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SETTLEMENT, GROWTH, AND SURVIVAL OF EASTERN OYSTERS ON ALTERNATIVE REEF SUBSTRATES

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ABSTRACT Restoration of the native eastern oyster (*Crassostrea virginica*) has been severely hindered by the dwindling supply and rising costs of fossil and new oyster shell (OS) for use in reef restoration. Consequently, emphasis has shifted to the use of alternative oyster reef materials, which need to be tested for their effectiveness as settlement substrate. Furthermore, low recruitment of wild larvae has also impeded restoration, indicating a need to assess the potential of field setting of cultured larvae. We experimentally examined oyster settlement, growth and survival on unconsolidated OS, vertically embedded oyster shell (ES) in concrete, and concrete Oyster Castles (OC) in field and mesocosm experiments. In addition, we examined settlement success of cultured larvae in the mesocosm experiment. In the field experiment, juvenile recruitment was 3× higher on castles and unconsolidated shell than on embedded shell. Castles retained 4× the number of oysters and hosted 5× the biomass than embedded shell, and retained 1.5× the oysters and hosted 3× the biomass than unconsolidated shell. The proportion of live oyster recruits on castles was 1.5× that on both embedded and unconsolidated shell. In the mesocosm experiment (90-d postlarval deployment), the castles recruited, retained, and hosted an oyster biomass 4× higher than that of unconsolidated and embedded shell. This study confirms that artificial reef materials, such as OC, are suitable alternative substrates for oyster restoration, and remote setting of larvae can be effective under controlled environmental conditions. Future restoration efforts should consider use of alternative reef substrates and field setting of larvae, where recruitment is limited, to maximize oyster recruitment, while simultaneously minimizing the cost of reef restoration.

KEY WORDS: oyster restoration, *Crassostrea virginica*, remote larval setting, artificial reefs, Oyster Castles

INTRODUCTION

The Chesapeake Bay native oyster, *Crassostrea virginica* (Gmelin, 1791), is an ecosystem engineer that performs critical ecological functions, including water filtration, sediment stabilization, and provision of nursery habitat for juveniles of diverse fish and invertebrate species (Kennedy et al. 1996). Prior to European colonization of North America, the native oyster population of the Chesapeake Bay was described as being so abundant that ''they lay as thick as stones'' throughout the Bay and its tributaries. As a result of overfishing, disease, and poor water quality, the native oyster population of the Chesapeake Bay currently stands at less than 1% of its historic population size (Rothschild et al. 1994, Wilberg et al. 2011). Additionally, human activities on land have increased the flow of sediment into the estuaries, which have weakened physiological health, lowered fecundity, and raised mortality of oysters (Newell 1988, Rothschild et al. 1994, Lenihan et al. 1999). Exacerbating the situation, the physical profile of reefs has been leveled by fishers exploiting oyster reefs (Rothschild et al. 1994), which places oysters lower in the water column where water flow is reduced and sediment accumulation rates are highest, thereby suffocating oysters (Newell 1988, Lenihan et al. 1999).

Efforts to restore native oyster populations have been extensive but largely ineffectual or unresolved (Ruesink et al. 2005, Kennedy et al. 2011). However, recent successful restoration efforts with natural shell reefs and alternative materials have defined promising approaches (Lipcius and Burke 2006, Taylor and Bushek 2008, Powers et al. 2009, Schulte et al. 2009, La Peyre et al. 2014). In the Chesapeake Bay as in other locations, availability and cost of reef substrate remains a significant hindrance to restoration progress (Kennedy et al. 2011). For instance, construction of sanctuary reefs in the Great Wicomico River oyster reef network required extensive use of dredged shell derived from buried fossil shell deposits (Schulte et al. 2009) at an estimated cost of nearly US$10,000 per ha per cm of reef. Cheaper substrates such as crushed concrete, limestone, and porcelain toilets have been used as alternative oyster reef materials (Soniat et al. 1991, Haywood III et al. 1999), but until recently their effectiveness has not been adequately tested against OS (La Peyre et al. 2014). In addition, enhancement of oyster recruitment through larval setting remotely on such structures has not been investigated experimentally in the eastern oyster.

Alternative substrate may be preferentially settled upon by oyster larvae due to the large amount of surface area of a suitable chemical composition available for larval settlement. Additionally, larval settlement may be facilitated by the ability of these alternative substrates to mimic the three-dimensional structure of natural oyster reefs. The three-dimensional nature of many alternative substrates allows the oysters to be oriented above the benthic floor and to escape some of the sedimentation and predation faced by those that settle on thin layers of OS.

In 2008, the Allied Concrete Corporation, in conjunction with The Nature Conservancy, developed an alternative reef substrate–Oyster Castle (OC). This prefabricated substrate features a parapet shape at the top of each block and is composed of limestone gravel, concrete, and crushed OS, all of which have

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been used in various forms to construct artificial oyster reefs. Hence, OC reefs can serve as a model system to examine the utility of alternative substrates in oyster reef construction. Moreover, due to the stackable nature of the block and low cost of ingredients, three-dimensional reef structures of variable heights can be constructed easily and cost effectively.

The objective of this study was to test the efficacy of alternative oyster reef substrates and remote setting of larvae in field and mesocosm experiments. Specifically, we compared settlement, size, and survival of oysters on OC, unconsolidated OS, and vertically embedded oyster shell (ES) in concrete as a function of natural recruitment and artificially enhanced recruitment through remote setting.

MATERIALS AND METHODS

Experimental Reef Substrates

Three reef substrates were tested in mesocosms and in the intertidal zone of the York River along the Virginia Institute of Marine Science (VIMS) in Gloucester Point, VA (Fig. 1). As part of a balanced analysis of variance (ANOVA) randomized block design (Underwood 1997), three sets of experimental blocks (larval subsidy) and three sets of control blocks (wild recruitment) were created using: (1) four OCs, (2) one tray of loose, unconsolidated OS containing 16 shells (~75 mm SH) per 0.25 m × 0.25 m quadrant, and (3) one tray of vertically ES in a 0.5 m × 0.5 m base of concrete (Quikrete underlayment concrete) per block. ES served as a control for substrate vertical relief, and all substrates occupied a bottom area of 0.5 m × 0.5 m. Substrates were conditioned in the intertidal zone for two months to ensure that the physicochemical and biological (development of a biofilm) characteristics of the substrate surface were suitable for larval settlement (Bonar et al. 1990).

Larval Deployment

Hatchery-reared eyed oyster larvae were obtained from the VIMS Aquaculture Genetics and Breeding Technology Center in Gloucester Point, VA. Approximately six million oyster larvae were concentrated into a larval ball and stored overnight in cheesecloth at ~5°C. Immediately prior to release, the larvae were removed from cold storage, allowed to equilibrate to ambient air temperature (~20 min), and then immersed in a beaker containing 2 L of river water. Light stirring was used to evenly distribute larvae in solution and then divided across both the field and mesocosm experimental blocks (~950,000 larvae per block).

Mesocosm Experiment

After the conditioning period, the experimental and control blocks were enclosed within three mesocosm tanks (Fig. 2). An air supply system was customized to ensure that the mesocosms remained well-oxygenated and that ambient, flow-through river water was circulated throughout each mesocosm. Prior to larval introduction, each mesocosm was partitioned with silt fence to separate experimental blocks from control blocks. Following a standard aquaculture industry protocol, a single coat of petroleum jelly was liberally applied to deter settlement of larvae on the mesocosm surface (Congrove et al. 2008). Prior to larval release, water flow in the mesocosms was halted and airflow was reduced.

Field Experiment

The reef substrates were placed in the intertidal zone along the northern shore of the York River for conditioning and the duration of the experiment (Fig. 3). After conditioning, the experimental blocks were enclosed with silt fence for a one-week larval settlement period, and the control blocks remained exposed.

Sampling

At fixed sampling intervals (15, 45, and 90 d), oyster settlement, shell height (SH), and survival were recorded. At each sampling interval, samples were randomly selected (Microsoft Excel

Figure 1. Photograph of the layout of experimental reef substrates used in the field experiments during the conditioning period, including Oyster Castle, unconsolidated oyster shell, and vertically embedded oyster shell in concrete.

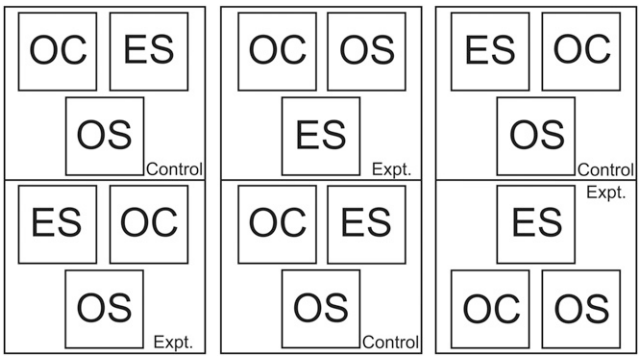

Figure 2. Layout of experimental and control blocks within the mesocosm experiment. Lines between blocks within mesocosms indicate silt fence barriers. ES, embedded oyster shell; OS, oyster shell; OC, Oyster Castle.

Random Number Generator) from one of the four quadrants within each replicate. SH was measured with calipers and mortality determined by gaping oyster spat or presence of oyster scars on the substrate.

Statistical Analysis

Analysis of variance models were used for both the field and mesocosm experiments using the 90-d sampling data. Ninety days was selected as the appropriate sampling interval for statistical analyses because this duration allowed for settlement of larvae, postsettlement mortality, and juvenile growth (Osman and Abbe 1994, Burke 2010). Response variables included: (1) Total Recruits (total number of live and dead recruits), (2) Live Recruits, (3) Live Recruit Biomass (ash-free dry oyster tissue mass), and (4) Proportion of Live Recruits (live recruits divided by total recruits). The two fixed factors were Reef with three levels (OC, OS, and ES) and Larval Subsidy with two levels (wild recruitment only, wild recruitment + larval subsidy). Location was a blocking factor with three levels. Levene's test was used to test the assumption of homogeneity of variance. The Student–Newman–Keuls (SNK) test was used to assess significant differences between factor levels.

Live oyster biomass, as ash-free dry mass (AFDM), was computed using the following power function (Lipcius, unpubl. data):

$$\text{AFDM} = 0.00002(\text{Shell Height})^{2.47}.$$

Akaike's Information Criterion (AIC) within an Information Theoretical approach (Anderson 2008) was used to evaluate five models (Table 1). AIC_c (a second-order bias correction estimator), Δ_i (a weighted measure of each model relative to the best-fitting model), and w_i (model probability) were used to compare model (g_i) fit. Analysis of variance tests were used to assess the goodness of fit of competing models.

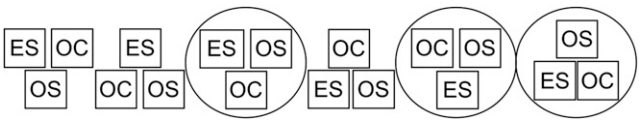

Figure 3. Layout of experimental and control blocks within the field experiment. Circles indicate silt fence enclosures surrounding experimental blocks. ES, embedded oyster shell; OS, oyster shell; OC, Oyster Castle.

TABLE 1.

Parameters for the candidate linear regression models.

			Effects			
Model	**Description**	***k***	**Subsidy**	**Reef**	**Subsidy × Reef**	**Block**
g_1	Full	8	×	×	×	×
g_2	Interaction	7	×	×	×	
g_3	Additive	5	×	×		
g_4	Reef	4	×			
g_5	Subsidy	3		×		

Response variables = total recruits, live recruits, live recruit biomass, and proportion of live recruits. k = number of parameters, including variance (σ^2) as a parameter.

The effect of recruit size on survival was analyzed with a log-linear model by using the frequencies of live and dead recruits as the response variable. Factors included recruit size (> or <30 mm SH), larval subsidy (yes, no), reef type (ES, OS, and OC), and experiment (field, mesocosm). There were significant interactions including experiment as a factor, so the log-linear models were conducted separately for the field and mesocosm experiments. Effects were integrated into the models with stepwise addition of effects using AIC as the criterion. All analyses were conducted using the R statistical software package (R Core Team 2014).

RESULTS

Mesocosm Experiment

Model g_3, the additive model including Reef and Subsidy (Table 1), provided the best fit for Total Recruits (AIC $w_i = 0.93$), Live Recruits (AIC $w_i = 0.93$), and Recruit Biomass ($w_i = 0.90$); Table 2 provides model selection results for all models from the mesocosm experiment. For these three variables, the Reef and Subsidy factors were always significant (Table 3). In addition, neither the global model (g_1) nor the interaction model (g_2) improved the fit significantly better than model g_3 (F test, $P > 0.27$ for all three variables).

The magnitude and direction of the Reef and Subsidy effects were equivalent for Total Recruits and Live Recruits (Table 4). To be concise, we only portray the patterns for Live Recruits (Fig. 4). Live recruit density (Fig. 4A) and biomass (Fig. 4C) were substantially and significantly (SNK test, $P < 0.05$) higher on OC than on OS; those on OS were significantly higher than those on ES (SNK test, $P < 0.05$). Recruit density and biomass were more than twice as high on OC than on OS; those on OS were nearly twice as high as on ES (Fig. 4A, C, Table 4). Recruit density was about 50% higher in the larval subsidy treatments (Fig. 4B, Table 4), whereas biomass was more than twice as high (Fig. 4D, Table 4). These differences were due to the earlier settlement and growth of cultured juveniles over wild juveniles, which resulted in a greater fraction of juveniles larger than 30 mm SH with larval subsidy (Fig. 5).

For the Proportion of Live Recruits, model g_4, the model that only included Reef (Table 1), provided the best fit (AIC $w_i = 0.81$), although model g_3 also had substantial support (AIC $w_i = 0.19$);

TABLE 2.

Complete model selection results for the mesocosm experiment.

Model	AIC*c*	Δ_i	w_i	r^2
Total recruits				
g_1	172.8	10.9	<0.01	0.91
g_2	169.2	7.2	0.02	0.90
g_3	161.9	0.0	0.93	0.88
g_4	168.3	6.3	0.04	0.78
g_5	190.6	28.6	<0.01	0.09
Live recruits				
g_1	172.6	11.0	<0.01	0.91
g_2	168.9	7.3	0.02	0.89
g_3	161.6	0.0	0.93	0.87
g_4	167.9	6.2	0.04	0.78
g_5	189.6	27.9	<0.01	0.10
Live recruit biomass				
g_1	127.1	12.5	<0.01	0.73
g_2	121.8	7.2	0.02	0.71
g_3	114.6	0.0	0.90	0.66
g_4	120.2	5.7	0.05	0.42
g_5	121.6	7.1	0.03	0.24
Proportion of live recruits				
g_1	86.0	16.4	<0.01	0.72
g_2	90.1	12.4	<0.01	0.67
g_3	99.6	2.9	0.19	0.66
g_4	102.4	0.0	0.81	0.64
g_5	87.7	14.7	<0.01	0.02

Table 2 provides model selection results for all models from the mesocosm experiment. The Reef factor was highly significant, whereas the effect of Subsidy was not significant (Table 3). In addition, neither the global model (g_1) nor the interaction model (g_2) improved the fit significantly better than model g_4 (F test, $P > 0.53$).

There was very high survival in all treatments (Fig. 5). The proportion of live recruits was nearly 0.998 in the ES treatment, and only dropped to 0.972 and 0.966 in the OC and OS treatments, respectively (Table 4). Although the effect of Reef was statistically significant (Table 3), the effect sizes were very small (Table 5) and not likely to be biologically significant (Fig. 5).

Field Experiment

We experienced storm conditions during our attempt to release hatchery-reared larvae in the field, which precluded a strong effect of larval subsidy in the field experiment (Table 5). Model g_4, the model that only included Reef (Table 1), provided the best fit for Total Recruits (AIC $w_i = 0.86$), Live Recruits (AIC $w_i = 0.78$), and Recruit Biomass ($w_i = 0.84$); Table 6 provides model selection results for all models from the field experiment. For these three variables, the Reef factor was always significant (Table 5). In addition, neither the global model (g_1) nor the interaction model (g_2) improved the fit significantly better than model g_3 (F test, $P > 0.34$ for all three variables).

Total recruit density (Fig. 6A) was approximately equal on OC and OS (SNK test, $P >> 0.05$), which had significantly higher recruit density than ES (SNK tests, $P < 0.05$). Total recruit density was about 3-fold higher on OC and OS than on ES (Table 7). Live recruit density (Fig. 6B) and biomass (Fig. 6C) were significantly higher on OC than on OS, and those on OS were significantly higher than those on ES (SNK test, $P < 0.05$).

TABLE 3.

Analysis of variance results for model g_3, the additive model with Reef and Subsidy as factors, from the mesocosm experiment.

Treatment	MS	df	*F*	*P*
Total recruits				
Subsidy	2838	1	10.75	0.005
Reef	11757	2	44.53	<0.0005
Error	264	14		
Live recruits				
Subsidy	2763	1	10.63	0.006
Reef	11045	2	42.52	<0.0005
Error	260	14		
Live recruit biomass				
Subsidy	187.5	1	9.87	0.007
Reef	162.0	2	8.53	0.003
Error	19.0	14		
Proportion of live recruits				
Subsidy	0.00011	1	0.85	0.37
Reef	0.0017	2	13.28	0.0006
Error	0.00013	14		

Although model g_4 was the best fitting model for Proportion of Live Recruits, we present the results for model g_3 because it shows the nonsignificant effect of Subsidy, and the results for models g_3 and g_4 did not differ in the significance of the factors.

Live recruit density and biomass were about 5-fold higher on OC than on ES; on OS they were slightly greater than those on ES (Table 7).

For the Proportion of Live Recruits, models g_3 (AIC $w_i = 0.38$) and g_5 (AIC $w_i = 0.49$) provided reasonable fits to the

TABLE 4.

Parameter estimates for model g_3, the additive model with Reef and Subsidy as factors, from the mesocosm experiment.

Parameter	Estimate	SE	*t*	*P*
Total recruits				
Intercept	14.94	7.66	1.95	0.07
Subsidy	25.11	7.66	3.28	0.005
Reef: Castle	84.33	9.38	8.99	<0.0005
Reef: Shell	18.83	9.38	2.01	0.06
Live recruits				
Intercept	15.11	7.60	1.99	0.07
Subsidy	24.78	7.60	3.26	0.006
Reef: Castle	81.50	9.31	8.76	<0.0005
Reef: Shell	17.50	9.31	1.88	0.08
Live recruit biomass				
Intercept	0.41	2.05	0.20	0.84
Subsidy	6.45	2.05	3.14	0.007
Reef: Castle	9.94	2.52	3.95	0.001
Reef: Shell	2.35	2.52	0.93	0.37
Proportion of live recruits				
Intercept	0.998	0.005	185.90	<0.0005
Subsidy	0.004	0.005	0.92	0.37
Reef: Castle	–0.026	0.007	–4.01	0.001
Reef: Shell	–0.032	0.007	–4.81	0.0003

The value for the Intercept represents the mean for the No Subsidy/Embedded Shell treatment. Other values represent the additional effect sizes due to the specific treatments, and are additive.

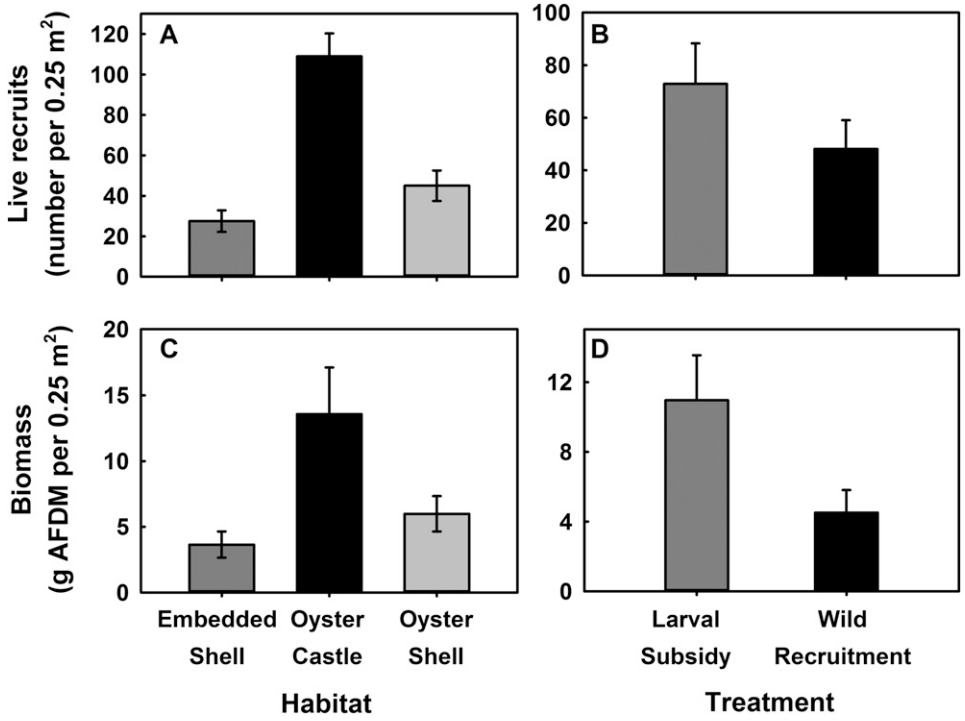

Figure 4. (A) Mean live recruits and (C) mean live recruit biomass on the various substrates, and (B) mean live recruits and (D) mean live recruit biomass in the experimental and control blocks, in the mesocosm experiment. Vertical bars represent one standard error of the mean.

data. We selected model g_3 due to its higher explanatory power as indicated by the r^2 values and due to the statistically significant effects of Reef and Subsidy (Tables 5 and 7). In addition, neither the global model (g_1) nor the interaction model (g_2) improved the fit significantly better than model g_3 (F test, $P > 0.47$ for all three variables); Table 6 provides model selection results for all models from the field experiment.

There was moderate to high survival of recruits in all treatments (Fig. 7). The proportion of live recruits was significantly higher with larval subsidy (Table 5), which increased survival by 47% (Fig. 7A, Table 7). Survival was also significantly higher

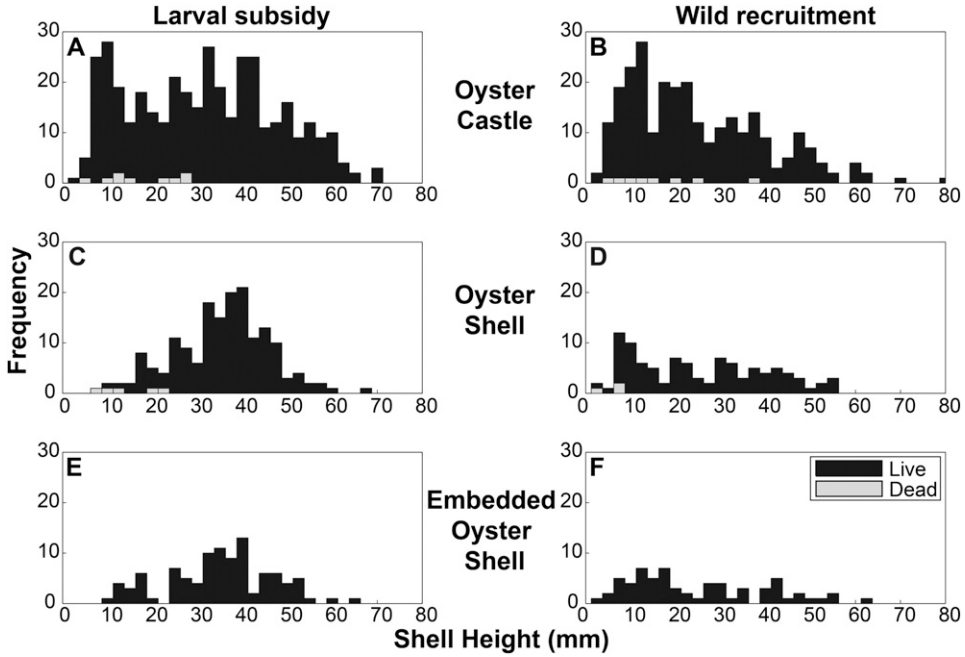

Figure 5. Size frequency histogram of live and dead oysters on Oyster Castles (A and B), oyster shell (C and D), and embedded shell (E and F) in the mesocosm experiment.

TABLE 5.

Analysis of variance results for model g_3, the additive model with Reef and Subsidy as factors, from the field experiment.

Treatment	MS	df	*F*	*P*
Total recruits				
Subsidy	2.72	1	0.05	0.83
Reef	385.39	2	6.81	0.009
Error	56.58	14		
Live recruits				
Subsidy	50.00	1	0.94	0.35
Reef	333.17	2	6.29	0.01
Error	52.98	14		
Live recruit biomass				
Subsidy	0.11	1	0.24	0.63
Reef	2.81	2	6.22	0.01
Error	0.45	14		
Proportion of live recruits				
Subsidy	0.32	1	5.71	0.032
Reef	0.18	2	3.22	0.071
Error	0.06	14		

Although model g_4 was the best fitting model for Total Recruits, Live Recruits, and Live Recruit Biomass, we present the results for model g_3 for consistency.

on OC than on OS and ES (SNK test, $P < 0.05$), ranging from about 60% on OS and ES to 91% on OC (Fig. 7B, Table 7). Mortality occurred primarily in juveniles less than 30 mm SH (Fig. 8), but it did not produce a greater fraction of juveniles greater than 30 mm SH, unlike the pattern for the mesocosm experiment (Fig. 5).

TABLE 6.

Complete model selection results for the field experiment.

Model	AIC*c*	Δ_i	w_i	r^2
Total recruits				
g_1	150.8	20.5	<0.01	0.51
g_2	144.2	13.8	<0.01	0.50
g_3	134.2	3.9	0.13	0.49
g_4	130.3	0.0	0.86	0.49
g_5	139.1	8.8	0.01	<0.01
Live recruits				
g_1	149.4	19.1	<0.01	0.51
g_2	142.8	12.5	<0.01	0.50
g_3	133.0	2.7	0.20	0.49
g_4	130.3	0.0	0.78	0.46
g_5	137.3	7.0	0.02	0.03
Live recruit biomass				
g_1	60.1	16.5	<0.01	0.58
g_2	55.8	12.2	<0.01	0.52
g_3	47.2	3.6	0.14	0.48
g_4	43.6	0.0	0.84	0.47
g_5	51.4	7.8	0.02	0.01
Proportion of live recruits				
g_1	24.4	15.2	<0.01	0.53
g_2	17.6	8.4	<0.01	0.53
g_3	9.7	0.5	0.38	0.46
g_4	11.9	2.7	0.13	0.25
g_5	9.2	0.0	0.49	0.22

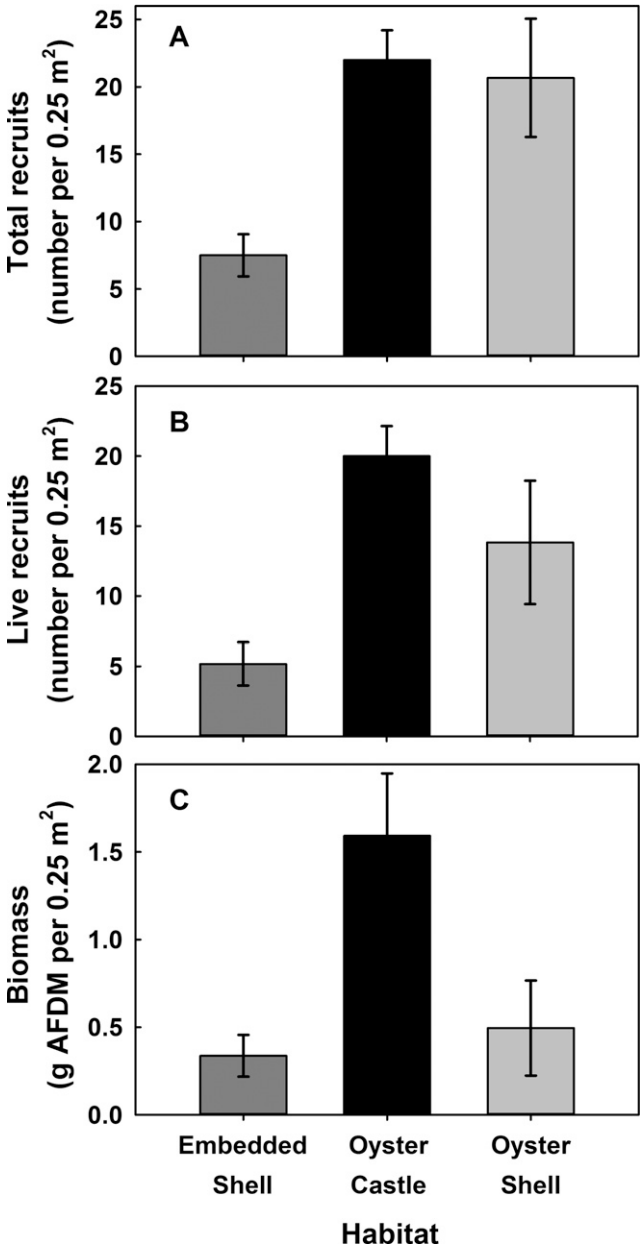

Figure 6. (A) Mean total recruits, (B) mean live recruits, and (C) mean live recruit biomass on the various substrates in the field experiment. Vertical bars represent one standard error of the mean.

Effect of Recruit Size on Survival

In the log-linear analysis including experiment as a factor, there was a significant interaction between Experiment and Reef type (AIC reduced by 14.6 with 2 df), so the analyses were run separately for the field and mesocosm experiments.

In the mesocosm experiment, the global log-linear model was significant (likelihood ratio $\chi^2 = 131.8$, df = 18, $P << 0.001$). The final reduced model with stepwise addition of effects using AIC did not include any significant two-way interactions, and only a significant main effect of size (likelihood ratio $\chi^2 = 513.5$, df = 20, $P << 0.001$). Overall, the probability of survival was 0.987 (Fig. 5). The only

TABLE 7.

Parameter estimates for model g_3, the additive model with Reef and Subsidy as factors, from the field experiment.

Parameter	Estimate	SE	*t*	*P*
Total recruits				
Intercept	7.11	3.55	2.01	0.06
Subsidy	0.78	3.55	0.22	0.83
Reef: Castle	14.50	4.34	3.34	0.005
Reef: Shell	13.17	4.34	3.03	0.009
Live recruits				
Intercept	3.50	3.43	1.02	0.33
Subsidy	3.33	3.43	0.97	0.35
Reef: Castle	14.83	4.20	3.53	0.003
Reef: Shell	8.67	4.20	2.06	0.06
Live recruit biomass				
Intercept	0.26	0.32	0.82	0.43
Subsidy	0.15	0.32	0.49	0.63
Reef: Castle	1.26	0.39	3.24	0.006
Reef: Shell	0.16	0.39	0.41	0.69
Proportion of live recruits				
Intercept	0.49	0.11	4.42	0.0006
Subsidy	0.27	0.11	2.39	0.03
Reef: Castle	0.29	0.14	2.12	0.05
Reef: Shell	–0.02	0.14	–0.15	0.88

The value for the Intercept represents the mean for the No Subsidy/Embedded Shell treatment. Other values represent the additional effect sizes due to the specific treatments, and are additive.

substantial difference in survival was due to size, with juveniles greater than 30 mm SH having a much higher probability of survival than juveniles less than 30 mm SH (odds ratio = 24.7). Of the 25 dead juveniles, only one was greater than 30 mm SH.

In the field experiment, the global log-linear model was significant (likelihood ratio $\chi^2 = 95.3$, df = 18, $P < 0.001$). The final reduced model with stepwise addition of effects using AIC did not include any significant two-way interactions, so the effect sizes were generated with the significant additive main effects model (likelihood ratio $\chi^2 = 34.1$, df = 14, $P << 0.005$). Overall, the probability of mortality was 0.063 (Fig. 8), which was nearly 5× higher than that in the mesocosms (0.013). The greatest difference in survival was due to size, with juveniles greater than 30 mm SH having a higher probability of survival than juveniles less than 30 mm SH (odds ratio = 12.6). The effect of reef type was also significant with oysters on OC having substantially higher survival probability than those on OS (odds ratio = 4.9) and ES (odds ratio = 4.4); there was no difference between ES and OS (odds ratio = 1.1). There was also a moderate effect of Subsidy increasing survival (odds ratio = 2.6).

DISCUSSION

Although there have been numerous studies demonstrating that oysters can recruit to and survive on artificial oyster reefs (Soniat and Burton 2005, Lipcius and Burke 2006, Nestlerode et al. 2007, Burke 2010, Dunn et al. 2014, La Peyre et al. 2014), this is among the first to test oyster settlement and survival experimentally (i.e., both field and mesocosm experiments) on alternative reef substrates (i.e., OC, ES, and OS reefs). In field and mesocosm experiments, alternative reef substrates recruited, retained, and hosted a greater oyster biomass than unconsolidated and ES. This study thus confirms that artificial reef materials are suitable alternative substrates for oyster restoration. Additionally, this study is among the first to demonstrate that field setting of hatchery-reared oyster larvae onto artificial substrates can be effective under controlled environmental conditions, thereby allowing for the possibility of enhancing settlement in locations that are limited by recruitment.

Alternative Substrates

Alternative reef substrates represent an effective means to enhance recruitment and survival of oysters in restoration efforts. In our mesocosm experiment, the OC recruited and hosted oyster density and biomass that were double that of OS; those on OS were nearly twice that of ES. These differences were due to recruitment, rather than survival, because survival was high in all treatments and only differed by less than 3% across the three reef types.

In the field experiment, the OC and OS were approximately equivalent in total recruit density, with the OC and OS retaining a total recruit density that was 3-fold higher than that on ES. Live recruit density and biomass were about 5-fold higher on

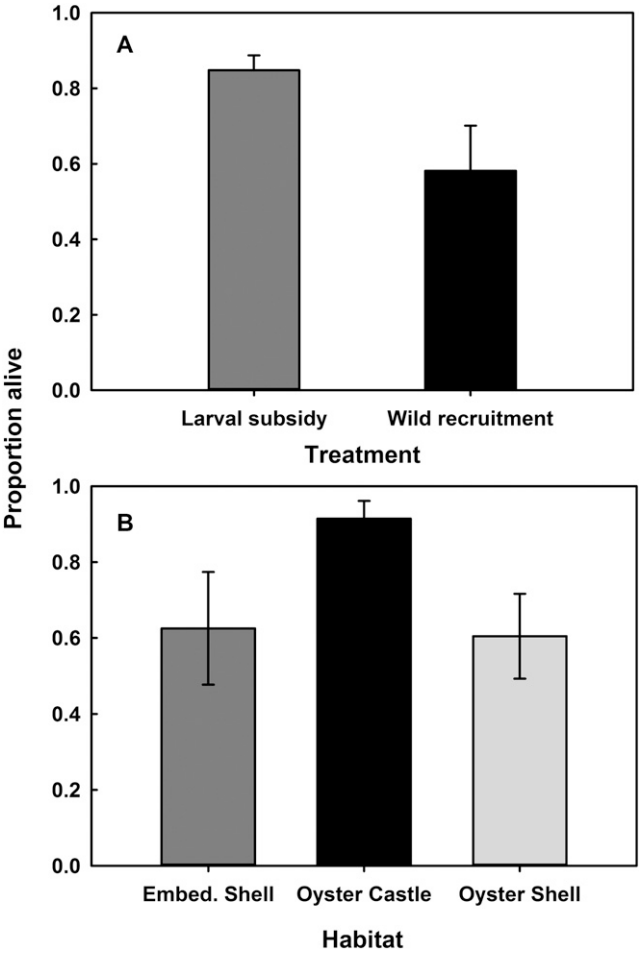

Figure 7. (A) Mean proportion of live recruits on experimental and control blocks, and (B) mean proportion of live recruits on the various substrates in the field experiment. Vertical bars represent one standard error of the mean.

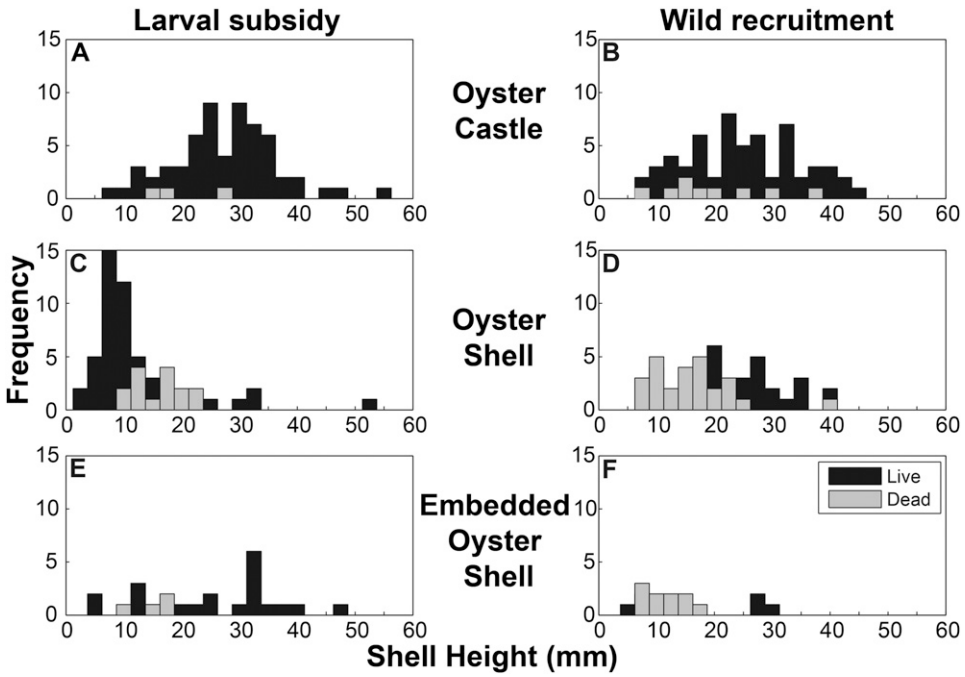

Figure 8. Size frequency histogram of live and dead oysters on Oyster Castles (A and B), oyster shell (C and D), and embedded shell (E and F) in the field experiment.

OC than on ES; on OS they were slightly greater than that on ES. The reduction in live recruit density on OS was due to the low survival of recruits—60% on OS and 91% on OC.

The performance of the OC was likely due to the enhanced vertical relief of the substrate (14 cm on OC compared with 7 cm for ES and 4 cm for OS), despite approximately equivalent surface area between reef treatment types. The higher relief would have allowed a greater percentage of substrate surface to remain above the sediment, out of hypoxic conditions, free from siltation, and in a fixed location. High levels of siltation were observed on the fixed ES, whereas OSs could be turned during periods of high wave activity (e.g., storm events), cleansed of silt, and remain exposed for settlement. Despite the propensity of OS to keep settlement surfaces exposed for recruitment, the turning of OSs also repositioned some existing recruits into suboptimal orientations (i.e., inducing burial of recruits) yielding reduced survival of recruits (60% on OS). The enhanced vertical relief provided by OC allowed for settlement surfaces to remain exposed, normoxic, and fixed in location, which, in part, allowed for a 5-fold higher oyster density and a 5-fold higher probability of survival on OC relative to OS and ES.

Elevation above the benthos, however, is not the only probable mechanism driving the differences in oyster density and biomass between reef treatments. OS provided a greater amount of horizontal OS surface area relative to the ES treatments, which consisted of a horizontal base surface of concrete into which the anterior end of the OS were vertically embedded. A flume study conducted by Soniat et al. (2004) identified that, in the absence of predators, oyster settlement was significantly higher on OS oriented horizontally as compared with shells oriented vertically. The authors posited that this may be due to larvae responding to the greater flow velocities over the horizontal shell surfaces which may enhance their feeding and growth (Soniat et al. 2004). In the case of vertically ESs, dense aggregations of shells oriented vertically can result in suboptimal flow velocities between shells. The combination of these factors likely explains how the OS hosted an oyster density and biomass that was double that of ES in the mesocosm experiment. OC provides a large amount of surface area for settlement, a design that limits impedance of water flow across settlement surfaces, and heightened vertical relief, all of which combine to enhance oyster recruitment and biomass. Despite the relative failure of the remote setting in the field experiment to artificially enhance recruitment on the various reef substrates, OC exhibited sufficient capacity to attract wild settlers at a level equivalent to, or greater than, OS.

Remote Setting

Field setting of larvae may be a useful approach to enhance bivalve recruitment in restoration efforts. In our mesocosm experiment, recruit density was about 50% higher in larval subsidy treatments, whereas biomass was more than twice as high. Recruits from the larval subsidy settled earlier and grew larger than wild recruits, which resulted in a greater fraction of juveniles greater than 30 mm SH in larval subsidy treatments. Moreover, within the field experiment, the proportion of live recruits was higher among larval subsidy treatments, which increased survival by 47%. This increase in survival amongst larval subsidy treatments is likely due to 'swamping' of local predators by the pulse of larval subsidy recruits (Seitz et al. 2001). This partial prey refuge may allow a greater portion of these juveniles to escape postsettlement predation (Newell et al. 2007) and to

Figure 9. Photograph captured five years postdeployment of a reef patch constructed using Oyster Castles from the mesocosm and field experiments within the intertidal zone of Indian Field Creek, a tributary of the Chesapeake Bay. Additional reef patches were constructed using embedded shell and unconsolidated shell and were similar in appearance.

grow to a size greater than 30 mm SH where they have a much greater probability of survival than juveniles less than 30 mm SH, similar to that observed for blue crab (*Callinectes sapidus* Rathbun, 1896) predation upon juvenile eastern oysters (Eggleston, 1990).

Previous studies that have used field remote setting methods have yielded success in maximizing bivalve recruitment. Arnold et al. (2005) used a coupled mesocosm-field study design to examine the efficacy of remote setting of laboratory-reared bay scallops (*Argopecten irradians* Lamarck, 1819) into controlled nurseries with subsequent transplantation into field locations and concluded that planting of cultured bay scallops was a successful strategy for increasing spawning stock density. Leverone et al. (2010) directly released hatchery-reared bay scallop larvae into two West Florida estuaries and concluded that larval remote setting can serve as an effective means to increase local scallop recruitment. In a 2006 VIMS study, a silt fence enclosure was used effectively to deter remotely set triploid oyster larvae from escaping the experimental replicates; only one in 60 oysters sampled outside the experimental replicates was a triploid (Burke 2010). Thus, under favorable field conditions, remote setting of hatchery-reared oyster larvae onto reef substrates could serve as an effective restoration strategy.

The mesocosms afforded optimal hydrodynamic conditions allowing for concomitant increases in oyster recruitment, growth, and survival relative to the field experiment; there was 5× greater recruitment, 10× greater biomass, and nearly 5× greater survival (probability of mortality reduced from 0.063 to 0.013). Given that the greatest difference in oyster survival within the field experiment was due to size (i.e., juveniles greater than 30 mm SH having greater probability of survival than juveniles less than 30 mm SH), efficient preseeding of reef substrates may serve an important role in bolstering their resiliency by enhancing juvenile oyster survival. Furthermore, remote setting of larvae onto reef substrates within mesocosms with growth to a mean juvenile size greater than 30 mm SH and subsequent transplantation into field restoration locations could serve to dramatically enhance local spawning stock biomass, especially in areas that are recruitment limited.

Implications for Oyster Restoration

OC, along with other similar alternative reef substrates continue to be used in both intertidal and subtidal reef restoration efforts by various governmental and nongovernmental agencies along the Atlantic and Gulf coasts, including a restoration effort conducted by us within a tributary of the Chesapeake Bay (Fig. 9) (McBride 2012). These reef substrates are suitable for use by municipalities, commercial landowners, and individual homeowners interested in shoreline stabilization (i.e., to attenuate wave energy) and erosion control (i.e., accretion of shoreline behind reef structure), especially given their ability to be constructed from readily available, inexpensive materials. The performance measures for alternative reef materials reported in this study will be essential for comparison and assessment of current and future restoration projects, which should consider use of alternative reef substrates and field setting of larvae to maximize oyster recruitment while simultaneously minimizing the cost of reef restoration.

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