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Settlement of fiddler crab megalopae on a North Carolina (USA) sandflat: species identification using multiplex PCR provides evidence for selective settlement

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ABSTRACT: Selection of settlement sites by planktonic larvae can have important impacts on adult population distributions. Three species of fiddler crabs — *Uca pugilator*, *U. pugnax*, and *U. minax* — commonly co-occur in mid-Atlantic estuaries of the USA. They share a common larval life history of export to coastal waters followed by reinvasion of the estuary as postlarvae (megalopae), but their adults occupy different habitats separated by salinity and sediment type. This separation of adults could be caused by differential larval supply, selective settlement, or by post-settlement processes. We examined the species composition of planktonic postlarvae delivered to an intertidal site with a monospecific population of *U. pugilator* and compared it to newly settled postlarvae and first-instar crabs at this site using a new multiplex PCR technique for species identification. We found that all 3 species were present in the plankton but that almost all settled megalopae were *U. pugilator*, indicating that selective settlement is important for maintaining the adult population distribution at this site. In addition, all first-instar crabs were *U. pugilator* except for a single *U. pugnax* individual, indicating that megalopae that initially settle in an inappropriate habitat can leave before metamorphosis. The multiplex PCR is faster and less expensive than existing molecular methods for identifying fiddler crab larvae and juveniles to species. Future experiments should examine the behavioral bases for the selective settlement of *Uca* spp. megalopae.

KEY WORDS: Larval transport · Larval settlement · Selective settlement · Multiplex PCR · Larval supply · *Uca*

INTRODUCTION

Fiddler crabs are important consumers in temperate and tropical salt marsh, sandflat, and mangrove ecosystems (Crane 1975). In estuaries of the mid-Atlantic coast of the USA, 3 species of fiddler crabs (genus *Uca*) commonly occur: *Uca pugilator* (Bosc, 1802), *U. pugnax* (Smith, 1870), and *U. minax* (Le Conte, 1855). In general, these 3 species share a common larval life history: their zoeae are exported to offshore waters where they develop into the postlarva or megalopa stage, and subsequently reinvade the estuary (Epifanio 1988). However, adults of these species are segregated by habitat in estuaries. *U. minax* is found in low-salinity areas, especially brackish marshes, *U. pugnax* in moderate to high-salinity marshes with muddy sediments, and *U. pugilator* in moderate to high-salinity sandflats and sandy

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areas of marshes (Teal 1958, Miller & Maurer 1973). Since larvae of all 3 species are presumably mixed in offshore waters yet adults inhabit different habitats, there must be one or more processes that lead to the separation of the adult populations. This segregation of fiddler crab adults at any individual site could be driven by (1) differential larval supply to that site, (2) selective settlement, or (3) random settlement followed by differential mortality or emigration of the settlers.

Each of these possibilities has been shown to be an important factor controlling the distributions of invertebrates with planktonic larvae. Oceanographic processes can vary the number of larvae transported to a particular site, contributing to adult population variations in barnacles (Roughgarden et al. 1988) and crabs (Shanks 1998). Planktonic transport of larvae is in turn affected by larval behaviors, such as diel or tidal migrations (reviewed by Sulkin 1984, Forward & Tankersley 2001). Once larvae are transported to a site, they must choose whether or not to settle there. Cues used for settlement site selection can range from the presence of adult conspecifics (Crisp & Meadows 1962, O'Connor & Judge 1999) to the presence of a preferred prey item (Hadfield & Pennington 1990), or the absence of a dominant space competitor (Young & Chia 1981) or predator (Welch et al. 1997). After settlement has occurred, post-settlement processes can affect the number of juveniles that remain in a habitat until the adult stage (Victor 1986, Osman & Whitlatch 1996). Additionally, while many studies have examined settlement of sessile species such as barnacles and bryozoans onto hard substrates (e.g. Johnson & Strathmann 1989, Maki et al. 1989), motile species have some ability to migrate to new habitats after initial settlement. For example, blue crabs *Callinectes sapidus* settle initially into seagrass or other complex vegetated habitats (Orth & van Montfrans 1987) but can subsequently undergo secondary dispersal to other habitats as juveniles (e.g. Forward et al. 2005, Reyns et al. 2006).

For *U. pugilator*, *U. pugnax*, and *U. minax*, it has been difficult to study the relative importance of these processes because their larvae, postlarvae, and juveniles are morphologically indistinguishable until at least the fifth crab instar (O'Connor 1990). Seasonal and spatial distributions of fiddler crab larvae in estuaries have been described (Pinschmidt 1963, Sandifer 1973), but details about particular species could not be determined at the time. The general life history of fiddler crabs has also been well described. Fiddler crab zoeae are released on nighttime ebb tides (Epifanio et al. 1988) and are carried down-estuary to the coastal ocean (e.g. Epifanio 1988, Epifanio et al. 1988, Levin & Bridges 1995, López-Duarte & Tankersley 2009), where they develop until metamorphosis to the megalopa stage. Late in zoeal development and after the molt to the megalopa stage, the larvae move to the bottom of the water column where they are transported towards estuaries by subtidal flow (Epifanio 1988). Megalopae move up-estuary using nocturnal flood tide transport (De Vries et al. 1994, Tankersley et al. 1995, Forward & Tankersley 2001) until they reach adult habitats, where they settle and metamorphose into benthic juvenile crabs (O'Connor 1993, O'Connor & Judge 1999). Some populations of *U. minax* are located far inland and their larvae may not reach the ocean (Borgianini et al. 2012, Staton et al. 2014), but larvae from populations closer to the mouths of estuaries most likely do reach offshore, since to date no studies have identified all zoeal stages of *Uca* spp. within estuaries. Presumably, larvae of all 3 species are mixed in the water column offshore and have some chance of being transported to the same habitats upon reinvasion of the estuary. The question becomes, then, whether the adult species distributions are driven by random settlement followed by post-settlement processes such as mortality and emigration, or by selective settlement, in which megalopae settle only in appropriate habitats occupied by conspecific adults. Prior studies (e.g. Behum et al. 2005, Brodie et al. 2005) provided some evidence for selective settlement in these species but examined settled postlarvae and juveniles without accounting for planktonic larvae supplied to the sites.

In addition to these descriptions of larval behavior and distribution, a number of studies have investigated physiological processes upon settlement of fiddler crab megalopae. For example, fiddler crabs accelerate metamorphosis in the presence of sediment with odors from conspecifics (e.g. Christy 1989, O'Connor 1991, O'Connor & Gregg 1998, O'Connor & Van 2006), water from adult habitats (O'Connor & Judge 1997, 1999, 2004), and extracts of adult crabs (O'Connor 2005). This physiological response indicates at least some role of larval settlement site choice in maintaining the observed adult distributions. All of these studies, however, were conducted using laboratory-reared larvae that were exposed to habitat cues either in the laboratory (Christy 1989, O'Connor 1991, O'Connor & Gregg 1998) or in field-deployed enclosures (O'Connor & Judge 1997, 1999, 2004). These procedures were necessary since the identity of field-caught larvae or megalopae could

not be known (O'Connor 1990). O'Connor (1993) also found that settlement of *U. pugilator* and *U. pugnax* closely matched their adult distributions in a North Carolina (USA) salt marsh, supporting the hypothesis of selective settlement for determining adult distributions. The megalopae in this study were field-collected, but had to be reared until the fifth crab instar to be identified. The time necessary for the rearing process has made additional studies like this difficult to pursue.

Recently, a restriction fragment length polymorphism (RFLP) was described that can identify individual *Uca* spp. zoeae or megalopae to species (Behum et al. 2005, Brodie et al. 2005). This identification technique represented a breakthrough for fiddler crab larval ecology, and it has been used to determine identities of field-caught larvae and juveniles in several studies (e.g. López-Duarte & Tankersley 2009, George et al. 2010). Using this technique to identify field-collected settlers, Brodie et al. (2005) also examined settlement by *U. pugilator*, *U. pugnax,* and *U. minax* in a South Carolina (USA) estuary. They found that all 3 species settled into habitats occupied by their adults, indicating some degree of selective settlement. However, *U. pugilator* also settled into habitats occupied by the other 2 species, whereas *U. pugnax* and *U. minax* did not, indicating some role for post-settlement processes in *U. pugilator*. Despite this initial evidence that selective settlement may be occurring in these 3 species, neither Brodie et al. (2005) nor O'Connor (1993) examined the planktonic supply of postlarvae to these sites. Therefore, they could not determine whether the patterns they identified resulted from selective settlement or from differences in larval supply.

In the present study, we introduce a new multiplex PCR method that substantially reduces time and cost compared to the method of Behum et al. (2005). We used this multiplex PCR method to compare the species distribution of the 3 species of fiddler crab megalopae in the plankton with the species distribution of settled megalopae and first-instar crabs at a site with a monospecific population of *U. pugilator*. We hypothesized that the species distribution of the settlers would be different from the species distribution of the plankton delivered to the site. Such differences would indicate that selective settlement by *Uca* spp. megalopae does occur at this location.

MATERIALS AND METHODS

Study area

The present study was conducted at the Rachel Carson National Estuarine Research Reserve near Beaufort, North Carolina, USA (Fig. 1). The reserve consists of a variety of substrate types, ranging from coarse sandy dunes and exposed sandflats to muddier areas that support *Spartina alterniflora* of varying density. The habitat in which we worked is an intertidal sandflat (34° 42.71′ N, 76° 40.47′ W) that supports a monospecific population of *Uca pugilator* that numbers in the millions (Reinsel 2004). The sandflat forms the northwestern shoreline of a large tidal embayment that is accessed by a narrow channel. The channel is the only source for water and plankton in the embayment. Other sites in the embayment (~350 m to the east) have populations of *U. pugnax*.

The Rachel Carson Reserve is located within the Newport River estuary, which contains numerous additional populations of the fiddler crabs *U. pugilator, U. pugnax,* and *Uca minax* (Pinschmidt 1963). All 3 species occur within 10 km of the estuary mouth, and megalopae of all 3 species are common in the plankton.

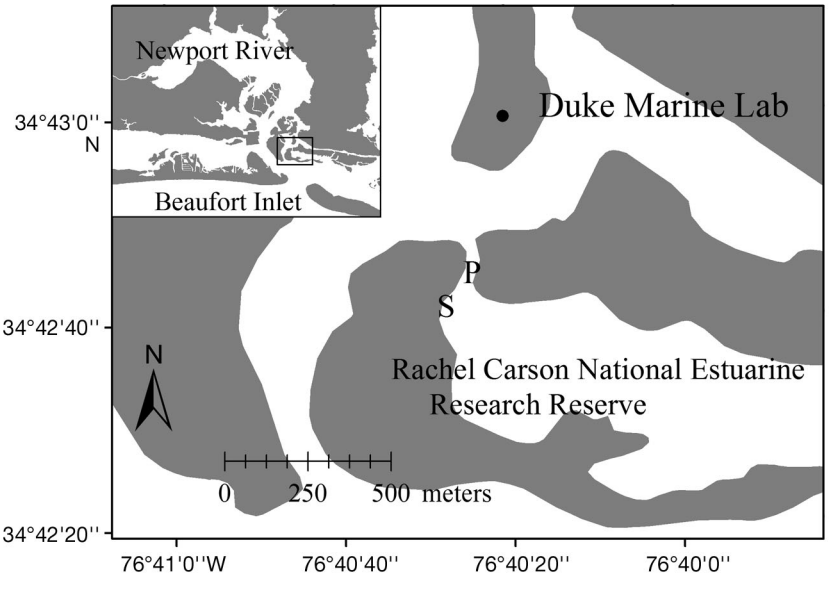

Fig. 1. Study site in Rachel Carson National Estuarine Research Reserve, North Carolina, USA. Locations of plankton and settler collections are indicated by P and S, respectively

Field collection of transporting megalopae and recent settlers

In order to assess the potential for selective settlement in the 3 species of fiddler crabs present in the Newport River Estuary, we compared the species composition of planktonic megalopae to that of recently settled megalopae. We collected these paired sets of samples a total of 9 times during August and September 2009 and July to September 2010.

We collected plankton samples from the channel that supplies water to our sample sandflat (Fig. 1). Megalopae use nocturnal flood tide transport for movement up estuaries to settlement sites in areas of adult populations (De Vries et al. 1994); they accomplish this by only swimming in the water column at night during rising tides (Tankersley & Forward 1994, Tankersley et al. 1995). Therefore, we sampled at night near the time of maximum flood currents. To maximize collection efficiency regardless of current flow through the channel, we collected plankton using a gasoline-powered plankton pump (Dayton model 1P986C), which was deployed from a boat anchored in the channel. Water was pumped through a 333 µm mesh plankton net for 10 min, which corresponds to 5.2 m^3 of water. Fiddler crab megalopae were sorted from the plankton sample and preserved in 95% ethanol for species identification.

To determine the species composition of settlers immediately following these nocturnal plankton collections, we collected settled megalopae and first-instar crabs during the daytime low tide following each nighttime plankton collection. We collected for approximately 30 min, or until at least 150 individuals were collected, whichever was shorter. Using forceps, we picked up all individuals observed on the exposed sediment adjacent to an area of short (<0.35 m), sparse *S. alterniflora*, below the zone of the adult burrows (Fig. 1). In the field, the megalopae and crabs were held in seawater; upon returning to the laboratory, they were preserved in 95% ethanol for species identification. Only juvenile crabs that were ≤1.3 mm carapace width, indicating that they were first instars (Hyman 1920), were used. First-instar crabs molt to the second instar in about 3 d (Hyman 1920), so older crabs would have had a longer period for post-settlement mortality to affect their distributions. First-instar crabs represent the earliest stage that has irreversibly settled in the location, since at metamorphosis they lose the ability to swim up into the water column and be transported away to a different site.

Species identification

DNA extraction

DNA was extracted from whole megalopae or juvenile crabs using Chelex extraction (Estoup et al. 1996). The individual megalopa was placed into a 1.5 ml microcentrifuge tube with 500 µl of a 10% solution of Chelex-100 resin (BIO-RAD) and 12 µl of Proteinase K (20 mg ml^{-1}), and incubated at 60°C for 60 min with constant shaking. The Proteinase K was then heat inactivated at 100°C for 20 min. The Chelex resin was pelleted by centrifugation at 13 000 × *g* for 1 min, and 2 µl of the supernatant were used as a template for multiplex PCR. Juvenile crabs were crushed with forceps as they were placed into the Chelex solution, since preliminary experiments showed that crushing improved the success of DNA extraction for juvenile crabs.

Multiplex PCR

A region of the internal transcribed spacer (ITS)-1 gene was amplified using multiplex PCR. In multiplex PCR, multiple primers are combined in a single reaction, but only the 'correct' primers bind due to sequence specificity. In some multiplex PCR methods (e.g. Hare et al. 2000, Larsen et al. 2005), complete primer pairs — both forward and reverse — are used. In this case, a common forward primer was used (ITS-1F, Schizas et al. 1999). Four new species-specific reverse primers were developed by examination of the ITS-1 sequences published by Behum et al. (2005, their Table 1). The reverse primers were designed to (1) bind to only 1 species, and (2) bind at different sites on the gene such that the amplicons would be of different lengths (Table 1, Fig. 2). Initial analyses used primer UPGR-2R for *U. pugilator*, but we determined that UPGR-1R generated an amplicon that was more easily distinguished from the other 2 species. Therefore, identifications performed since April 2013 (approximately 30% of data presented) used UPGR-1R instead of UPGR-2R.

PCR was performed in a 20 µl reaction volume using 2 µl of template DNA, 5 pmol of each primer, 10 µl of Promega Master Mix (Promega, M7505), and nuclease-free water. The PCR was performed in an Eppendorf Mastercycler Gradient thermal cycler and used the following reaction conditions: initial denaturing for 3 min at 96°C, followed by 35 cycles of 94°C for 15 s, 57°C for 45 s, and 72°C for 60 s. The sizes of amplified DNA fragments (amplicons) were

Table 1. Primers used in multiplex PCR to identify species of fiddler crab (*Uca* spp.) megalopae and juveniles. ITS: internal transcribed spacer region

Primer name	Species	Sequence (5′–3′)	Approximate size (bp) of amplicon with ITS-1F	Reference
ITS-1F	Most crustaceans	CACACCGCCCGTCGCTACTACCGATT		Schizas et al. (1999)
UPGR-2R	*U. pugilator*	CCGTGCCGACTGACAAGACATTCCG	500	This study
UPGR-1R	*U. pugilator*	GGGCTCGTCGCCCAGGAAGTTTTGGTGTGA	250	This study
UPX-2R	*U. pugnax*	GCTGCGGTGGCAGCCGCGACAAC	300	This study
UMX-1R	*U. minax*	ACCGAAAGCTCTGCCCGGTATATCT	420	This study

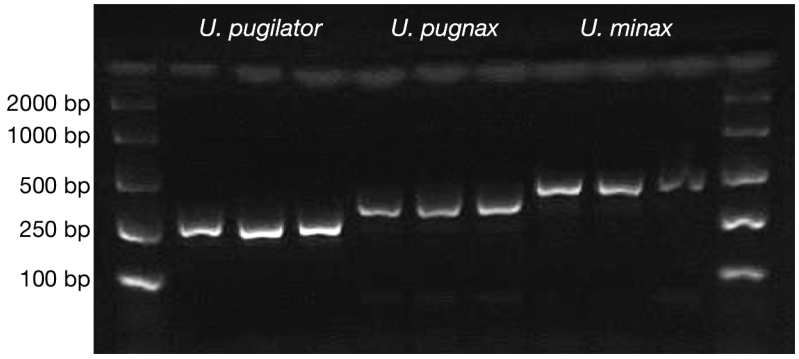

Fig. 2. Banding patterns of multiplex PCR products of 3 species of fiddler crabs (*Uca* spp.) using primers ITS-1F, UPGR-1R, UPX-2R, and UMX-1R. Note that the amplicons for *U. pugilator*, *U. pugnax*, and *U. minax* are ~250, ~300, and ~420 bp, respectively. Ladder (first and last lanes) is BioLine EasyLadderI

determined by gel electrophoresis using 4 µl of PCR product in a 1.5% agarose gel with GelRed™ stain (Biotium). Images were captured using a Syngene InGenius gel documentation system with UV transillumination. Sizes of fragments were determined using either a 100 bp ladder (Fisher Scientific, BP2573-100) or EasyLadder I (BioLine).

Data analysis

When available, at least 40 megalopae were selected from each collection of plankton and settlers for identification. However, due to variation in number available in field collections and number successfully identified, sample sizes ranged from 11 to 66 individuals. For the dates that first-instar crabs were present, between 19 and 39 were identified. The species composition of the plankton and settled megalopae were compared using chi-squared goodness of fit tests (Sokol & Rohlf 1981), with the distribution in the plankton as the expected distribution.

RESULTS

The species composition of recently settled megalopae was significantly different from that of planktonic megalopae on all sampling dates ($\chi^2_1 > 3.841$; $p < 0.05$ for all tests; Fig. 3). In general, the plankton samples comprised 50 to 80% *Uca pugilator*, <30% *U. pugnax*, and very few (<10%) *U. minax*. However, on both 4 August and 5 September 2010, there were higher proportions of *U. pugnax* (55 and 50%, respectively) megalopae in the plankton than on the other sampling dates (Fig. 3). In addition, 14 September 2009 was unusual in that there were higher proportions of both *U. pugnax* (36%) and *U. minax* (18%) in the plankton, but only 1 settled megalopa was collected (*U. pugilator*; data not shown in Fig. 3 since only 1 megalopa was collected). In contrast to the plankton, the settled megalopae were dominated by *U. pugilator* on all dates; on 6 out of the 8 sampling dates on which megalopae were collected, *U. pugilator* made up between 96 and 100% of the collected megalopae (Fig. 3). The few settlers that were not *U. pugilator* were exclusively *U. pugnax*; no *U. minax* were identified among the settlers on any sampling date. Of 116 first-instar crabs that were collected on 4 separate sampling dates over a period of 2 yr, only a single individual was *U. pugnax* (Table 2).

DISCUSSION

Adult fiddler crabs in the Newport River estuary occupy distinct habitats delineated by salinity and sediment type (Teal 1958, Pinschmidt 1963). Our results suggest that selective settlement by the mega-

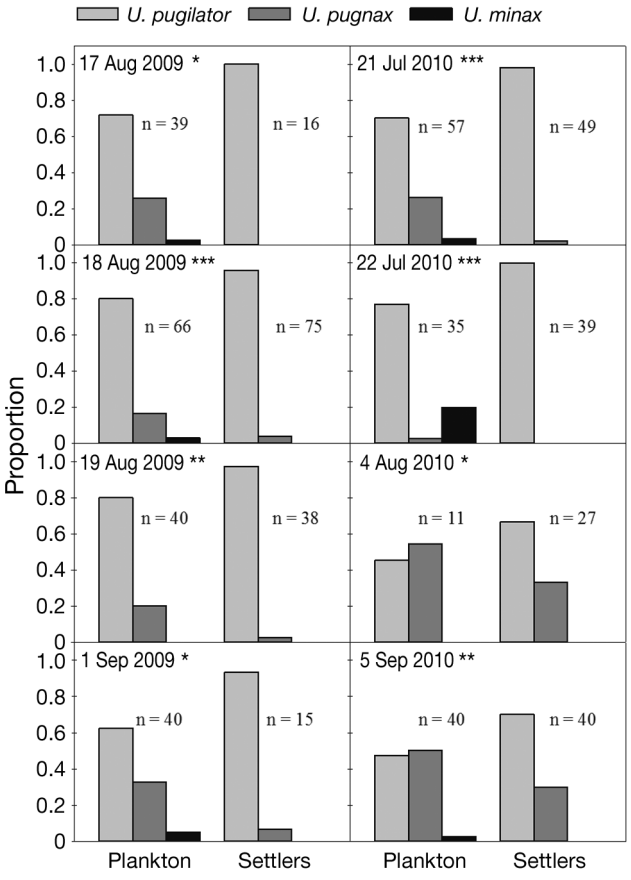

Fig. 3. Species composition of *Uca* spp. megalopae collected from plankton and recently settled megalopae and first crabs for 8 sampling dates in 2009 and 2010. *p < 0.05; **p < 0.01; ***p < 0.001 by chi-squared goodness of fit test. n = total number of individuals identified on each sampling date. Planktonic megalopae were also collected on 14 September 2009 (data given in the 'Results'), but because only a single settled megalopa was collected, that sampling date was omitted from this figure

Table 2. Species composition of *Uca pugilator* and *U. pugnax* first-instar crabs collected on 4 sampling dates in 2009 and 2010. No *U. minax* juveniles were identified

Date	*Uca pugilator*	*Uca pugnax*
17 August 2009	33	1
1 September 2009	25	0
14 September 2009	39	0
21 July 2010	19	0

lopae likely plays an important role in determining the adult distributions of these species. The study site had a monospecific population of *Uca pugilator* adults. Nevertheless, the plankton collected during rising tide at night adjacent to the study site consisted of a mixture of megalopae of all 3 species. The megalopae collected on the subsequent daytime low tide at the *U. pugilator* population site (settlers), however, were between 67 and 100% *U. pugilator* (Fig. 3). On all dates, the proportion of *U. pugilator* among the settlers was significantly higher than their proportion in the plankton. Additionally, all but one of the >100 first-instar crabs identified were *U. pugilator* (Table 2). These differences between plankton and newly settled megalopae and first-instar crabs indicate that selective settlement is occurring in at least 1 of the species: *U. pugilator* could be stimulated to select this site, or the other 2 species could either not be stimulated to settle, or actively avoid this location. Other studies have examined settlement of fiddler crab megalopae (O'Connor 1993, Brodie et al. 2005), and found that each *Uca* species settled primarily into habitats with their own adult conspecifics, although Brodie et al. (2005) found some *U. pugilator* settlers in habitats dominated by other species. However, neither of these studies examined the species distribution in the plankton on nocturnal flood tides at their sites. Thus, the patterns they observed could have been due either to differences in planktonic supply or to selective settlement. Our study demonstrates that the settlers are different from the plankton delivered to the site, so some form of selection must be occurring.

The significantly higher proportions of *U. pugilator* among the settlers could indicate that megalopae of this species actively selected this settlement site. In order for an individual megalopa to remain at a settlement site and not continue to transport during the next nocturnal flood tide, it would need to change its behavior to interrupt the flood tide transport process, settle on the substrate, and molt fairly quickly into the juvenile stage. Christy (1989) and O'Connor (1991) found that *U. pugilator* megalopae molted more quickly to the first crab instar when reared over sediment from adult habitats. However, in their studies, induction of the metamorphic molt required exposure to the habitat cue for at least 24 h (O'Connor & Judge 1997). Since this is longer than a megalopa would remain at a site without a behavioral change to stop its nocturnal flood tide transport swimming behavior, it is unlikely that accelerated molting alone could drive the selection process. A rapid behavioral change that allowed the megalopae to remain in the habitat until they molt would also be required (Hadfield & Koehl 2004, Koehl & Hadfield 2004). Since most of the individuals we collected were megalopae, it seems likely that some sort of behavioral mechanism is also involved.

While it is possible that *U. pugilator* selected this settlement site, the patterns we observed could also occur if fewer of the other 2 species settled there, either because the habitat cues were not stimulatory, or because they actively avoided the location. The salinity at this site (35‰) is inappropriate for *U. minax* (Teal 1958), so we did not expect to find them in great numbers in our samples. This salinity should not be problematic for *U. pugnax*; adults are common in nearby habitats with the same salinity (O'Connor 1990, 1993). However, the sediment type at the collection site is inappropriate for *U. pugnax*, since they lack the spoon-tipped setae necessary to scrape food off the sand particles (Miller 1961, O'Connor 1990). We therefore do not find many *U. pugnax* adults at this site, and it is not surprising that we did not collect many settled megalopae of *U. pugnax*. Without a cue from conspecifics to accelerate molting (O'Connor & Judge 1997, 1999, 2004, O'Connor & Van 2006) or change their behavior (Hadfield & Koehl 2004) to stop flood tide transport, *U. pugnax* and *U. minax* megalopae could simply remain in the water column as the tide recedes and be transported away from the intertidal zone.

It is also possible that *U. pugnax* and *U. minax* megalopae actively avoided settling at this site. Negative selection is less common and more difficult to demonstrate than positive selection, but it has been observed in several cases, in both sessile and mobile species. For example, Young & Chia (1981) demonstrated that bryozoan larvae can avoid settling in locations with a dominant space competitor. In addition, barnacle larvae have been shown to avoid settling on surfaces that have chemical cues from a predatory whelk (Johnson & Strathmann 1989). Settling blue crab megalopae, which remain mobile after settlement, avoid settling on hog's hair collectors containing their predators (Welch et al. 1997). It is possible that the presence of *U. pugilator* in the large densities found at this site provides a negative chemical cue for *U. pugnax* and *U. minax* megalopae. This cue could cause them to continue swimming even after the end of flood tide so they are transported away from the intertidal area.

Avoiding a potential settlement site could also be accomplished by continued swimming in the plankton and delaying metamorphosis into the benthic juvenile. Fiddler crab megalopae (*U. pugnax*) can delay metamorphosis to the juvenile stage when exposed to cues from potential predators (O'Connor 2005), and megalopae of all 3 species take longer to metamorphose in the absence of cues from conspecifics than in their presence (O'Connor 1991, 2005, O'Connor & Judge 1997, 2004, O'Connor & Gregg 1998, O'Connor & Van 2006). If they did happen to settle briefly in an inappropriate site, they would likely not molt to the benthic first crab and subsequently lose their ability to swim in the water column; they would instead continue their nocturnal flood tide transport at the next opportunity. Our data indicate that this process did occur in our study. Although we collected some *U. pugnax* megalopae from the sediment surface, only one of the first-instar crabs was *U. pugnax* (Table 2), indicating that any remaining settled megalopae swam away before metamorphosis.

Regardless of the mechanisms involved, our data suggest that some settlement site choice (either positive or negative) is occurring in one or more of these species. Additional field collections like the ones presented here, conducted in locations with monospecific populations of the other species, as well as in locations with mixed populations, would provide additional insight into which species can effectively select their settlement sites and what cues they may use to do so. The molecular techniques (Behum et al. 2005 and this study) to identify fiddler crab larvae and juveniles to species make these additional studies feasible.

The present study also presents a new, more efficient molecular technique to identify fiddler crab larvae. Prior to the development of molecular tools to identify fiddler crab larvae, field-caught individuals had to be reared in the laboratory until the fifth crab instar, when they could be identified by setation on the maxillipeds (O'Connor 1990, 1993). The first molecular method to identify fiddler crab larvae to species — the RFLP technique (Behum et al. 2005) — was a breakthrough for fiddler crab larval ecology. For the first time, field-caught megalopae could be readily identified to species, making it feasible to answer many basic questions of settlement site selection and behavior. However, their method used individual DNA extraction kits and disposable microtube pestles, resulting in a cost per sample of over $5.00. Our method uses the Chelex method of Estoup et al. (1996), which is faster than the commercial DNA extraction kits used by Behum et al. (2005), and eliminates the restriction digest, resulting in a time savings of nearly 50% and an 80% reduction in cost per larva. Thus, the multiplex PCR method presented here increases the promise of molecular identification of fiddler crab larvae by making it practical to identify relatively large numbers of larvae in a short time at manageable expense. This will allow future researchers in fiddler crab larval ecology to effec-

tively answer questions about fiddler crab larval settlement in the field and conduct behavioral experiments using animals that have experienced field conditions rather than laboratory-reared animals. This is important, given that Welch & Epifanio (1995) found differences in rates of growth and survival between laboratory-reared larvae and those reared in field-deployed enclosures, indicating that there are likely behavioral differences as well.

The present study is the first that we are aware of to directly compare the species composition of plankton delivered to a site with the fiddler crab settlers at that site, thus directly demonstrating some form of selective settlement by fiddler crab megalopae. The mechanism for that selection is yet undetermined. Future studies should examine behavioral responses to adult and/or habitat cues in field-caught larvae that may underlie the selective settlement observed here. These behavioral changes should occur on shorter time scales (Hadfield & Koehl 2004) than the acceleration of molting identified by previous investigators (e.g. O'Connor 1991, O'Connor & Judge 1997, 2004). Possible mechanisms could include directed swimming toward odors of adult conspecifics or habitat cues, or cessation of the flood tide transport swimming behavior. These behavioral responses can now be examined using field-caught megalopae, with their species identities being determined after the experiment using the multiplex PCR method described here.

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