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The Role of Adult Fiddler Crab Environmental Acoustic Cues and Chemical Cues in Stimulating Molting of Field-Caught Megalopae

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Abstract
In mid-Atlantic estuaries, three fiddler crab species, Uca pugilator, Uca pugnax and Uca minax co-occur, with their adults occupying different habitat types distinguished by salinity and sediment size. Some evidence exists that selective settlement is responsible for this separation but the mechanism is largely unknown. We tested the hypothesis that field-caught megalopae would accelerate metamorphosis in the presence of adult species-specific environmental acoustic cues and conspecific chemical cues. We placed megalopae in seawater with and without adult chemical cues, exposed them to one of three sound treatments for 8 days, and recorded the time each megalopa took to metamorphose. In the absence of adult chemical cues, very few megalopae molted regardless of sound treatment. Molting in the presence of habitat sound and chemical cues varied by species. Many U. pugilator molted in all sound and odor combinations, including no odor/sound. U. pugnax was stimulated to molt by chemical cues from either U. pugilator or U. pugnax, but molting was similar across sound treatments. Our results do not support the hypothesis that sound stimulates molting by fiddler crab megalopae, but support the role of chemical odors from adults as molting cues.

Keywords
fiddler crabs, megalopae molting, chemical cues, acoustic cues

Disciplines
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Comments
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The role of adult fiddler crab environmental acoustic cues and chemical cues in stimulating molting of field-caught megalopae

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Abstract

In mid-Atlantic estuaries, three fiddler crab species, *Uca pugilator, Uca pugnax* and *Uca minax* co-occur, with their adults occupying different habitat types distinguished by salinity and sediment size. Some evidence exists that selective settlement is responsible for this separation but the mechanism is largely unknown. We tested the hypothesis that field-caught megalopae would accelerate metamorphosis in the presence of adult species-specific environmental acoustic cues and conspecific chemical cues. We placed megalopae in seawater with and without adult chemical cues, exposed them to one of three sound treatments for 8 days, and recorded the time each megalopa took to metamorphose. In the absence of adult chemical cues, very few megalopae molted regardless of sound treatment. Molting in the presence of habitat sound and chemical cues varied by species. Many *U. pugilator* molted in all sound and odor combinations, including no odor/sound. *U. pugnax* was stimulated to molt by chemical cues from either *U. pugilator* or *U. pugnax*, but molting was similar across sound treatments. Our results do not support the hypothesis that sound stimulates molting by fiddler crab megalopae, but support the role of chemical odors from adults as molting cues.

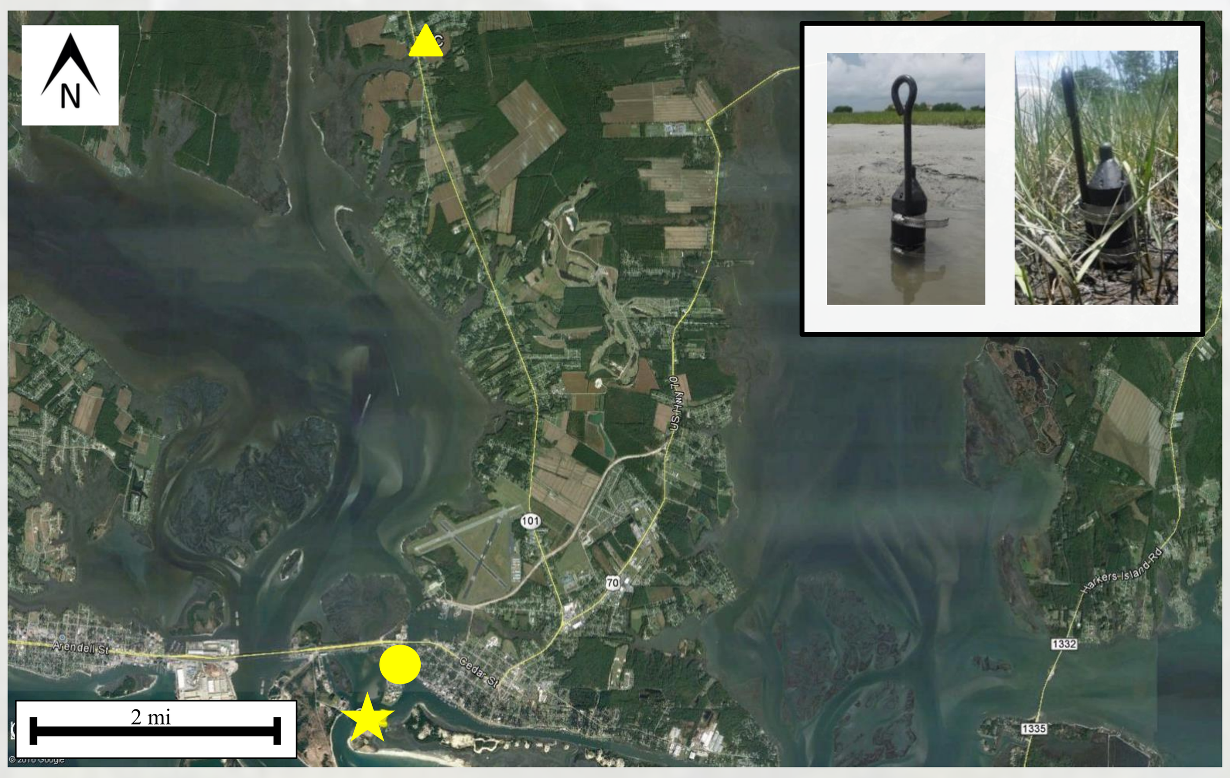

Figure 1. Sample sites in Beaufort NC: Carrot Island sandflat (star) and Bell Creek Marsh (triangle). Oval represents location of Duke Marine Lab. Inset is a picture of the SoundTrap in each sample site.

Background

- The sand fiddler crab, *Uca pugilator*, the mud fiddler crab, *Uca pugnax* and the red-jointed fiddler crab, *Uca minax* commonly co-occur in mid-Atlantic estuaries (Crane 1975) but occupy different microhabitats (Teal 1958, Miller & Maurer 1973)
  - *U. pugilator* occupies moderate to high salinity sandflats and sandy areas of salt marshes.
  - *U. pugnax* occupies moderate to high salinity salt marshes with muddy sediments.
  - *U. minax* occupies low salinity salt marshes.
- Fiddler crab zoeae of all 3 species develop offshore; megalopae reinvade estuaries using flood-tide transport (DeVries et al. 1994).
- Some evidence exists for selective settlement (Brodie et al. 2005, Welch et al. 2015), but the mechanism driving the process is unknown.
- Cues from favorable habitat may stimulate and/or accelerate molting by megalopae to the benthic first crab instar, whereas cues from unfavorable habitat may inhibit and/or delay metamorphosis.
- Odors of adult conspecifics and/or adult habitat have been shown to accelerate molting in lab-reared megalopae of all 3 species (e.g. Christy 1989, O'Connor 1991, O'Connor & Judge 2004, O'Connor & Van 2006) and stimulate settlement in field-caught megalopae (Welch et al. 2016).
- Recent studies have determined that environmental acoustic cues trigger settlement behavior in some common coral species, oyster, and reef fish larvae (e.g. Vermeij et al. 2010, Lillis et al. 2014, Barth et al. 2015) and coastal crab megalopae (Stanley et al. 2011).
- It is important to study acoustic cues because: sound travels farther underwater and can be detected at greater distances than chemical cues; *Uca* spp. rely on sound for mating rituals; and the spatial extent and intensity of anthropogenic sound in the ocean is increasing.

Hypothesis

Field-caught megalopae will be stimulated to molt faster when exposed to the specific sound of their adult habitat and chemical cues from adult conspecifics.

Methods

- We deployed a SoundTrap in a sandflat and salt marsh to record the soundscape of adult settlement sites (Fig. 1) over a full tidal cycle, with a flood tide occurring between 2-4am, when fiddler crab megalopae are transported inshore.
- Using Adobe Audition, we clipped a 60-minute sound segment during the peak of the flood tide from each recording and amplified the signals so megalopae in each experimental container in the were exposed to ~115dB re: 1 µPa (~20dB louder than sounds in the control tanks: ~97dB re: 1µPa).
- To prepare chemical cue odor water, we collected adult *U. pugilator* from the Rachel Carson Estuarine Research Reserve on Carrot Island in Beaufort, North Carolina (USA) and adult *U. pugnax* from the Bell Creek Salt Marsh, approximately 10 km from the Duke University Marine Laboratory (Fig 1). We soaked 50 g of adult crabs in 1000 ml of filtered seawater for 1 hour (Fig. 2a).
- We collected megalopae with a plankton net near the Duke Marine Lab in Beaufort, North Carolina on nocturnal flood tides in July 2016 (Fig. 2b).
- We placed 26 megalopae in 400 ml of either filtered estuarine seawater or one of the 2 odors for 8 days at 25º C and a 14:10 light:dark cycle in one of the three sound treatments. We changed the water and fed the megalopae *Artemia* nauplii daily (Fig. 2c).
- Molt status was monitored 4 times daily (0600, 1200, 1800, 2400). We preserved any megalopae that molted into crabs or died in 95% ethanol for identification. After the 8 days, we preserved all remaining megalopae.
- Because the three species cannot be visually distinguished at the megalopa or first crab stage (O'Connor 1991), we identified all individuals to species using the multiplex PCR method of Welch et al. (2015) (Fig. 2d).

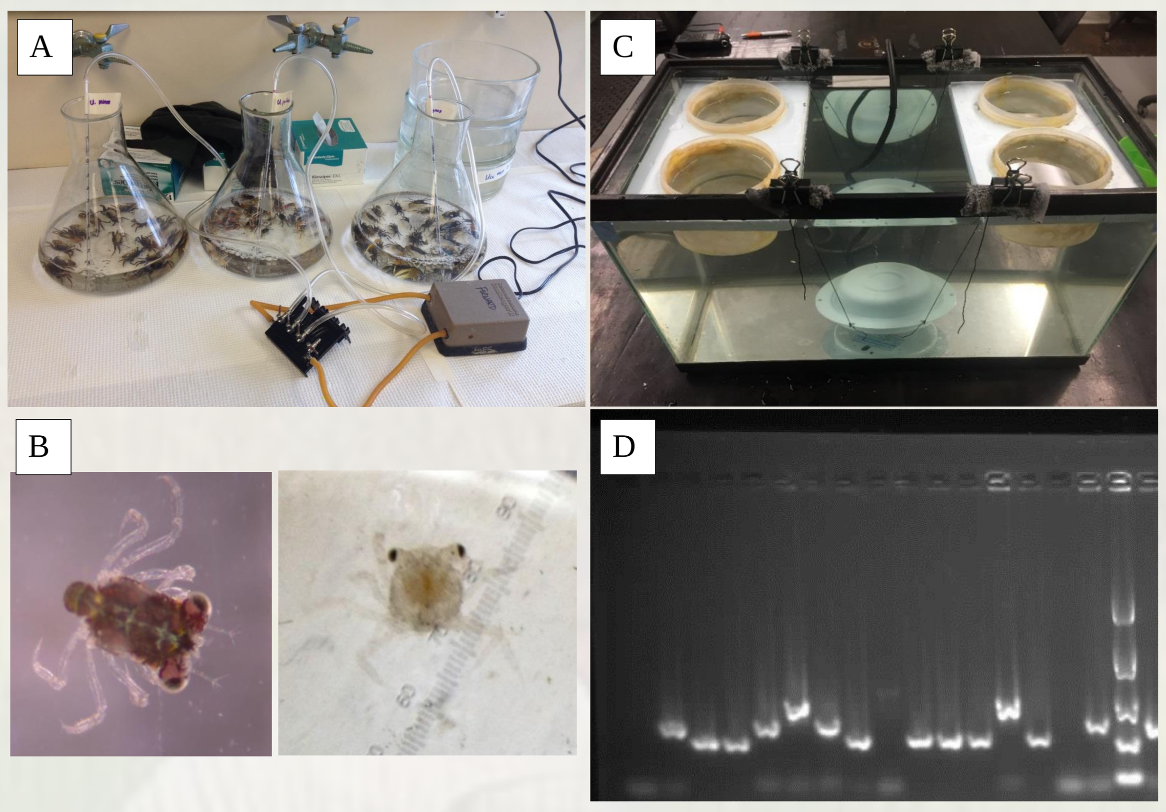

Figure 2. Adult crabs soaking to make odor waters (A), fiddler crab megalopa (left) and first crab instar (right) (B), individual tank setup (C), and picture of a gel to ID species (D).

Uca pugilator

Uca pugnax

Uca minax

Sound treatment did not appear to stimulate molting in megalopae of any of the fiddler crab species.

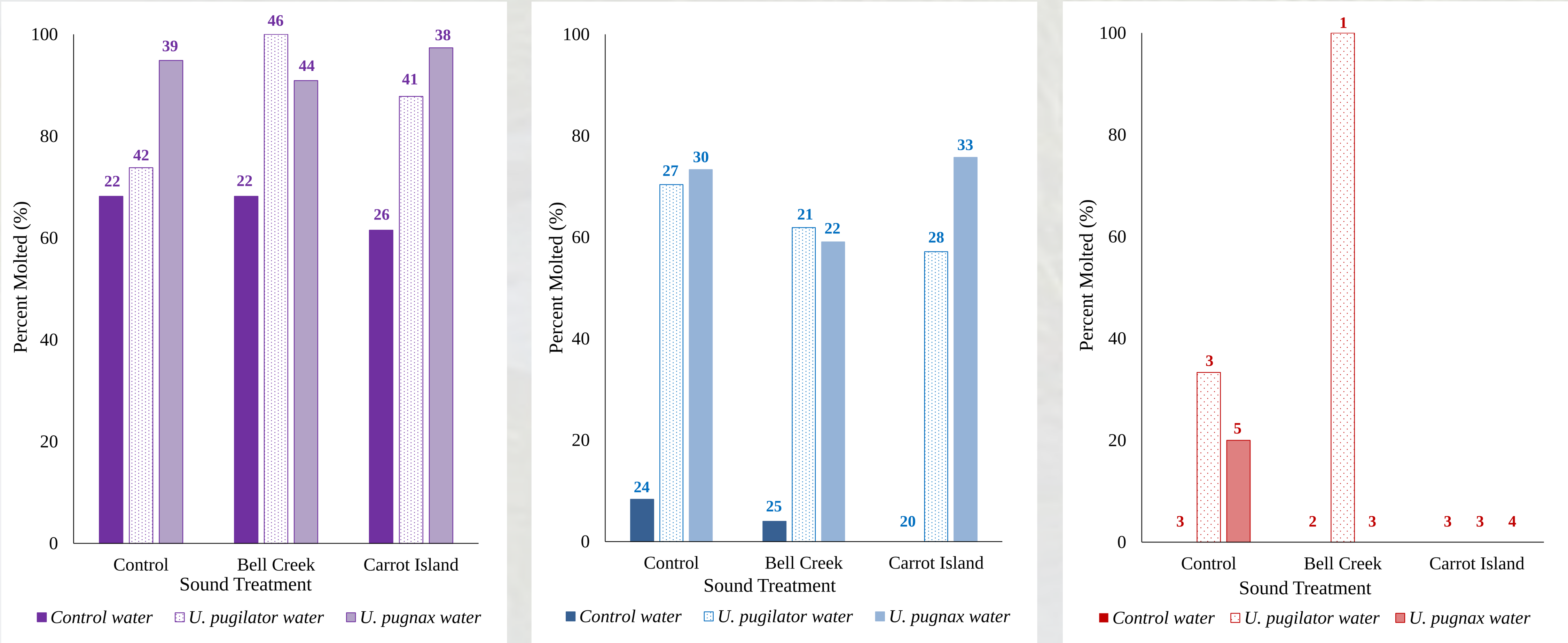

Figure 3. The proportion of each species of fiddler crab (genus *Uca*) megalopae that molted in each water treatment—control (no odor), *U. pugilator* odor water, and *U. pugnax* odor water—while being exposed to one of three sound treatments—control (no sound), Bell Creek sound treatment, and Carrot Island sound treatment. Number above indicates each sample size.

Results

Sound does not appear to affect settlement behavior of *Uca* spp. megalopae (Fig 3).

- Over half (>60%) of *U. pugilator* megalopae molted in each sound-chemical cue treatment combination. In the control sound treatment, a significantly higher proportion of megalopae molted in *U. pugnax* odor water than control water ($z=-2.82$; $p<0.005$) or conspecific water ($z=2.58$; $p=0.010$). In the Bell Creek and Carrot Island sound treatments, significantly more molting occurred when the megalopae were exposed to congeneric water than control water. A higher proportion of *U. pugilator* megalopae molted in the Bell Creek sound treatment (100%) than in the Carrot Island sound treatment (87.8%) when the megalopae were exposed to conspecific odor water ($z=-2.44$; $p=0.015$).
- Fewer than 10% of *U. pugnax* molted in the control water treatments in each of the sound treatments, but they molted significantly more (>55%) in each of the sound treatments when exposed to either *U. pugilator* or *U. pugnax* odor water. Exposure to conspecific odor water compared to exposure to *U. pugilator* odor water, however, did not result in a higher proportion of molts in any of the sound treatments. There was also no difference between the proportion that molted in *U. pugnax* odor water in the control sound treatment and the proportion that molted in the Bell Creek or Carrot Island sound treatments.
- Very few of the *U. minax* megalopae exposed to any sound-chemical cue treatment combination molted during the experiment (3 out of 27; 11.1%). There was no significant difference between the proportion that molted in the control sound treatment and the proportions that molted in the Bell Creek and Carrot Island sound treatments, even when odor water treatment was taken into consideration. A much larger sample size is needed before we can determine whether sound influences settlement behavior in *U. minax* megalopae.

Conclusion

Fiddler crab megalopae do not appear to be stimulated to molt by the sound cues of the habitat that their adults inhabit. Instead, they appear to rely on chemical cues released by their adult conspecifics or congenerics as a settlement cue.

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