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Larvae of native and non-native crabs behave differently in response to chemical cues from potential fish predators and adult crabs

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ABSTRACT

The behavior of planktonic larval invertebrates can play an important role in predator avoidance. Megalopae, the last larval stage in crab development, are strong swimmers yet are vulnerable to predation by fish. The behaviors of megalopae of the native mud crab *Dyspanopeus sayi* (Smith, 1869) and the Asian shore crab *Hemigrapsus sanguineus* (De Haan, 1835) in flowing seawater were analyzed in response to chemical cues from potential fish predators and adult crabs. Fish predators consisted of cunner (*Tautogolabrus adspersus* [Walbaum, 1792]), tautog (*Tautoga onitis* [Linnaeus, 1758]), and mummichog (*Fundulus heteroclitus* [Linnaeus, 1766]). Larvae were reared to early and late stages of megalopal development. Individual megalopae were dropped into a horizontal glass tube containing a stream of flowing artificial seawater (control) or seawater containing chemicals from fish or adult crabs. Behaviors of each megalopa were observed and categorized as swimming, walking, tumbling, or inactive. In control seawater, megalopae of both species swam upstream more than in seawater with fish or crab cues. Mud crab megalopae were inactive in the presence of chemicals from all fish species whereas Asian shore crab megalopae were inactive more in the presence of cues from tautog and cunner than from mummichog, a species unrelated to fish predators in its native range. Late-stage *D. sayi* megalopae walked on the bottom of the tube in response to confamilial adult cues more than *H. sanguineus*. Both species can therefore detect chemical cues in flowing water, even early in megalopal development, and *H. sanguineus* can distinguish cues from different potential fish predators.

1. Introduction

Many benthic invertebrates have planktonic larval development that leads to dispersal from adult habitats. At the end of planktonic development, larvae must locate an area suitable for settlement and juvenile growth. During dispersal and settlement, the risk of predation by predators, especially coastal and estuarine fishes and pelagic gelatinous invertebrates, is high (Morgan, 1995), and predation is a major selection pressure. Antipredator mechanisms of larvae include chemical and morphological defenses and behaviors that might lessen predation, such as flaring of spines and flexing of the body as well as swimming and sinking (Morgan, 1995).

Water-soluble chemicals are often cues for metamorphosis of brachyuran crab larvae (Epifanio and Cohen, 2016; Forward et al., 2001) but behavioral responses of larvae to water-soluble chemicals, especially those from potential predators, are less well known. Because crab megalopae, the last larval stage in the brachyuran life cycle, are strong swimmers compared to larvae that rely on ciliary action for locomotion (Chia et al., 1984; Luckenbach and Orth, 1992), behavioral responses could be effective in predator avoidance. For example, late-stage larvae of some decapod species respond to chemical cues from predators and adult habitats by orienting and swimming toward or away from the source of the cue (Boudreau et al., 1993a; Diaz et al., 1999; Forward et al., 2003). Less well understood is how different species respond to the same predator cue, the variety of behavioral responses possible, and whether behaviors change as larvae mature.

The purpose of the study was to determine if the behavioral responses of early and late stage crab megalopae change when chemical cues from potential fish predators and adult crabs are added to flowing seawater. The crab species tested were *Dyspanopeus sayi*, which is native to the western North Atlantic Ocean, and *Hemigrapsus sanguineus*, an invasive species from the western North Pacific Ocean that has successfully colonized the Atlantic coast of North America as well as Europe (Epifanio, 2013; Gothland et al., 2013). The mud crab *D. sayi* inhabits estuaries (Williams, 1984) and its larval stages are common in estuarine water (Sandifer, 1975). The Asian shore crab *H. sanguineus* is common in rocky intertidal habitats along estuaries and open coasts (Epifanio, 2013; O'Connor, 2014) and its larval stages have behaviors

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that could facilitate export from estuaries (Cohen et al., 2015; Park et al., 2004, 2005). Megalopae of both species molt in response to odors from conspecific adults (Kopin et al., 2001; Steinberg et al., 2007), which indicates the ability of both species to detect and physiologically respond to chemical cues. Moreover, both species transition from the plankton to the benthos in coastal and estuarine waters, where potential fish predators are common. Potential fish predators include cunner (*Tautogolabrus adspersus*) and tautog (*Tautoga onitis*), species in the family Labridae that prey on invertebrates and have confamilial species in the western North Pacific Ocean (Rasch and O'Connor, 2012), and mummichog (*Fundulus heteroclitus*) a species in a family (Fundulidae) native to the Atlantic coast of North America but absent in the Pacific Ocean.

2. Materials and methods

2.1. Collection and housing of fish and crabs

Cunner (*T. adspersus*, 6–12 cm total length [TL]) and tautog (*T. onitis*, 8–15 cm TL) were collected along the rocky coastline of New Bedford, Massachusetts (41.600443, − 70.901347) and Fairhaven, Massachusetts (41.606044, − 70.831950) with the use of a seine net. Mummichogs (*F. heteroclitus*, 4–9 cm TL) were collected using baited traps or a seine net in a brackish stream in Dartmouth, Massachusetts (41.526410, − 70.983783) and along the Westport River estuary in Massachusetts (41.502564, − 71.032557). Fish were collected in the summer of 2013 and 2014 under Commonwealth of Massachusetts Division of Marine Fisheries scientific permit #054542.

Fishes were housed separately by family or species in mesh baskets (35.6 cm diameter) or plastic aquaria (40.7 L) with rocks and shells, and placed in a 727.5 L flow-through tank (15.2 cm high water level) with natural seawater (salinity 31) at ambient temperature. All fish were fed a diet of marine fish flakes (Ocean Nutrition Formula One®) daily. A few days prior to experimentation, fish separated by species were moved to 18.9 L containers with natural seawater (salinity 30) and air stones and fed small marine pellets (OMEGA ONE Natural Protein Formula®). After the fish were used in experiments, they were returned to their respective containers with natural seawater and kept for up to four days before being used again. Seawater was changed in each of the containers every two to three days, or after a fish death. After they were used twice in experiments, they were returned to flow-through tanks until they were needed again for more experiments. Fishes were housed and maintained under the University of Massachusetts Dartmouth Institutional Animal Care and Use Committee Protocol #13-03.

Adult Asian shore crabs, *Hemigrapsus sanguineus* and the mud crabs *Dyspanopeus sayi*, *Eurypanopeus depressus*, and *Panopeus herbstii* were collected by hand from rocky intertidal shorelines of southern Massachusetts and Rhode Island. Mixed species of mud crabs were used to generate chemical cues because identifying each crab to species requires manipulating mouth appendages (Williams, 1984), possibly causing stress chemicals to be produced. Tanks holding adult crabs contained aerated artificial seawater (salinity 30, Instant Ocean®) and clean rocks for shelter. Adult crabs were fed OMEGA ONE® small marine pellets daily and water was changed frequently (every 1–3 days).

2.2. Culture of crab larvae

Egg-bearing females of the Asian shore crab *Hemigrapsus sanguineus* and the mud crab *Dyspanopeus sayi* (identified post-mortem) were housed separately by species in aerated natural seawater until they were close to releasing larvae, at which time females were placed individually in covered glass fingerbowls (10.5 cm diameter) with 200 ml artificial seawater (salinity 30, Instant Ocean®) and the antibiotics sodium penicillin G (21.9 mg l^{-1}) and streptomycin sulfate (36.5 mg l^{-1}) to reduce bacterial growth. Females were not fed during this time. The female crabs were kept in an environmentally controlled chamber, at 25 °C ± 1 °C, with a 14 h light:10 h dark cycle. Females were transferred to new fingerbowls with new artificial seawater and antibiotics daily until larvae hatched.

Larvae were placed in glass fingerbowls (19 cm diameter) with 750 ml artificial seawater and antibiotics in groups of approx. 150 individuals. Larvae were fed newly-hatched brine shrimp nauplii (*Artemia* sp., Platinum Grade Argentemia®) daily. The larvae were incubated in the environmentally controlled chamber, and moved to clean fingerbowls with new seawater and food daily. Zoeae were reared to the megalopal stage, at which time density was reduced to approx. 75 individuals per bowl. The day of molting to the megalopal stage was considered to be day zero. Each brood of megalopae was used in experiments at both the "early" stage of megalopal development (day 1 for *D. sayi*, day 2 for *H. sanguineus*) and the "late" stage (day 4 for *D. sayi*, day 5 for *H. sanguineus*). Different days were chosen for each species because megalopae of *D. sayi* molt to the first crab stage earlier than *H. sanguineus* megalopae.

2.3. Preparation of chemical cues

Twenty-four hours prior to experimentation, fish or adult *H. sanguineus* or mixed species of mud crabs (*D. sayi*, *E. depressus* and *P. herbstii*) were rinsed twice with artificial seawater, and placed in groups in covered aquaria (30.5 cm L × 15.25 cm W × 17.75 cm H) containing aerated artificial seawater (salinity 30). The aquaria were standardized by a volume ratio of 50 ml of animal: 1950 ml of artificial seawater (Rasch and O'Connor, 2012). Tanks holding adult crabs also contained clean rocks for shelter. Fish and crabs were not fed. After 24 h, the water was passed through a net to collect the animals and remove any large particles. The crabs or fish were returned to their housing tanks, and the artificial seawater with the specific chemical cue was used in behavioral assays within 20 min. Test water with chemical cues was made with as many different individual crabs and fish as possible (Table 1).

2.4. Behavioral assay

A Chemical Rheotaxis Induction System (CRIS), designed by R.A. Tankersley (formerly at the Florida Institute of Technology), was used to determine megalopal behavior in response to the various chemical cues in flowing water. The CRIS consisted of a 42.5 cm long glass tube (6.35 mm inner diameter), with three openings: an inflow opening, an outflow opening, and a 5 cm long vertical tube halfway between, into which the megalopae were dropped to enter the flow of water (Fig. 1). The average flow speed of water was 1.16 cm·s^{-1} (range 1.05–1.26 cm·s^{-1}) for *D. sayi* and 2.11 cm·s^{-1} (range 1.90–2.32 cm·s^{-1}) for *H. sanguineus*. These flow speeds were chosen because they allowed the megalopa to actively swim into flowing

Table 1
Summary of the number of broods of larvae of each crab species tested in each cue, as well as the number of different groups of crabs or fish and the number of individuals per group used to create each type of chemical cue.

Crab Species	Cue	# of broods	# of groups	# of indiv/group
D. sayi	Control	6		
	Adult crabs	4	2	18–21
	Cunner	4	2	8–13
	Tautog	4	2	1–5
	Mummichog	2	1	29–33
H. sanguineus	Control	5		
	Adult crabs	4	2	14–17
	Cunner	3	2	8–13
	Tautog	5	3	1–5
	Mummichog	4	3	9–21