SPATIOTEMPORAL VARIATION IN OYSTER FECUNDITY AND REPRODUCTIVE OUTPUT IN A NETWORK OF NO-TAKE RESERVES

RAYMOND M. MROCH III,[†] DAVID B. EGGLESTON* AND BRANDON J. PUCKETT

North Carolina State University, Department of Marine, Earth and Atmospheric Sciences, Center for Marine Sciences and Technology, 303 College Circle, Morehead City, North Carolina 28557

ABSTRACT   Adult fecundity and reproductive potential can be critical determinants of subsequent larval supply and juvenile recruitment, and important determinants of placed-based management, especially when selecting sites for marine reserves where larval export is an expected outcome. We quantified spatiotemporal variation in fecundity and reproductive potential of female oysters (*Crassostrea virginica*) within a network of no-take oyster broodstock reserves by sampling over 3 y at 3 spatial scales: (1) per capita, (2) per square meter, and (3) per reserve. A total of 2,596 oysters were collected using scuba from six reserves in Pamlico Sound, NC, during 2006 to 2008 and processed in the laboratory for fecundity. Per-capita fecundity ranged from 0–340,500 eggs, and increased exponentially with oyster size, peaking in May of all years. In general, per-capita fecundity was highest at more inland mesohaline reserves, whereas reproductive potential per square meter and reserve reproductive potential were highest at more seaward polyhaline reserves as a result of a combination of relatively high density, and large oyster size and reserve areas. All 3 reproductive metrics increased in general over time. These results suggest that inland broodstock reserves should be prioritized for stock enhancement/seeding—and more seaward reserves for reserve expansion—and highlight the need to consider spatiotemporal variation and the scale at which a key demographic rate (fecundity and reproductive potential) is expressed when assessing the efficacy and conservation/restoration targets of marine reserves.

KEY WORDS: fecundity, marine reserves, broodstock, scale dependence, habitat restoration, oyster, *Crassostrea virginica*, Pamlico Sound

INTRODUCTION

Fecundity (individual reproductive capacity) and reproductive potential (fecundity scaled to a unit area) can be key determinants of distribution and abundance patterns of early juvenile recruits, particularly for marine benthic populations (Hughes et al. 2000). Recognition of the link between spawning stock biomass, a proxy for reproductive potential, and subsequent recruitment has been used to justify the use of spawning or broodstock reserves closed to fishing for intensively fished species (Bohnsack 1998, Lipcius et al. 2003, Dew & McConnaughey 2005, Nemeth 2005, Lambert et al. 2006, Eggleston et al. 2009). By directly protecting individuals within a reserve from fishing, broodstock reserves— and no-take marine reserves in general—are expected to increase organism density, average size, and age of reserve inhabitants (i.e., conserve spawning stock biomass) (Halpern & Warner 2002, Halpern 2003, (Lester et al. 2009, Puckett & Eggleston 2012). Increased fecundity of older/larger individuals (Mann & Evans 1998, Berkeley et al. 2004, Birkeland & Dayton 2005), combined with increased adult densities in areas protected from fishing, should lead to increased reproductive potential from reserves, some of which is presumably exported across reserve boundaries into adjacent fished areas or other reserves, in the case of reserve networks (reviewed in Sobel and Dahlgren (2004) Cudney-Bueno et al. (2009) Pelc et al. (2009), and Haase et al. (2012)).

The efficacy of a given broodstock reserve to enhance fisheries and/or subsidize reserves within a network depends on spatio-temporal variation in reserve reproductive potential and subsequent larval connectivity, both of which can vary drastically over spatiotemporal scales. For example, at relatively small spatial scales (e.g., <1 m^2), changes in the density and degree of spawner aggregation altered fertilization success dramatically in free-spawning sea urchins, which in turn could alter larval supply to downstream populations (Levitan et al. 1992). At relatively large spatial scales (>300 km), fecundity of free-spawning corals (the proportion of colonies on each reef that contained eggs) on the Great Barrier Reef varied from 15–100% which in turn modified the strength of spawning stock–recruit relationships (Hughes et al. 2000). In general, however, we know relatively little about spatiotemporal variation in fecundity and reproductive potential from marine benthic invertebrates (but see Lester et al. (2007) and Jack and Wing (2010)) because of the difficulties of measuring recruitment, abundances, and fecundities at large spatial scales that approach or exceed the extent of larval dispersal (Hughes et al. 2000, Sanford & Menge 2007). The goals of this study were to quantify spatiotemporal variation in per-capita fecundity and reproductive potential of the Eastern oyster (*Crassostrea virginica* Gmelin; hereafter referred to as oyster) within a network of marine no-take broodstock reserves.

The spatial scale at which fecundity is measured has important management implications. For example, per-capita fecundity provides information on how individual performance might vary over space and time, but does not account for differences in population density and reserve area, both of which affect reproductive potential, and thus the function of broodstock reserves. Reserves containing organisms with high per-capita fecundity might be good candidates for stock enhancement and seeding to augment local densities, particularly in locations that have strong larval connectivity within and among reserves (i.e., nursery role function (*sensu* Beck et al. 2001)). Conversely, reserves containing individuals with relatively low per-capita fecundity but high densities, large size structure, or large reserve footprints may have higher overall reproductive potential (i.e., effective nursery area (*sensu* Dahlgren et al. 2006)) and warrant reserve expansion. Sessile species such as oysters are ideal to

examine patterns and processes underlying spatiotemporal variation in fecundity and reproductive potential because they are subjected to local conditions, thereby removing any confounding artifacts associated with movement among habitats of differing quality.

Oysters have long been recognized as a keystone species that supports hundreds of species in estuarine ecosystems (Wells 1961, Zimmerman et al. 1989, Tolley & Volety 2005) by providing habitat (Wells 1961, Bahr & Lanier 1981, Lenihan & Peterson 1998, Eggleston et al. 1999, Lehnert & Allen 2002) and water quality ecosystem services (Langefoss 1975, Palmer 1980, Lewitus et al. 1998, Jackson et al. 2001, Pietros & Rice 2003). Oysters were once highly abundant in the estuaries of the middle Atlantic states of the United States, but declined in abundance sharply during the 20th century (Chestnut 1951, Chestnut 1955, Bahr & Lanier 1981, Hargis & Haven 1988, Rothschild et al. 1994, Lenihan & Peterson 1998, Mann & Evans 1998, Breitburg et al. 2000, Jackson et al. 2001, Kirby & Miller 2005) as a result of a combination of factors, including eutrophication, disease, and destructive fishing practices (Dealteris 1988, Hargis & Haven 1988, Rothschild et al. 1994, Lenihan & Peterson 1998, Jackson et al. 2001, Kirby & Miller 2005, Luckenbach et al. 2005). To combat declines in abundance, efforts are underway to restore native oyster populations worldwide (Coen & Luckenbach 2000, Powers et al. 2009, Schulte et al. 2009, Beck et al. 2011). Typically, enhancement and restoration programs consist of supplementing hard substrata with a thin veneer of shell material, transplanting disease-resistant stocks, effecting stock enhancement with hatchery-reared cohorts, or constructing 3-dimensional artificial reefs protected from harvest (i.e., reserves) (Coen & Luckenbach 2000).

We present the results of a 3-y study that quantified spatiotemporal variation in fecundity and reproductive potential at 6 oyster reserves in Pamlico Sound, NC. At each reserve, we quantified per capita fecundity (number of eggs per individual across 6 oyster size classes); investigated the relationship between oyster size, sex ratio, and per-capita fecundity; quantified reproductive potential per square meter (number of eggs per square meter) as the sum of size-specific per-capita fecundity scaled to size class abundance in 1 m^2 of hard substrate; and quantified reserve reproductive potential (number of eggs per reserve) as reproductive potential per square meter scaled to the reserve footprint.

MATERIALS AND METHODS

Focal Species

Oysters are protandrous, sequential hermaphrodites, initially spawning as males and transitioning to functional females at ~30 mm left valve length (LVL) (Galtsoff 1930, Thompson et al. 1996), which enables oysters to spawn potentially millions of eggs at older ages (Brooks 1891, Churchill 1920, Nelson 1928, Galtsoff 1930, Davis & Chanley 1956, Cox & Mann 1992, Mann & Evans 1998) and is an evolutionary adaptation to offset the high mortality of oyster larvae (Thompson et al. 1996). There is little information on spatiotemporal variation in fecundity of oysters in any region (but see Cox & Mann (1992)). Available data in Pamlico Sound on potential larval dispersal (Haase et al. 2012), density and growth (Powers et al. 2009, Puckett & Eggleston 2012), and settlement (Ortega & Sutherland 1992)

of oysters (Fig. 1; see Study System) exhibit a high degree of spatiotemporal variation. During this study, oyster settlement was concentrated spatially along the Outer Banks near Hatteras and Ocracoke Inlets (see Study System below), with a temporal peak in midsummer and a secondary peak in early fall (Eggleston & Puckett, unpubl. data). 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 (Etherington & Eggleston 2003, Reyns et al. 2006).

Study System

Pamlico Sound is a primarily wind-driven, shallow lagoonal estuary that is separated from the Atlantic Ocean by a string of barrier islands known as the Outer Banks (Fig. 1) (Lin et al. 2007). Pamlico Sound is the largest component of the Croatan–Albemarle–Pamlico Sound estuarine system (CAPES), the largest lagoonal estuarine system in the United States (Epperly & Ross 1986, Luettich et al. 2002). The North Carolina Division of Marine Fisheries designated 10 reserves for oyster reef restoration in meso- and polyhaline waters of Pamlico Sound that historically contained subtidal oyster reefs (Fig. 1) (Winslow 1889). Reserves were intended to function as broodstock supplying larvae to one another and areas in Pamlico Sound open to harvest. In this study, we quantified fecundity and reproductive output at 6 of 10 broodstock reserves in Pamlico Sound: Bluff Point, Crab Hole, Deep Bay, Hatteras, Ocracoke, and West Bay (Fig. 1) because these reserves are currently the target of very active oyster restoration efforts and span the length/width axes of Pamlico Sound. Within reserve boundaries, subtidal artificial reefs consisting of limestone rip-rap mounds spaced ~25 m apart were constructed to provide hard substrate for oyster larval settlement. Reserves differed in area of boundary and artificial reef footprint (Table 1).
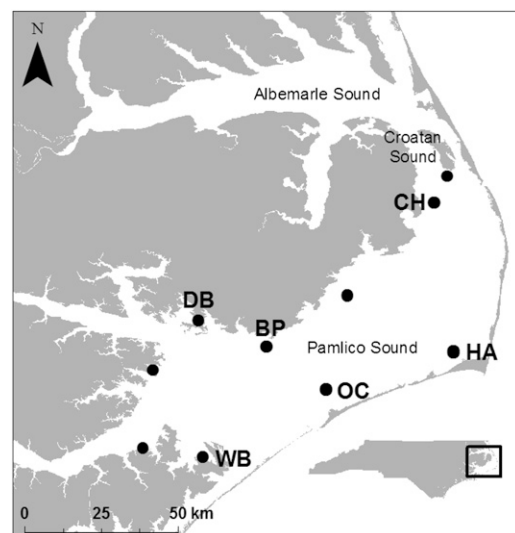

Figure 1. Map of the Croatan–Albemarle–Pamlico Sound estuarine system. Location of oyster reserves in Pamlico Sound are depicted by closed circles. Circles not drawn to scale. Labeled reserves—West Bay (WB), Ocracoke (OC), Hatteras (HA), Crab Hole (CH), Bluff Point (BP), and Deep Bay (DB)—were studied. Map of North Carolina inset for reference.

TABLE 1.

Oyster reserves in Pamlico Sound, NC, sampled from 2006 to 2008.

Reserve	Year created	Mounds (n)	Quadrats (n)	n	Area of reserve boundary (km^2)	Area of hard substrate (m^2)	Mean salinity (SE)
West Bay	1996	14	3	477	0.03	2,725	20.1 (1.2)
Ocracoke	2004	20*	4	466	0.11	4,384	23.0 (1.1)
Hatteras	1996	15	3	479	0.19	5,783	23.6 (1.4)
Crab Hole	2003	20	4	287	0.13	13,648	18.8 (0.8)
Deep Bay	1996	14	3	431	0.07	6,278	16.6 (0.7)
Bluff Point	2005	26†	5	456	0.08	5,325	18.7 (0.5)

* Mounds created in 2005. † Mounds created in 2006.
Number of mounds are those created in 2003 (i.e., those sampled with quadrats) unless otherwise noted. Number of quadrat replicates was allocated proportional to the number of mounds created during 2003 in 2006 and 2007. In 2008, 3 quadrat replicates were conducted at all reserves. The number of oysters processed for per-capita fecundity at each reserve (n) was pooled across size classes and sampling periods. Area of hard substrate within each reserve was estimated from side-scan sonar surveys (Ballance & Eggleston 2008). For reserve locations, see Figure 1.

Field Methods

Oysters were collected from each of the 6 broodstock reserves (Fig. 1) by scuba divers using a 0.25-m^2 quadrat. To minimize variation in fecundity resulting from age of an oyster reef, we collected oysters from artificial reefs created during 2003 when possible. Ocracoke and Bluff Point reserves were not established until 2005 and 2006, respectively, so mounds created during the first year of each reserve's existence (2005 and 2006, respectively) were sampled. Because each reserve contained varying numbers of mounds, sampling effort was normalized by the number of mounds within a reserve, resulting in 3–5 mounds sampled during each sampling trip (Table 1). Samples were collected in June 2006 and August 2006, May 2007 and August 2007, and May 2008 based on previous years' back-calculated temporal peaks in observed settlement in Pamlico Sound (Eggleston & Puckett, unpubl. data).

All oysters were removed from the substrate and counted; a randomly selected subsample ($\geq 1/8$) of the 0.25-m^2 quadrat was then measured for LVL to the nearest 0.1 mm.

Measured oysters were separated into 6 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 subsampled for a given reserve, 10 oysters from the 2 smallest size classes and 20 oysters from each of the remaining size classes were selected randomly, 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. There were no significant differences in the concentration of oyster eggs for a given oyster size class between ethanol and formalin preservation techniques (t-test: $df = 29$, $P = 0.3$).

Laboratory Methods

During each sampling month, oysters from each reserve in each size class were processed individually to determine the total egg contents (i.e., per-capita fecundity) by following the general procedures in Cox and Mann (1992). After recording the displacement volume of the oyster meat, the gonad and visceral mass were sliced front and back with a scalpel and rinsed with 1 μm filtered seawater until the entire gonad was
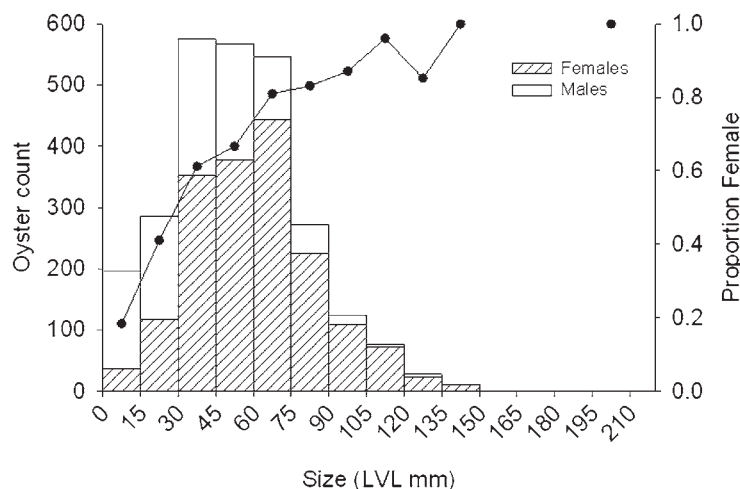

Figure 2. Counts of male and female oysters in 15-mm left valve length (LVL) size bins pooled among all reserves during all sampling dates (June 2006, August 2006, May 2007, August 2007, and May 2008). The proportion of female oysters in each 15-mm size class is depicted by the line. A total of 2,596 oysters were collected over all sampling dates.

stripped of its contents. The egg suspension was then passed through a 90-μm mesh sieve into a 2-L beaker. After the volume was recorded, a 1-mL sample was taken while stirring with an Eppendorf pipette. The eggs in the sample were counted using a FlowCam flow cytometer (e.g., Buskey & Hyatt 2006). Three 1-mL samples were counted for each oyster, and the average per-capita fecundity (F_{ind}, eggs per oyster) of the 3 subsamples was estimated as

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

where E_{mean} is the mean egg count from 3 subsamples and V_{rinse} is the total rinse volume from the 2-L beaker.

Reproductive potential per square meter (RP_{area}) was estimated for each quadrat replicate at each reserve during each sampling month as

$$RP_{area} = \sum_{sc=1}^{6} \sum_{1}^{n} (F_{sc}) + (SD_{sc} \times R),$$

where sc is the number of size classes ($n = 6$), n is the count of oysters per square meter in a size class obtained from a replicate quadrat 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, SD_{sc} is the standard deviation of mean per-capita fecundity of oysters in a size class
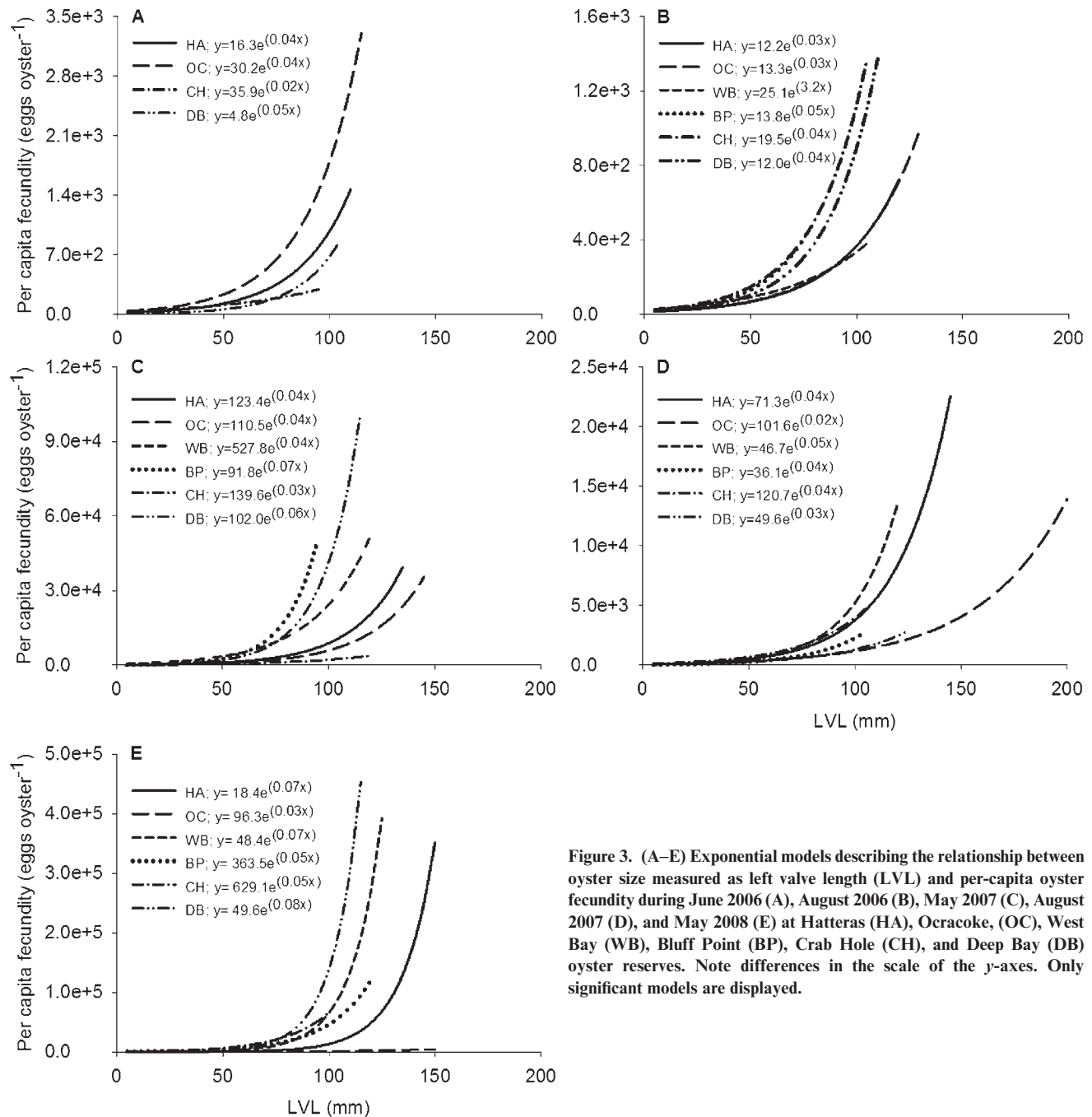

Figure 3. (A–E) Exponential models describing the relationship between oyster size measured as left valve length (LVL) and per-capita oyster fecundity during June 2006 (A), August 2006 (B), May 2007 (C), August 2007 (D), and May 2008 (E) at Hatteras (HA), Ocracoke, (OC), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB) oyster reserves. Note differences in the scale of the y-axes. Only significant models are displayed.

at a reserve during a sampling month, and R is a random number between –1 and 1 generated from a uniform distribution. If the deviation term caused fecundity to be negative for an individual, fecundity for that individual was set equal to 0. To estimate total reproductive potential for a given broodstock reserve, estimates of reproductive potential per square meter were multiplied by the total surface area of hard substrate within each reserve (Table 1), determined with field maps generated via side-scan sonar surveys (Ballance & Eggleston 2008).

Statistical Analyses

A 2-way ANCOVA model was used to test whether mean per-capita fecundity (eggs per oyster) of female oysters varied significantly ($\alpha = 0.5$) according to month and reserve, with oyster LVL measured in millimeters as the covariate, and month and reserve as factors. Linear least-squares regression was used to fit exponential models to the relationship between oyster size and log-transformed eggs per oyster. Two-way ANOVA models were used to test whether mean reproductive potential per square meter and reserve reproductive potential varied significantly according to month and reserve. Data were $\log(x + 1)$ transformed to satisfy assumptions of normality and homogeneity of variance (reserve, $P > 0.1$; month, $P > 0.1$). Multiple pairwise comparisons between treatment levels were conducted with a Student's Newman-Keuls test.

RESULTS

Spatiotemporal Variation in Per-Capita Fecundity

A total of 2,596 oysters were collected for fecundity analysis from the 6 broodstock reserves over 5 sampling periods from 2006 to 2008 (Table 1). Per-capita fecundity ranged from 0 (i.e., males) to 340,500 eggs. Egg-bearing females were present in all size classes (Fig. 2). The sex ratio switched from predominately male to female in the 30–45-mm size class and was more than 80% female at 60–75 mm LVL (Fig. 2). Mean per-capita fecundity increased exponentially with oyster LVL (Fig. 3). Average per-capita fecundity of female oysters varied significantly by month, reserve, and oyster size; however, a significant 3-way interaction precluded contrasts across the main effects (LVL × reserve × month, $P < 0.0001$; Table 2). Temporally, per-capita fecundity within a reserve was generally higher in May than in June or August and tended to increase over time. Spatially, per-capita fecundity during a sampling date was often 2–3-fold higher at the more inland reserves (i.e., lower salinity) at West Bay and Bluff Point than at more seaward reserves (i.e., higher salinity) at Ocracoke (Fig. 3).

Spatiotemporal Variation in Reproductive Potential per Square Meter

Mean reproductive potential per square meter varied significantly by month and reserve ($P < 0.0001$; Table 2). A significant reserve-by-month interaction precluded contrasts across the main effects ($P < 0.0001$; Table 2). In general, temporal comparisons of reproductive potential per square meter within a reserve were significantly higher in May than in June or August, and in May 2008 and August 2007 than during the same months the preceding year. Spatial comparisons of reproductive potential per square meter during a given month were generally higher at more seaward reserves such as Hatteras and lower at more inland reserves such as Deep Bay (Fig. 4), which is opposite the pattern observed for per-capita fecundity. Differences in spatial patterns of per-capita fecundity and reproductive potential per square meter were driven by spatial differences in oyster density and LVL among reserves (Fig. 5) (Puckett & Eggleston 2012). Reserves with both relatively high oyster density and LVL ranked high in reproductive potential per square meter, whereas reserves with either high mean oyster density and small mean LVL (e.g., Crab Hole) or larger mean LVL and lower mean oyster density (e.g., Deep Bay) ranked lowest in reproductive potential per square meter (Figs. 4 and 5).

TABLE 2.

Results of analysis of covariance for the effects of reserve (Bluff Point, Crab Hole, Deep Bay, Hatteras, Ocracoke, and West Bay), 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 per oyster + 1)), reproductive potential per square meter (log(eggs per square meter + 1)), and reserve reproductive potential (log(eggs per reserve)).

| Scale	Variation source	SS (df)	Mean square	F	P
Per capita fecundity	Reserve	37.7 (5)	7.5	4.5	0.0004
	Month	142.3 (4)	35.6	21.4	<0.0001
	LVL	1,259.1 (1)	1,259.1	757.8	<0.0001
	Reserve × month	98.3 (20)	4.9	3.0	<0.0001
	LVL × reserve	43.2 (5)	8.6	5.2	<0.0001
	LVL × month	70.5 (4)	17.6	10.6	<0.0001
	LVL × reserve × month	132.3 (20)	6.6	4.0	<0.0001
Reproductive potential/m²	Reserve	61.5 (5)	12.3	8.2	<0.0001
	Month	684.3 (4)	171.1	114.2	<0.0001
	Reserve × month	165.5 (20)	8.3	5.5	<0.0001
Reserve reproductive potential	Reserve	120.2 (5)	24.0	4.4	0.0016
	Month	704.1 (4)	176.0	31.8	<0.0001
	Reserve × month	278.5 (20)	14.4	2.6	0.0015
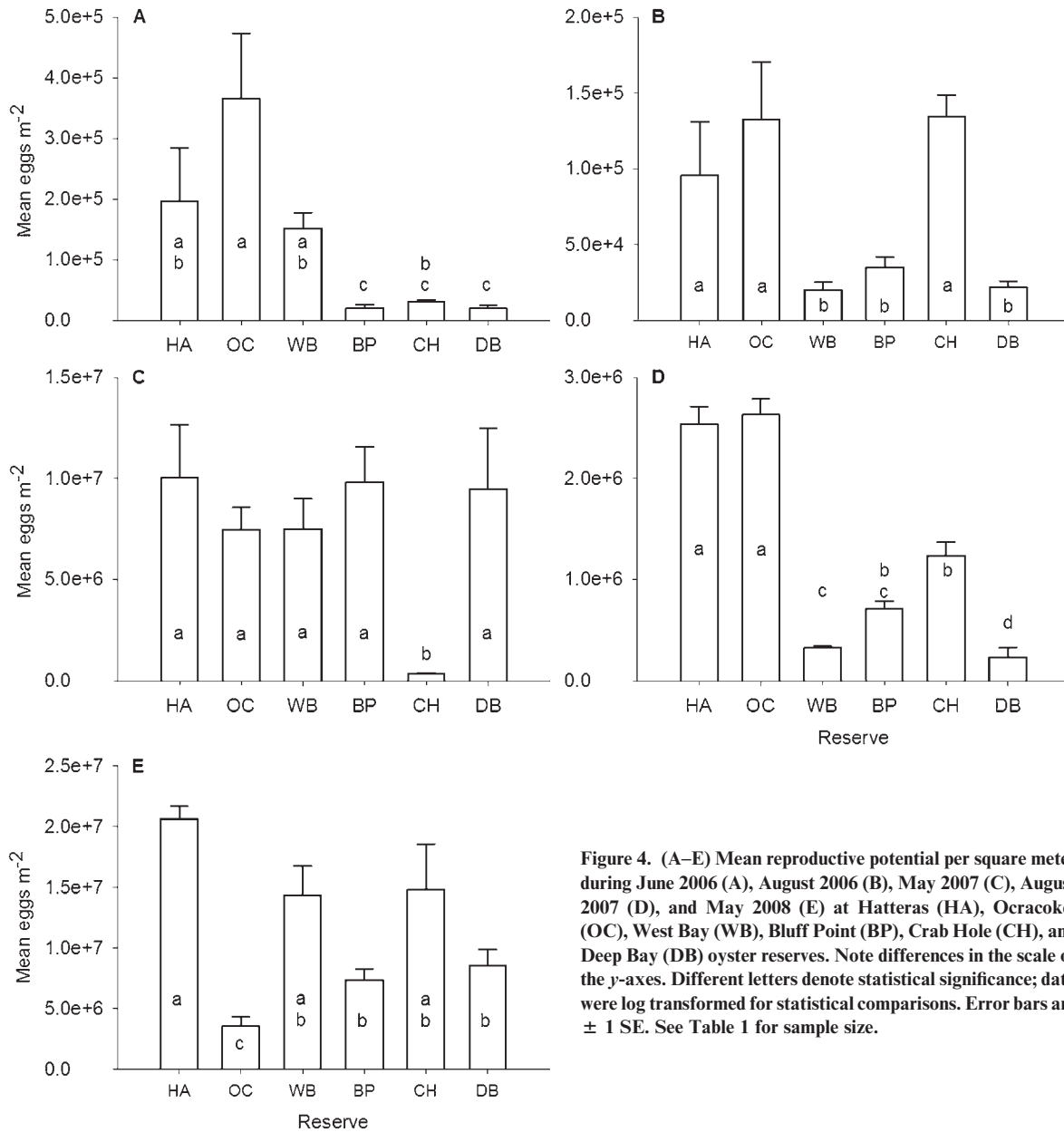
Figure 4. (A–E) Mean reproductive potential per square meter during June 2006 (A), August 2006 (B), May 2007 (C), August 2007 (D), and May 2008 (E) at Hatteras (HA), Ocracoke, (OC), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB) oyster reserves. Note differences in the scale of the *y*-axes. Different letters denote statistical significance; data were log transformed for statistical comparisons. Error bars are ± 1 SE. See Table 1 for sample size.

Spatiotemporal Variation in Reserve Reproductive Potential

Mean reproductive potential within a reserve varied significantly by month, reserve, and their interaction ($P < 0.0016$; Table 2). Temporal comparisons of reserve reproductive potential were similar to patterns observed in per-capita fecundity and reproductive potential per square meter, with peaks in May and a general increase over time. Although the general trend of peak reproductive potential in May 2007 and 2008 was true for most reserves, there were some exceptions, such as Crab Hole, where reserve reproductive potential increased significantly during each sampling date. Spatially, patterns of reserve reproductive potential during a sampling period were similar to patterns in reproductive potential per square meter in which more seaward reserves had the highest reserve reproductive potential and more inland reserves the lowest (Fig. 6). A notable exception was Crab Hole, the largest reserve, which ranked consistently low in reproductive potential per square meter and consistently high in reserve reproductive potential (Fig. 4 vs. Fig. 6).

DISCUSSION

This study quantified a comprehensive suite of 3 indices of fecundity and reproductive potential that can be applied to rank reserve broodstock function according to specific management goals: (1) per-capita fecundity, (2) reproductive potential per square meter, and (3) reserve reproductive potential. For example, management goals may involve adding more robust individuals (e.g., disease resistant in the case of oysters) to the population, either through stock enhancement programs or reducing natural mortality within reserves. In this case, management efforts should be allocated to reserves with the highest per-capita fecundity, such as the more inland reserves at Bluff Point and West Bay in our Pamlico Sound study system. If the
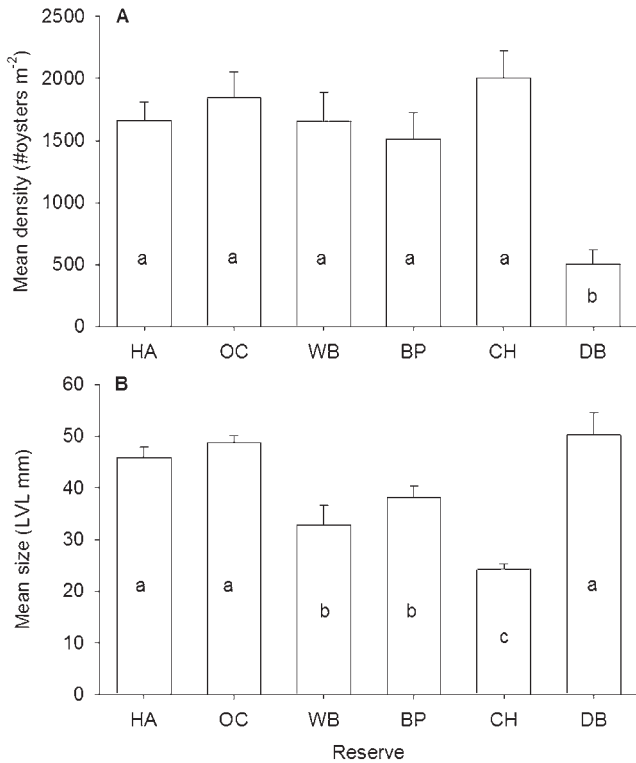
Figure 5. (A, B) Spatial variation in mean oyster density (A) and size (B) measured as left valve length (LVL) among oytster reserves: Hatteras (HA), Ocracoke (OC), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB). Data were pooled across sampling dates. Different letters denote statistical signficance. Error bars are ± 1 SE and n = 106 quadrats.

goal is to maximize the impact of reserve expansion on reproductive potential, then reserves that combine the joint effects of relatively high population density, adult size, and per-capita fecundity (i.e., high reproductive potential per square meter) should receive priority. In this study, more seaward reserves such as Ocracoke and Hatteras would receive priority for reserve expansion. Last, managers may wish to rank the broodstock function of current reserves. Highly ranked reserves would be those that combine the joint effects of relatively high reproductive potential per square meter and large reserve area, thus providing the greatest reserve reproductive potential (e.g., seaward reserves in this study). As demonstrated in this study and that of Puckett and Eggleston (2012), demographic data applied to various management goals can, however, be contradictory, depending on the demographic rates considered and the spatiotemporal scale of the response variable. Accordingly, using multiple demographic rates, including a comprehensive suite of reproductive metrics, is an effective approach to guide comprehensive restoration and management, not only in our study system of oyster broodstock reserves, but in any reserve or reserve network aimed at enhancing or restoring populations.

Three lines of evidence from our study support the efficacy of marine reserves in restoring oyster broodstock function. First, the sex ratio of oysters in our study system was more than 4:1 female to male at sizes of ≥ 60–75 mm. Typically, the sex ratio of natural populations that are exposed to fishing

pressure is ~1:1, even for oysters larger than 75 mm (Thompson et al. (1996) and references therein). The commercial oyster size limit in North Carolina is 76 mm LVL, suggesting that commercial harvest is comprised largely of females. By preventing harvest, reserve protection should lead to an increase in females (i.e., spawning stock biomass), and thus greater reproductive potential (assuming sperm is not limiting). Second, the exponential increase in per-capita fecundity as a function of oyster LVL suggests that documented increases in age/size structure (Puckett & Eggleston 2012) within reserves should result in greater reproductive potential with time (Jack & Wing 2010). Third, the observed temporal increases in fecundity and reproductive potential provide empirical evidence that reserve broodstock function is responding to reserve implementation in relatively rapid time frames.

Temporal Variation in Fecundity and Reproductive Potential

All 3 metrics—per-capita fecundity, reproductive potential per square meter, and reserve reproductive potential—were highest in May for nearly every reserve, which when accounting for an average planktonic larval duration of 2–3 wk (can range from 14–60 days depending on temperature and food availability (Dekshenieks et al. 1996, Kennedy 1996)), coincides with peak soundwide settlement (Eggleston & Puckett, unpubl. data). Thus, the relatively high fecundity and reproductive potential measured in this study in May 2007 and May 2008 are likely the result of sampling just prior to mass spawning whereas the relatively low reproductive values in June 2006 are likely the result of sampling just after mass spawning. If so, it does not appear that oysters spawn all their eggs during a single event. Our results support previous research suggesting that oysters undergo gametogenesis during fall and early spring prior to a first massive spawning event in mid May, followed by less synchronized spawning over the remainder of the reproductive season (Galtsoff 1930, Choi et al. 1994, Thompson et al. 1996). The single exception to this rule was Crab Hole, where reproductive potential increased throughout the duration of the study (i.e., August 2007 > May 2007), likely because of the combined influences of increased density and number of older/larger individuals during the time period studied (Puckett & Eggleston 2012).

In support of the large body of empirical evidence suggesting that the density, age/size structure, and biomass of fishery species increases within no-take reserve boundaries over relatively short (1–5 y) time spans (Halpern & Warner 2002, Halpern 2003, Lester et al. 2009, Jack & Wing 2010), we observed a general increase in 3 key response variables regarding broodstock function—particularly, reproductive potential per square meter as a function of time (i.e., May 2008 > May 2007 and August 2008 > August 2007). Increases in reproductive potential per square meter over time is a function of temporal increases in per-capita fecundity and oyster density, which increased ~400%, on average, at each reserve during the study period (Puckett & Eggleston 2012). In a management context, it is interesting to consider the rate of change in fecundity and reproductive potential in terms of site prioritization. For example, Ocracoke may currently have the highest reproductive potential per square meter, but it may be increasing at a slower rate over time than at Bluff Point, such that in a few years, Bluff Point will surpass Ocracoke in reproductive potential per square meter.
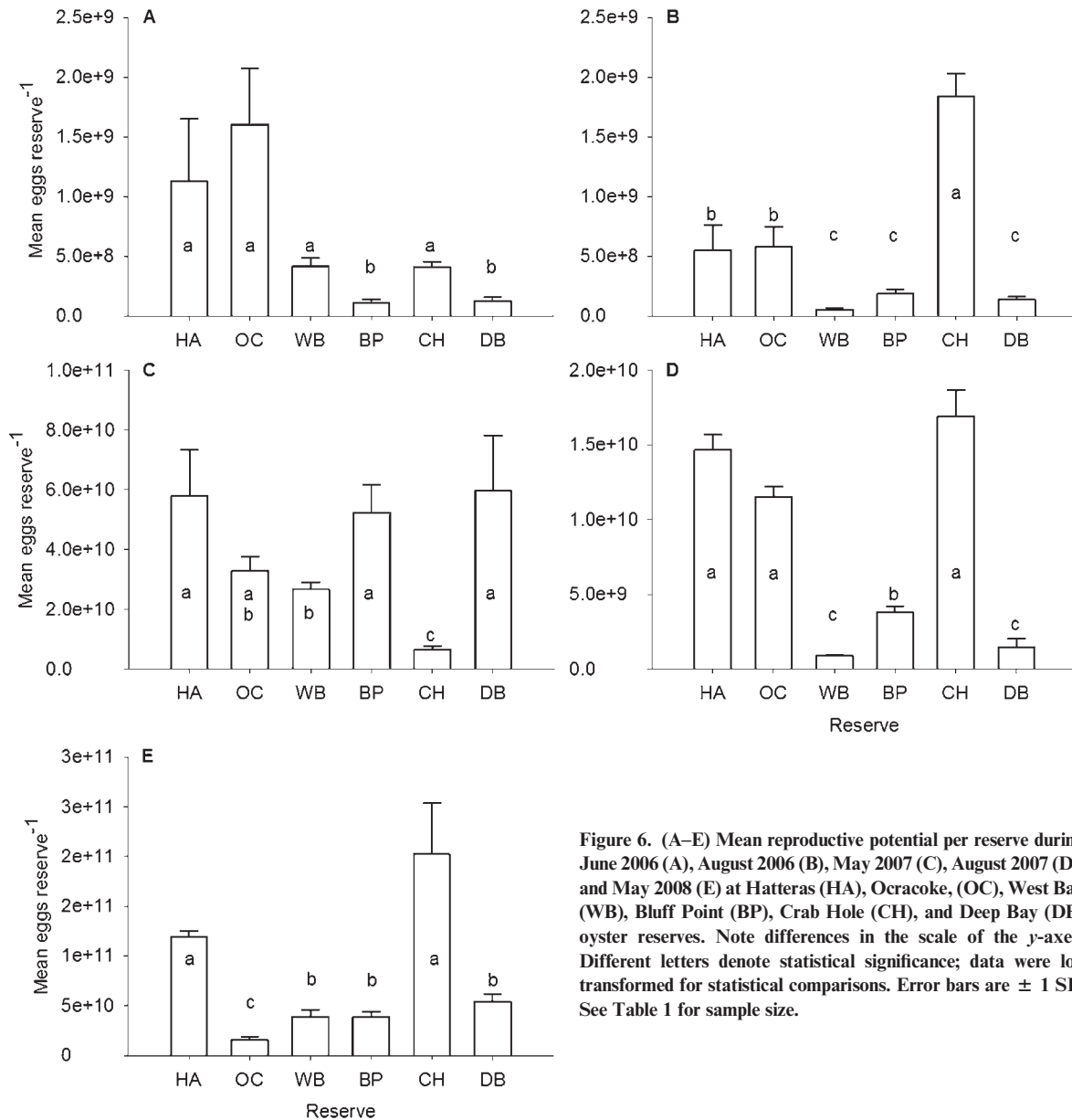
Figure 6. (A–E) Mean reproductive potential per reserve during June 2006 (A), August 2006 (B), May 2007 (C), August 2007 (D), and May 2008 (E) at Hatteras (HA), Ocracoke, (OC), West Bay (WB), Bluff Point (BP), Crab Hole (CH), and Deep Bay (DB) oyster reserves. Note differences in the scale of the *y*-axes. Different letters denote statistical significance; data were log transformed for statistical comparisons. Error bars are ± 1 SE. See Table 1 for sample size.

Thus, managers and researchers should strive to reassess the functioning of each reserve within a network of reserves because demographic rates will likely change over space and time.

Spatial Variation in Fecundity

Per-Capita Fecundity

Despite containing, on average, smaller oysters (Fig. 5B), mean per-capita fecundity was higher at more inland reserves where salinity was lower, such as Bluff Point and West Bay (Fig. 3) than more seaward reserves, with the notable exception of Crab Hole. The relatively low rate of increase in per-capita fecundity with LVL observed at more seaward reserves such as Ocracoke and West Bay may be the result of increased prevalence of common oyster parasites, such as pea crabs (*Zaops ostreum*), MSX (*Haplosporidium nelsoni*), and Dermo

(*Perkinsus marinus*) in warm, high-salinity waters (Villalba et al. 2004). Pea crabs, for example, were present inside oysters almost exclusively at Ocracoke and Hatteras, and were completely absent from other reserves. Similarly, the rate of increase in per-capita fecundity with LVL at Crab Hole (relatively low salinity) was the lowest of all reserves in this study. Crab Hole was unique for 2 reasons: (1) it was the only reserve where mean LVL was less than the size at which the sex ratio is predominately female and (2) it had extremely high densities of Atlantic ribbed mussels (*Geukensia demissa*), a sessile suspension feeder, that may have increased competition for space and food. Further studies determining the infection rates by pea crabs, relative impacts of parasites on oyster health and fecundity, and impacts of interspecific competition on oyster growth and survival (Lohse 2002, Boudreaux et al. 2009) in Pamlico Sound are needed for a more rigorous assessment of these potential impacts on fecundity.

Reproductive Potential Per Square Meter

Broodstock reserves with the highest reproductive potential per square meter 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 as a result of 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 (Haase et al. 2012, Eggleston & Puckett, unpubl. 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 & Puckett, unpubl. data). Consequently, a disproportionate amount of oysters settle in the seaward reserves of Pamlico Sound (e.g., Hatteras and Ocracoke). We expect that relatively high oyster settlement at Hatteras and Ocracoke will be offset by increased predation-induced mortality at these more saline reserves over time (Wells 1961, Puckett & Eggleston 2012).

Reserve Reproductive Potential

Despite the spatiotemporal variation in mean oyster density and LVL, reproductive potential was fairly homogenous among reserves. At the reserve level, much of the spatial variation in reproductive potential was reduced because of 2 factors. First, there was a high degree of variance in reproductive potential per square meter that was exacerbated by multiplying the differing reserve hard substrate surface areas (Table 1). Second, some of the reserves that had a high reproductive potential per-square-meter measurement were also smaller reserves. For example, Ocracoke generally had the highest reproductive potential per square meter, but had the second smallest amount of hard substrate surface area. These differences in reserve substrate area (up to 5 times) dampen any spatial variation in reproductive potential per square meter when scaled to reserve size.

CONCLUSIONS

Improved capability to predict conditions leading to enhanced settlement, survival, growth, and fecundity is likely to improve restoration success (Powers et al. 2009). Moreover, estimates of fecundity can provide a simple cost/benefit assessment in terms of egg production as a function of dollars spent on restoration and conservation. This cost/benefit assessment can be a powerful tool for determining the best use of finite resources for conservation projects. Understanding the dynamics of oysters and populations of other species linked by larval dispersal requires a better understanding of the importance of scale on key demographic response variables, as well as the need to consider a comprehensive suite of response variables to inform specific management goals more effectively.

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