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LONG-TERM OYSTER RECRUITMENT AND GROWTH ARE NOT INFLUENCED BY SUBSTRATE TYPE IN CHINA: IMPLICATIONS FOR SUSTAINABLE OYSTER REEF RESTORATION

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ABSTRACT Successful and sustainable oyster reef restoration relies on suitable substrate material that is both readily available and encourages long-term recruitment and growth of oysters. China is increasing oyster reef restoration, however, little information is available to guide sustainable practices under local conditions and on ecologically relevant time scales. This study examines the effects of four substrate materials (oyster shell, clam shell, limestone, and clay brick) on community demographics for the Kumamoto oyster (*Crassostrea sikamea*) and associated macrofauna over a 3-y period in Xiangshan Bay, China. During the first 2 y, oyster and clam shell had similarly high recruitment and abundance of live oysters when compared with limestone and clay bricks. All substrate types, however, ended up with similar oyster abundances and size distributions after 3 y. Similar trends existed with regard to structural complexity (weight and volume) of substrate and any differences at the onset of the experiment were no longer apparent by the end. Abundance and community structure of associated macrofauna did not differ among the four substrate types regardless of time. These results indicate that different types of substrate material may be used for oyster reef restoration in China given projects have a scope longer than 2 y. These restored reefs can be expected to support viable and self-sustaining oyster populations with increased structural complexity and vibrant macrofaunal communities. Restoration practitioners using the Kumamoto oyster in China may use local materials as substrate for reefs and look forward to success where oyster recruitment is adequate and other factors such as predation and sedimentation are low.

KEY WORDS: ecosystem engineer, cultch, population dynamics, nekton, Xiangshan Bay, ecosystem services

INTRODUCTION

Oyster reefs are important temperate and subtropical biogenic habitats that provide a variety of economic and ecological functions. These include fishery production, water quality improvement, and erosion control (Coen & Grizzle 2007, Kellogg et al. 2014, La Peyre et al. 2015, zu Ermgassen et al. 2015). Oyster reefs, however, are in peril globally and have declined by more than 85% in the past century due to environmental pollution, habitat change, overfishing, and disease (Beck et al. 2011). Consequently, oyster habitats are increasingly restored to mitigate the degradation of coastal ecosystems in places like the United States and Europe, and now increasingly in China (Quan et al. 2009, 2012a, 2012b, Quan & Wang 2013). The restoration and rehabilitation of oyster reef habitats, however, requires substrate or cultch that is practical, locally available, and biologically suitable.

Oyster shell is the most suitable and preferable substrate for settlement and survival of oyster larvae (Soniat & Burton 2005, Brumbaugh & Coen 2009). Many alternative substrates have been investigated for oyster production and ecosystem-based restoration due to absence of oyster shell in some areas, which include but are not limited to clam shell, concrete, limestone, bamboo, wood, plastics, rubber, and even crab cages (Soniat et al. 1991, Soniat & Burton 2005, Brumbaugh & Coen 2009, George et al. 2015). In general, hard and calcium-based substrates show higher attractiveness to setting larvae than noncalcium materials (Chatry et al. 1986, Brumbaugh 2000, Furlong 2012, Brown et al. 2014). The majority of experiments use the eastern oyster *Crassostrea virginica* (Gmelin, 1791), but in China, the Kumamoto oyster *Crassostrea sikamea* (Amemiya, 1928) is native. It is unknown whether oyster recruitment and settlement patterns, as well as associated macrofauna, differ between these oyster species. Local restoration of oysters in China is increasing, and it is important for managers and practitioners to better understand the limitations of alternative substrate materials given that oyster shell is not abundant in many places.

Oyster larvae can show differential recruitment and growth rates through time (George et al. 2015). Substrate type may influence these population trajectories, thus affecting structural complexity and the long-term sustainability of restored oyster reefs (e.g., Brumbaugh 2000, O'Beirn et al. 2000, Nestlerode et al. 2007, Quan et al. 2012b). Most studies, however, monitor oyster recruitment, growth, and survival of alternative substrate in early stages of reef development or for 1 y (Chatry et al. 1986, Soniat et al. 1991, Haywood & Soniat 1992, George et al. 2015). This may result in premature conclusions that some substrates are better than others without taking into account temporal scales relevant to restoration success (>1 y). It has been shown that temporal trajectories for ecosystems services of restored oyster reefs may differ (La Peyre et al. 2014). So far, few studies have examined the effect of substrate type on reef functions in late stages (3–5 y) of reef development.

This study aims to determine the effects of substrate material on the settlement, survival, population establishment, and reef

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development of the Kumamoto oyster to better inform the selection of potential alternative substrates for oyster reef restoration in China. Specifically, field experiments were conducted to explore the substrate preferences at the time of settlement and examine the response of oyster and associated macrofauna population development 3 y postconstruction.

MATERIALS AND METHODS

Substrate Materials

This study tested different substrate types for their ability to act as substrate and attract oyster spat (i.e., settlement), as well as promote oyster survival and growth. The following substrates were tested: (1) clay brick fragments, (2) limestone pieces, (3) oyster (*Crassostrea sikamea*) shell, and (4) clam [*Meretrix meretrix* (Linnaeus, 1758)] shell. The clay brick and limestone pieces came from a local source in Fenghua City, Zhejiang Province, China, and were rectangular in shape, either 2 × 3 cm or 6 × 8 cm, respectively. Oyster shells collected were approximately 6 mo old, and with shell heights of 4.3 ± 1.2 cm. The clam shells ranged 3.4 ± 0.6 cm in shell height and were collected from the neighboring aquaculture zone in Qidong county, Jiangsu Province. All substrate materials were cleaned of any fouling organisms, allowed to dry in the sun, and visually inspected before use in the experiment.

To ensure that each substrate type had comparable structural characteristics at the onset of the experiment, the following metrics were measured using a standard amount of 1.9 l of dry material as a proxy for structural complexity: weight (kg), surface area (cm^2), and volume (ml). The weight was measured using an electric balance with 0.1 g accuracy. The volume of the experimental reefs was measured using water displacement in a plastic container, marked to the nearest milliliter. To measure surface area, aluminum foil was wrapped around each piece or shell and then the foil was measured to the nearest square centimeter based the relationship between weight and surface area of the foil (Haywood & Soniat 1992, Bergey & Getty 2006).

Each substrate type was bagged separately using plastic mesh bagging (mesh size: 1.5 cm, bag length: 60 cm, bag diameter: 11 cm). Each bag was divided into three smaller compartments called "lobes" to prevent substrate from settling at the bottom of each large bag. This method of dividing mesh bags into lobes is common for reef restoration in China. Using a cylinder PVC pipe (diameter: 11 cm, height: 20 cm), each lobe was filled with 1.9 l of dry substrate material.

Experimental Design

To ensure adequate larval supply, the experiment was conducted alongside commercial oyster (*Crassostrea sikamea*) hatcheries and aquaculture farms located in the Xiangshan Bay, Zhejiang Province (29° 30′ 34.98″ N, 121° 28′ 32.14″ E; Fig. 1). The water temperature in the bay ranged from 10.32°C to 27.96°C, and salinity varied between 23.17 and 26.28 psu from 2011 to 2014. In July 2011, a total of 200 bags (4 substrate types × 50 replicate bags) was hung on a constructed bamboo frame (15 × 3 × 1.5 m, LWH) within the intertidal zone (Fig. 2).

Samples at the experimental site were initially collected in September and November 2011, as well as February, July, and December 2012. This sampling regime was designed to capture immediate trends in oyster settlement, as well as seasonal patterns, for more than 1 y. The experiment then ran for 2 y, and sampling was carried out again in October 2014 to determine whether demographics changed in the long term (>3 y). At each sampling event, five replicate bags per substrate type were retrieved and live oysters were counted and measured. A random subset of 20 oysters in each bag was used when

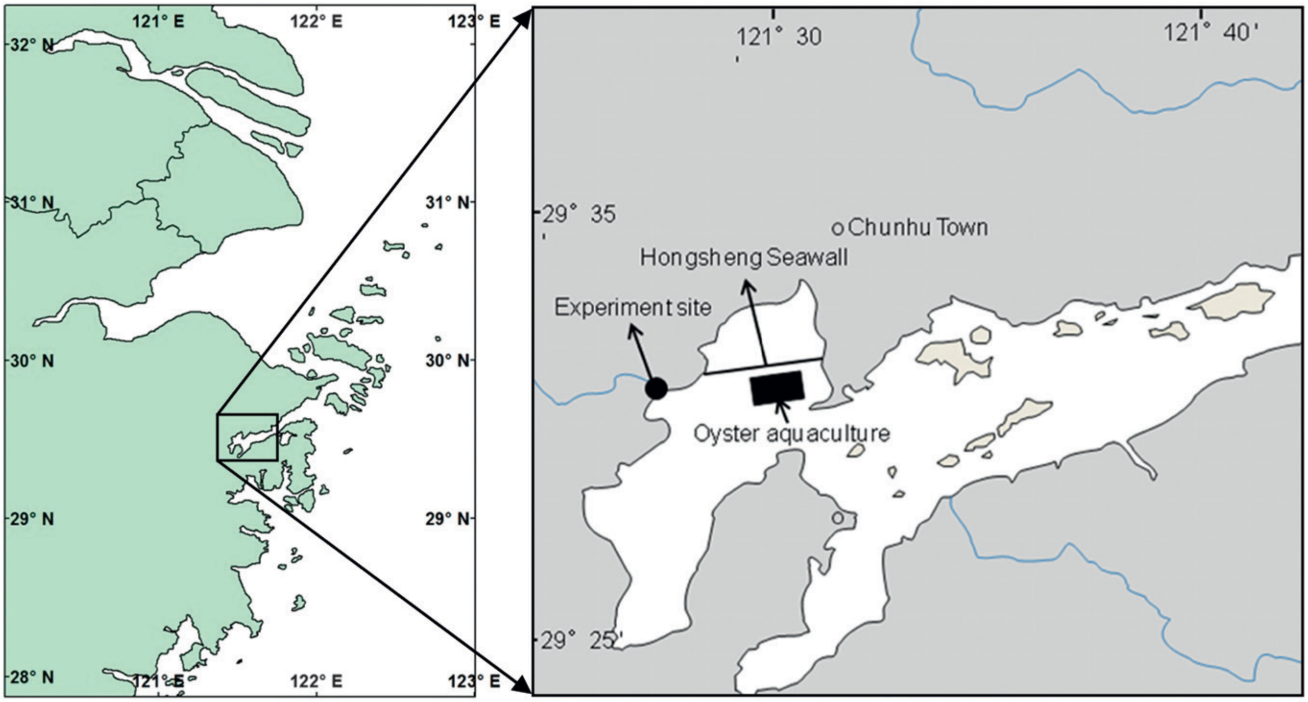

Figure 1. The study location within Xiangshan Bay, Zhejiang Province, China.

Figure 2. The experimental bamboo frame and substrate bags within the intertidal zone adjacent to active oyster aquaculture farms.

measuring shell height (to the nearest 0.1 mm with calipers). In December 2012 and October 2014, the bags were dried and weighed and also the volume of the material was determined (to the nearest 10 ml) using the water displacement method described above (Haywood & Soniat 1992, Bergey & Getty 2006). The net increases in total weight and wet volume in bagreef were estimated through subtracting the initial value of total weight and wet volume for each bag. These metrics were used as proxies for structural complexity. Resident macrofauna associated with each substrate type was also collected in the final sampling event (October 2014). To do this, the contents of each bag was sieved (mesh size 0.5 mm) and organisms were identified to species level.

Statistical Analyses

To determine if the physical properties of the different substrate materials differed from one another at the onset of the experiment, a one-way analysis of variance (ANOVA) was run on dry weight (g), surface area (cm^3), and volume (ml) as factors. To standardize units, number of oysters per substrate type were converted to individuals per square centimeter. Two-factor ANOVA was carried out to examine differences in oyster abundance as a result of substrate type and time, as well as their interaction. Similar two-way ANOVAs were also carried out on structural complexity [oyster weight (kg) or wet volume (l)]. Using functional groupings, a one-way ANOVA was used to compare abundances (ind./bag) of resident macrofauna of the different substrate types at the final sampling event. Prior to all analyses, data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Cochran's test). If necessary, the data were log transformed. All significant effects were followed with *post hoc* pairwise comparisons on least-squared means using Tukey's honest significant difference ($P < 0.05$).

To examine community composition of macrofauna, a Bray–Curtis similarity matrix on square-root-transformed abundance data (using species with relative abundance >1%) was developed to produce a nonmetric multidimensional scaling ordination plot. Nonparametric analysis of similarity (ANOSIM) was used to determine if differences in macrofaunal communities existed among the four substrate types at the final sampling event.

RESULTS

Physical Properties of Substrate Materials

At the onset of the experiment, oyster shell (0.21 kg/dry liter) was the lightest substrate whereas limestone (0.92 kg/dry liter) was the heaviest (Table 1). Clay bricks (664 cm^2/dry liter) had the least surface area per dry liter of substrate whereas clam shell (2,993 cm^2/dry liter) had the greatest. The wet volume of four substrate types ranked in the following order: limestone > clay bricks > clam shell > oyster shell ($P < 0.05$; Table 1).

Substrate weight and wet volume varied significantly according to material and date ($P < 0.05$; Table 2). Individual comparisons at each sampling event revealed that limestone and oyster shell had significantly greater net increases in weight and wet volume than those of clay bricks after 16 mo ($P < 0.05$; Fig. 3). The weight and wet volume of the reefs, however, showed no significant differences among the four substrate types by the final sampling event ($P > 0.05$; Fig. 3).

Oyster Population Metrics

Oyster abundances were significantly affected by substrate type, sampling period, and their interaction (Table 3). Based on live oyster abundance per lobe (~1.9 dry liters), the oyster shell and clam shell treatments had similar abundances of oysters, but attracted significantly greater spat than the limestone and clay bricks treatments in the first five sampling events (Fig. 4A). In the last sampling event (October 2014), however, there were no significant differences among the four substrate types with regard to oyster abundance (Fig. 4A).

When oyster abundances were expressed as live oyster per square centimeter of substrate, the attraction of the four substrate types to oyster larvae was ranked in the following order: limestone = oyster shell > clam shell > clay bricks (Fig. 4B). In February 2012, the number of spat per square centimeter in the

TABLE 1.

Physical properties of the four substrate materials (mean ± SE) at the beginning of this study.

	Clay bricks	Limestone pieces	Oyster shell	Clam shell
Dry weight (kg)	0.63 ± 0.01^{a}	0.92 ± 0.02^{ab}	0.21 ± 0.00^{bc}	0.47 ± 0.01^{c}
Surface area (cm^2)	664 ± 26^{a}	742 ± 28^{a}	$1{,}969 \pm 60^{b}$	$2{,}993 \pm 192^{c}$
Volume (ml)	303 ± 14^{a}	340 ± 14^{b}	126 ± 6^{c}	232 ± 9^{d}

Letters indicate homogeneous subgroups ($P < 0.05$).

TABLE 2.

Results from two-way ANOVAs testing whether substrate weight (kg) or wet volume (l) of reefs varied as a function of substrate type and date. The bold *P* value indicated statistically significant differences ($P < 0.05$).

	df	Mean square	*F*	*P*
Dry weight (kg)				
Substrate type	3	0.045	3.382	**0.035**
Date	1	0.444	32.135	**<0.001**
Substrate type × date	3	0.022	1.557	0.227
Residual	40	0.013		
Volume (ml)				
Substrate type	3	0.067	4.132	**0.018**
Date	1	0.331	20.536	**<0.001**
Substrate type × date	3	0.010	0.620	0.609
Residual	40	0.016		

TABLE 3.

Results from two-way ANOVAs testing whether oyster abundance (ind./cm^2) at reefs varied as a function of substrate type and date. The bold *P* value indicated statistically significant differences ($P < 0.05$).

	df	Mean square	*F*	*P*
Abundance (ind./lobes)				
Substrate type	3	3.330	195.589	**<0.001**
Date	5	1.438	84.443	**<0.001**
Substrate type × date	15	0.247	14.527	**<0.001**
Residual	120	0.017		
Abundance (ind./cm^2)				
Substrate type	3	0.030	23.138	**<0.001**
Date	5	0.176	134.175	**<0.001**
Substrate type × date	15	0.008	6.237	**<0.001**
Residual	120	0.013		

four substrate types decreased to the same level after overwintering. During the following recruitment period (July–August 2012), oyster shell and clam shell attracted significantly greater spat than did limestone and clay bricks. The oyster abundances in each of the four substrate types, however, were back to similar values at the end of the growing season in December 2012. In the next 2 y, oyster shell and clam shell treatments experienced higher mortality than did those at clay bricks and limestone ($P < 0.05$; Fig. 4B), which lead to greater oyster abundances per square centimeter of substrate for the clay bricks and limestone treatments than the oyster shell and clam shell treatments.

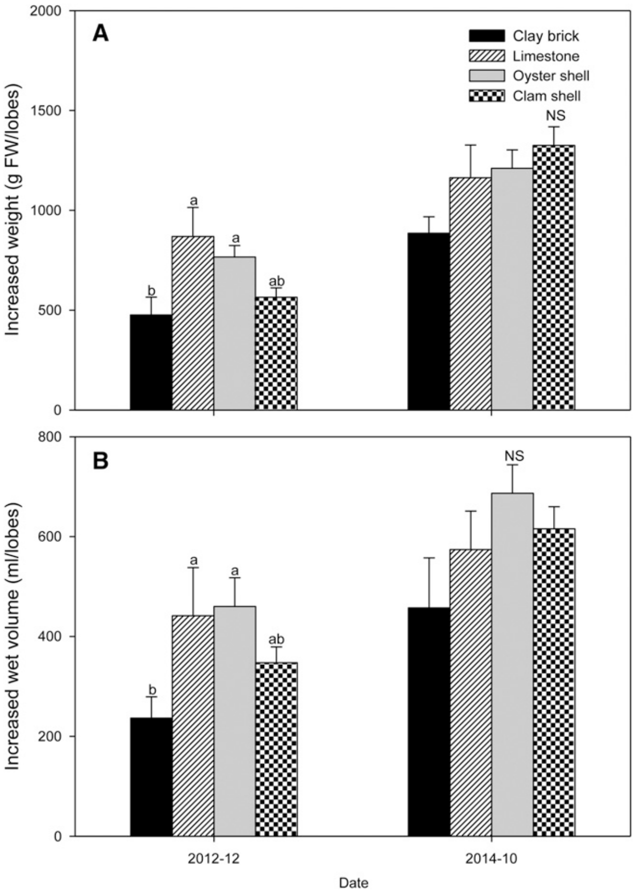

Figure 3. Total weight (A) and wet volume (B) of the four substrate materials in December 2012 and October 2014, 18 and 40 mo postconstruction. Different letters indicate significant differences at α = 0.05 level. NS = no significance.

The mean size of oyster spat at the start of the experiment remained similar among the four substrate types (Fig. 5). In the next four sampling periods (from November 2011 to December 2012), the mean size of oysters increased from a mean size of 7.7 to 21.2 mm. Additionally, significantly greater growth rates of oysters were observed in the clay brick and limestone treatments than in the oyster shell and clam shell treatments (Fig. 5). After 2 y, oyster populations in the four substrate types returned to similar size structure configurations (Fig. 5).

Macrofaunal Assemblages

A total of 13 species were recorded in the samples of resident benthic macrofauna, representing three crab species, three bivalve species, three gastropod species, and four polychaete species. The total abundances of benthic communities, and 12 of the 13 species, did not differ among the four substrate types ($P > 0.05$; Table 4). Minor differences were found for the clam (*Trapezium* sp.), and there was significantly greater abundances in the clam shell treatment than the clay bricks and oyster shell treatments ($P < 0.05$). The most abundant species included crabs *Hemigrapsus penicillatus* and *Metopograpsus quadridentatus*, bivalves *Barbatia virescens* and *Modiolus flavidus* (Dunker, 1857), and the polychaete worm *Perinereis nuntia*. Multidimensional scaling analysis also indicated that there were similar resident benthic macrofaunal communities among the four substrate types (one-way ANOSIM, $P > 0.05$; Fig. 6).

DISCUSSION

Effect of Substrate Type on Oyster Settlement and Recruitment

This study compared the attractiveness of four substrate materials on recruitment and settlement of oysters. Two months after reef deployment, the three calcium substrate treatments

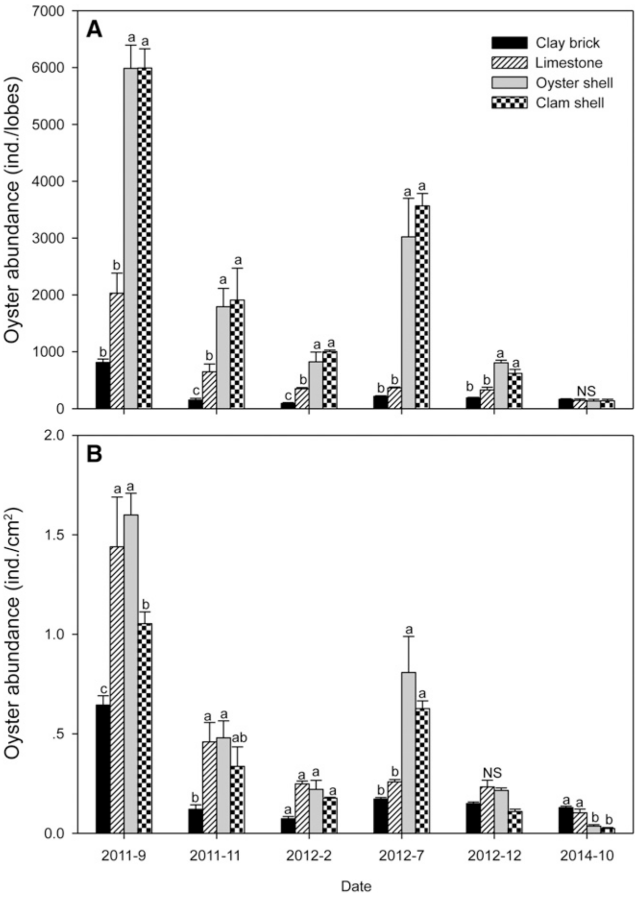

Figure 4. Comparison of oyster abundance expressed as live oyster per lobes (A) and cm^2 of substrate (B) in four substrate types through time. Different letters indicate significant differences at $\alpha = 0.05$ level. NS = no significance.

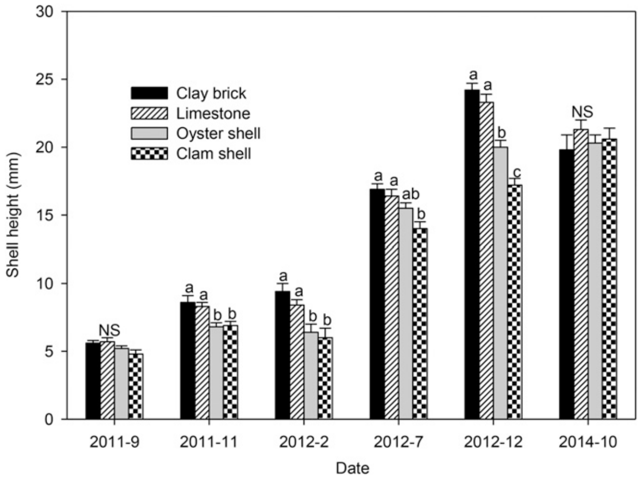

Figure 5. Mean (SE) shell height of oyster *Crassostrea sikamea* found on the four substrate types. Different letters indicate significant differences at $\alpha = 0.05$ level. NS = no significance.

(oyster shell, limestone, and clamshell) attracted greater spat than the clay brick treatment. Previous studies using the eastern oyster (*Crassostrea virginica*) report similar results with differences in larval recruitment according to substrate material (Table 5). Many of these studies have shown that limestone and concrete are a preferred alternative to oyster or clam shell as substrate for successful oyster recruitment (e.g., Chatry et al. 1986, Soniat et al. 1991, Haywood et al. 1999, Furlong 2012). In contrast, this study found that differences in oyster settlement and recruitment were no longer significant as the experiment progressed, and after 3 y oyster abundance was similar across all substrate types.

Substrates high in calcium content may induce oyster larvae to settle through either the direct effect of surface chemistry (chemical composition and texture) (Anderson & Underwood 1994, Bavestrello et al. 2000) or as a response to chemicals associated with biofilm (Keough & Raimondi 1995, Soniat & Burton 2005). As biofilm development itself, however, is also regulated by surface chemistry of substrate material (Faimali et al. 2004), it is possible that chemical composition was the major cue to settlement of oyster larvae; clay brick has a similar chemical composition to sandstone and gravel, and low recruitment in silicon materials confirmed it was an unfavorable substrate. This present study monitored the oyster spat abundances in four substrate types after 2 mo, and thus it is difficult to separate larval responses to substrate chemistry from responses mediated by biofilm.

Effect of Substrate Types on Oyster Growth, Survival, and Population Establishment

This study found that live oysters associated with the clam and oyster shell treatments showed much higher mortality rates than those recruiting to the limestone and clay bricks treatments during reef development. One possible explanation is that the oyster and clam shell pieces packed tightly together and thus provided limited interstitial space for oyster colonization. Additionally, the structure of the two shell substrates more easily accumulated sediment within cultch materials than the two other substrates (limestone and clay bricks). The largest oysters were found in the clay brick and limestone treatments, which may also reflect the lower sediment accumulation rates and thus physiological stress.

The success of oyster reef restoration depends on oyster recruitment, growth, and survival. Although there were significant effects of substrate types on initial oyster recruitment in this study, the abundances and size of oysters sampled from the four substrate materials remained similar after 3 y. George et al. (2015) also reported a consistent pattern that spat recruitment densities in five substrates (concrete, limestone, porcelain, river rock, and oyster shell) had no differences after reef deployment. In contrast, Manley et al. (2010) found that oysters in PVC and steel crab trap treatments had greater oyster densities and shell height, and lower mortality rate than those in mesh bags treatment (oyster and whelk shell). The vertical structure of the crab traps reduced oyster mortality by providing refuge from predation and physical stress from sedimentation (Manley et al. 2010).

Effect of Substrate Types on Reef Function

Limited information is available on the effect of substrate types on habitat complexity and functioning of oyster reefs (but see Manley et al. 2010, Brown et al. 2014, George et al. 2015). This study demonstrated that limestone and oyster shell produced

TABLE 4.

Abundance (ind./bag) of resident benthic macrofauna associated with the experimental substrate during the final sampling event at 3 y (mean ± SE).

Taxa	Species	Clay bricks	Limestone	Oyster shell	Clam shell
Crab	*Hemigrapsus penicillatus*	31.6 ± 4.8^{a}	45.6 ± 8.9^{a}	35.6 ± 5.2^{a}	46.8 ± 16.5^{a}
Crab	*Metopograpsus quadridentatus*	1.2 ± 0.4^{a}	2.6 ± 1.2^{a}	2.0 ± 1.1^{a}	1.6 ± 0.8^{a}
Crab	*Macromedaeus distinguendus*	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.4 ± 0.4^{a}
Bivalve	*Barbatia bistrigata*	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.0 ± 0.7^{a}	0.0 ± 0.0^{a}
Bivalve	*Barbatia virescens*	1.2 ± 0.7^{a}	1.0 ± 0.6^{a}	2.2 ± 1.3^{a}	5.0 ± 2.4^{a}
Bivalve	*Trapezium* sp.	**0.0 ± 0.0^{a}**	**1.2 ± 0.7^{ab}**	**0.0 ± 0.0^{a}**	**10.8 ± 6.5^{b}**
Gastropod	*Littorina scabra*	0.2 ± 0.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.4 ± 0.4^{a}
Gastropod	*Littorinopsis inbermedia*	0.2 ± 0.2^{a}	0.0 ± 0.0^{a}	0.2 ± 0.2^{a}	0.0 ± 0.0^{a}
Gastropod	*Nerita albicilla*	0.2 ± 0.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.2^{a}
Polychaete	*Perinereis nuntia*	2.8 ± 0.9^{a}	2.2 ± 0.9^{a}	1.8 ± 1.2^{a}	5.4 ± 2.7^{a}
Polychaete	*Mysta tchangsii*	0.6 ± 0.3^{a}	0.0 ± 0.0^{a}	0.2 ± 0.2^{a}	0.8 ± 0.3^{a}
Polychaete	*Harmothoe imbricata*	0.2 ± 0.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.2^{a}
Polychaete	*Marphysa sanguinea*	0.4 ± 0.3^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.2^{a}
Total		38.6 ± 4.9^{a}	52.6 ± 9.5^{a}	43.0 ± 5.1^{a}	71.6 ± 27.9^{a}

Different lowercase letters in superscript indicate significant differences among substrate types ($P > 0.05$), which was only detected for the bivalve *Trapezium* sp. as shown in bold.

significantly higher habitat complexity than clay brick and clam shell in the initial stages of reef development, which was a response to greater oyster spat densities in these two substrates. The habitat complexity across the four substrate types, however, were not significantly different by the end of experiment. Over the longer term, it was found that substrate types did not influence reef development for the Kumamoto oyster in this study.

Structured habitat can increase species abundance and diversity (Harding & Mann 2001, Luckenbach et al. 2005, Humphries et al. 2011, Quan et al. 2012a). Oyster reefs typically support abundant and dense assemblages of resident and transient macrofauna because they provide nursery and foraging habitat (Luckenbach et al. 2005, Rodney & Paynter 2006, Quan et al. 2012b). In the present study, there was no effect of substrate type on total densities and community structure of resident benthic macrofauna, which is likely a function of the similar structural complexities between substrate types. Brown et al. (2014) concluded that restored reefs supported similar nektonic and benthic assemblages as natural reefs, regardless of substrate material, which is supported by our findings. George et al. (2015) reported similar patterns that substrate type had no or little effect on nekton density and community composition. The present study also supported the conclusion that presence of structure, regardless of substrate type, was an important factor influencing macrofaunal density and community structure (Humphries et al. 2011, Brown et al. 2014, George et al. 2015).

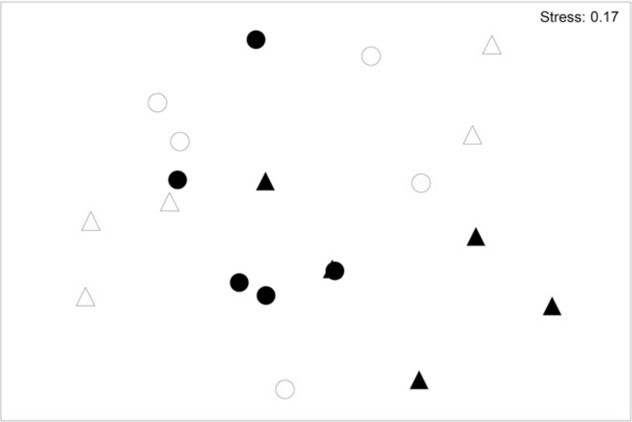

Figure 6. Nonmetric multidimensional scaling ordination of resident benthic macroinvertebrate communities associated with clay brick (○), limestone (●), oyster shell (△), and clam shell (▲). The result from ANOSIM shows no significant difference among four substrate types.

Restoration Implications

There are increasing efforts to restore oyster reefs globally and more recently in China. Currently, there are no standardized methods to determine size, shape, vertical relief, and substrate type used to build the reefs. The difference in environment and site location play important roles in determining oyster reef restoration methods, and in particular, the availability and suitability of substrate materials. This study indicates that substrate type had no effect on oyster population establishment and reef development over longer time scales beyond the first 2 y of reef development. These results showed that "nontraditional" substrates (such as clay brick) can be used as base materials for restoring oyster reefs in China, given there are no recruitment limitations. These findings contrast with others that found substrate materials other than oyster shell are not suitable for restoration due to inadequate interstitial space to serve as refugia from predation (O'Beirn et al. 2000, Nestlerode et al. 2007).

The experimental duration of this study provides an important temporal perspective into the population dynamics of oysters using different substrate materials for reef restoration in China. The study found that oyster recruitment, growth, survival, and reef development may be similar across a variety of substrate materials. Thus, availability of substrate should dictate what materials are used for reef restoration in China. Future restoration design of oyster reefs in China should aim to integrate both geophysical (e.g., siltation, wave energy) and biological (e.g., predator, recruitment rate) mechanisms.

TABLE 5.

Comparison of efficiencies of different substrate materials in oyster settlement, survival, and growth.

Sources	Study site	Experiment design	Oyster species	Metrics	Rank as settling hard substrate
Brown et al. (2014)	Northern GOM	Field experiment	*Crassostrea virginica*	Oyster density after several years	Rock (limestone, concrete) = oyster shell
Brumbaugh (2000)	Chesapeake Bay	Field experiment	*C. virginica*	Spat settlement	Limestone marl = oyster shell
Chatry et al. (1986)	Gulf of Mexico	Field experiment	*C. virginica*	Spat settlement	Limestone > clam shell
Furlong (2012)	Gulf of Mexico	Field experiment	*C. virginica*	Adult oyster density Hard substrate volume	Limestone/concrete > oyster shell
George et al. (2015)	St. Charles Bay, Texas	Field experiment	*C. virginica*	Spat settlement after 4 mo since reef deployment	Concrete = limestone = porcelain = river rock = oyster shell
Greene and Grizzle (2005)	Great Bay Estuary	Laboratory/field experiment	*C. virginica*	Spat settlement, oyster survival and growth	Concrete/rock = oyster shell
Haywood et al. (1999)	Grand Terre, Lesser Antilles	Laboratory/field experiment	*C. virginica*	Spat settlement	Limestone = concrete > clamshell > gravel (field experiment)
Soniat et al. (1991)	Texas A&M oyster hatchery				Limestone = clam shell > concrete > gravel (laboratory experiment) Gypsum = clam shell
Manley et al. (2010)	Sapelo Island, Georgia	Field experiment	*C. virginica*	Oyster recruitment, densities, biomass, shell height	Spat stick = oyster shell = whelk shell
Nestlerode et al. (2007)	York River, Virginia	Field experiment	*C. virginica*	Spat settlement, survival, and growth	Oyster shell > clam shell
O'Beirn et al. (2000); Coen and Luckenbach (2000)	Fisherman's Island	Field experiment	*C. virginica*	Spat settlement, survival, and growth	Oyster shell > surf clam shell = coal ash
Tamburri et al. (2008)	VIMS, Virginia	Laboratory experiment	*C. virginica*	Spat settlement	Oyster shell = granite > fiberglass, PVC, steel
This study	Xiangshan Bay, China	Field experiment	*Crassostrea sikamea*	Oyster density and size after several years	Limestone = oyster shell = clam shell = clay brick

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