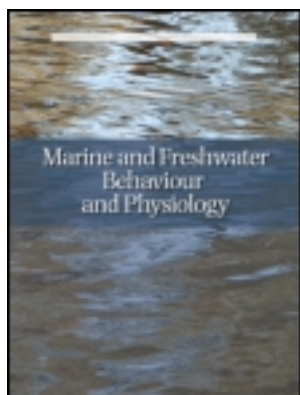


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## SHORT COMMUNICATION

### Conditioning the crab *Carcinus maenas* against instinctive light avoidance

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*Carcinus maenas* is an invasive species found in Northern American waters overtaking the less adaptable species and responding to various forms of conditioning. In this study, we conditioned *C. maenas* to reverse its innate light avoidance behavior. Within 6 days of testing, 21 out of 30 crabs were successfully trained to enter a beam of light to receive food, although their instincts are to seek shelter from predators in dark areas. Some took as little as 2 days to reverse their light aversion. They also responded faster once trained. Larger crabs had faster response times than smaller ones, while there was no difference between sexes. The conditioned response lasted for at least 4 days without reinforcement. The rapid learning abilities observed during this experiment may help to explain the pervasive success of *C. maenas* as an invasive species.

**Keywords:** conditioning; crustacea; *Carcinus maenas*; light attraction; invasive species

Behavioral research has been conducted on crustaceans as models for basic memory and learning systems (Abramson and Feinman 1987, 1988; Hermitte and Maldonado 1991). Relative to their body size, crustaceans have small brains and are not considered to have complex cognitive recognition (Carew and Sahley 1986; Fanenbruck et al. 2004). However, their brains are complex and contain several integration centers (Sandeman et al. 1992). Various conditioning procedures focusing on classic learning paradigms have been explored in the European shore crab, *Carcinus maenas*, such as negative reinforcement (Abramson and Feinman 1987, 1988; Abramson et al. 1988; Feinman et al. 1990). In one study, *C. maenas* showed conditioning capabilities by decreasing their acclimation time in lighted chambers (Dimant and Maldonado 1992). Another study conditioned the crab *Chasmagnathus granulatus* by applying a mild electric shock to the walking legs of the crab in a dark environment. *Chasmagnathus granulatus* began to associate darkness with shocks and were eventually trained to display an enhanced running response whenever exposed to darkness (Hermitte and Maldonado 1991).

*Carcinus maenas* is an invasive species widely distributed in North America along both the Atlantic and Pacific coasts (Carlton and Cohen 2003). It is commonly found in sheltered marine and estuarine habitats on mud, sand, and rock substrates, as well as in cordgrass marshes (Crothers 1968, 1970; Berrill and Berrill 1981). They are

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known to aggregate under rocks and other debris on sand during low tide and often inhabit burrows in *Spartina* banks (Edwards 1958; Crothers 1968; Ropes 1968; Klein Bretler 1976; Dare and Edwards 1981). If given a choice, *C. maenas* will live in a dimly lit environment (Palmer 1964) and avoid brightly lit areas presumably to evade predation. Common predators include birds, fish, shorebirds, and octopuses (Hacunda 1981; Malpass 1992).

The goal of our study was to demonstrate the conditioning abilities of *C. maenas* to new behaviors contrary to their instincts. We chose to train the crabs to directly move toward light to receive food, against their natural preference to hide from predators in dark, covered areas. Specifically, we hypothesized that the crabs could be conditioned to approach a light field without direct food incentive.

We held 30 *C. maenas*, collected from Little Sippewissett Marsh, Massachusetts in the artificial sea water system at Boston University for 26 days. Size ranged from 3.4 to 6.2 cm carapace width (mean size 4.9 cm). We categorized all crabs with a carapace width greater than 4.8 cm to be large and less than 4.8 cm to be small. Of the 21 crabs that completed all the phases of the experiment, 13 were female and 8 male. The crabs were randomly split into two groups and fed on alternate days to keep them motivated to look for food during trials. Each crab underwent 6 days of trials as they were only subjected to testing on days they were not fed. Thus we carried out all trials over a 12-day period. Trials were performed in a  $38 \times 32 \times 14 \text{ cm}^3$  white plastic bin (Figure 1) located in a dark room. A grid with  $13 \times 11 \text{ cm}^2$  cells was drawn on the bottom of the bin (Figure 1). During trials, a beam of bright white incandescent light from a MiniMag™ flashlight was introduced over a randomly selected grid cell (Figure 1). The  $89 \text{ cm}^2$  circular area

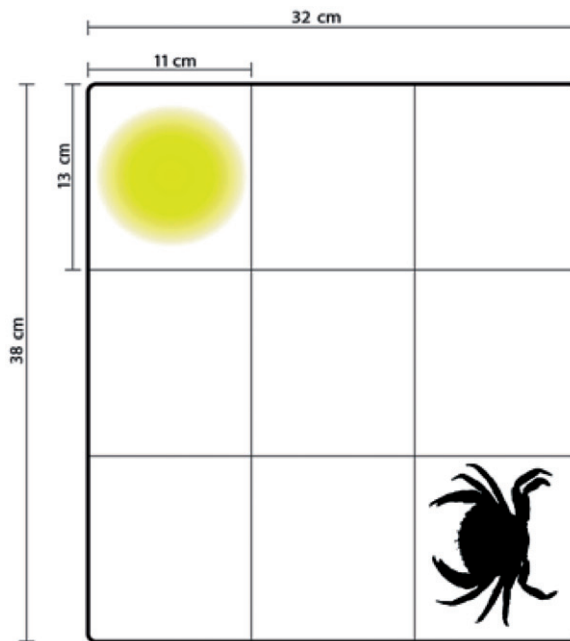


Figure 1. Container used during trials with average crab size relative to grid and location of light beam.

of the beam was focused on the center of the cell (Figure 1). Approximately 0.1 g of thawed frozen shrimp was used as food for all trials.

An increasingly challenging four-phase training process was developed for this experiment. Each day, all crabs were tested until they either completed or failed three consecutive trials. Crabs were immediately advanced to the next phase after successful completion of three consecutive trials of the previous phase. Thus, crabs could move through more than one phase during a test day; they could also spend several test days in one phase before reaching the criterion of three consecutive successes to move to the next phase. During the first phase, we introduced food into the container and dispersed the food odor with a pipette to alert the crab to the presence of food. This phase was performed in complete darkness and light was only introduced after the crab had located the food. Once the crab found the food, we shone the light on the crab until it finished eating. Phase-1 was considered a training stage and individual trials were not timed. For the second phase, light and food were introduced simultaneously, and again we dispersed the food odor. For Phase-3 trials, we also introduced food and light simultaneously, but did not disperse the odor. Dye studies were performed without the crabs present solely to demonstrate that food odor did not naturally disperse throughout the bin during the 5-min trials. Dispersal of odor cues was therefore generated by the crabs' gill currents (Atema 1985). During the fourth and final phase, we only introduced light and the crab was expected to approach the center of the light field to receive food. For each trial, we moved the light to a new location to ensure that the crab had been conditioned for the light rather than for location.

Trial times were measured in seconds beginning when the crab was placed in the container and ending once the crab reached the center of the light field; if the entire carapace had not reached the light field within 5 min trials, were terminated. For data analysis, we evaluated search times during criterion trials, i.e. for each phase, we added the last three individual crab search times and averaged them among crabs. We evaluated the effects of sex and size on search times in criterion Phase-4 using a Student's *t*-test. After reaching Phase-4 criterion, we continued to measure the response times of successful crabs in subsequent days.

Over a period of 12 days, we successfully taught 21 out of 30 *C. maenas* (70%) to reverse their dark preference and enter a bright beam of light shone in their container without food or odor present. The number of crabs that had successfully completed Phase-4 increased over the test period from 2 on day 2 to 21 on day 6 (Figure 2). Subsequent data analysis is based on these 21 crabs.

The number of trials required to reach criterion and search times during criterion trials (sum of last three successful search times) were not significantly different among Phases (ANOVA,  $F=0.40$ ,  $p=0.67$ ): Phase-2,  $536 \pm 77$  s over  $4.7 \pm 0.4$  trials; Phase-3,  $460 \pm 95$  s over  $5.5 \pm 0.5$  trials; Phase-4,  $458 \pm 116$  s over  $5.5 \pm 1.0$  trials (Figure 3A–C). However, when the crabs reached criterion in Phase-4, they searched significantly faster ( $181 \pm 23$  s over three trials; ANOVA,  $F=4.1$ ;  $p=0.011$ ; Figure 3D); this efficiency was maintained in the subsequent days.

In the three Phase-4 criterion trials, a size effect was evident with smaller crabs searching  $103 \pm 17$  s per trial and larger crabs  $69 \pm 6$  s per trial (*t*-test,  $t=2.3$ ,  $p=0.017$ ). There was no significant difference in search times between females ( $80 \pm 10$  s per trial) and males ( $82 \pm 14$  s per trial). All crabs that completed Phase-4 before day 6 of the experiment showed memory retention and continued succeeding

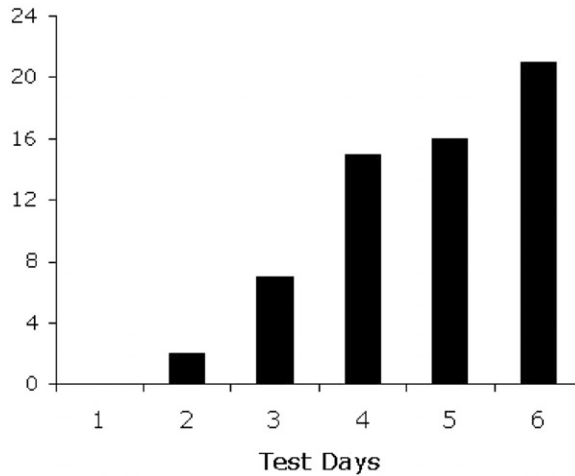


Figure 2. The number of crabs that were successfully trained consistently increased over the course of the experiment.

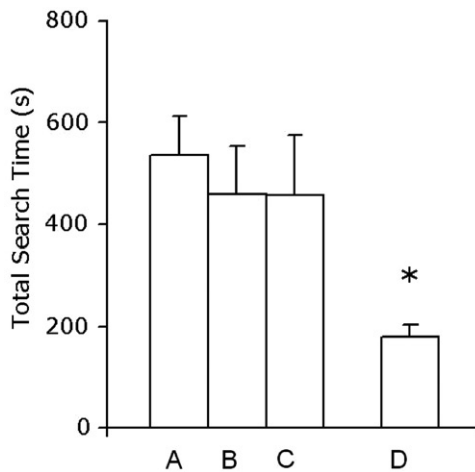


Figure 3. Search times. A, B, and C: summed search times of all trials of test Phases 2, 3, and 4 respectively. D: summed search times of three successive successful trials of Phase-4. Mean ( $n=21$ ) and standard error. D is significantly less than A, B, and C (ANOVA,  $p=0.011$ ).

in subsequent Phase-4 trials for up to 4 more days. The fastest crab took 14 s on average to reach the light spot on test day 6.

During our 12-day experiment, 70% of *C. maenas* were conditioned to approach a light (Figure 2). These results show that *C. maenas* can be rather quickly conditioned to look for food within a light field despite their natural tendency to hide and seek shelter in darker habitats. This result suggests that it is possible to teach *C. maenas* and perhaps other crustaceans and marine invertebrates to deviate from or even reverse innate behaviors. Crabs may respond more strongly to a light stimulus when paired with an unconditioned stimulus such as electric shock

(Smith and Baker 1960). However, in the more natural task of this study, *C. maenas* demonstrated impressive learning capabilities in response to light as the sole sensory stimulus that signified the likelihood of food. Since, in the final test, food was introduced after the crab had arrived in the light field, only light and not food odor was necessary and sufficient for attraction. Previous studies have shown that crustaceans are capable of rapid learning (individual odor memory: Karavanich and Atema 1998; Dimant et al. 2002; Gherardi and Atema 2005). The significance of this study is the rapidity of learning observed in *C. maenas* reversing their strong innate dark bias; light had become an attractant.

We found that larger *C. maenas* showed faster response times in Phase-4 trials than smaller crabs. Since carapace width increases with age (Paul and Paul 2000), this result implies that older crabs are capable of learning this behavioral reversal faster than younger ones. We did not test if they were learning faster or running faster once learned. Larger *C. maenas* have been shown to eat more mollusks than smaller crabs, which generally prefer *Spartina*, a developmental shift from grazing to hunting (Cohen et al. 1995), and we used a piece of shrimp as bait. Conceivably, larger crabs have fewer predators and take greater risks, although in many species the reverse is seen.

The evolutionary success of organisms can be dependent on their ability to hide from predators (Palma and Steneck 2001). Marine decapods such as *C. maenas* are particularly vulnerable to predators when exposed in open water and therefore seek refuge within their respective habitats (Stein and Magnuson 1976; Wilson 1989; Wahle and Steneck 1992; Beck 1995; Stachowicz and Hay 1996; Moksnes 2002). The methodology for this experiment was designed for simplified conditions: there was an absence of predators and no available shelter for the crabs. We used intentionally simplified conditions to test *C. maenas* for its ability to reverse its response to a single stimulus: light. A subsequent series of experiments could test if habitat complexities representative of field conditions such as predation and shelter would prevent or delay the reversal process.

Rapid learning may play an important role of *C. maenas* as an invasive species. They have a wider salinity and temperature tolerance, an omnivorous diet, and a higher reproductive rate than native crabs (Roudez et al. 2007). It would be interesting to test native crabs for their ability to reverse innate responses. Rapid learning may allow *C. maenas* to adapt to and take advantage of natural variability and compete favorably with native crabs.

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