

AVOIDANCE OF CONSPECIFIC INJURY-RELEASED CHEMICAL CUES BY FREE-RANGING *Gammarus lacustris* (CRUSTACEA: AMPHIPODA)

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Abstract—Behavioral responses to chemical cues have been demonstrated for a range of aquatic animals. Injury-released chemical alarm cues from conspecifics are released when a prey's predator is actively foraging. Detection of these cues elicits antipredator behaviors that reduce the probability of predation. Amphipod crustaceans in the genus *Gammarus* are widespread denizens of ponds and streams. Antipredator responses by *Gammarus* to conspecific alarm cues, and subsequent reduction of predation risk, are known from experiments in the laboratory. Here, we verify laboratory findings by demonstrating an avoidance response to alarm cues using a field population of *G. lacustris*. We used small traps baited with sponge blocks containing either water (control) or injury-released cues from *Gammarus*. We repeated the experiment twice. In both experiments, significantly fewer *Gammarus* were captured in traps with alarm cue sponges than in traps with water sponges. Predatory leeches *Dina parva* were attracted to *Gammarus* traps in the first experiment but not the second experiment. In the second experiment, we measured the individual weight of captured amphipods. Two size classes were present; small (1–5 mg) and large (35–108 mg). Both sizes contributed to the avoidance response. Within the large size class, small individuals were significantly less responsive to the alarm cue than large individuals, implying that small adult *Gammarus* may have different cost/benefit decision criteria for risk assessment than large *Gammarus*.

Key Words—*Gammarus*, amphipod, crustacea, alarm cue, alarm signal, predator–prey, avoidance behavior, leech, *Dina parva*.

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INTRODUCTION

Predator-prey interactions in aquatic environments are mediated, in part, by chemical cues released from various sources including disturbed and/or injured prey, and predators (Smith, 1992; Chivers and Smith, 1998; Kats and Dill, 1998; Tollrain and Havell, 1999; Wisenden 2000). Injury-released chemical alarm cues are released after a successful predatory attack and indicate imminent and proximate danger to conspecifics of the prey (see Chivers and Smith, 1998, for review). Aquatic taxa ranging from protozoa to amphibia use alarm cues to detect and avoid predators. Most of these studies have been conducted in the laboratory setting. While laboratory studies provide a carefully controlled environment in which to test specific hypotheses, the results lack the realism of the natural habitat. Field verification of laboratory findings is an important part of the scientific study of chemical ecology.

Amphipods in the genus *Gammarus* occur commonly in aquatic environments. They are small (<2 cm) detritivores that associate closely with the substratum and fall prey to a wide variety of predators. The substratum is often highly structured and water clarity often limited, reducing the reliability of visual information in assessing predation risk. *Gammarus* are well suited for the study of chemically mediated predator-prey interactions because they can be studied in the laboratory and in the field.

Laboratory populations of *Gammarus* show antipredator behavior in response to injury-released alarm cues of conspecifics (Williams and Moore, 1985; Mathis and Hoback, 1997; Wudkevich et al., 1997). In a laboratory study, latency to first capture by predatory green sunfish *Lepomis cyanellus* was significantly longer for *Gammarus* exposed to conspecific alarm cues than *Gammarus* exposed to water or heterospecific alarm cues (Wisenden et al., 1999).

It has not yet been established that free-living *Gammarus* respond with antipredator behavior to conspecific alarm cues in their natural habitat. We tested a field population of *Gammarus* for an avoidance response to alarm cues modeled after similar experiments on small fishes (e.g., Mathis and Smith, 1992; Chivers and Smith, 1994; Wisenden et al., 1995). Small sponge blocks soaked in water (control) or injury-released alarm cues of *Gammarus* were placed in small invertebrate traps. If field *Gammarus* avoid alarm cues, then we predicted that traps with alarm cues would catch fewer *Gammarus* than traps with water.

Recognition of conspecific alarm cues as indicators of risk generally occurs at a very early stage of development and requires no previous experience (Pfeiffer, 1963; Waldman, 1982; Magurran, 1989). However, there is some evidence from fishes that large individuals avoid alarm cues more effectively than small individuals (Mathis and Smith, 1992; Chivers and Smith, 1994; Chivers et al., 1995). Large, old individuals are more experienced than small, young individuals and may improve or acquire recognition of danger cues with age, particularly for responses to heterospecific alarm cues (Chivers et al., 1995). Alternatively, larger individuals

may be more vulnerable to predation and, thus, exhibit a lower response threshold than small individuals (Mathis and Hoback, 1997).

To investigate the effect of size on recognition of conspecific alarm cues we weighed captured *Gammarus*. We predicted that if aversion of alarm cue was acquired with age, or if cost/benefit decision criteria change with size, then large *Gammarus* would be caught in alarm cue traps in lower proportions than small *Gammarus*.

METHODS AND MATERIALS

Experiment 1. Alarm cue was prepared from adult *Gammarus lacustris* sampled from Erhard Pond in fall of 1997, located approximately 70 km SE of Moorhead, Minnesota, USA (46°30'N, 96°05'W). Erhard Pond does not contain any fish species. A stock solution of alarm cues was prepared by reducing 15 adult *Gammarus* (mean weight \pm SE = 52.1 \pm 1.6 mg, N = 15) to a fine pulp with mortar and pestle and diluting to a final volume of 37.5 ml with dechlorinated tap water. Small blocks of cellulose sponge (2 \times 2 \times 1.5 cm) each received 1.5 ml of the stock solution of alarm cues. This method of stimulus preparation is similar to the one successfully used by Wudkevich et al. (1997) and Wisenden et al. (1999). We thus prepared 15 sponges of *Gammarus* alarm cue and froze them at -20°C . Fifteen additional sponge blocks were soaked in 2.5 ml of dechlorinated tap water to control for the effect of sponge and water and then frozen at -20°C . Sponges were kept on ice during transport to the study site and remained frozen until used in the experiment to guard against any degradation of the cue.

We placed 30 invertebrate traps in Erhard Pond approximately 5 m apart along the shore, at a depth of about 0.5 m. The traps consisted of a 1-q (946-ml) wide-mouth Mason jar fitted snugly with a plastic funnel inserted into the jar opening with the apex of the funnel directed inward. The funnel was held in place by rubber bands attached to hooks on the outside of the funnel and the jar. Traps were laid on their side on the pond bottom. Inside each jar we placed a sponge soaked in either alarm cue or water. Traps were set in pairs (one control trap and one experimental trap simultaneously) at 5-min intervals, then pulled exactly 1 hr later at 5-min intervals in such a way that time in the water was held constant and equal among trap pairs and sponge types. Trap contents were stored and returned to the laboratory for counting.

Experiment 2. We repeated the experiment in spring 2000 at the same study site using similar methods. Adult *Gammarus* to be used for alarm stimulus were collected a few days prior to the field test and brought to the laboratory. Some of the adults were coupled in precopulatory amplexus. For each sponge (3.5 \times 3 \times 2 cm), we individually crushed a male-female pair (mean \pm SE combined weight of each male-female pair = 176.9 \pm 4.2 mg; N = 17) to a fine pulp with a mortar and pestle and diluted to a final volume of 10 ml with dechlorinated

water. Thus, stimulus strength in the second experiment was about four times stronger than in the first experiment. We prepared each of 17 sponges with 10 ml of alarm cue and another 17 sponges each with 10 ml of dechlorinated water (control). All sponge blocks were frozen at -20°C , and transported to the study site on ice to keep them frozen until needed.

Thirty-four traps were placed in the littoral zone within 1 m from the water's edge along the shoreline of Erhard Pond at approximately 5-m intervals. The traps used in the second experiment were commercial traps (MT3 minnow traps, Aquatic Ecosystems Inc.) and differed slightly from those used in the first experiment. The commercial traps consisted of 1-q (946-ml) wide-mouth Mason jars with inverted plastic funnels adapted to thread directly onto the jar mouth. The traps were laid on their side on the pond bottom as in the first experiment. Each trap contained one sponge block (control or alarm cue) and was set and retrieved in control-alarm pairs at 5-min intervals as for the first experiment. Time in the water was exactly 1 hr. Trap contents were returned to the laboratory to be counted and weighed to the nearest milligram. Large *Gammarus* were weighed individually. *Gammarus* <5 mg were too small to weigh individually. Small *Gammarus* for each trap were combined and weighed en masse to determine average weight.

Statistical Analysis. Statistical tests for avoidance behavior are one-tailed because we predicted a priori that *Gammarus* would avoid conspecific alarm cue. All other statistical tests are two-tailed.

RESULTS

Experiment 1. The median number (and 25th percentiles) of *Gammarus* per trap was 90 (50–117.5) and 40 (30–66.5) for control and alarm cue traps, respectively (Figure 1). Significantly more *Gammarus* were captured in control traps than alarm cue traps (Wilcoxon Mann Whitney test: $z = 1.95$, $P < 0.026$). These data are consistent with an avoidance response to conspecific alarm cues.

Leeches *Dina parva* entered traps with *Gammarus* alarm cue significantly more frequently than control traps (Figure 2). There was a median (and 25th percentiles) of 0 (0–1) and 2 (0.5–3) leeches in control and alarm cue traps, respectively (Wilcoxon Mann Whitney test: $z = 2.57$, $P = 0.025$). Five of 15 control traps caught at least one leech, whereas 11 of 15 alarm cue traps caught at least one leech (Figure 3). There was no correlation between number of *Gammarus* and number of leeches per trap for all traps combined ($R^2 = 0.0003$, $F = 0.007$, $P = 0.931$), within alarm cue traps ($R^2 = 0.0022$, $F = 0.029$, $P = 0.866$) or within control traps ($R^2 = 0.0007$, $F = 0.008$, $P = 0.927$).

Experiment 2. The median number (and 25th percentiles) of *Gammarus* per trap was 6 (5–9) and 3 (2–5) for control and alarm traps, respectively (Figure 4). Significantly more *Gammarus* were captured in control traps than in alarm cue traps (Wilcoxon Mann Whitney test: $z = 2.84$, $P = 0.002$). These data, collected

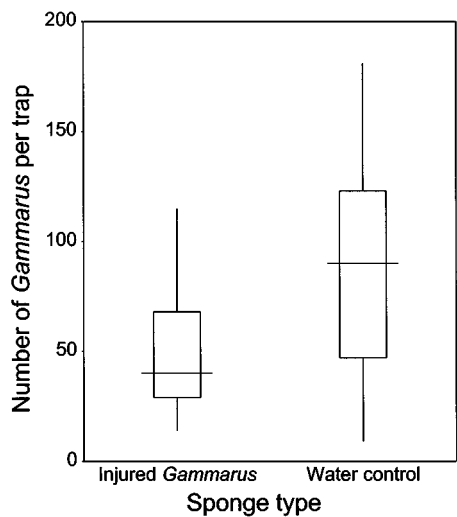


FIG. 1. Box and whisker plot of median, 25th percentiles, and range of number of *Gammarus* caught in the first experiment. Traps were baited with sponges containing either *Gammarus* injury-released alarm cues or water (control).

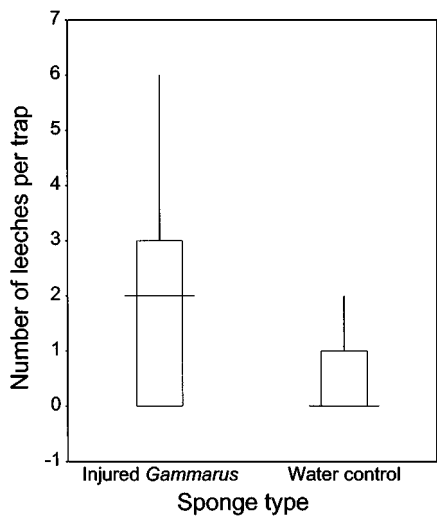


FIG. 2. Box and whisker plot of median, 25th percentiles, and range of the number of leeches caught in the first experiment. Traps were baited with sponges containing either *Gammarus* injury-released alarm cues or water (control).

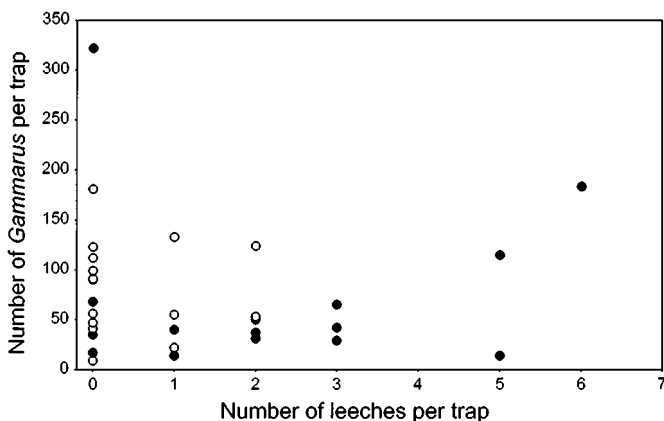


FIG. 3. Number of *Gammarus* per trap plotted against the number of leeches per trap in the first experiment. Open symbols, traps with water sponges; closed symbols, traps with alarm cue sponges.

at a different time of year and with slightly different gear and stimulus strength, corroborate data from the first experiment. Only four of 17 alarm cue traps and three of 17 control traps caught leeches. Because the majority of traps caught no leeches, the median (± 25 th percentiles) number of leeches entering both trap types was 0 (0–0) (Wilcoxon Mann Whitney test: $z = 1.29$, $P = 0.197$).

There were two distinct size classes in the catch (Figure 4). Small (1–5 mg) and large (35–108 mg) *Gammarus* both contributed to the overall avoidance response.

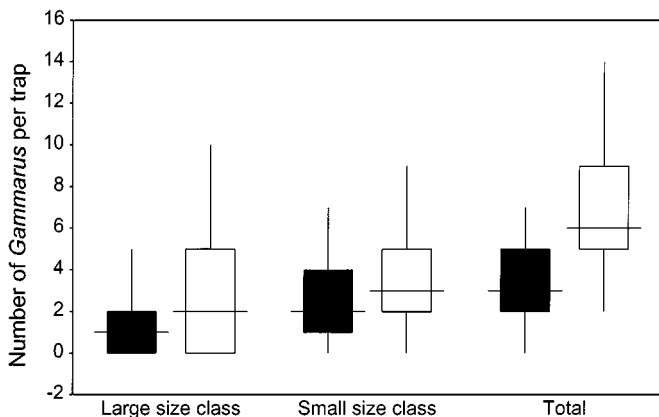


FIG. 4. Box and whisker plot of median, 25 percentiles and range of the number of *Gammarus* per trap in the second experiment. The large size class ($N = 72$) ranged from 32 to 108 mg, the small size class ($N = 104$) ranged from an average of 1 to 5 mg. Solid bars, alarm cue traps; open bars, control traps.

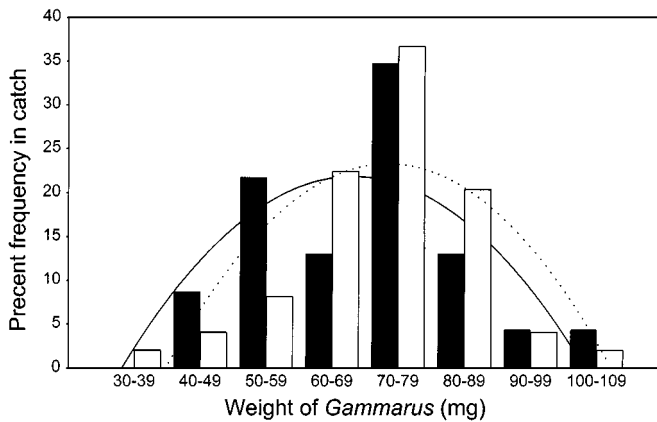


FIG. 5. Percent of the catch in each trap type in the second experiment represented by the large size class of *Gammarus* ranging from 32 to 108 mg. Fitted lines are second-order polynomial functions. Alarm cue traps, solid bars and solid line; control traps, open bars and dashed line.

However, the effect of alarm cue was not significant within the small size class (Wilcoxon Mann Whitney test: $z = 1.09$, $P = 0.276$) or (barely) the large size class (Wilcoxon Mann Whitney test: $z = 1.95$, $P = 0.051$) partly due to the low overall numbers of animals captured in the second experiment.

Within the large age class, small individuals did not avoid traps containing alarm cue as much as large individuals (Figure 5). We divided the size range of *Gammarus* into equal thirds creating small (32–57.3 mg) and large (82.7–108 mg) size categories with the adult size class. Small *Gammarus* represented 30.4% of the individuals caught in alarm cue traps, whereas small *Gammarus* represented only 10.2% of the catch in control traps ($\chi^2 = 4.44$, $P < 0.05$). Large *Gammarus* represented 17.4% and 20.4% of catches in alarm cue and control traps, respectively ($\chi^2 = 0.09$, $P > 0.9$). Similar analysis of length distribution of the small size class (<5 mg) was precluded by the low accuracy of weight measurements at the limit of the range of our electronic balance. We do not have individual weights for small *Gammarus*.

DISCUSSION

These data provide an important verification of laboratory findings. Field populations of *Gammarus lacustris* avoided injury-released conspecific alarm cues in two separate experiments at different times of year and by using slightly different methods. Chemical alarm cues serve as an important indicator of predation risk

for *Gammarus* in the field, and presumably antipredator behavioral responses such as avoidance behavior serve to decrease the probability of predation (Hews, 1988; Mathis and Smith, 1993; Wisenden et al., 1999).

Responses by very small *Gammarus* contributed to the overall avoidance response, suggesting that recognition of conspecific injury-released cues as indicators of predation risk is either innate or acquired at a very early stage of development. Innate responses to conspecific alarm cues are thought to be the case for various fish species (Pfeiffer, 1963; Waldman, 1982; Magurran, 1989), but to our knowledge this is the first such evidence for an aquatic arthropod.

Within the adult size class of *Gammarus*, small individuals were disproportionately represented in alarm cue traps. We do not interpret this to indicate acquired recognition of conspecific alarm cues over time and experience (cf. Chivers et al., 1995) because very small juvenile *Gammarus* responded to the alarm cue.

Large *Gammarus* may respond most strongly to conspecific alarm cues because they have a greater cost-benefit trade-off than small *Gammarus*. Large adult *Gammarus* are more responsive to risk of fish predation than small adult *Gammarus* (Mathis and Hoback, 1997). Although fish predators are absent from our study site, a variety of waterfowl use the site and presumably prey on *Gammarus*. Large *Gammarus* would be easier to detect by tactile probing of the vegetation and provide more energy per unit of foraging time.

Alternatively, cost-benefit trade-offs in risk assessment could depend on foraging opportunities for individuals at varying levels of social hierarchy, competitive ability, or somatic resources. Trade-offs between risk and foraging are well known (e.g., Milinski and Heller, 1978; Milinski, 1985; Godin and Sproul, 1988; Lima and Dill, 1990). Hungry (Smith, 1981; Brown and Smith, 1996), and low condition (Wisenden, Rush, and Sargent unpublished) fish do not respond to conspecific alarm cues with an overt behavioral response. Small *Gammarus* may tolerate more predation risk while foraging because of subordinate status or weak competitive abilities. Social interactions, dominance hierarchies, and territorial behavior among *Gammarus* are not well understood with respect to risk avoidance.

Leeches are significant predators of *Gammarus* and detect prey by chemical and tactile means (Dahl and Greenberg, 1997; Dahl, 1998). Leeches were attracted to traps with injury-released *Gammarus* cues, leaving open the possibility that *Gammarus* avoided alarm cue traps because of the presence of leeches rather than recognition of alarm cues per se. However, there was no correlation between the number of leeches and the number of *Gammarus* caught per trap. Moreover, leeches were attracted to the *Gammarus* cue in the first experiment only, and not in the second experiment, where cue concentration was four times higher. Thus, *Gammarus* aversion to the alarm cue in the first experiment was likely attributable to the alarm cue and not leeches.

Ostariophysan fishes possess specialized epidermal cells that contain an alarm pheromone. This pheromone is released when the skin is damaged; it signals alarm

to conspecifics and also serves as an attractant to predators (Mathis et al., 1995). Attracting predators can benefit the individual sending the signal because secondary predators attempt piracy or cannibalism that afford the prey an opportunity for escape (Chivers et al., 1996). The predator attraction hypothesis is the only mechanism for the evolution of ostariophysan alarm substance cells to receive experimental support. Leech attraction to injury-released cues of *Gammarus* raises the possibility that a similar alarm signal/predator attractant cue may be released from injured *Gammarus*. Leeches do not engulf their prey. Presumably, there is the potential for large amounts of internal fluids from *Gammarus* to be released in the process of being consumed by a leech, inadvertently informing other *Gammarus* of predation risk, but also attracting other leeches. Although special glands or cells that may contain a specialized pheromone are not known for *Gammarus*, chemical deterrents are known to occur in a number of aquatic arthropods in the insect orders Coleoptera and Hemiptera (Scrimshaw and Kerfoot, 1987). Further study is required to determine if *Gammarus* possess specialized structures and the nature of competitive foraging among two or more leeches.

In addition to verifying laboratory results and revealing potential size-dependent behavioral decision criteria, these data establish a convenient field technique for further testing the role of chemical cues in mediating predator prey interactions in aquatic invertebrates. It is our hope that future work will be stimulated in this direction.

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