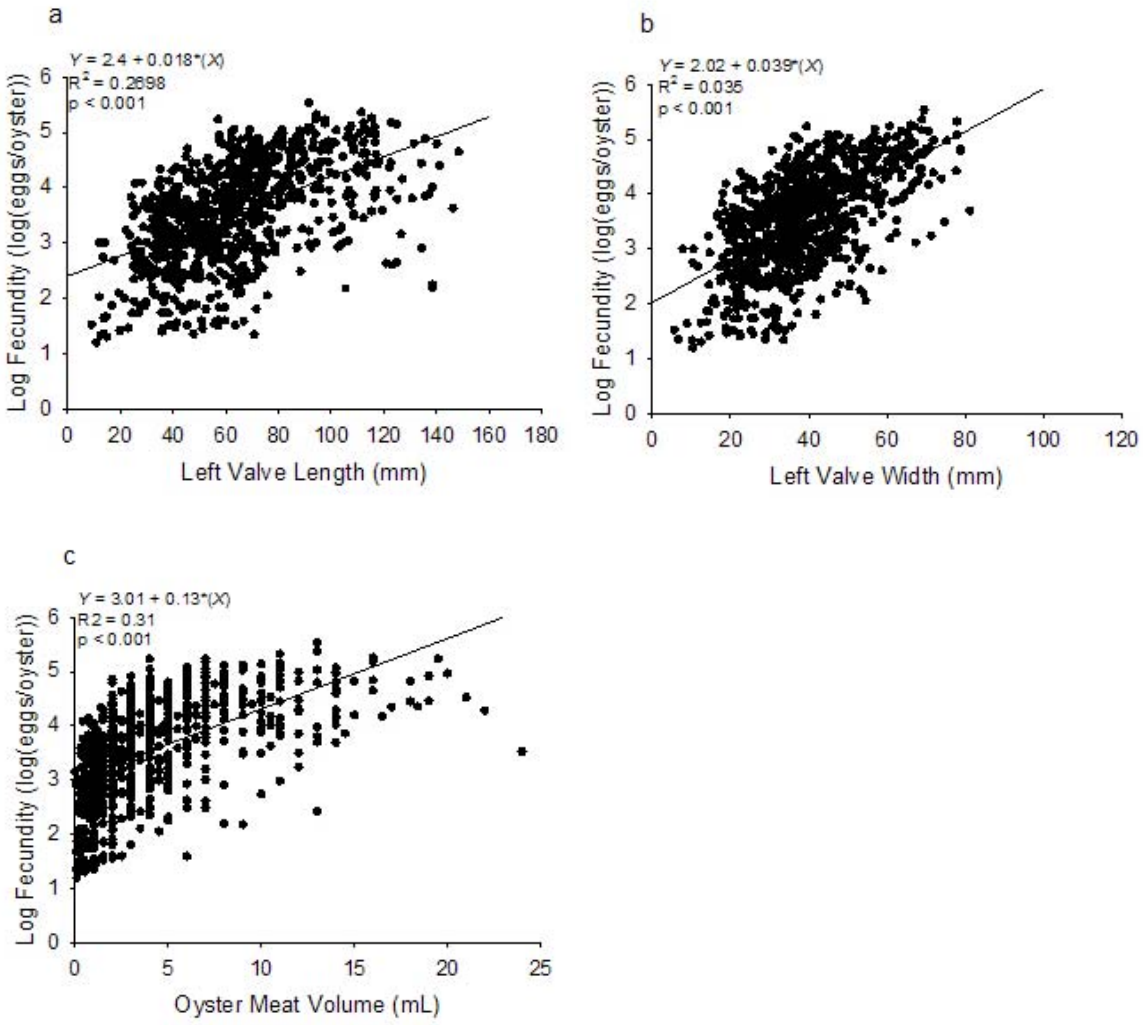

Figure 17: Relationship between a) left valve length, b) left valve width, and c) meat volume and per capita fecundity (log(eggs/oyster)) for May 2007 and 2008 only. Mean per capita fecundity (n = 824) measurements were log(x+1) transformed to satisfy the assumptions of a linear model.

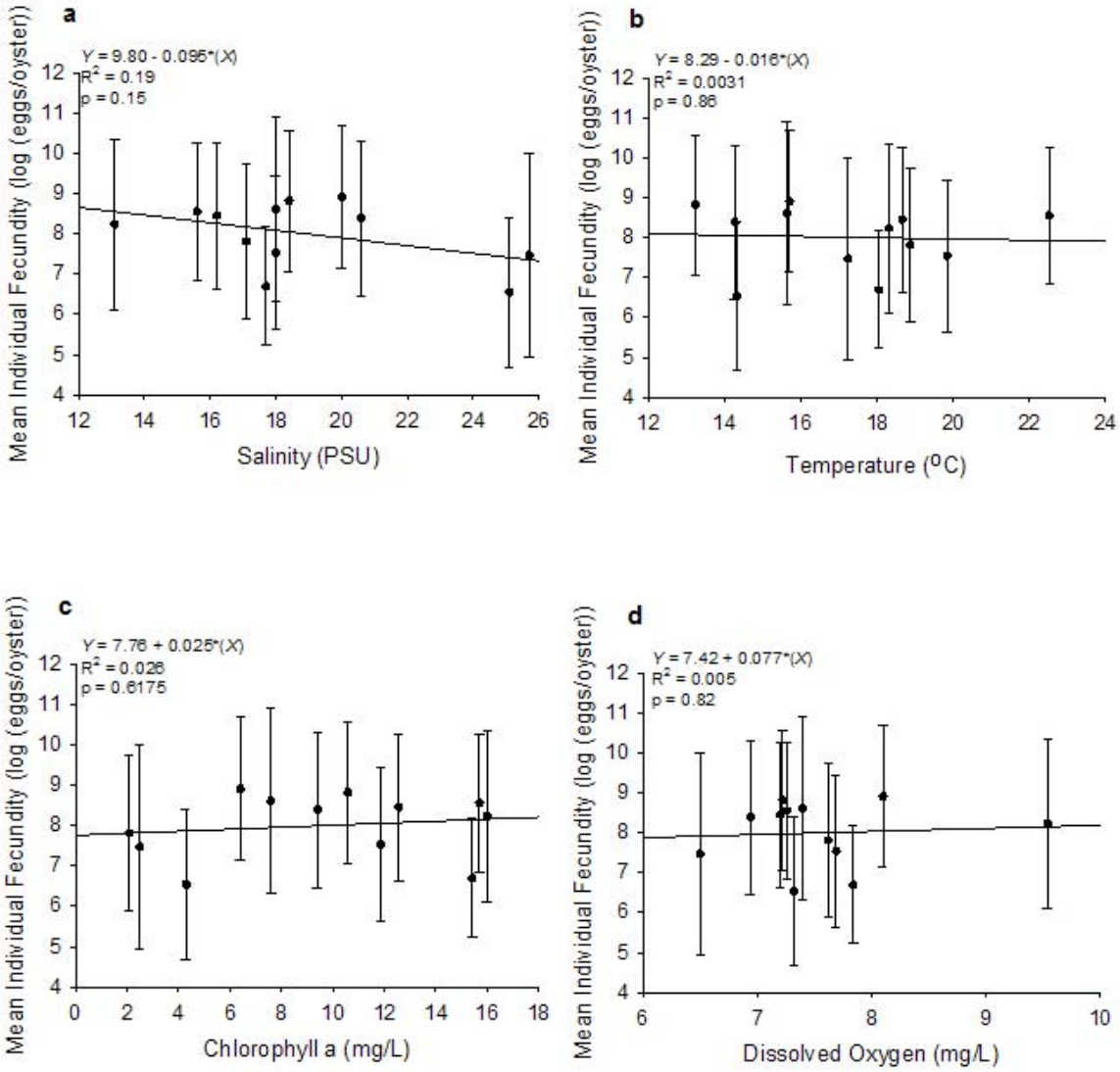

Figure 18: Relationship between a) salinity, b) temperature, c) chlorophyll α, and d) dissolved oxygen and mean per capita fecundity (log(eggs/oyster)) for May 2007 and 2008 only. Mean per capita fecundity (n = 824) measurements were log(x+1) transformed to satisfy the assumptions of a linear model. Error bars are ± 1 SE.

APPENDIX

Appendix: Fecundity estimates for each oyster size class from each reserve and sampling event. Table includes a count of oysters collected for fecundity processing (N(S)), count of female oysters used for fecundity calculations (N(F)), mean (mean) and standard deviation (Stdev) of mean individual egg counts, log-transformed mean (mean(log)) and standard deviation (Stdev(log) of mean egg counts , and results of Student Newman Keuls multiple comparisons test (SNK grouping). Size classes with the same letter are statistically similar within a sampling month.

Bluff Point	Size Class	N (S)	N (F)	Mean	Stdev	Mean (log)	Stdev (log)	SNK Grouping
June 2006	0-15	0	.	.	.	.	.	.
	15.1-30	11	3	55.10	59.78	3.65	1.03	A
	30.1-45	20	8	81.99	89.43	4.04	0.88	A
	45.1-60	19	14	84.46	87.42	3.99	0.98	A
	60.1-75	12	9	241.50	410.64	4.62	1.35	A
	75+	0	.	.	.	.	.	.
Aug 2006	0-15	10	5	21.98	17.76	2.88	0.84	C
	15.1-30	10	3	51.63	45.38	3.67	0.99	BC
	30.1-45	22	8	143.34	104.47	4.65	0.97	AB
	45.1-60	19	10	343.53	331.48	5.29	1.22	A
	60.1-75	15	13	268.72	173.87	5.29	0.98	A
	75+	0	.	.	.	.	.	.
May 2007	0-15	3	1	43.30	.	3.79	.	.
	15.1-30	10	4	237.73	179.96	5.21	0.86	C
	30.1-45	20	17	2682.61	2989.54	7.11	1.51	B
	45.1-60	21	20	8015.53	10552.83	8.18	1.36	AB
	60.1-75	19	19	18058.65	17671.73	9.38	1.04	A
	75+	20	19	26426.21	17979.43	9.97	0.68	A
Aug 2007	0-15	10	1	20.70	.	3.08	.	.
	15.1-30	10	5	135.60	91.30	4.75	0.64	B
	30.1-45	20	7	323.24	263.64	5.45	0.89	AB
	45.1-60	20	16	301.63	233.51	5.40	0.84	AB
	60.1-75	20	13	948.46	746.26	6.43	1.08	AB
	75+	20	13	2284.74	2021.56	7.15	1.37	A
May 2008	0-15	10	1	994.00	.	6.90	.	.
	15.1-30	10	4	2434.60	2216.38	7.25	1.38	B
	30.1-45	21	18	4015.81	4326.98	7.66	1.30	AB
	45.1-60	19	18	7022.16	7879.27	8.21	1.21	AB
	60.1-75	20	19	17537.87	18901.46	9.14	1.32	A
	75+	20	20	87038.46	82630.81	10.88	1.15	A

Appendix, Continued:

Crab Hole	Size Class	N (S)	N (S)	Mean	Stdev	Mean (log)	Stdev (log)	SNK Grouping
June 2006	0-15	10	.	.	.	.	.	.
	15.1-30	10	3	4.11	94.27	92.17	1.20	A
	30.1-45	21	11	4.38	71.48	102.01	0.81	A
	45.1-60	19	10	5.26	243.64	292.53	1.13	A
	60.1-75	21	10	5.00	326.49	258.75	1.11	A
	75+	15	6	5.21	419.76	380.92	1.47	A
Aug 2006	0-15	10	6	2.39	6.83	11.38	0.55	C
	15.1-30	10	7	4.28	299.08	163.66	1.15	B
	30.1-45	20	18	5.04	271.21	272.46	1.23	B
	45.1-60	20	19	5.08	233.49	266.94	1.18	B
	60.1-75	20	19	5.46	195.45	294.80	0.73	AB
	75+	13	12	6.32	913.94	955.88	1.24	A
May 2007	0-15	10	.	.	.	.	.	.
	15.1-30	10	1	6.19	.	486.00	.	.
	30.1-45	20	6	6.86	1070.68	1359.12	1.02	A
	45.1-60	20	8	5.61	642.14	577.78	1.47	A
	60.1-75	20	13	6.78	3445.95	2177.82	1.40	A
	75+	13	11	7.35	7143.72	4268.49	1.54	A
Aug 2007	0-15	10	2	4.55	187.17	161.65	1.61	C
	15.1-30	10	8	6.32	821.95	938.39	1.2	AB
	30.1-45	20	13	5.79	637.00	568.68	1.14	B
	45.1-60	20	7	6.20	399.02	629.51	0.87	AB
	60.1-75	13	11	7.38	3034.27	2558.76	0.99	A
	75+	13	13	7.76	2602.01	3128.67	0.78	A
May 2008	0-15	10	1	0	.	0	.	.
	15.1-30	9	7	7.69	5173.31	5147.47	1.76	B
	30.1-45	21	21	7.96	7110.12	6340.44	1.66	A
	45.1-60	20	18	9.35	19955.57	17185.61	0.93	A
	60.1-75	18	18	9.80	25402.96	29529.01	1.26	A
	75+	7	8	9.45	1.71	30000.88	1.71	A

Appendix, Continued:

Deep Bay

	Size Class	N (S)	N (F)	Mean	Stdev	Mean (log)	Stdev (log)	SNK Grouping
June 2006	0-15	1	0	.	.	.	.	.
	15.1-30	9	0	.	.	.	.	.
	30.1-45	19	8	38.69	19.76	3.56	0.54	B
	45.1-60	21	8	94.84	91.01	4.17	0.96	B
	60.1-75	19	10	268.28	452.69	4.64	1.35	B
	75+	21	13	680.24	448.00	6.21	1.02	A
Aug 2006	0-15	9	2	23.65	0.49	3.20	0.02	CD
	15.1-30	10	4	12.80	7.94	2.52	0.48	D
	30.1-45	21	9	131.78	103.14	4.63	0.74	B
	45.1-60	20	10	164.70	375.06	4.05	1.22	BC
	60.1-75	16	14	303.04	259.24	5.34	0.98	B
	75+	19	14	1027.19	1023.98	6.54	0.90	A
May 2007	0-15	0	.	.	.	.	.	.
	15.1-30	10	3	442.77	330.47	5.72	1.23	C
	30.1-45	19	17	2788.39	2644.91	7.32	1.45	BC
	45.1-60	20	19	17023.25	41637.12	7.44	2.23	BC
	60.1-75	18	17	17260.62	23459.03	8.44	2.06	AB
	75+	20	19	39946.57	34098.46	10.08	1.25	A
Aug 2007	0-15	4	3	140.33	79.12	4.79	0.76	CD
	15.1-30	10	5	71.94	35.06	4.20	0.46	D
	30.1-45	20	19	320.55	344.84	5.42	0.87	BCD
	45.1-60	20	18	545.96	742.70	5.66	1.18	BC
	60.1-75	20	19	1006.95	1087.60	6.26	1.34	AB
	75+	20	16	1371.73	985.55	6.94	0.83	A
May 2008	0-15	0	0	.	.	.	.	.
	15.1-30	10	6	213.72	161.44	5.07	0.88	C
	30.1-45	19	18	2314.41	2451.88	7.15	1.29	B
	45.1-60	21	19	5012.26	7325.73	7.27	1.96	B
	60.1-75	20	20	31007.11	20038.66	10.07	0.85	A
	75+	20	19	75056.66	39187.44	10.92	1.11	A

Appendix, Continued:

Hatteras	Size Class	N (S)	N (F)	Mean	Stdev	Mean (log)	Stdev (log)	SNK Grouping
June 2006	0-15	4	.	.	.	.	.	.
	15.1-30	6	1	46.50	.	3.86	.	A
	30.1-45	24	12	59.21	44.86	3.79	0.85	A
	45.1-60	21	9	489.72	510.00	5.54	1.28	A
	60.1-75	20	17	450.12	423.58	5.64	1.06	A
	75+	26	25	1547.13	1915.47	6.39	1.66	A
Aug 2006	0-15	9	1	10.30	.	2.42	.	.
	15.1-30	11	3	45.67	36.00	3.55	1.04	AB
	30.1-45	20	5	56.04	33.98	3.88	0.66	AB
	45.1-60	19	7	218.41	291.96	4.56	1.37	AB
	60.1-75	21	16	150.44	198.27	4.52	0.93	AB
	75+	20	15	852.55	873.99	6.06	1.50	A
May 2007	0-15	8	2	300.65	360.13	5.08	1.75	A
	15.1-30	10	1	474.70	.	6.16	.	.
	30.1-45	20	4	720.75	571.2637	6.39	0.68	A
	45.1-60	20	11	1872.85	2081.53	6.78	1.43	A
	60.1-75	20	17	17496.94	33489.90	7.93	2.03	A
	75+	20	20	19021.03	25032.98	8.93	1.49	A
Aug 2007	0-15	2	.	.	.	.	.	.
	15.1-30	10	.	.	.	.	.	.
	30.1-45	17	12	495.41	719.42	5.45	1.24	B
	45.1-60	21	14	1349.16	1228.78	6.66	1.29	A
	60.1-75	18	16	2457.03	3372.46	7.05	1.31	A
	75+	20	18	5729.17	8142.01	7.81	1.44	A
May 2008	0-15	2	1	3.10	.	22.30	.	.
	15.1-30	10	2	216.65	269.62	2.00	4.65	BC
	30.1-45	22	15	646.51	973.58	5.38	1.61	BC
	45.1-60	18	13	1370.97	1356.65	6.34	1.77	B
	60.1-75	20	19	399.60	6840.75	7.47	1.69	B
	75+	20	20	47920.67	40527.48	10.31	1.19	A

Appendix, Continued:

Ocracoke	Size Class	N (S)	N (F)	Mean	Stdev	Mean (log)	Stdev (log)	SNK Grouping
June 2006	0-15	0	0	.	.	.	.	.
	15.1-30	12	9	280.39	342.00	4.96	1.26	B
	30.1-45	19	12	126.95	114.67	4.46	0.96	B
	45.1-60	19	16	215.14	170.48	5.09	0.83	B
	60.1-75	19	17	933.48	631.02	6.62	0.70	A
	75+	11	9	3007.82	3007.95	7.16	1.75	A
Aug 2006	0-15	4	1	10.20	.	2.42	.	.
	15.1-30	10	4	33.60	16.06	3.43	0.59	AB
	30.1-45	20	9	73.31	85.40	3.81	1.04	AB
	45.1-60	16	9	242.79	458.73	4.56	1.33	AB
	60.1-75	17	4	85.52	69.14	4.14	0.97	AB
	75+	23	15	641.09	682.96	5.81	1.34	A
May 2007	0-15	10	3	349.60	559.84	4.49	2.11	A
	15.1-30	10	2	259.15	53.53	2.55	0.21	AB
	30.1-45	20	10	391.44	263.49	5.77	0.68	ABC
	45.1-60	20	9	2483.52	2485.02	7.31	1.26	BCD
	60.1-75	20	14	5990.49	7474.86	7.74	1.64	CD
	75+	20	20	18519.75	27826.27	9.06	1.26	D
Aug 2007	0-15	7	.	.	.	.	.	.
	15.1-30	10	1	30.30	.	3.44	.	.
	30.1-45	20	2	35.65	4.74	3.60	0.13	B
	45.1-60	21	6	743.62	680.04	6.16	1.15	A
	60.1-75	19	9	1824.30	3857.39	6.39	1.50	A
	75+	20	20	3312.52	5416.59	7.49	1.01	A
May 2008	0-15	9	1	21.30	.	3.10	.	.
	15.1-30	12	2	386.00	44.06	5.42	1.60	AB
	30.1-45	19	6	348.93	183.34	5.71	0.65	AB
	45.1-60	20	9	663.16	891.08	5.41	1.69	AB
	60.1-75	19	14	3072.04	4825.91	6.75	1.89	AB
	75+	20	18	5394.62	7517.39	7.57	1.61	A

Appendix, Continued:

West Bay	Size Class	N (S)	N (F)	Mean	Stdev	Mean (log)	Stdev (log)	SNK Grouping
June 2006	0-15	10	.	.	.	.	.	.
	15.1-30	11	10	294.90	291.10	5.14	1.18	AB
	30.1-45	22	18	182.30	117.86	4.99	0.72	AB
	45.1-60	15	4	159.32	185.13	4.61	1.10	AB
	60.1-75	23	18	97.97	90.92	4.24	0.86	B
	75+	19	15	616.18	901.54	5.69	1.23	A
Aug 2006	0-15	10	2	8.25	0.35	2.22	0.04	B
	15.1-30	9	3	34.10	42.63	3.04	1.22	AB
	30.1-45	14	5	242.98	294.90	4.77	1.45	A
	45.1-60	15	6	255.95	257.34	5.08	1.20	A
	60.1-75	14	10	589.27	1078.55	5.21	1.53	A
	75+	23	15	339.27	447.32	5.26	1.08	A
May 2007	0-15	10	2	74.25	42.36	4.23	0.60	C
	15.1-30	9	8	2025.96	2177.30	6.91	1.51	B
	30.1-45	21	16	6521.16	9466.81	8.04	1.27	AB
	45.1-60	20	19	10036.33	14104.74	8.58	1.21	AB
	60.1-75	20	19	15584.28	24814.24	8.91	1.41	A
	75+	20	20	33325.00	40995.26	9.69	1.50	A
Aug 2007	0-15	10	1	41.70	.	3.75	.	.
	15.1-30	9	5	66.10	36.15	4.18	0.44	B
	30.1-45	20	13	689.99	510.90	6.10	1.16	A
	45.1-60	20	15	986.64	902.88	6.33	1.27	A
	60.1-75	12	8	1548.01	1126.83	7.02	0.99	A
	75+	11	11	4529.72	4680.33	7.86	1.19	A
May 2008	0-15	10	.	.	.	.	.	.
	15.1-30	10	3	521.00	489.77	5.52	1.88	D
	30.1-45	20	15	1692.91	1954.68	6.86	1.15	C
	45.1-60	20	21	3013.43	3714.03	7.48	1.01	C
	60.1-75	22	21	14179.15	16311.00	8.81	1.46	B
	75+	18	18	70359.81	62021.88	10.73	1.05	A