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RECRUITMENT PREFERENCES OF NON-NATIVE MUSSELS: INTERACTION BETWEEN MARINE INVASIONS AND LAND-USE CHANGES

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ABSTRACT

Preferential patterns of settlement of marine invertebrate larvae may significantly affect the expansion of introduced populations when coupled with anthropogenic changes to available substrates in marine environments. In the near-shore marine environments of the southeastern USA the addition of hard substrates into primarily salt marsh habitats may provide necessary settlement sites for recently introduced marine invertebrates such as the Asian green mussel, *Perna viridis*, and the South American charru mussel, *Mytella charruana*. Preliminary studies in Florida suggest that *M. charruana* adults are predominantly found on man-made substrates, especially docks. The present study investigated whether *P. viridis* and *M. charruana* preferentially settle on substrates commonly used in man-made structures such as plexiglass, wood and rock, or on natural substrates like the shells of oyster (*Crassostrea virginica*), green mussels or charru mussels. Frames containing a mixture of these six substrates were deployed at two locations in Florida and retrieved after 1, 2 and 3 months. Over 1-month deployments, both species preferentially recruited to natural substrates. While this pattern was repeated on substrates in the field for 2 months in *M. charruana*, there were no differences in *P. viridis* spat density among any of the substrates in the field for 2 or 3 months. These results suggest that man-made hard substrates will likely not affect expansion of these two species by providing preferred settlement sites. Man-made substrates may still, however, provide better locations for survival.

INTRODUCTION

Anthropogenic changes in land use such as construction of roads and fragmentation of forests have recently been cited as increasing the abundance of many terrestrial invasive species (Parks *et al.*, 2005; Bradley & Mustard, 2006; Ohlemuller, Walker & Wilson, 2006; Thiele & Otte, 2006). This suggests that human activities not only transfer nonnative species into novel habitats, but may also facilitate the success of an invasion postintroduction (Vitousek *et al.*, 1997). Whether similar anthropogenic activities result in a greater probability of establishment and expansion of nonnative species in marine environments has received less attention.

Benthic marine invertebrates preferentially settle according to a variety of substrate parameters including topography (Crisp, 1961; Walters & Wethey, 1996), microbial films (Morse, Froyd & Morse, 1984; Bonar *et al.*, 1990), the presence of conspecifics (Hidu, William & Veitch, 1978; Pawlik, 1986; Walters, Hadfield & del Carmen, 1997), water flow velocity at the substrate water interface (Wethey, 1986) and chemical inducers associated with prey (Steneck, 1982) or predators (Raimondi, 1988; Rodriguez, Ojeda & Inestrosa, 1993). Settlement preferences such as these can be especially important when dealing with newly introduced species since the presence of suitable substrates is the first step in the invasion process that ultimately determines establishment and range expansion. Furthermore, anthropogenic land-use changes that affect substrate availability in marine environments may play a direct role in successful establishment of introduced species, and may also be used as a management tool to limit population expansions. For example, the increasing availability of man-made hard substrates in areas of native soft-bottomed salt marsh may provide the necessary requirements for establishment and expansion of a variety of sessile invertebrates that only recruit on hard substrata.

Such is the case for much of the Atlantic and Gulf coasts of the southeastern USA. Coastal areas are dominated by soft-bottomed marshes inhabited primarily by marsh grasses like *Spartina* and *Juncus* species. Oysters engineer the only hard substrate in these areas, but this habitat is rapidly declining (Beck *et al.*, 2009). Marshes in the southeastern USA have become increasingly influenced by man-made structures, including docks, bridges and jetties. In fact, the number of permits for construction of docking facilities in the central and northern east coast counties of Duval and Volusia in Florida (where the current study was conducted, see below) has averaged 67.4 and 76.2 per year, respectively, over the last decade (Florida Department of Environmental Protection, Environmental Resource Permitting, Permit Application database). Obviously, a steady increase in the number of docks and associated structures has the potential to provide additional substrate for a variety of sessile, marine invertebrates.

Two species of mussels recently introduced to coastal waters of the southeastern USA provide an excellent system in which to study potential effects of the addition of man-made, hard substrates to soft-bottomed habitat on the establishment of nonnative species. The Asian green mussel, *Perna viridis* (Linnaeus, 1758), and the Central/South American charru mussel, *Mytella charruana* (d'Orbigny, 1846), were both introduced to the United States within the last 25 years (Benson *et al.*, 2001; Ingrao, Mikkelson & Hicks, 2001; Boudreaux &

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Walters, 2006a). *Perna viridis* is native to the tropical waters of the Indo-Pacific while *M. charruana*'s native range includes subtropical and tropical portions of the Pacific and Atlantic coasts of Central and South America (Gillis *et al*., 2009). *Perna viridis* was first discovered in Tampa Bay, Florida (Benson *et al*., 2001), and is currently found on both the Gulf and Atlantic coasts of Florida and shells have been found as far north as Charleston, SC (SC Department of Natural Resources News Release, October 16, 2006). *Mytella charruana* was introduced to the Atlantic coast of north Florida in 1986, and considered extirpated in 1987 until it was documented over 100 km further south along the east coast of central Florida in 2004 in Mosquito Lagoon waters (Boudreaux & Walters, 2006a). *Mytella charruana* also currently ranges northward into South Carolina, a few kilometres south of Charleston (E.A. Hoffman, unpubl.). Both mussels are known to recruit on hard substrates in their native ranges and similar observations have been made in introduced populations. In fact, a preliminary assessment of *M. charruana* abundance in Mosquito Lagoon, Florida, involving equal search time among different types of substrates, found that the vast majority (73%) of mussels were attached to man-made substrates (wooden dock pilings, cinder blocks, PVC pipe; Boudreaux & Walters, 2006b). The rest were found in crevices of live, native oyster (*Crassostrea virginica*) clusters (22%), with the remaining 5% split among mangrove roots, disarticulated oyster shells and driftwood. The preponderance of *M. charruana* adults on man-made substrates suggested that either *M. charruana* larvae are preferentially settling on these substrates, or that they are experiencing lower survival on naturally occurring hard substrates (oyster reefs). Similar data are not available for *P. viridis* although Baker, Fajans & Berquist (2003) suggested this animal could devastate intertidal oyster reefs; individuals covering the bottoms of floating docks, boat hull, bridge pilings and rock jetties are common (M. R. Gilg & L. J. Walters, personal observations).

Here, we test the hypothesis that both *M. charruana* and *P. viridis* larvae preferentially settle on man-made substrates at two locations in Florida in order to explore the interaction between marine invasions and land-use changes. Sessile marine invertebrates were given three different substrates that are typically used to construct man-made structures in Florida, as well as the hard shells of three different bivalve species to represent natural substrates. The density of all settling invertebrates on each substrate was determined and compared over a 3-month period.

METHODS

Frames (72 cm × 72 cm) constructed of 1.27-cm diameter PVC pipe were covered with a plastic mesh (Vexar, 1-cm diameter openings). Five replicates of each of six substrates, including untreated 3-cm thick wood, 1-cm thick clear plexiglass that was lightly roughened with sandpaper, 1-cm thick wire-cut tile and disarticulated shells of *Crassostrea virginica*, *Perna viridis* and *Mytella charruana*, were attached to each frame in a randomly generated arrangement by drilling two holes through each substrate (except *M. charruana* shells which had a single hole) and attached them to the mesh with plastic cable ties. The wood, plexiglass and tile substrates all measured 9 cm × 9 cm and were considered man-made substrates, since each is predominantly associated with man-made structures in Florida. Wood was pressure-treated pine advertized for use in marine applications and tiles were Sunshine Pavers® wire-cut terracotta, which is a cement tile often used to study coral recruitment (Kuffner *et al*., 2006). While plexiglass is not a typical structure used in marine systems, it was chosen for its smooth finish better to replicate the surface of boat hulls. The disarticulated shells were considered natural substrates and their surface area was calculated by tracing around the perimeter of the shell and determining the area inside the perimeter with an area metre (CID Inc. CI-202).

Frames were deployed at two locations in Florida: Mosquito Lagoon near New Smyrna Beach (28.9069°N, 80.8206°W) and Sister's Creek Marina near Jacksonville (30.4195°N, 81.4194°W). Frames were attached underneath floating docks such that the substrates were facing down (away from the bottom of the dock) and macroaglal growth would be minimal. Six frames were deployed at each site on May 16, 2007. Frames were deployed in pairs for retrieval after 1, 2 or 3 months. After 1 month, two 1-month frames were retrieved and replaced with two new 1-month frames. After 2 months, two 1-month and two 2-month frames were retrieved and replaced with two new 1-month frames. Unfortunately, four of six frames were lost in the first month at the Sister's Creek Marina site. The two remaining frames were collected for use as 1-month samples and six new frames were deployed on June 16, 2007 with a collection schedule as described above. Thus, all frames had been retrieved from Mosquito Lagoon by August 16, 2007, and from Sister's Creek Marina by September 16, 2007.

After collection, the substrates and attached fauna were allowed to air-dry prior to analysis. No sessile organisms were lost or damaged during this drying process. Each substrate was then examined under a dissecting microscope and all attached *P. viridis* and *M. charruana* were counted and reported as number of individuals/cm^2. In situations where considerable silt covered the substrates, an initial scan was performed and any visible mussels were removed. The substrate was then soaked in a water bath for 3 min followed by gentle brushing with a soft bristle paint brush to remove the silt. The substrate was then examined a second time to record all additional mussels present. Each mussel collected was also measured for maximum shell length (SL) using an ocular micrometre attached to a dissecting microscope. Data were checked for normality and all statistical comparisons of density among substrates and over time were performed using SPSS 16.0 software.

RESULTS

Neither *Perna viridis* nor *Mytella charruana* spat were observed on any of the substrates on any collection date from the Mosquito Lagoon sampling site. On the other hand, spat of both mussel species were found in collections from Sister's Creek. Since *P. viridis* and *M. charruana* spat were only found at the Sister's Creek site, all subsequent analyses of mussel density refer only to collections made at that location.

A two-way analysis of variance (ANOVA) revealed significant differences in both main effects of month and substrate for both *P. viridis* (Table 1) and *M. charruana* (Table 2) on frames that were in the field for 1 month. For *P. viridis*, spat density was greatest in the months of June and August, while *M. charruana* showed consistently increased spat density between June and August followed by a sharp decline in September (Fig. 1). In both species of mussels, overall settlement tended to be greater on shells than on any of the other substrates (Fig. 1, Tables 1 and 2). Spat of *P. viridis* reached significantly greater

Table 1. Two-way ANOVA testing density of *Perna viridis* spat collected from Sister's Creek with main effects of substrate and month.

Source	Type III SS	df	MS	*F*	*P*
Month	0.236	3	0.079	23.374	<0.001
Substrate	0.100	5	0.020	5.915	<0.001
Month × substrate	0.134	15	0.009	2.654	0.001
Error	0.610	181	0.003		

Table 2. Two-way ANOVA testing density of *Mytella charruana* spat collected from Sister's Creek with main effects of substrate and month.

Source	Type III SS	df	MS	*F*	*P*
Month	0.066	3	0.022	5.076	0.002
Substrate	0.051	5	0.010	2.344	0.043
Month × substrate	0.048	15	0.003	0.743	0.739
Error	0.780	181	0.004		

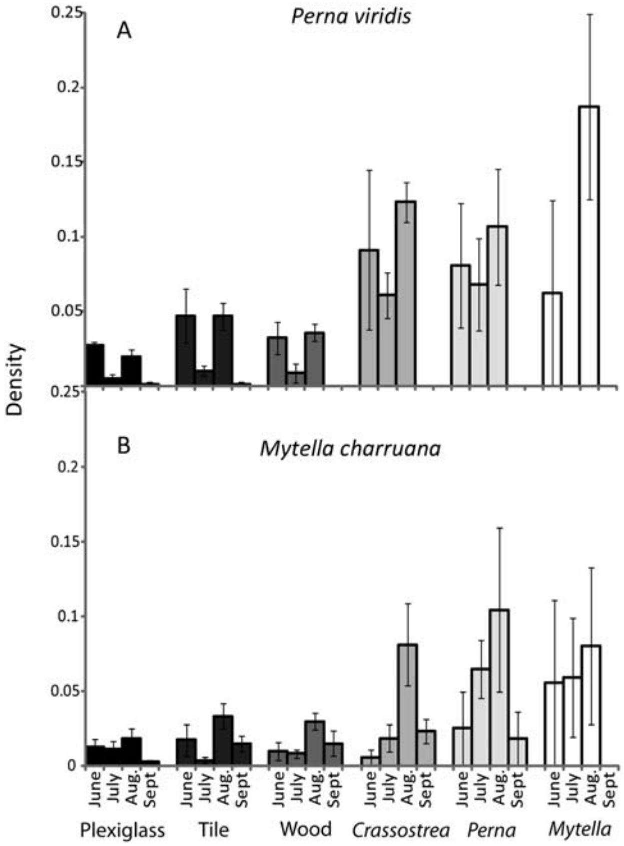

Figure 1. Mean ($\pm$SE) spat density (individuals/cm^2) over time and on different substrates for *Perna viridis* (**A**) and *Mytella charruana* (**B**). Substrates were collected monthly.

densities on both disarticulated *Crassostrea virginica* and *P. viridis* shells than on nearly all of the man-made substrates (Table 3). The comparison between density on *P. viridis* shells and tile, while marginally insignificant, showed the same trend. While a similar trend was observed with *M. charruana* spat, only density on *P. viridis* shells was significantly greater than the density on plexiglass (Table 3). When *M. charruana* spat densities on all disarticulated shells were pooled and compared to densities on all man-made substrates, however, significantly greater densities are observed on disarticulated shells ($t = 3.29$, df $= 108.47$, $P < 0.001$).

Frames that were in the field for 2 and 3 months, however, showed very different mussel density patterns than the 1-month collections. For example, whereas *P. viridis* spat densities differed significantly among substrates that were collected after 1 month in the field, there was no significant difference in spat densities among substrates that were in the field for either 2 ($F_{(5,45)} = 0.793$, $\mathrm{MS}_{\mathrm{error}} = 0.003$, $P = 0.561$; Fig. 2A) or 3 ($F_{(4,41)} = 0.953$, $\mathrm{MS}_{\mathrm{error}} = 0.002$, $P = 0.444$; Fig. 2B) months. Likewise, the density of *M. charruana* spat collected after 2 or 3 months in the field showed no significant differences among substrates (2 months: $F_{(5,45)} = 2.291$, $\mathrm{MS}_{\mathrm{error}} = 0.028$, $P = 0.061$, Fig. 3A; 3 months: $F_{(4,41)} = 1.187$, $\mathrm{MS}_{\mathrm{error}} = 0.002$, $P = 0.331$; Fig. 3B). When spat densities on disarticulated shells are pooled and compared to pooled spat on man-made substrates, *P. viridis* spat show no significant difference in density between the two substrate groupings after deployment for either 2 ($t = 0.09$, df $=$ 25.28, $P = 0.927$) or 3 ($t = -0.383$, df $= 25.92$, $P = 0.705$) months. Conversely, *M. charruana* spat again showed greater density on pooled shells than on pooled man-made substrates in the 2-month samples ($t = -2.11$, df $= 21.39$, $P = 0.047$; Fig. 3A) and a nonsignificant trend in the same direction in the 3-month samples ($t = -1.60$, df $= 16.50$, $P = 0.129$; Fig. 3B).

Since *P. viridis* spat showed a strong preference for shells in the 1-month samples, but since the pattern was not repeated in either the 2- or 3-month samples, we tested whether the lack of difference among substrates in the 2- and 3-month collections was due to greater recruitment on the man-made substrates than expected, or to lower recruitment on the shells

Table 3. Results of a Tukey's pairwise *post hoc* analysis of spat density on different substrates collected after 1 month in the field.

Plexi.	Tile	Wood	*C.v.*	*P.v.*	*M. c.*
Plexi.	0.949	0.997	**0.002**	**0.005**	0.067
Tile	0.999	0.998	**0.035**	0.076	0.403
Wood	0.999	1.000	**0.010**	**0.025**	0.200
C.v.	0.595	0.834	0.820	1.000	0.899
P.v.	**0.042**	0.119	0.114	0.779	0.974
M.c.	0.290	0.528	0.512	0.995	0.972

Comparisons of *Perna viridis* spat density are shown above the diagonal, while comparisons of *Mytella charruana* spat density are below the diagonal. All mussels were collected from Sister's Creek. Abbreviations: Plexi., plexiglass; *C.v.*, *Crassostrea virginica*; *P.v.*, *Perna viridis*; *M.c.*, *Mytella charruana*. Bold indicates $P < 0.05$.

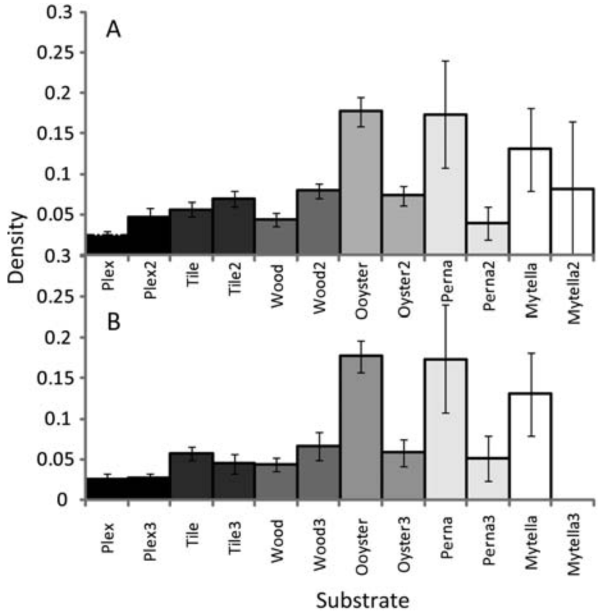

Figure 2. Comparison of mean ($\pm$SE) spat density (individuals/cm^2) of *Perna viridis* on various substrates. For each substrate the first bar represents spat density summed across three consecutive monthly collections. The second bar represents spat density on substrates in the field for 2-month (**A**) and 3-month (**B**) periods.

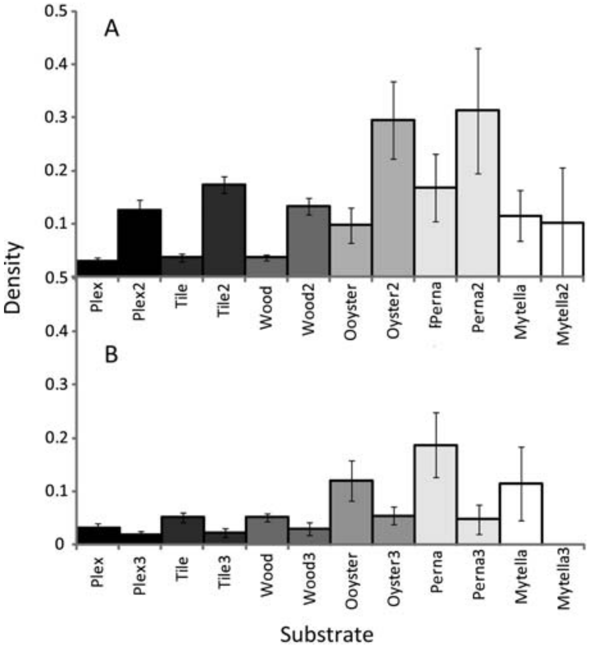

Figure 3. Comparison of mean (±SE) spat density (individuals/cm^2) of *Mytella charruana* on various substrates. For each substrate the first bar represents spat density summed across three consecutive monthly collections. The second bar represents spat density on substrates in the field for 2-month (**A**) and 3-month (**B**) periods.

than expected. The 2-month samples consisted of frames that were deployed in mid-June and collected in mid-August. Therefore, all else being equal, spat density on these substrates should be the sum of the recruitment that occurred over those 2 months. Similarly, the 3-month samples that were in the field from mid-June through mid-September should contain all the spat that settled in each of those months. Because we collected frames that had been in the field for a single month during each of these time periods, we were able to compare the sum of the spat densities from each of those 1-month collections to their corresponding 2- and 3-month samples in an effort to determine whether density on the man-made substrates has increased or density on the shells has decreased.

Comparisons of mean *P. viridis* spat density summed over the July and August monthly collections to mean spat density on substrates collected after the same 2-month period in the field showed some increases and some decreases depending on the substrate (Fig. 2A). For example, wood substrates that were in the field for 2 months had significantly higher spat density than the sum of the 1-month wood substrates from the same time period ($t = 2.85$, df = 18, $P = 0.011$). On the other hand, the density of spat was significantly lower on oyster shells deployed for 2 months compared to the summed 1-month substrates ($t = 4.54$, df = 18, $P < 0.001$) and an nonsignificant trend in the same direction was observed on *P. viridis* shells ($t = 1.65$, df = 15, $P = 0.120$). Similar patterns were observed when the 3-month samples were compared to the sum of settlement over the monthly collections during July, August and September, with only the decrease in density on oyster shells being significant ($t = 4.70$, df = 18, $P < 0.001$; Fig. 2B).

When mean *M. charruana* spat density summed over the July and August monthly collections was compared to spat density on substrates collected after being in the field for the same 2-month period, a very different pattern emerged. Spat density increased significantly for all 2-month substrates except shells of *P. viridis* and *M. charruana* (plexiglass: $t = 4.42$, df = 18, $P = 0.001$; tile: $t = 7.96$, df = 18, $P < 0.001$; wood: $t = 5.68$, df = 18, $P < 0.001$; *Crassostrea*: $t = 2.47$, df = 18, $P = 0.029$; *Perna*: $t = 1.16$, df = 15, $P = 0.309$; *Mytella*: $t = 0.13$, df = 12, $P = 0.917$; Fig. 3A). Interestingly, when the comparison between the sum of three consecutive monthly recruitment periods was compared to substrates deployed continuously for 3 months, the opposite trend was found. *Mytella charruana* spat density tended to be lower on substrates in the field for 3 months, but this was only significant on tile ($t = 2.27$, df = 18, $P = 0.036$; Fig. 3B).

Mean SL of *P. viridis* spat differed significantly among substrates for the 1-month collections but not the 2- or 3-month collections (data not shown). A Tukey's *post hoc* test revealed that the difference was due to the presence of significantly larger spat on wood when compared to either plexiglass or tile (data not shown). The 2- and 3-month collections did not show similar trends. Comparisons of mean SL of *P. viridis* spat across the 1-, 2- and 3-month collections showed a steady increase in mean size (Fig. 4) over each month. Mean SL increased each month on all substrates, except for the spat that settled on *P. viridis* shells which did not differ in size between months 2 and 3. When spat were pooled across substrates, the mean SL of spat was *c.* 3.8 mm (±1.5), 6.5 mm (±2.5) and 9.4 mm (±2.8) after the 1-, 2- and 3-month deployments, respectively.

Differences in mean SL among spat that settled on different substrates were also apparent for *M. charruana* in both 1- and 3-month collections, but were not significant for the 2-month collection. The relationship between size and substrate was inconsistent over time. In the 1-month collections spat on wood substrates were significantly smaller than spat on both *P. viridis* and *C. virginica* shells, and spat on tile were

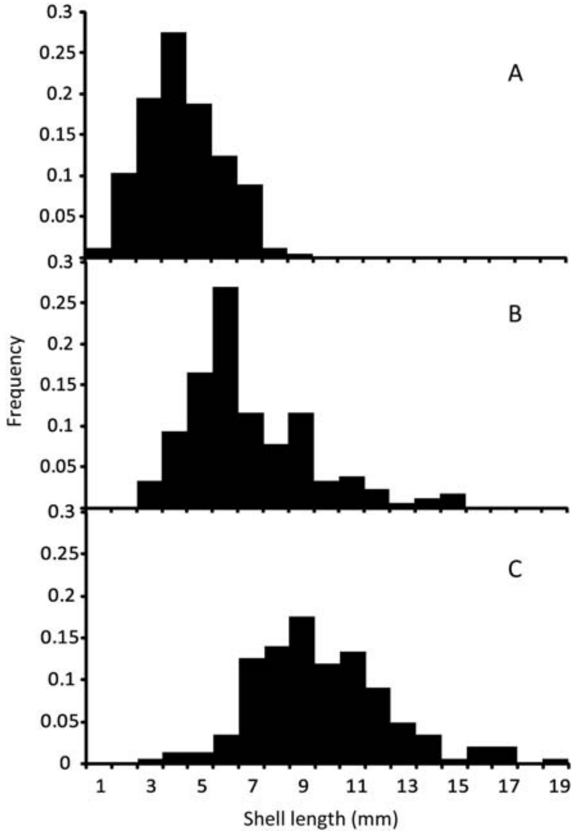

Figure 4. Size/frequency histograms of *Perna viridis* spat pooled from all substrates after 1 (**A**), 2 (**B**) and 3 months (**C**) in the field.

Table 4. Mean mussel size (mm) ± SE by substrate and month.

Time in field	Plexiglass	Tile	Wood	*Crassostrea*	*Perna*	*Mytella*	Significance
Perna viridis							
1 month	3.43 (1.59)	3.49 (1.45)	4.47 (1.59)	3.84 (1.38)	3.73 (1.05)	3.96 (7.19)	0.002
2 months	6.89 (2.47)	6.41 (2.31)	6.48 (2.69)	6.03 (2.13)	6.77 (4.30)	NA	0.729
3 months	9.36 (2.49)	9.27 (2.85)	9.91 (2.51)	8.93 (3.27)	6.44 (3.15)	NA	0.084
Significance	<0.001	<0.001	<0.001	<0.001	<0.001	NA	
Mytella charruana							
1 month	2.98 (1.37)	2.68 (1.55)	2.45 (1.30)	3.60 (1.75)	4.02 (1.40)	NA	0.002
2 months	6.89 (2.47)	6.41 (2.31)	6.49 (2.69)	6.03 (2.13)	6.77 (4.30)	NA	0.075
3 months	9.37 (2.49)	9.27 (2.85)	9.91 (2.51)	8.93 (3.27)	6.44 (3.15)	NA	<0.001
Significance	<0.001	<0.001	<0.001	<0.001	0.010	NA	

Significance column represents results of one-way ANOVAs comparing mean mussel lengths among substrates, while significance rows provide results of one-way ANOVAs comparing mean mussel lengths across time periods in the field. All mussels were from Sister's Creek.

significantly smaller than those on *P. viridis* shells (Table 4). The smallest spat in the 3-month collections, however, were those on *P. viridis* shells. These spat were significantly smaller than those on all other substrates. Neither of these trends was apparent in the 2-month collections. Mean SL for *M. charruana* spat tended to increase each month on all substrates. Size increased every month on oyster, wood and plexiglass substrates but significant differences were only observed between 1- and 2-month collections on *P. viridis* shells and between 1-month and both 2- and 3-month collections on tile. *Mytella charruana* spat pooled across substrates increased in mean SL from *c.* 3.2 mm (±1.6), 6.5 mm (±1.9) and 7.6 (±2.5) mm in the 1-, 2- and 3-month collections, respectively (Fig. 5).

DISCUSSION

Our data provide unequivocal support for the conclusion that the nonnative mussels *Perna viridis* and *Mytella charruana* do not preferentially settle on the man-made substrates used in this experiment. Neither of these mussels had greater spat densities on any of the man-made substrates compared to the natural substrates. In fact, the opposite was often true. *Perna viridis* spat were found at greater densities on shells than on the man-made substrates that had been in the field for a single month. *Mytella charruana* spat were present at significantly higher densities on shells than on man-made substrates that were collected after 1 and 2 months in the field, with a similar trend after 3 months in the field. These data suggest that, especially in *M. charruana*, settlement tends to be greater on disarticulated shells than on any other substrates used in this experiment. This relationship was not observed with *P. viridis* where densities tend to equilibrate over all substrate types when in the field for 2 or 3 months.

The consistently lower densities of *M. charruana* spat on man-made substrates than on disarticulated shells is intriguing, since the opposite pattern was found in previous sampling of adults in Mosquito Lagoon (Boudreaux & Walters, 2006b). In these previous observations, equal man-hours were spent looking for *M. charruana* on all available natural (live oyster clusters, disarticulated oyster shells, driftwood, red and black mangrove roots, bases of cordgrass *Spartina alterniflora*) and man-made substrates (cement blocks, submerged PVC, submerged wooden and tiled pilings). More *M. charruana* were found on man-made substrates like docks and few were ever found on either disarticulated shells or live oyster reefs. These opposing patterns suggest that, while *M. charruana* spat may preferentially settle on the shells of other mollusks, man-made substrates may provide a better habitat for longer-term survival. Decreased survival could be explained by competition with other mussels, barnacles or oysters when settling amongst live animals. In fact, interspecific competition affecting survival and growth in intertidal and near-shore subtidal communities has been observed on numerous occasions (Steffani & Branch, 2005; Rius & McQuaid, 2006; Ruesink, 2007). On the other hand, the difference between the two experiments could also be explained by the fact that the substrates used in the present experiment were placed under floating docks and therefore in a subtidal habitat, while oyster reefs and portions of pilings are intertidal. Therefore, the lack of adult *M. charruana* on oyster reefs may simply be due to lower survival in intertidal habitats than in subtidal habitats.

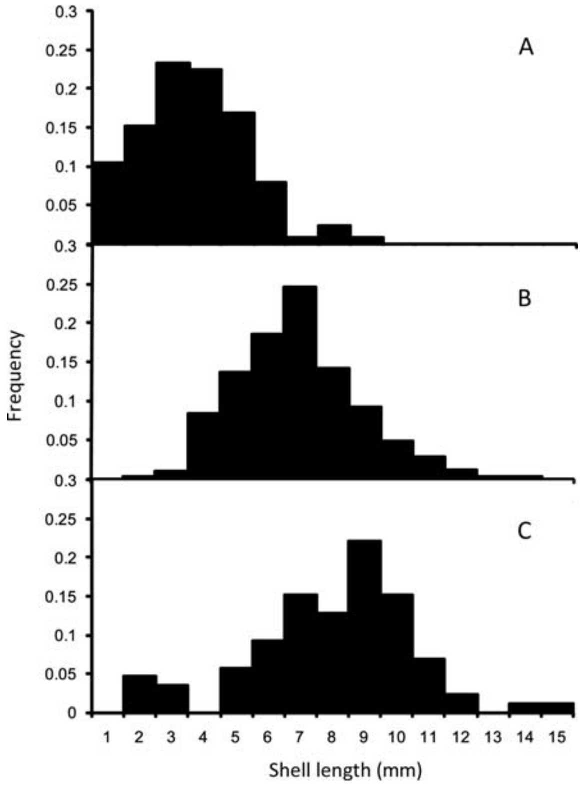

Figure 5. Size/frequency histograms of *Mytella charruana* spat pooled from all substrates after 1 (**A**), 2 (**B**) and 3 months (**C**) in the field.

In *P. viridis*, the greater spat density on shells when compared to man-made substrates disappears on substrates that have been in the field for periods of time greater than one month. When summed monthly collections were compared to substrates in the field during the same 2- or 3-month period, the lack of difference among substrates in the 2- and 3-month collections appears to be due both to an increase in spat density on man-made substrates and to a decrease in spat density on shells relative to the 1-month samples (Fig. 5). The increase in *P. viridis* spat density on man-made substrates that have been in the field for longer periods of time may be explained by initial changes in biofilms or the accumulation of other fouling organisms, such as barnacles, that may influence subsequent *P. viridis* recruitment. Biofilms have been associated with increased larval settlement in polychaetes (Kirchman *et al.*, 1982), bryozoans (Maki *et al.*, 1989), abalone (Morse *et al.*, 1984), oysters (Bonar *et al.*, 1990), barnacles (Maki *et al.*, 1988) and sea urchins (Pearce & Scheibling, 1991). In some mussel species, larval settlement often takes place on filamentous algae (Bayne, 1964; Gilg & Hilbish, 2003; Alfaro, 2006) which could also increase on substrates left in the field over longer periods of time. Why the biofilm or algae would only affect settlement on the man-made substrates, however, is unknown. Settlement can also be induced by the presence of conspecifics and by prey species (Rodriguez *et al.*, 1993), but these seem to be unlikely explanations for the observed patterns since conspecifics were not present on any of the substrates and mussels are filter feeders that do not ingest larvae of organisms associated with the substrates.

The differences in size among spat collected after different lengths of time in the field, however, suggest that differences in spat density among substrates may be due to a mechanism other than accumulation of biofilm, conspecifics or fouling organisms. If a buildup of other organisms was necessary prior to significant settlement by *P. viridis* spat, then the man-made substrates in the field for 2 months should contain relatively recent settlers that are similar in size to those typically found on 1-month substrates. This does not appear to be the case, since spat size seems to be similar on nearly all the substrates and the spat on each substrate tend to be larger the longer the substrates have been deployed. There was also a distinct lack of the smallest size classes of spat (<3 mm) in the 2- and 3-month collections for *P. viridis*. These data suggest that the spat settled relatively early on the substrates and had additional time to grow when the substrates remained in the field for longer time periods. These early settlers may have interfered with later settlement events in some way (e.g. competition for space), resulting in the lack of spat in the smaller size classes.

The decrease in density of *P. viridis* spat on shells may be due either to an intraspecific or an interspecific competitive interaction with other spat on the same substrate. *Perna viridis* spat density seemed to equilibrate at *c.* 0.05 individuals/cm^2, regardless of how long the substrates were in the field. In fact, greater average densities were found in the 1-month samples than in the 2- or 3-month samples. This suggests that spat density on these substrates may be density dependent. The density-dependence hypothesis is also supported by the lack of small size classes of spat on substrates in the field for 2 and 3 months, suggesting that additional settlement either could not be supported, or was inhibited by the presence of other organisms. Crisp (1961) showed that several species of barnacles reached an equilibrium density of new settlers over time, but other examples of this are unknown to the authors.

Mytella charruana spat densities, on the other hand, tended to increase after 2 months in the field when compared to the sum of consecutive 1-month collections. Density then declined in the 3-month collections. These data suggest that *M. charruana* spat survival or recruitment may also be density-dependent, but that it took more time (or more individuals) to reach the tipping point than in *P. viridis*. The similarity in the patterns of SL for both *M. charruana* and *P. viridis* again suggests that new settlement was limited during the latter part of a deployment on substrates that were in the field for 2 or 3 months, although some 2–4 mm spat were found in these collections.

Another intriguing finding of the study was the complete lack of *M. charruana* and *P. viridis* settlement at the Mosquito Lagoon site. While *P. viridis* is currently only common in the northernmost reaches of Mosquito Lagoon, *M. charruana* has been consistently found in Mosquito Lagoon for years. Indeed, the preliminary assessment of adult *M. charruana* density on different substrates was taken in Mosquito Lagoon. Thus, the lack of *M. charruana* recruitment in the collections from Mosquito Lagoon was surprising. The fact that few *M. charruana* mussels are currently found within the Mosquito Lagoon suggests that ecological conditions in the lagoon that may have initially been favorable are no longer supportive of long-term survival in this species. Studies are currently underway to investigate this question.

The findings of this study suggest that, while settlement of *P. viridis* and *M. charruana* is not greater on man-made substrates than on disarticulated shells, man-made substrates that have been in the field for even short periods of time can be attractive to settling larvae. This is especially true for *P. viridis*. Therefore, the increase in construction and deployment of man-made hard structures in the coastal areas of the southeastern USA will, at the very least, provide additional habitat for these two introduced species. Whether survival to adulthood is greater on man-made substrates than on shells or live organisms remains to be seen. Furthermore, the fact that disarticulated shells often received greater settlement than man-made substrates suggests that these mussels may act as substrate engineers (Gutierrez *et al.*, 2003), thereby resulting in a positive feedback for further population expansion. Therefore, the mussels themselves may provide a greater risk to their expansion than the addition of man-made hard substrates.

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