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
Ecology and Evolutionary Biology | Environmental Studies | Marine Biology | Terrestrial and Aquatic Ecology

Comments
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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. 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.

Methods
• We deployed a Sound Trap 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 inland.
• 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: 1μPa.
• To prepare chemical cues, 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 25°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 megalopa 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 stage we identified all individuals to species using the multiplex PCR method of Welch et al. (2015) (Fig 2d).

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 combination. In the control sound treatment, a significantly higher proportion of megalopae molted in U. pugnax odor water than control water (p=2.82, p=0.005) or conspecific water (p=2.58, p=0.010). In the Bell Creek and Carrot Island sound treatments, significantly more megalopae molted when the megalopae were exposed to conspecific 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 (87.5%) when the megalopae were exposed to conspecific odor water (p=2.44, p=0.015).
• Fewer than 10% of U. pugnax molted in the control sound 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 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 a given sound treatment and the proportion of megalopae 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.

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 (Crate 1975) but occupy different microhabitats (Teal 1938, Miller & Mason 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.
• Fiddler crab zoae of all 3 species develop offshore; megalopa reinvade estuaries using flood-tide transport (DeVetes 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 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, oysters, 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; Uuc 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.

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 Sound Trap in each sample site. (Figure 1)

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

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. Note above indicates each sample size. (Figure 3)

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Literature Cited