

Planktonic ingress of fiddler crab megalopae to the Newport River Estuary, NC: evidence for semilunar periodicity in *Uca pugnax* and species-specific sampling bias by hog's hair larval collectors

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ABSTRACT: We examined the planktonic ingress of fiddler crab megalopae into the Newport River Estuary, NC, using hog's hair collectors to determine whether there were differences in patterns of ingress among the 3 *Uca* species in this estuary. In 2 consecutive summers, we documented peaks in settlement of fiddler crab megalopae onto passive larval collectors at the quarter moons (neap tides), corresponding with longer durations of flood tides during darkness. However, molecular identifications revealed that over 80% of the megalopae settling on collectors were *U. pugnax*, even though there are millions of *U. pugilator* on sandflats within 1 km of the collection site and *U. minax* are common in the estuary. Plankton net samples taken on the same nights as collector settlement confirmed that megalopae of all 3 *Uca* species are common in the plankton. The proportions of species in the plankton and settling on collectors were significantly different. These differences may reflect species-specific differences in thigmotactic behavior, or may indicate differences in times of transport within a tide, since collectors reflect settlement at the end of rising tide while plankton nets sample the abundance at the time of maximum flood tide current. Thus, hog's hair collectors may not be an unbiased sampling method for fiddler crab megalopae.

KEY WORDS: *Uca* · Larval settlement · Larval supply · Hog's hair collectors · Larval collectors · Lunar phase · Nocturnal flood tide transport

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INTRODUCTION

Fiddler crabs are important consumers in estuarine communities (e.g. Crane 1975, Robertson et al. 1980, Hoffman et al. 1984, Dye & Lasiak 1987, Reinsel 2004, Kristensen & Alongi 2006, Cuellar-Gempeler & Munguia 2013, Hughes et al. 2014). In estuaries on the Atlantic coast of the US, the 3 species *Uca pugilator* (Bosc, 1802), *U. pugnax* (Smith, 1870), and *U. minax* (Le Conte, 1855) commonly co-occur; their adult distributions are determined by sediment type and salinity (Teal 1958). *U. pugilator*, the sand fiddler

crab, primarily inhabits sandflats in areas of moderate to high salinity, but can sometimes be found in sandy areas of marshes (Teal 1958, O'Connor 1993). *U. pugnax*, the mud fiddler crab, lives in muddy areas and marshes, also in moderate to high salinity (Teal 1958). *U. minax*, the red-jointed or brackish-water fiddler crab, lives primarily in marshes, ranging from near fresh to moderate-salinity water (Teal 1958, Brodie et al. 2005). It is unclear what proximal mechanism maintains this separation of the 3 species into different habitats—larval supply (Roughgarden et al. 1988), selective settlement (Young & Chia 1981,

Hadfield & Pennington 1990, Brodie et al. 2005), or post-settlement processes (Osman & Whitlatch 1996).

These 3 *Uca* species share a common larval life history. Zoea larvae are released on nocturnal spring tides (Christy 1982) and are swept rapidly out of the estuary (Christy 1982, López-Duarte & Tankersley 2007) to offshore waters, where they develop through 5 zoeal stages (Hyman 1920, Epifanio 1988) followed by the postlarval or megalopa stage. As they develop, the zoeae, and later the megalopae, swim successively deeper in the water column and are returned to the estuary by the landward residual flow (Epifanio 1988, Epifanio et al. 1988). Once in the estuary, the megalopae use flood tide transport (De Vries et al. 1994, Forward & Tankersley 2001) to move upstream in estuaries to settlement sites. In fiddler crabs, flood tide transport is controlled by an endogenous rhythm in activity that cues the megalopae to swim on flood tides and remain inactive on ebb tides (Tankersley & Forward 1994). This behavioral rhythm is suppressed by light, so megalopae only transport during nighttime flood tides (Tankersley et al. 1995). Since all 3 species share this common larval life history and they co-occur in estuaries, it is highly likely that they are all present in the coastal ocean and return to estuaries in a similar manner.

A number of studies have examined ingress of fiddler crab megalopae into mid-Atlantic estuaries, primarily coincident with studies on blue crab settlement using hog's hair collectors (Boylan & Wenner 1993, Jones & Epifanio 1995, Metcalf et al. 1995, van Montfrans et al. 1995). In general, they found (as did Christy 1982) that ingress of megalopae is highest on neap tides. However, none of these studies could

identify the megalopae to species because zoeae and megalopae of *U. pugilator*, *U. pugnax* and *U. minax* are morphologically identical; individuals can only be identified morphologically to species after growth to the fifth crab instar (O'Connor 1990). Therefore, studies performed on field-caught fiddler crab larvae refer to them as *Uca* spp. In 2005, Brodie and colleagues developed a molecular technique based upon a restriction fragment length polymorphism (RFLP) that could identify field-caught fiddler crab megalopae to species (Behum et al. 2005, Brodie et al. 2005). Since that time, the method has been used to examine settlement of fiddler crab megalopae into adult habitats (Brodie et al. 2005) and to confirm the identities of field-caught larvae in rhythm studies (López-Duarte & Tankersley 2009). Using a revision of this method, Welch et al. (2015) also found evidence of selective settlement by fiddler crab megalopae of these species. However, to date no studies have examined the species-specific ingress of these fiddler crab megalopae into estuaries.

We conducted daily sampling of *Uca* spp. megalopa settlement onto hog's hair collectors (Metcalf et al. 1995) followed by identification of the megalopae to species (Welch et al. 2015), in order to determine whether there were species-specific differences in timing of ingress to the estuary on either seasonal or lunar time scales.

MATERIALS AND METHODS

Study area

The Newport River Estuary (North Carolina, USA; Fig. 1) is an ideal site in which to conduct a study of the ingress of fiddler crab megalopae after offshore development. It is a relatively small estuary with a convenient sampling site located at the Duke University Marine Laboratory, approximately 2 km from the inlet. There are large populations of all 3 species of fiddler crabs within the estuary and its tributaries (Pinschmidt 1963, Reinsel 2004) and *Uca* spp. megalopae are common in the plankton during the summer (De Vries et al. 1994).

Larval collections were conducted at the Duke Marine Laboratory in Beaufort, North Carolina on Pivers Island (Fig. 1). Adult populations of all 3 local fiddler crab species are common within 10 km of the study site. The Rachel Carson National Estuarine Research Reserve, which is less than 1 km away, supports a population of primarily *Uca pugilator* that numbers in the millions, with densities as high

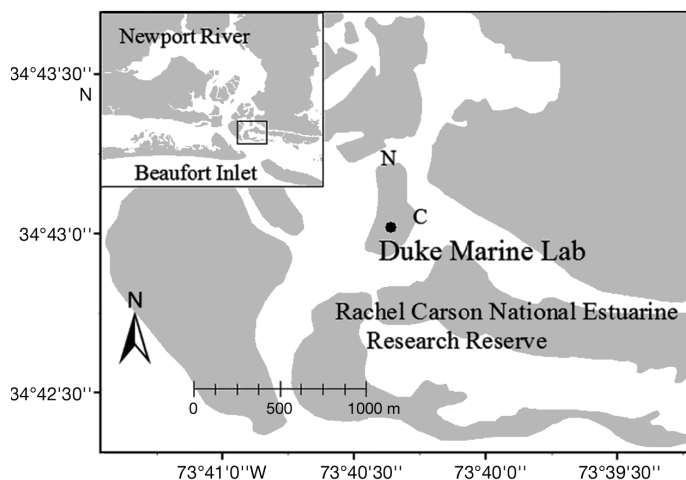


Fig. 1. Study area; locations of hog's hair collector sampling and plankton net sampling are represented by C and N, respectively

as 200 m^{-2} (Reinsel 1995). Upstream of the site there are numerous *Spartina alterniflora* marshes that support *U. pugnax* and *U. minax* populations, both separately and in combination (Pinschmidt 1963, O'Connor 1993). This site has semidiurnal tides, with spring tides occurring on new and full moons, and neap tides occurring on the quarter moons. Tidal currents range from near 0.4 m s^{-1} on neap tides to 0.75 m s^{-1} on spring tides (Welch 1998). For a full description of the hydrodynamic and climactic variables at this site, see De Vries et al. (1994).

Daily megalopa collection

Fiddler crab megalopae were collected using cylindrical passive hog's hair larval collectors in the style of Metcalf et al. (1995). Three collectors were deployed from the south end of the main dock at the Duke Marine Lab in Beaufort, North Carolina ($34^{\circ} 43.02' \text{ N}$, $76^{\circ} 40.25' \text{ W}$; Fig. 1). Collectors were hung at the surface, approximately 0.3 m below the mean low tide. Water depth at the collection site is $\sim 7 \text{ m}$. Collectors were sampled daily from 10 June to 12 August and 20 August to 18 September 2010, and from 11 June to 9 August and 2 September to 8 November 2011. Each day (typically between 08:00 and 10:00 h) hog's hair sleeves were removed from the collectors and replaced with sleeves that had been soaking in running estuarine water for 24 h.

To harvest collected megalopae from the collector, hog's hair sleeves were rinsed with a freshwater hose, with the rinse water collected in a 20 l bucket (Metcalf et al. 1995). Sleeves were allowed to soak in the rinse water in the bucket for 20 min. Following the soak they were rinsed again with fresh water for 5 min; this rinse liquid and the soak water were poured through a $500 \mu\text{m}$ sieve and organisms collected on the sieve were transferred to estuarine water. Processed sleeves were soaked in estuarine water until they were redeployed the following day. *Uca* megalopae were sorted from the samples, counted and preserved in 95 % ethanol for later identification. Samples from the 3 collectors were pooled for analysis because the individual collectors caught different numbers of megalopae each night.

Plankton net collection

In order to compare the species composition of the megalopae on the passive collectors to that available in the plankton, additional samples were taken with

a plankton net on 2 dates in June and July 2010, and 5 dates in June, July and August 2011. On each sample date, a 0.75 m diameter $333 \mu\text{m}$ net was deployed from a platform under the Pivers Island Bridge in Beaufort, NC ($34^{\circ} 43.20' \text{ N}$, $76^{\circ} 40.40' \text{ W}$) for approximately 1 h surrounding the time of maximum nocturnal flood current. The current held the net just below the surface of the water; De Vries et al. (1994) found no difference between surface and bottom abundances of crab larvae at this site, so the surface crab larval abundance should be representative of the water column. *Uca megalopae* were separated from the organisms collected in the net and preserved in 95 % ethanol for species identification.

Species identification

From each daily collector sample, 40 megalopae were haphazardly selected for identification to species. For days on which fewer than 40 megalopae were collected, all animals were identified. After identification, proportions of each species in the subsamples were extrapolated to generate total settlement for each species per day. For plankton net samples, either 40 or 80 megalopae were haphazardly selected for identification. *Uca* spp. megalopae were identified to species using the multiplex PCR method of Welch et al. (2015). DNA was extracted from individual megalopae using the Chelex method of Estoup et al. (1996). The extracted DNA was used as a template for PCR with a single forward primer (ITS-1F; Schizas et al. 1999) and 3 species-specific reverse primers (UPGR-2R, UPX-2R, and UMX-1R; Welch et al. 2015) that result in different-length fragments of the ITS-1 gene. Only the correct reverse primer binds to the template, and the length of the amplified fragment allows the determination of the species of the megalopa (Welch et al. 2015). Late in the analysis, the primer UPGR-2R was replaced with UPGR-1R, which provides a clearer separation of *U. pugilator* samples from the other 2 species.

Multiplex PCR was performed in a $20 \mu\text{l}$ reaction volume using $2 \mu\text{l}$ of template DNA, 5 pmol of each primer, $10 \mu\text{l}$ of Promega Master Mix (Promega, M7505), and nuclease-free water. The PCR was performed in an Eppendorf Mastercycler Gradient 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 were determined by gel electrophoresis using $4 \mu\text{l}$ 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 100 bp ladder (Fisher Scientific, BP2573-100) or EasyLadder I (BioLine).

Data analysis

Periodicity in time series of megalopal settlement onto the collectors were analyzed using maximum entropy spectral analysis (MESA; Levine et al. 2002). Because there was a gap in sampling during August of each year due to availability of personnel, only the period from early June to early August of each year could be analyzed for periodicity. Single missing sample dates were replaced using linear interpolation. The phase of periodic variations relative to the spring/neap cycle was determined using the cross-correlation (Wing et al. 1995) between megalopal settlement and the height of the nocturnal high tide. All MESA and cross-correlation analyses were per-

formed in MATLAB (Mathworks) using m-files provided by J. Cohen at the University of Delaware (pers. comm.).

The species composition of megalopae from the daily collectors was compared with that of plankton net samples for 7 dates in 2010 and 2011 using chi-squared goodness of fit tests, with the distribution of the plankton net samples as the expected distribution (Sokal & Rohlf 1981). When sample sizes were unequal, the expected distribution was normalized to the total number of settlers identified on that date.

RESULTS

In 2010, total settlement of *Uca* spp. megalopae onto the 3 collectors ranged from 3 to 2548 megalopae d^{-1} (Fig. 2a). Settlement was variable, with generally low abundance (≤ 200 megalopae d^{-1}) punctuated by periodic peaks of $\geq 300 d^{-1}$. The largest peak in settlement, a 3 d period of over

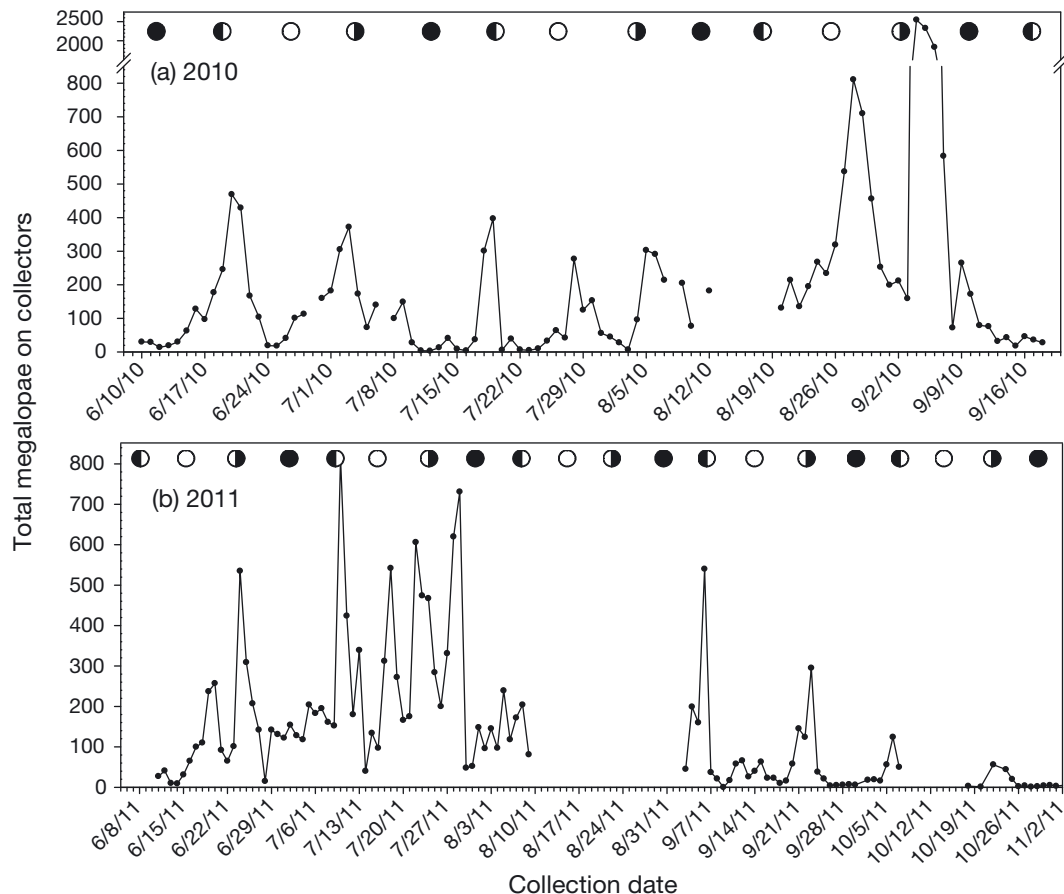


Fig. 2. Total *Uca* spp. megalopal settlement onto 3 hog's hair passive collectors in (a) 2010 and (b) 2011. Missing data indicate that collectors were not deployed on that date. Open, closed and half-filled circles represent phases of the moon

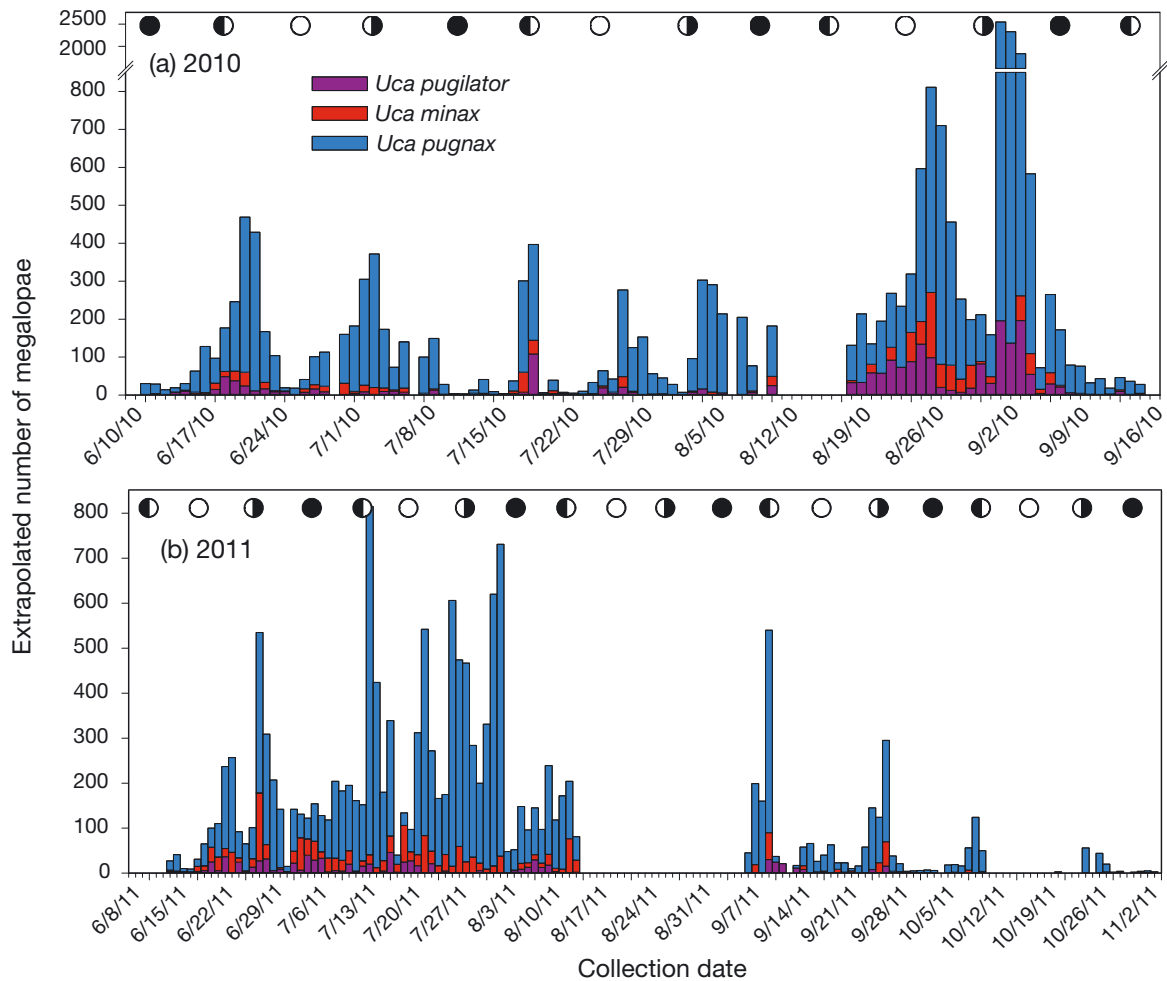


Fig. 3. Species distributions of *Uca pugilator*, *Uca pugnax* and *Uca minax* settlers on hog's hair collectors in (a) 2010 and (b) 2011. If $n \leq 40$ settlers, distribution represents all larvae identified from that sample. If $n \geq 40$ settlers, totals are extrapolated from the proportions identified in a subsample of 40 individuals. Open, closed and half-filled circles represent phases of the moon

1800 megalopae d^{-1} , occurred in early September just after the third quarter moon (Fig. 2a). In 2011 the pattern was very similar, but without the very large September peak (Fig. 2b). Sampling continued later into the fall in 2011 than in 2010. The magnitude of settlement peaks declined from about 400 megalopae d^{-1} in early September to 250 d^{-1} in late September to 150 d^{-1} in early October, with one final peak in late October with fewer than 50 megalopae d^{-1} . Background settlement also declined throughout the fall, to near zero except on the peak days.

Settlement of each of the 3 species was similar to the general pattern of the group, with periods of fairly low settlement and periodic peaks (Fig. 3). However, in both years settlement was dominated by *Uca pugnax* (Fig. 3), which represented 83.6% of the total megalopae that settled on the collectors in each year. *U. pugilator* represented 10.2 and 5.0%, and

U. minax represented 6.2 and 11.4% of settlement in 2010 and 2011, respectively (Fig. 3).

To determine whether the unusually high proportion of *U. pugnax* found on the passive collectors reflected the available larvae in the plankton, species compositions from plankton net samples on 7 dates were compared to the collector data for those days. The species distribution of megalopae from the collectors was significantly different from that of plankton net samples on all dates except 7 July 2011 ($\chi^2_1 > 3.841$, $p < 0.05$ for all significant tests; $\chi^2_1 = 0.53$, $p > 0.05$ for 7 July 2011; Fig. 4). In general, the plankton net samples contained a mixture of all 3 species, each comprising $\sim 10\%$ or more of the megalopae identified. The few exceptions were 21 July 2010, when there were no *U. minax*, and 3 August 2010, when there were 2.5% *U. pugilator* present in the plankton net samples (Fig. 4). *U. pugnax* was generally most

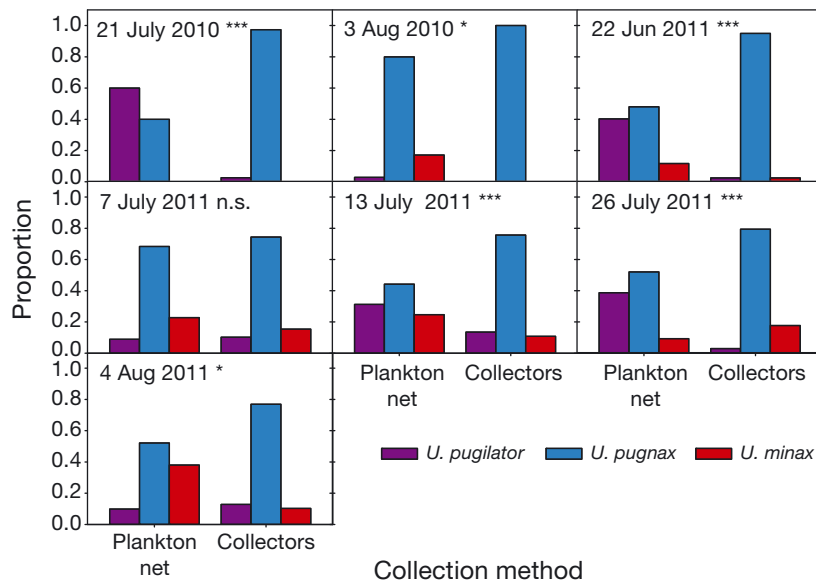


Fig. 4. Species distributions of *Uca* spp. megalopae from plankton net samples and hog's hair collectors for 7 sample dates in 2010 and 2011. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significantly different ($p > 0.05$) by chi-squared goodness of fit test

abundant, comprising between 40 and 80% of the individuals identified from the net samples. The megalopae from the collectors on these dates were also dominated by *U. pugnax*, but the proportions were much higher; in all cases over 75% of the megalopae on the collectors were *U. pugnax* (Fig. 4).

MESA analysis for *U. pugnax* indicated that there was significant periodicity in both years ($p < 0.05$). In 2010, the dominant MESA-periodicity was 9.3 d, with a smaller but significant semi-lunar periodicity at 14.3 d. In 2011, the dominant MESA-periodicity was close to a semi-lunar timeframe at 17.9 d. In both years, there were significant cross-correlations between settlement onto the collectors and the height of the nocturnal high tides. Correlations were negative at time lags corresponding to spring tides, which occur on full and new moons at this location (2010: $r^2 < -0.38$ for lags of 1, 2, 17 and 31 d, all $p < 0.05$; 2011: $r^2 = -0.41$ at a lag of 1 d; $r^2 = -0.28$ at a lag of 17 d, $p < 0.05$). Correlations were positive at time lags corresponding to neap tides, on the quarter moons (2010: $r^2 = 0.30$ for lags of 9 and 10 d, $r^2 = 0.43$ for lags of 23 and 24 d, all $p < 0.05$; 2011: $r^2 = 0.29$ for a lag of -7 d, $p < 0.05$). A lag of 0 d corresponds to the night of the lunar month with the highest amplitude nocturnal high tide (i.e. spring tides). Because of the large number of days on which there were 0 individuals of *U. pugilator* and/or *U. minax* megalopae that settled on the collectors (Fig. 3), MESA analysis could not be performed for either of those species.

DISCUSSION

Several prior studies have examined ingress of *Uca* spp. megalopae into estuaries using hog's hair collectors (Boylan & Wenner 1993, Jones & Epifanio 1995, van Montfrans et al. 1995). However, this is the first study using these collectors that also identified the megalopae to species using newly available molecular techniques (Welch et al. 2015). We looked for species-specific patterns in timing of ingress to the Newport River Estuary, but we found no evidence for a shift in the dominant species over the course of the season. In fact, we found that settlement of fiddler crab megalopae onto the collectors was overwhelmingly dominated by *Uca pugnax* at all times of the summer and fall (Fig. 3). We did not analyze patterns of settlement for *U. pugilator* and *U. minax* because we believe that their

settlement onto the collectors was not representative of their presence in the plankton (Fig. 4).

In both years, settlement onto the collectors was highest on neap tides, which occur on the quarter phases of the moon at this location (Fig. 2). This pattern is consistent with the behavioral model for flood-tide transport by fiddler crab megalopae (Tankersley & Forward 1994, Tankersley et al. 1995, Forward & Tankersley 2001). Swimming behavior of fiddler crab megalopae is controlled by a circatidal rhythm with the active period during flood tide (Tankersley & Forward 1994). However, swimming activity is suppressed by a photoreponse during the day (Tankersley et al. 1995), so they only transport on nighttime flood tides. Since neap tides are associated with lower tidal amplitude and therefore weaker tidal current, it might be predicted that settlement onto passive collectors would be lower during neap tides simply because of the lower volume of water that passes by the collectors. However, in North Carolina during the summer, the longest duration of flood tide at night occurs during neap tides (Forward et al. 2004). Therefore, maximum transport of fiddler crab megalopae should take place when the entire period of flood tide occurs during the night, which occurs during neap tides; settlement onto the collectors was in fact highest during this predicted time of maximum nocturnal transport (Fig. 2). This pattern became especially apparent during the fall of 2011, when background settlement onto the collectors was

smaller. In September and October, there were distinct peaks in settlement just after each quarter moon, although the magnitude of each peak declined through the fall. In addition, the 2011 sampling established the end of *Uca* spp. settlement in the fall as late October/early November.

Jones & Epifanio (1995) did not find a lunar pattern in settlement of *Uca* spp. megalopae in Delaware Bay. However, the overall settlement of fiddler crabs in their study was much lower than in the present study and there were many days when no fiddler crabs settled on the collectors. It is possible that these densities were too low to detect a pattern. Our results are consistent with those of Christy (1982) and Boylan & Wenner (1993), who found maximum immigration of *Uca* spp. megalopae into South Carolina estuaries on the waning quarter moon. In addition, Christy & Morgan (1998) found that maximum ingress of *Uca* spp. in the Gulf of Mexico occurred on the maximum amplitude nocturnal flood tides. This pattern is consistent with our results, since in North Carolina the maximum duration of nocturnal flood tides occurs during neap tides. Our results are most consistent with those of Forward et al. (2004), in which the settlement of blue crab megalopae onto collectors was greatest during neap tides. Although swimming behavior in blue crabs (Welch & Forward 2001) is regulated differently than in fiddler crabs (Tankersley & Forward 1994), the outcome is the same—nocturnal flood tide transport. Both their study and ours support the hypothesis that ingress of these crab megalopae is maximal when the entire flood tide occurs in darkness.

The overwhelming abundance of *U. pugnax* on the collectors (Fig. 3) was unexpected. All 3 species of fiddler crabs are common as adults in the Newport River Estuary (Hyman 1920, Pinschmidt 1963), and there is a very large (i.e. millions of individuals) population of *U. pugilator* within 1 km of the sampling site (Reinsel 2004). In addition, all 3 species were common in our plankton net samples (Fig. 4), so we would have expected to see them in similar proportions on the collectors, but we did not. In our study, hog's hair collectors clearly sampled *U. pugnax* much more effectively than the other 2 species.

There could be several explanations for this species-specific difference; two of these involve behavioral responses of larvae to the collectors themselves. First, these passive settlement collectors function largely because of thigmotactic (the tendency to cling to structure) behavior by the larvae (Wolcott & De Vries 1994, Metcalf et al. 1995). *U. pugnax* megalopae may be more thigmotactic than the other 2 spe-

cies, making those larvae more likely to settle onto the collectors. This sort of strong thigmotaxis would be adaptive for *U. pugnax* since it settles in marsh habitats, where there is abundant cordgrass *Spartina alterniflora* providing structure to cling to. A number of authors have discussed thigmotaxis in blue crab megalopae (Metcalf et al. 1995, Morgan et al. 1996, Hasek & Rabelais 2001), but no studies have examined species-specific differences in thigmotaxis among these fiddler crab species. Measurements of the magnitude of thigmotaxis in each of the species should be conducted to evaluate the relative importance of thigmotaxis in determining settlement patterns in these species. In addition, it is possible that the thigmotaxis varies among developmental stages within a species (Moksnes & Wennhage 2001); i.e. that premolt megalopae may be more thigmotactic, and thus more likely to cling to the collectors than intermolt megalopae. However, Hasek & Rabelais (2001) tested this hypothesis for blue crab megalopae and found no difference in the frequency of different molt stages in plankton net samples versus on collectors. Also, our sample site was very near the mouth of the estuary (<3 km from the mouth), and Metcalf & Lipcius (1992) found that molt stage in blue crab megalopae advanced from the shelf to the bay mainstem to secondary estuaries. There is no *a priori* reason to expect that the 3 *Uca* species would enter the estuary in different molt stages, with the possible exception of *U. minax*, which is found as adults in lower salinity habitats farther up the estuary and thus may enter at an earlier developmental stage.

The second behavioral possibility is that *U. pugilator*, which typically settles on sandflats, is adapted to settle onto predominantly horizontal surfaces, rather than the vertical surfaces provided by the collector or the stems of cordgrass. If that were the case, *U. pugilator* megalopae may avoid the collectors merely because of their vertical structure; perhaps collectors that provided a horizontal collection surface would collect *U. pugilator* more effectively.

Alternatively to interactions of larvae with the collectors, these interspecific differences in collection could be due to the timing of collection. Collectors and plankton nets are sampled at different times of the tide (Hasek & Rabelais 2001). The collectors sample to some degree throughout the tide, but settlement onto the collectors largely occurs at slack water after flood tide (Tankersley et al. 2002). On the other hand, plankton net samples are generally taken during maximum flood current to maximize water flow through the net. If the 3 species have behaviors that cause them to transport at different times within the

tide, then these 2 methods would potentially sample different larval populations.

Another possible explanation for our results could be that chemical cues at the collection site affected settlement onto the collectors, possibly attracting *U. pugnax* to the collectors. However, this is unlikely because the collection site (dock) was in deep water (~7 m) and was not immediately adjacent to any fiddler crab habitat. The collectors were relatively close to the dock pilings, but there were no adult fiddler crabs on the pilings to provide chemical cues for settlement. Therefore, we expect the chemical cues from the dock piling community to be neutral for fiddler crab settlement, or to affect the 3 species equally.

No matter which of these alternatives is correct, it is clear that the hog's hair collectors attracted *U. pugnax* to a greater degree than the other 2 species. This finding has important implications for past and future studies using these collectors. It is likely that what we know from previous collector experiments about *Uca* spp. (Boylan & Wenner 1993, Jones & Epifanio 1995, van Montfrans et al. 1995) is more reflective of *U. pugnax* than of the other 2 species. In locations where *U. pugnax* is the dominant species (e.g. Jones & Epifanio 1995), this may not present a problem. However, in locations where all 3 species coexist, sampling using hog's hair collectors may not be representative of the larval population as a whole.

In summary, sampling of settlement of fiddler crab megalopae onto hog's hair collectors over 2 consecutive summers was dominated by *U. pugnax*. Settlement was highest on neap tides, consistent with maximal transport of fiddler crab megalopae occurring during nocturnal flood tides. *U. pugnax* megalopae occurred in much higher proportion on the collectors than in plankton samples at the site. Thus, hog's hair collectors are likely not an unbiased sampling method for *Uca* spp. megalopae. Future experiments should examine differences in thigmotaxis and other behavioral differences among the 3 species.

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