Ethology **105,** 407—414 (1999)

ISSN 0179–1613

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Survival Benefit to Antipredator Behavior in the Amphipod *Gammarus minus* (Crustacea: Amphipoda) in Response to Injury-released Chemical Cues from Conspecifics and Heterospecifics

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Wisenden, B. D., Cline, A. & Sparkes, T. C. 1999: Survival benefit to antipredator behavior in the amphipod *Gammarus minus* (Crustacea: Amphipoda) in response to injury-released chemical cues from conspecifics and heterospecifics. Ethology **105,** 407—414.

Abstract

A wide range of aquatic taxa use environmental chemical cues for the assessment of predation risk. We examined whether *Gammarus minus* (Crustacea: Amphipoda) exhibit antipredator behavior in response to injury-released chemicals from conspecifics or heterospecifics (Crustacea: Isopoda). We then examined whether behavioral responses to these cues conferred survival benefits to the amphipods. In the first part of this study, we tested the behavioral response of *G. minus* to the following treatments: 1. water containing injury-released cues of conspecifics; 2. water containing injury-released cues of a sympatric isopod crustacean, *Lirceus fontinalis*; or 3. water containing no cues (control). Relative to the control, *Gammarus* responded to the conspecific cue by moving to the substratum and decreasing activity. In contrast, *Gammarus* responded to the heterospecific cue by moving up into the water column and increasing activity. In the second part of this study, we tested if the behavioral response to these cues confers a survival benefit to *Gammarus* when exposed to a predator. A green sunfish (*Lepomis cyanellus*) was retained behind a partition in the test tanks. Two minutes after the introduction of the chemical cues in the first test, the barrier was lifted and predation events recorded. Relative to the control, the time to the first attack increased for *Gammarus* exposed to conspecific cues and decreased for those exposed to heterospecific cues. These data indicate that *Gammarus* distinguish between chemical cues from conspecific and heterospecific crustaceans, and that the antipredator response to conspecific cues confers a fitness benefit (i.e. increased survival due to increased time to the first attack).

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Introduction

Water is an excellent medium for the solution and dispersal of chemical signals (Hara 1992; Dodson et al. 1994). In many aquatic habitats, visual information is unavailable due to turbidity or highly structured environments (plants, sticks, detritus, etc.). Thus, many aquatic animals have evolved the ability to assess environmental information using chemical cues (Hara 1992; Smith 1992; Dodson et al. 1994; Wisenden et al. 1995). One important area of environmental assessment is information pertaining to the presence of predation risk. A wide range of aquatic taxa use injury-released chemical cues of conspecifics as an indicator of predation risk (for a review see Chivers & Smith (1998)). These cues are typically released only during predatory attacks. In some instances antipredator behavior has been shown to occur in response to injury-released chemical cues of heterospecifics (Snyder 1967; Smith 1982; Smith et al. 1991; Mathis & Smith 1993a; Chivers et al. 1995, 1996; Wisenden et al. 1994, 1995, 1997). These results support the hypothesis that sympatric species benefit from responding to each other's alarm signals because an alarm signal of one member of a 'prey guild' indicates risk to all members.

A number of studies document antipredator behavior by prey species in response to conspecific and heterospecific cues. Rarely is the subsequent survival benefit examined; that is, does a behavioral response to cues result in increased survival when exposed to predators (Hews 1988; Mathis & Smith 1993b)? We tested *Gammarus minus* (Crustacea, Amphipoda) for an antipredator response to injury-released cues from conspecifics and from a sympatric crustacean, the isopod *Lirceus fontinalis*. We compared the behavioral response to the isopod cue with the response to the conspecific cue to determine the presence of antipredator behavior. In the second part of this study we tested whether behavioral responses by *Gammarus* conferred a survival benefit when exposed to a predator.

Materials and Methods

Gammarus are ubiquitous freshwater crustaceans in streams, ponds and lakes. They are subject to heavy predation by fish (Andersson et al. 1986; Holomuzki & Hoyle 1990; Andersen et al. 1993; Boates et al. 1995; Mathis & Hoback 1997; Dahl & Greenberg 1997) and exhibit antipredator behavior in response to injury-released chemical cues from conspecifics (Williams & Moore 1985; Wudkevich et al. 1997; Dahl et al. 1998). Thus, *Gammarus* is well suited to serve as a test species for this study.

Gammarus minus and *L. fontinalis* were collected from Grier Creek, 40 km west of Lexington, KY, USA and transported to a wading pool in the laboratory facilities at the University of Kentucky. Grier Creek contains banded sculpins (*Cottus carolinae*) but does not contain sunfish. *Lirceus* are eaten by sculpins (Sparkes 1996) and, thus, there is the potential for cross-species reactions to injury-released cues between *Gammarus* and *Lirceus*. Green sunfish (*Lepomis cyanellus*), an important predator of *G. minus* in nearby streams (Holomuzki & Hoyle 1990), were collected from a pond at Spindle Top Farm, located on the extended campus of the University of Kentucky. Sunfish (≈ 9 cm in length, $n = 6$) were held in laboratory aquaria for 3 wk before use in trials and maintained on a diet of earthworms and mosquitofish.

Test aquaria (75 × 30 × 30 cm) were divided into three compartments modeled after the methods used by Mathis & Smith (1993b). One end compartment, 37.5 cm of the tank's length, contained 10 *Gammarus*, a thin layer of natural-colored gravel, five shelter objects, an airstone and an injection tube. The injection tube was a second airline tube through which stimuli could be injected. The injection tube ended just above the airstone, such that stimuli were quickly dispersed throughout the *Gammarus* compartment by water currents created by the airstone. The shelter objects were constructed from ceramic tile and measured 5.75 × 2.8 cm, supported by legs 1 cm high. A grid of black lines (the cells measured 7.5 × 7.5 cm) was drawn on to the front pane of the *Gammarus* section to quantify activity. The barrier between the *Gammarus* compartment and the center compartment was opaque and formed a tight seal. The center compartment was 7.5 cm wide and was the section where a fresh supply of dechlorinated water entered the test tank. The other end compartment, 30 cm of the tank's length, was the sunfish section. The barrier between the center and sunfish compartments was made of open rigid plastic mesh that freely allowed water entering the center section to flow to an outflow drain at the end of the sunfish section. The sunfish compartment contained a thin layer of natural-colored gravel, an overturned clay flowerpot and the drain siphon.

The purpose of the flow-through system was to flush away chemical cues released by the sunfish that might serve to warn *Gammarus* of the presence of a predation risk. It is noteworthy that the *Gammarus* used in this experiment were collected from a location where sunfish are absent. This reduced the likelihood that *Gammarus* would recognize sunfish odor as dangerous if some odor managed to cross the opaque barrier into the *Gammarus* section. Recognition of predator odors is a learned response in many aquatic organisms (Göz 1941; Magurran 1989; Mathis & Smith 1993c; Chivers & Smith 1994; Wisenden et al. 1997). In addition, we were careful not to feed *Gammarus* or *Lirceus* to the sunfish before or during the trials because chemical cues released from the diet of a predator may signal danger even to naive prey (Mathis & Smith 1993d; Brown et al. 1995; Chivers et al. 1996).

The trials lasted for 21 min. For 8 min, we recorded amphipod behavior: the number swimming in the water column, crawling on the bottom, stationary, and occupying a shelter and the number of *Gammarus* in the lower, middle and upper horizontal zones. An index of vertical distribution was calculated by multiplying the number of *Gammarus* in the upper zone by 3, middle zone by 2 and the lower zone by 1. Counts were recorded every 30 s over the 8 min prestimulus period. Over the ninth minute, we injected a test stimulus (water control, *Gammarus* or *Lirceus* cues). We recorded *Gammarus* activity every 10 s for the next 2 min. After the 2 min poststimulus period (i.e. at the end of 11 min since the start of the trial), we removed the barriers between the sunfish and *Gammarus* compartments. This allowed the sunfish to enter the *Gammarus* section and feed on them. We recorded the time to the first attack by the sunfish, the number of successful and unsuccessful attacks, and calculated the percentage of capture success as [(number eaten)/(total number of attacks in 10 min)] × 100. The trials were ended 10 min after the removal of the barriers, or until the sunfish successfully captured all of the *Gammarus*.

The stimuli were prepared by crushing five medium-sized *Gammarus* or *Lirceus* to a thin paste with a mortar and pestle. Death was instant and humane. Five milliliters of dechlorinated tap water was added to the resulting pulp and held for later use. Control tests used 5 ml of dechlorinated tap water to control for the effect of stimulus injection. Sixty milliliters of tank water was drawn through the injection tube into a syringe, discarded to rid the tube of stale water and a second 60 ml was withdrawn and retained. The volume of the injection tube was 30 ml. Stimuli were injected at the appropriate time by first injecting the 5 ml of stimulus into the injection tube, then slowly flushing the stimuli into the test tank using the 60 ml of tank water previously retained.

A total of 45 trials were conducted; 15 trials in each stimulus treatment (water, *Gammarus* and *Lirceus* cues). Data were analyzed using Kruskal–Wallis one-way ANOVAS comparing responses to the three treatments: water (control), *Gammarus*, and *Lirceus* (Siegel & Castellan 1988). Post-hoc pairwise comparisons among treatment medians (alpha-corrected) were performed using methods outlined in Siegel & Castellan (1988; p. 213).

Results

Gammarus minus responded to injury-released cues from conspecifics with a typical antipredator response, but responded to the *Lirceus* cues with increased activity (Fig. 1). In response to the test stimuli, there was an overall change in the number of *Gammarus* swimming ($KW = 31.08$, $p < 0.001$), the number of *Gammarus* stationary ($KW = 18.12$, $p < 0.001$) and vertical distribution ($KW = 25.70$, $p < 0.001$), but no change in the number of *Gammarus* crawling ($KW = 0.22$, $p = 0.894$) or using shelter objects ($KW = 1.29$, $p = 0.524$). In response to injured conspecifics, *Gammarus* significantly decreased their vertical distribution (i.e. moved to the bottom), reduced swimming activity and increased stationary behavior relative to the controls. In response to the *Lirceus* cue, *Gammarus* significantly increased movement into the water column, resulting in an increase in the number swimming and a decrease in the number stationary relative to the controls.

Overall, there was a significant effect of treatment on the time to the first attack ($KW = 6.15$, $p = 0.046$, Fig. 2). The $\bar{X} \pm$ standard error (SE) time to the first attack after the introduction of the *Gammarus* cue was 57.67 ± 9.4 s, 38% longer than the time to the first attack in control trials (41.67 ± 4.5 s) ($p < 0.05$). After the introduction of the *Lirceus* cue, the time to the first attack was 34.87 ± 2.8 s, 16% shorter than in control trials ($p < 0.05$).

Once a green sunfish had initiated an attack, the percentage of capture success was very high ($\bar{X} \pm SE = 81.1 \pm 1.3$). Capture success did not differ among treatments ($KW = 0.65$, $p = 0.723$, Fig. 2). All 10 *Gammarus* were consumed in every

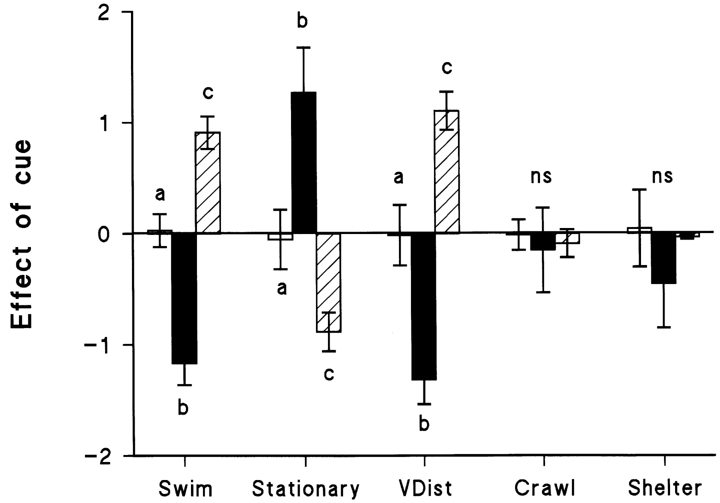

Fig. 1: Median (± 25 percentiles) change (poststimulus minus prestimulus) in the number of *Gammarus* amphipods engaged in swimming, stationary behavior, crawling or using a shelter object. Vdist is an index of vertical distribution that increases with height in the water column. Open bars, water controls; solid bars, injured conspecific *Gammarus* cue; hatched bars, injured *Lirceus* isopod cue. The letters above the bars indicate the results of alpha-corrected multiple comparisons among treatments, different letters indicate treatments that differ by $p < 0.05$. ns, no significant differences ($p > 0.05$)

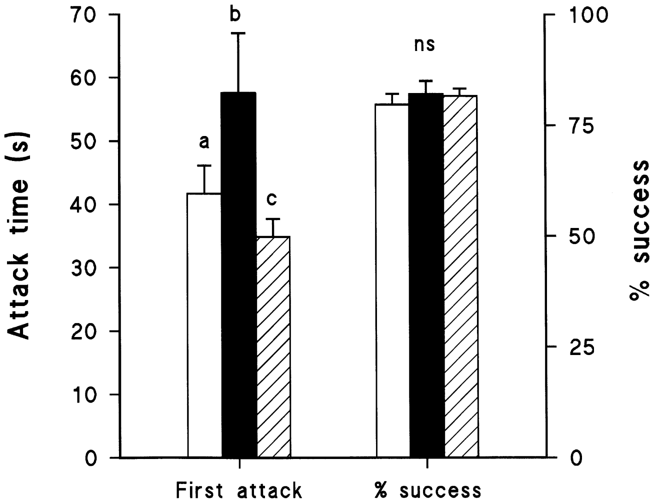

Fig. 2: The $\bar{X}$ ± standard error (SE) time (s) to the first attack and overall attack success by green sunfish after amphipods were 'warned' with water (open bars), injured conspecific *Gammarus* cue (solid bars), or injured *Lirceus* isopod cue (hatched bars)

trial except for three (one trial in the *Gammarus* treatment, two trials in the controls). The $\bar{X} \pm SE$ of the total number of attacks were 11.6 ± 0.57, 12.33 ± 0.27 and 12.0 ± 0.54 for *Gammarus*, *Lirceus* and water control, respectively (KW = 0.312, p = 0.856). When only trials in which all 10 *Gammarus* were consumed are considered, the $\bar{X} \pm$ SE of the total number of attacks were 12.1 ± 1.27, 12.33 ± 1.05 and 12.62 ± 1.39, respectively (KW = 0.901, p = 0.637).

A delay in the time to the first attack in the *Gammarus* trials indicates an antipredator benefit to *Gammarus* that leave the water column, move to the substratum and become stationary. An increase in the time to the first attack in *Lirceus* trials indicates a fitness cost to responding to the *Lirceus* cue in the presence of a predator.

Discussion

Our study has two novel findings. First, the antipredator response to injured *Gammarus* is not a general response to injured crustaceans, nor a general response to small sympatric crustaceans that may be subject to the same predators as *Gammarus*. Second, the behavioral changes that occurred following the introduction of injured *Gammarus* resulted in an increased time to the first attack by the predatory sunfish. This latter finding verifies that the commonly observed behavioral response to injury-released cues from conspecifics is adaptive in that they seem likely to reduce the individual's risk of predation. The difference between escape and capture in a predator–prey encounter can be decided by a split-second interaction. We demonstrated a 16 s delay in the time to the sunfish attack. This would give *Gammarus* plenty of time to find refuge from attack in nature. Access to refuge is a major determinant of *Gammarus* vulnerability to predation to brown trout *Salmo trutta* (Dahl et al. 1998). The ultimate foraging success of sunfish in this study was an artifact of the confined area of the test aquaria.

These findings corroborate earlier findings by Hews (1988) and Mathis & Smith (1993b) in establishing that the behavioral changes in response to chemical cues confer an antipredator benefit. This is the first such demonstration for an aquatic arthropod. The specific behaviors exhibited, reduction in activity and movement to the bottom, are similar to those found in previous studies of *Gammarus* alarm behavior (Williams & Moore 1985; Wudkevich et al. 1997) and are common antipredator strategies in a variety of animals (Lima & Dill 1990). In the study by Wudkevich et al. (1997), *G. lacustris* tended to move out of the water column in response to the odor of a fish predator (*Esox lucius*) but not in response to the odor of larval dragonflies. This suggests that the adaptive value of movement to the bottom is contingent on the hunting style of the specific predator (Wooster & Sih 1995).

The absence of an antipredator response to the *Lirceus* cue may stem from the fact that *Gammarus* are predatory on isopods (Bengtsson 1982). Some invertebrates respond to injury-released cues from heterospecifics (Snyder 1967; Mathis & Smith 1993a; Chivers et al. 1996; Wisenden et al. 1997), but in these examples, the heterospecific cues were not also a prey item of the test animal. We propose that the increased activity in response to *Lirceus* can be interpreted as a feeding response by *Gammarus* and that in nature *Gammarus* would have to tradeoff conflicting selection pressures. In the presence of the *Lirceus* cue they should increase activity but in the presence of fish cues (absent in this experiment) they should decrease activity. Foraging activity increases *Gammarus* visibility to predators and may also distract *Gammarus* from predator vigilance. However, further studies will be required to understand cross-species reactions in *Gammarus*.

This study extends the range of aquatic taxa known to exhibit antipredator behavior in response to injury-released cues from heterospecifics. It is novel in that it suggests that cross-species reactions to alarm cues within a prey guild cannot necessarily be inferred simply by habitat sympatry and diet overlap of predators. Moreover, this study confirms that chemical information is a very important source of environmental information for the assessment of predation risk, and that the behavioral response to this information is adaptive in reducing the risk of predation.

Acknowledgements

We thank A. Sih for use of his laboratory space and equipment. B. D. Wisenden was supported by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada.

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Received: April 29, 1998

Initial acceptance: September 11, 1998

Final acceptance: October 22, 1998 (J. Brockmann)