Comparison of Substrates for Eastern Oyster (Crassostrea virginica) Spat Settlement in the Loxahatchee River Estuary, Florida

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COMPARISON OF SUBSTRATES FOR EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) SPAT SETTLEMENT IN THE LOXAHATCHEE RIVER ESTUARY, FLORIDA

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ABSTRACT The eastern oyster [*Crassostrea virginica* (Gmelin, 1791)] is an important epibenthic species in estuarine and coastal marine ecosystems, providing habitat for commercially valuable species and enhancing ecosystem function. One way to assess oyster population structure and the potential suitability of oyster restoration sites is through deployment of adult oyster shells or other substrates, and quantifying oyster spat settlement. The suitability of travertine tiles versus axenic adult oyster shells for *C. virginica* settlement was compared by deploying shellstrings with tiles and shells in four different locations across two seasons (fall or spring) in the subtropical, Loxahatchee River estuary, FL. There was no significant difference in spat densities on oyster shells compared with tile tops and bottoms, although there was significant spatial and temporal variation in spat settlement. Spat were slightly more abundant on the top of deployed tiles compared with the bottom, which differs from typical *C. virginica* settlement behavior. One possible explanation may be the presence of other fouling organisms on the bottom of tiles which decrease oyster settlement rates. Results show that oyster spat settlement was indistinguishable between travertine tiles and oyster shells and thus suggest that travertine tiles are preferable to axenic oyster shells because spat settlement can be precisely quantified per unit area.

KEY WORDS: oyster spat settlement, *Crassostrea virginica*, estuary, fouling community, Loxahatchee River, tile

INTRODUCTION

The eastern oyster *Crassostrea virginica* (Gmelin, 1791) is a common mollusc found within the intertidal zone of estuarine habitats from Canada and western Atlantic to the Gulf of Mexico. Analogous to coral reefs, *C. virginica* are ecosystem engineers, creating biogenic reefs capable of supporting diverse populations of fauna (Breitburg 1999, Tolley & Volety 2005, Stunz et al. 2010), and facilitating benthic–pelagic food web coupling (Yeager & Layman 2011, Smyth et al. 2013). Expansive *C. virginica* reefs also enhance ecosystem function, for instance, they filter particulate matter from the water column, thus increasing light penetration (Coen et al. 2007, Grabowski et al. 2012, Layman et al. 2014). While *C. virginica* reefs provide numerous ecosystem services and are an important resource for benthic and pelagic food webs, *C. virginica* populations have declined, largely as a result of overharvesting and associated habitat degradation (Rothschild et al. 1994, Lenihan & Peterson 1998, MacKenzie 2007), reduced water quality (Lenihan & Peterson 1998, Jackson 2001), and introduced diseases (Chu & Hale 1994, Andrews 1996). To this end, managers have increasingly focused on oyster restoration efforts, including improved water management and habitat restoration (Coen et al. 2007).

A key approach of many successful oyster restoration projects is to provide substrate (cultch) in close association with extant oyster reefs so that spat from the existing reefs colonize the newly available substrate. One of the most commonly used methods to monitor oyster spat recruitment and the availability of spat at potential restoration sites is the shellstring method (Bartol & Mann 1997, Southworth & Mann 1998). This method uses strings of axenic oyster shells with each shellstring constructed by drilling a hole in the center of adult oyster shells and threading those shells, inner surface down, on heavy gauge wire (Haven & Fritz 1985). Shells are then suspended off the bottom in the shallow intertidal or subintertidal zone. Subsequent to collection, the top and bottom shells are discarded and settled spat are enumerated on the underside of the remaining shells (Haven & Fritz 1985, Wilson et al. 2005, Volety et al. 2009). Among the greatest challenges of using the shellstring method is obtaining a consistent and reliable supply of shells for constructing the shellstrings which necessitates ~10 adult oyster shells per string (i.e., sample) for each deployment (Haven & Fritz 1985). Refuse shells from restaurants are ideal, but coordinating logistics with local restaurants can be difficult. These shells also require a quarantine time of at least 1 mo to reduce the potential introduction of pathogens such as *Perkinsus marinus*, which leads to diseased oysters and potential mortality (Chu & Hale 1994, Bushek et al. 2004). Finally, shells are not reused due to the difficulties associated with cleaning fouling communities from the inner shell surface.

An alternative method is the deployment of shellstrings with unglazed tiles instead of axenic oyster shells as a substrate for spat settlement. Unglazed tiles are more readily available and allow for a uniform surface area to quantify spat. Despite the apparent benefits of using tiles in lieu of oyster shells for monitoring spat settlement, there is a paucity of data comparing the relative suitability of axenic oyster shells and tiles (but see Nalesso et al. 2008). This study explored whether travertine tile, a commercially available calcium carbonate-based material typically used for construction, would be a suitable substrate for *Crassostrea virginica* spat settlement. Results of this study frame a discussion on the suitability of travertine tiles in research and monitoring programs on temporal and spatial patterns of oyster spat settlement.

MATERIALS AND METHODS

Samples were collected in the Northwest and Southwest Forks of the Loxahatchee River estuary (26° 57′ N, 80° 06′ W); a subtropical system located in southeast Florida that drains a 620 km^2 watershed and connects to the Atlantic Ocean through the Jupiter Inlet (Fig. 1; South Florida Water Management

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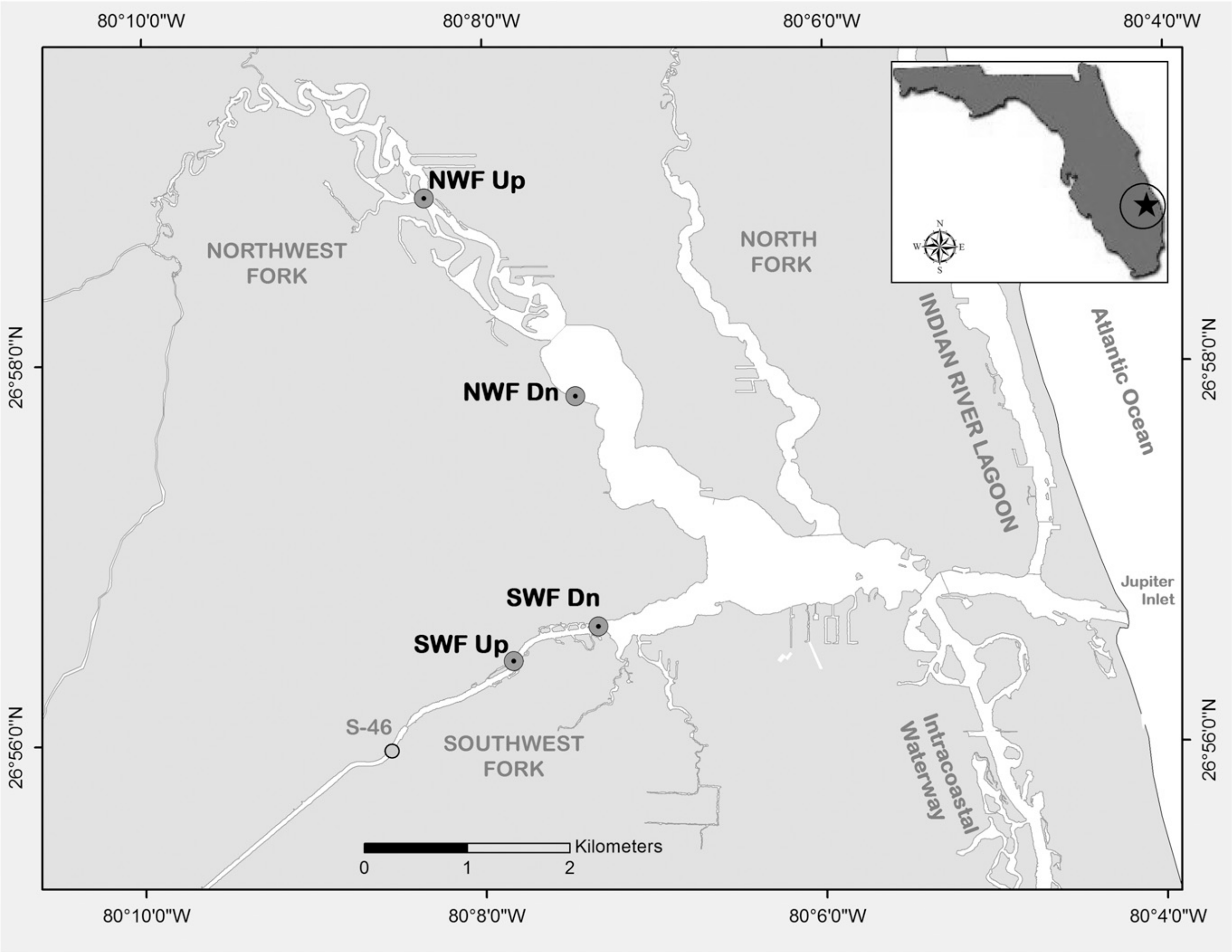

Figure 1. Map of the Loxahatchee River estuary showing locations of the four sampling sites. Sampling sites are in close proximity to extant oyster beds.

District 2006). Freshwater is supplied to the system continuously through the Northwest Fork which is the primary source of freshwater to the system. Flow of freshwater to the Southwest Fork is controlled by a gated water control structure which controls upland flooding during major rainfall events (South Florida Water Management District 2006). The Loxahatchee River is highly stratified with a salt-wedge boundary that oscillates upstream and downstream in both river forks. Throughout much of the 20th century, the hydrology of the system has been greatly altered to accommodate development and agriculture to the area (McPherson et al. 1982). These changes have redefined the spatial extent in which viable oyster reefs occur, i.e., the Northwest and Southwest Forks of the estuary, where salinity ranges are favorable for oyster spawning, settlement, and survival (Woodward-Clyde International-Americas 1998, South Florida Water Management District 2006).

Sites used for this study were selected based on proximity to extant oyster reefs. The sampling points were located in the nearshore shallow subtidal zone of both the upstream and downstream Northwest Fork and Southwest Forks (hereafter, NWF Up, NWF Down, SWF Up, SWF Down). The experiment was run for a period of 28 days in August to September 2014 (fall) and again in April 2015 (spring) based on previously documented bimodal oyster spat settlement peaks within the Loxahatchee River estuary (Loxahatchee River District, unpublished data). Salinity and temperature were continuously recorded in the Northwest Fork (W 80.1279°, N 26.9720°) and Southwest Fork (W 80.1218°, N 26.9433°) during both sampling periods using datasondes (600XLM, YSI Hydrodata), which helped characterize differences between physical parameters across site and season (Table 1).

Paired shellstring arrays were deployed (modified from Haven & Fritz 1985) in which one string contained seven oyster shells and the paired string contained seven travertine tiles as settlement substrate. Square travertine tiles were 7.5 × 7.5 cm, with a 4-mm hole drilled through the center and a 6-mm thick and 12.5-mm diameter nylon washer placed between them. Thus, tiles were evenly spaced 6 mm apart and each had an available surface area of 51.3 cm^2 for oyster spat settlement. Paired shellstring arrays were constructed with 1.7-mm diameter weed trimmer line and were suspended below the ends of the horizontal cross arm of a 2.54-cm PVC "T" hanger that was anchored into the substrate. Shellstrings were suspended in the shallow subintertidal zone. Three shellstring arrays were

TABLE 1.

Mean temperature (°C) and salinity values for the fall and spring sampling periods in the Northwest Fork (NW Fork) and Southwest Fork (SW Fork) collected continuously every 15 min using datasonde instruments.

Season	Temperature	Salinity
Fall		
NW Fork	30.3	19.9
SW Fork	29.8	28.1
Range fall values (minimum–maximum)	27.3–33.9	1.9–33.5
Mean fall values	30.0	23.5
SD (±)	0.91	5.3
Spring		
NW Fork	27.7	20.6
SW Fork	27.8	31.1
Range spring values (minimum–maximum)	24.9–30.8	6.0–35.1
Mean spring values	27.7	25.8
SD (±)	0.70	5.8
Range NW Fork values (minimum–maximum)	25.1–33.9	1.9–32.0
Mean NW Fork values ± SD	28.9 ± 1.5	19.7 ± 2.8
Range SW Fork values (minimum–maximum)	24.9–32.6	16.8–35.1
Mean SW Fork values ± SD	28.8 ± 1.1	29.5 ± 3.0

Data reported are the daily means of all measurements for each site.

deployed at each of the four sample sites across both seasons ($n = 24$ oyster T replicates).

Following shellstring retrieval, a stereo microscope (Parco XMZ series) was used to enumerate oyster spat on the top and bottom of tiles, and the bottom of shells. The surface area of each oyster shell used in this study was approximated ($n = 119$ shells; 120 shells were deployed but one was broken and was therefore excluded from analysis) with http://www.sketchandcalc.com, a web-based application that enables the user to calculate the area of irregular shapes. Spat density on each substrate type was standardized to 1 m^2 for all statistical calculations.

Each shellstring was treated as a discrete sample in which mean standardized spat densities were calculated across all five oyster shells for each oyster "T". Strings with tiles were treated likewise, except the top of the tile was also sampled and treated as a distinct sample. Spat densities/m^2/T were natural log (x + 1) transformed to meet assumptions of normality and homoscedasticity. A nested analysis of variance was used to test for potential differences in oyster spat densities on replicate oyster Ts (random factor) among the monitoring treatments (shell, tile bottom, or tile top), accounting for the statistical variation caused by sampling site (NWF Up, NWF Down, SWF Up, SWF Down) and season (fall or spring). Where significant effects were detected, Tukey's honestly significant difference test was performed to examine differences across treatments and sites (SPSS IBM v. 23.0).

RESULTS

Oyster spat were observed at all four sampling sites during both fall 2014 and spring 2015 recruitment events and were present on 92.5% of the oyster shells, 85.8% travertine tile bottoms, and 88.8% tile tops. No difference in spat density was detected among oyster T replicates within site or across treatments (Table 2); mean untransformed spat densities (±SD) on shells had 6,120.8 ± 6,073.1 spat/m^2 present, tile bottoms had 6,809.6 ± 5,833.6 spat/m^2 present, and tile tops had 9,491.5 ± 8,719.5 spat/m^2 present (Fig. 2). The number of spat recruits varied substantially across the four sites (Table 2). Specifically, spat densities were highest in the NWF Down site (mean ± SD shell = 12,387.7 ± 2,991.6 spat/m^2, mean ± SD tile bottom = 16,413.2 ± 3,320.0 spat/m^2, mean ± SD tile top = 16,783.6 ± 7,789.4 spat/m^2) and lowest in the SWF Down site (mean ± SD shell = 2,741.0 ± 2,613.5 spat/m^2, mean ± SD tile bottom = 4,230.0 ± 4,397.1 spat/m^2, mean ± SD tile top = 2,631.5 ± 2,573.1 spat/m^2; Fig. 2). Spat density also differed between seasons; total untransformed mean spat density was found to be ~129% higher in the spring than fall (Fig. 2).

DISCUSSION

The results of this study indicate that travertine tile is a suitable replacement for axenic oyster shell when quantifying oyster spat recruitment using shellstrings. These findings mirror results from an oyster spat recruitment study conducted in estuarine habitats in Brazil, in which Nalesso et al. (2008) sought to elucidate mangrove oyster (*Crassostrea* spp.) recruitment patterns on various recycled materials, including oyster shells, PET bottles, car tires, and unglazed clay tiles using the shellstring method. The authors found that mean spat densities were highest on oyster shells (2,040.9/m^2) and tiles (1,886.8/m^2) compared with other substrate types (Nalesso et al. 2008). It is not surprising that spat may recruit in similar densities on shell versus tile, as travertine is a form of precipitated carbonate comparable to oyster shell which can induce similar settlement behavior as a result of comparable surface chemistry (Soniat & Burton 2005). In addition, *Crassostrea virginica* spat have been found to settle on a wide variety of substrates including cement, limestone, granite, and plastic (Soniat & Burton 2005, Manley et al. 2008, Nalesso et al. 2008).

Although there was no significant difference in spat density across tile bottoms, tile tops, and shells, *Crassostrea virginica* spat were observed to be more abundant on the tops of tiles than tile bottoms. This was surprising, as previous oyster recruitment studies found that spat preferentially attach to substrate bottom (Ortega & Sutherland 1992, Nalesso et al. 2008), likely because oyster larvae exhibit negative phototropism (Baker & Mann 1998). Other fouling organisms settling on tiles in this study may have influenced the settlement of oyster spat on tile tops. For instance, abundant acorn barnacles (*Balanus* sp.) and calcareous tube-forming serpulid worms were observed on tile bottoms, potentially limiting the space available to oyster spat (Sutherland & Karlson 1977, Nalesso et al. 2008). Further research using this tilestring method might elucidate effects of interactions between conspecifics and other fouling organisms on spat density and recruitment rates.

Overall, mean spat settlement was greater in the spring, although water temperatures were slightly cooler in April than in August/September (Table 1). This was unexpected, as *Crassostrea virginica* recruitment in Florida has been found to increase in the late summer–fall following maximum summer water temperatures (Parker et al. 2013). Salinity was slightly lower in the fall as a result of increased precipitation during the rainy season in southeast Florida, and salinity was greater in the

TABLE 2.

Summary of nested analysis of variance evaluating effects of oyster T replicates (random factor) within site (fixed factor), treatment (fixed factor; shell, tile bottom, tile top), site, and season (fixed factor; fall or spring) on mean oyster (*Crassostrea virginica*) density.

Source of variation	df	*F*	*P*
Treatment	2,57	0.46	0.63
Site	3,8	25.5	**0.000**
Season	1,57	76.0	**0.000**
Oyster T (site)	8,57	0.63	0.74
Error	57		

Spat densities were averaged across treatments per replicate, and data were log (x + 1) transformed. Significant values (at $\alpha = 0.05$) are highlighted in bold.

spring when settlement was higher (Table 1). Typically, *C. virginica* exhibit decreased survivorship, growth rates, and spawning in low salinity environments due to physiological constraints, but this may only be in areas with very low salinity (<10) (Wilson et al. 2005, Parker et al. 2013). Salinity also varied across sampling region where it was higher in the SWF location than the NWF location. High salinity in the SWF location may explain reduced spat settlement compared with the NWF location (Table 1). Although eastern oysters may exhibit a wide salinity tolerance range (1.2–36.6 in Apalachiocola Bay, FL; Menzel et al. 1966), their optimal salinity tolerance range for the same system was found to be in the low-mid-20s, which is lower than the maximum salinities recorded in the SWF in this study (Wang et al. 2008, Parker et al. 2013). More work is necessary to determine whether these mechanisms are drivers of spat settlement in this system, as the primary focus of this study was to compare spat density differences between oyster shell and travertine tile.

The results obtained from this study support the use of travertine tiles when monitoring oyster larval settlement. Because travertine tiles are readily available, relatively inexpensive, have an easily quantifiable surface area, and are bioavailable habitat for oyster spat, natural resource managers and agencies may be able to effectively use tilestrings. In doing so, resource managers may be able to better quantify oyster spat settlement, which will facilitate placement of oyster reef restoration projects and allow for improved monitoring of wild oyster population dynamics.

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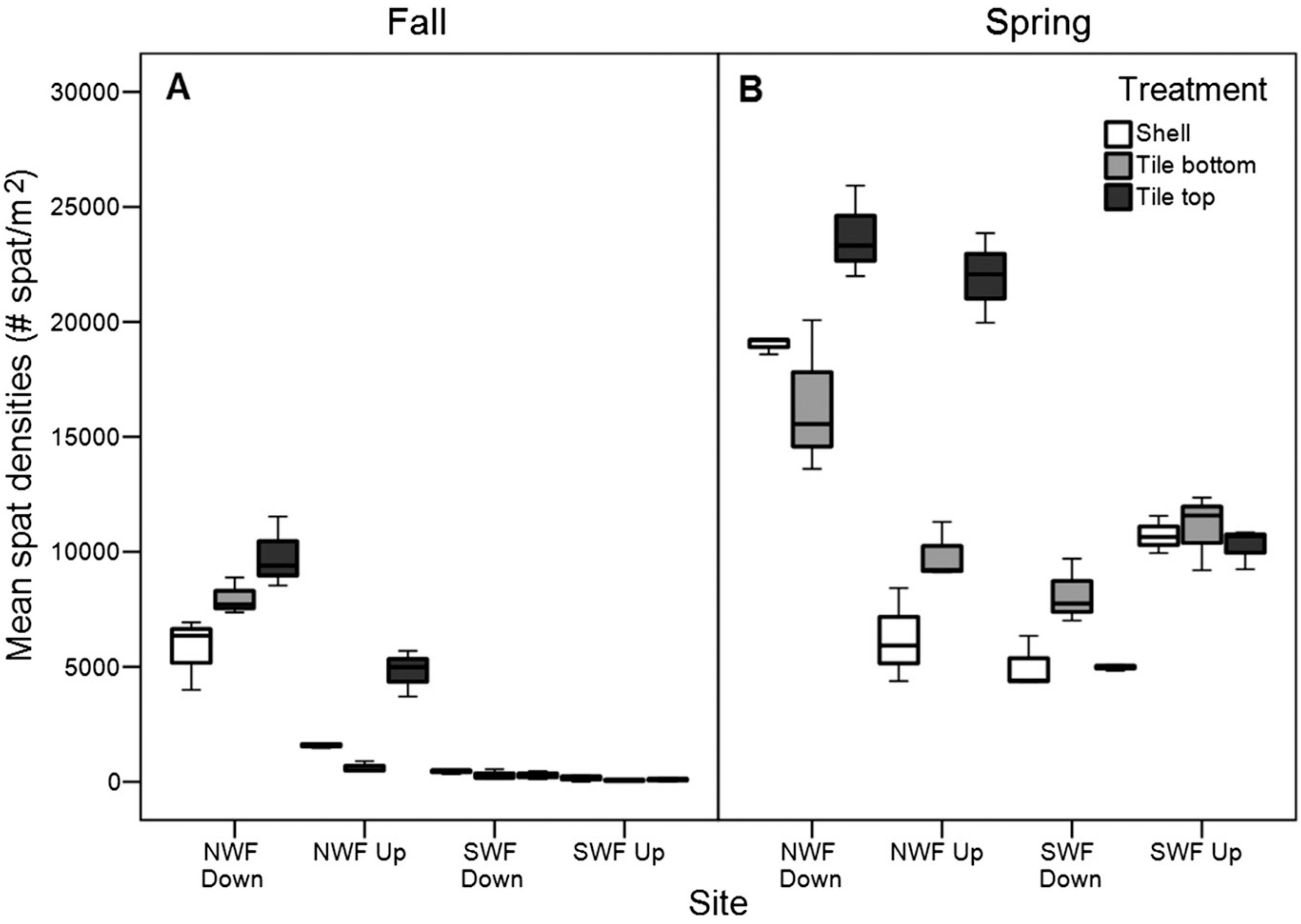

Figure 2. Mean spat density of *Crassostrea virginica* per square meter on the three different treatments (oyster shells, travertine tile bottoms, and travertine tile tops) in (A) the fall and (B) the spring, across the four sampling sites: Northwest Fork downstream (NWF Down), Northwest Fork upstream (NWF Up), SWF downstream (SWF Down), and SWF upstream (SWF Up).

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