



Amphipods as potential prey of the Asian shore crab *Hemigrapsus sanguineus*: Laboratory and field experiments

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ABSTRACT

The invasive crab *Hemigrapsus sanguineus* has a generalist diet, consuming primarily macroalgae and sessile and sedentary invertebrate prey. To assess the dietary importance of motile prey, predation by *H. sanguineus* on the amphipod *Hyale plumulosa* was examined in laboratory and field experiments. Male and female crabs 7–27 mm in carapace width were offered *H. plumulosa* in laboratory microcosms with and without sediment (sand and rocks) present. Despite sex differences in claw morphology, male and female *H. sanguineus* consumed similar numbers of amphipods. Large crabs of both sexes consumed fewer *H. plumulosa* than small crabs, and consumption was lower when sediment was present. Crab abundance was then manipulated in the field in randomized complete block experiments in the lower rocky intertidal zone. In three 14-day experiments, *H. sanguineus* had no negative effect on amphipod abundance. In fact, excluding crabs from cages led to lower amphipod densities. The results of the 2-species predator–prey laboratory experiments did not transfer to the natural environment, suggesting that complex interactions exist between *H. sanguineus* and motile amphipod prey in rocky intertidal areas.

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1. Introduction

Documenting specific impacts of nonindigenous species in marine environments can be challenging. Impacts can include direct and indirect interactions between native and non-native species resulting in either negative or positive outcomes (Bruno et al., 2005). Of the diverse potential ecological effects of invading animal species, predation of native species is of great concern. Predation could significantly affect native prey species if the number, biomass, or foraging rate of the invading species is large.

A successful marine invader in both North America and Europe is the Asian shore crab *Hemigrapsus sanguineus*. In less than a decade, the species went from being rare to extremely common in rocky intertidal habitats in southeastern New England (Kraemer et al., 2007; O'Connor, 2014). *H. sanguineus* is not a large crab, with maximum carapace width ~ 40 mm (Fukui, 1988), but it can reach very high densities (> 100 per m²) (Kraemer et al., 2007; O'Connor, 2014). Gut analyses show that it is omnivorous, consuming algae and invertebrates (Ledesma and O'Connor, 2001; Lohrer et al., 2000). Laboratory studies of predation have focused on impacts of Asian shore crab foraging on sessile prey such as macroalgae and bivalve molluscs (Bourdeau and O'Connor, 2003; Brousseau and Baglivo, 2005; Brousseau et al., 2001;

DeGraaf and Tyrrell, 2004; Griffen and Delaney, 2007). Field experiments have documented impacts on mussels and barnacles (Griffen and Byers, 2009; Lohrer and Whitlatch, 2002; Tyrrell et al., 2006), yet predatory effects of *H. sanguineus* on motile prey have been less well studied.

Common motile macroinvertebrates in rocky intertidal habitats are amphipods, small crustaceans that consume macroalgae (Duffy and Hay, 2000) and are eaten by fish (Clark et al., 2006; Savaria and O'Connor, 2013; Stoner, 1979) and birds (Grant, 1981). An epifaunal amphipod species often found in protected embayments in New England is *Hyale plumulosa*, which lives under rocks and in crevices, where it crawls about (Bousfield, 1973). Amphipod remains have been found in the guts of *H. sanguineus* (McDermott, 1999) and the crabs *Cancer irroratus* and *Carcinus maenas* (Donahue et al., 2009).

Because of their small size, high abundance, and epifaunal habitat, amphipods are potential prey of *H. sanguineus*. McDermott (1999) observed *H. sanguineus* actively pursue and capture *H. plumulosa* in preliminary laboratory experiments. Amphipods (*Gammarus* spp.) in laboratory microcosms with rocks or algae were eaten by both *C. maenas* and *H. sanguineus* (Griffen and Byers, 2006). In the present study, laboratory experiments were conducted to determine the ability of male and female *H. sanguineus* of various sizes to consume *H. plumulosa*. The effect of *H. sanguineus* on the abundance of *H. plumulosa* was then tested in field experiments in which crab density was manipulated and other prey species were available to *H. sanguineus*.

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2. Materials and methods

2.1. Collection of crabs and amphipods for experimentation

Quadrats were sampled to determine densities and sizes of the *H. sanguineus* population during the late spring and summer at the field experimental site located in Clark's Cove, New Bedford, Massachusetts, USA (41.594856, –70.911341), along a west-facing coastline with a tidal range of 1.1 m. Sediment at the site consisted of coarse sand with cobble rock cover of ~95%. Little macroalgae was present in the intertidal zone but it occurred subtidally on large rocks.

A 0.25 m² quadrat was randomly placed near the low tide mark, each rock within the quadrat was removed, and all crabs found were captured. The underlying sediment was probed to recover any buried crabs. Male and female crabs were then separated into the following four size classes based on carapace width (CW) for use in both laboratory and field experiments: 1) 7–11.9 mm, 2) 12–16.9 mm, 3) 17–21.9 mm, and 4) 22–26.9 mm. These size classes had the highest representation at the study site; few *H. sanguineus* above 26.9 mm in size were collected.

Crabs were placed according to size class into 13 L holding tanks containing rocks and aerated natural seawater and fed a diet of small blue mussels (*Mytilus edulis*), green algae including *Ulva* spp. and *Enteromorpha* spp., and 2.5 mm commercial fish pellets (Corey Aquafeed). The water had been filtered (ambient seawater passed through two 25 µm pore sand filters and ultra-violet filtration) and was replaced once a week. During the experiments, the seawater was ~22 °C and had a salinity of ~32.

Amphipods (*H. plumulosa*) for laboratory experiments were collected at the same location. Sediment containing amphipods was placed into a 19 L bucket filled with seawater. The contents were swirled by hand, causing any amphipods in the sediment to swim up into the water column. The water was then decanted onto a 500 µm sieve and any resulting amphipods were collected. In the laboratory, the amphipods were kept in aerated natural seawater under conditions described above, and fed green algae (*Ulva* spp. and *Enteromorpha* spp.).

2.2. Laboratory feeding trials

The ability of male crabs in 4 size classes (7–11.9, 12–16.9, 17–21.9, and 22–26.9 mm CW) to capture and consume amphipods (*H. plumulosa*) 2.5–12.0 mm in length was tested. Feeding trials were conducted in 20.3 cm diameter glass culture bowls. Trials were conducted with and without sediment (sand and rocks), to assess sediment as a refuge from predation. A 1 cm layer of coarse sand (500–2000 µm) and three rocks, one small (253–624 cm²), one medium (654–1593 cm²), and one large (1895–4341 cm²), were added to half of the experimental replicates to provide refuge for the amphipods. Predation of amphipods by female crabs in the smallest and largest size classes (7–11.9 and 22–26.9 mm CW) was tested without sediment, and sediment was used in trials with the largest size class.

Experimental replicates consisted of one *H. sanguineus* and ten amphipods per bowl, equivalent to ~310 amphipods and 31 crabs per m². A total of 40 replicates with sediment and 40 replicates without sediment were conducted for each size class of crab. Only crabs with intact chelae were used in experiments, and each crab was used only once. Both crabs and amphipods were held without food for 24 h prior to experiments.

At the beginning of a trial, 20 bowls were filled with 1 L of filtered (25 µm) seawater (salinity approx. 32). The water was aerated and bowls were covered with clear plexiglas to prevent crabs from escaping. The crab and amphipods were allowed to interact for 24 h under a summer day:night cycle, in natural light supplemented with fluorescent light during the daytime. Controls consisted of bowls with only amphipods present (with and without sediment) to account for natural

mortality. One control bowl was run during each feeding trial; all 10 amphipods were always found in controls at the end of the experiment.

At the end of each trial, each bowl was uncovered, the crab was removed, and the number of amphipods remaining was counted. For replicates with sediment, each rock was rinsed to ensure that no amphipods remained attached to it, and the sand in the bowl was carefully searched for amphipods. Missing amphipods were considered to have been consumed. A few crabs (≤ 3 per treatment) molted during the experiment and were excluded from the analysis.

2.2.1. Statistical analysis

Data did not meet assumptions of parametric analysis even with transformation so nonparametric analyses were conducted. For male crabs, differences in the number of amphipods missing from the bowls in each crab size class were examined using a Kruskal–Wallis test followed by the Dunn's post-hoc test for multiple comparisons. Experiments with and without sediment were tested separately. For female crabs, Mann–Whitney Rank Sum tests were used to compare consumption by small (7–11.9 mm CW) and large crabs (22–26.9 mm CW), and trials with large crabs with and without sediment.

2.3. Field experiments

Randomized complete block caging experiments were used to determine if *H. sanguineus* would have an impact on amphipod abundance in the natural environment. Three experiments were done in Clark's Cove in 2002: from 24 May–12 June, 4–23 July, and 16 August–4 September. Experiments were conducted in the lower third of intertidal zone; the average intertidal elevation was 27 cm above MLW, with experiments conducted at higher elevation in August (34 cm) than May (19 cm).

Treatments consisted of an open plot (0.25 m²) marked by 4 stakes (= natural control), a partial cage (= cage control), and cages stocked with *H. sanguineus* between 7 and 26.9 mm CW at densities representative of the field site (= 1X), double the natural density at the site (= 2X), or without *H. sanguineus* (= 0X). Cages at normal and double field densities were stocked with *H. sanguineus* at densities and sizes reflecting numbers found in surveys at the site before each experiment. Each block (n = 5) contained one replicate of each treatment randomly arranged in a linear fashion parallel to the shore, with each experiment running along 64–67 m of shoreline. Each experiment was conducted in undisturbed intertidal areas.

Cages were constructed of 2.54 cm diameter white PVC pipe and 3-way elbows in a cube-shaped frame that measured 0.5 m wide, 0.5 m long, and 0.5 m high, circumscribing a benthic area of 0.25 m². The PVC frame was filled with stones to add weight. Black plastic (HDPE) mesh (6.35 mm openings) was attached to all 6 sides of the frame using plastic cable ties. The mesh allowed amphipods to move into and out of the cage while preventing entrance or exit of all but the smallest *H. sanguineus*, which would be unable to prey on many amphipods. Partial cages had only two sides of the frame covered with mesh, as well as a top and bottom mesh panel.

2.3.1. Experimental procedure

Experimental set-up required a total of 5 days, with each block requiring one day for deployment. A 0.25 m² square quadrat was placed over the substrate, rocks were moved to a large bin, and all *H. sanguineus* in the quadrat were removed. The top 5 cm of sediment within the quadrat was also removed and placed into another bin. The cage was fitted into the quadrat, excavated sediment and rocks were replaced inside the cage, and the outside of the cage was secured to metal rods embedded in the substrate. The area around the cage was returned as close as possible to the original condition. Each treatment was spaced 1 m from the adjacent treatment. Once all the cages had been buried into the ground, the tops were closed with cable ties. The 0.25 m²

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