

BEHAVIOURAL RESPONSES BY RED-BACKED SALAMANDERS TO CONSPECIFIC AND HETEROSPECIFIC CUES

by

AARON M. SULLIVAN^{1,2)}, DALE M. MADISON¹⁾ and JASON R. ROHR^{1,3,4)}

(¹ Department of Biological Sciences, Binghamton University (State University of New York), Binghamton, New York 13902-6000)

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Summary

Chemical cues released from injured prey are thought to indicate the proximity of a predator or predation event, and therefore, an area of elevated predation risk. Prey often avoid chemical cues released from injured heterospecifics, but there is little evidence to determine whether this is due to homologous cues among phylogenetically related species, or avoidance of injured syntopic species that experience predation from the same predators. The purpose of this study was to examine the response of terrestrial red-backed salamanders (*Plethodon cinereus*) to chemical cues from non-injured and injured members of their prey guild that vary in their relatedness to *P. cinereus*. In the laboratory, *P. cinereus* avoided chemical cues from injured conspecifics, injured and non-injured slimy salamanders (*P. glutinosus*), and injured confamilial dusky salamanders (*Desmognathus ochrophaeus*). Red-backed salamanders did not avoid rinses from non-injured conspecifics and dusky salamanders, or cues from injured and non-injured earthworms (*Lumbricus sp.*), a more distantly related prey guild member. These results cannot be fully explained by either phylogenetic relatedness (among plethodontid salamanders) or prey guild membership alone. We suggest that a combination of these factors, and perhaps others, likely influenced the evolution of heterospecific alarm cue avoidance in the red-backed salamander.

²⁾ Corresponding author's e-mail address: asulliv1@binghamton.edu

³⁾ Present address: 101 Morgan Building, Department of Biology, University of Kentucky, Lexington, Kentucky 40506-0225

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Introduction

Predation influences the ecology and evolution of nearly every organism, and is important in regulating the size and species composition of natural communities (Hairston *et al.*, 1960; Paine, 1966; Sih *et al.*, 1985; Lima & Dill, 1990; Lima, 1998). Prey can incorporate two general defense mechanisms in the face of such widespread predation: predator avoidance, which reduces the probability of encountering a predator, and antipredator behavior, which reduces the likelihood of successful predation after predator encounter (Brodie *et al.*, 1991). Predator avoidance mechanisms, such as avoidance of chemicals signaling the proximity of a foraging predator, may be especially advantageous since prey may eliminate or reduce predator encounters altogether and hence the chance of injury or death (Edmunds, 1974; Endler, 1986; Sih, 1987; Lima & Dill, 1990).

Many species use chemical cues from predators or predation events to reduce predation risk (reviewed by Weldon, 1990; Chivers & Smith, 1998; Kats & Dill, 1998), because chemical cues can provide more benefits than visual or acoustic cues in many predator-prey contexts due to their mode of delivery, efficiency, and duration (Wilson, 1975; Bradbury & Vehrencamp, 1998). For instance, chemical cues can be detected in turbid aquatic environments and around obstacles where visual cues would be less effective (Smith, 1992; Dodson *et al.*, 1994). Their efficiency may result from their economic biosynthesis (*e.g.* the use of nitrogenous waste products as disturbance cues in *Orconectes* crayfish; Hazlett, 1990), or their effectiveness in minute quantities (*e.g.* alarm cues released from 1 cm² of *Pimephales promelas* skin may generate an active space of 58,000 l; Lawrence & Smith, 1989). Chemical cues also may persist in the environment longer than both acoustic and visual cues, conveying information to prey species after the predator has left the area, which may be especially beneficial if predators revisit the same foraging areas.

Chemical substances released from damaged individuals may serve as alarm cues and indicate high-risk areas where predation has recently occurred. These cues have been studied in a variety of species dating back to von Frisch's discovery of the ostariophysan '*Schreckstoff*' (as cited in Smith, 1977). An interesting and complicating aspect of chemical alarm cue detection involves risk assessment using damage-release chemical cues deposited by injured heterospecific organisms because, unlike responses to

conspecific alarm cues, responses to heterospecific alarm cues may be influenced by phylogenetic relatedness and ecological relatedness (Chivers *et al.*, 1997; Chivers & Mirza, 2001). The phylogenetic relatedness hypothesis states that responses to heterospecific alarm chemicals are due to the homology of alarm cues among closely related species (*e.g.* the ostariophysan fishes; Smith, 1992; Brown *et al.*, 2000). The ecological relatedness hypothesis suggests that species benefit from recognizing alarm cues from syntopic members of the same prey guild that may or may not be closely related. Because of the complexity of community interactions, it is likely that these hypotheses work together to produce the broad chemical sensitivity seen in many species (Kats & Dill, 1998), but there is little evidence to support this claim.

The evolutionary and ecological roles of alarm chemicals among aquatic vertebrates have been well studied (see reviews in Smith, 1992; Chivers & Smith, 1998; Kats & Dill, 1998; Chivers & Mirza, 2001), but only recently have these chemicals been established in terrestrial vertebrates, especially among terrestrial amphibians (Lutterschmidt *et al.*, 1994; Chivers *et al.*, 1996, 1997; Hucko & Cupp, 2001). Salamanders, in particular, are good candidates for production of, and response to, alarm chemicals because they have a highly glandular integument (Williams & Larsen, 1986; Jaeger & Gabor, 1993; Simons *et al.*, 1994; Hamning *et al.*, 2000), use chemical cues to mediate a wide variety of behavior (Madison, 1972, 1975, 1977; Dawley, 1984; Jaeger, 1986; Mathis, 1990), and release defensive secretions when attacked (Arnold, 1982; pers. obs.)

The importance of chemical cues and plethodontid salamanders in the community ecology of forests in the eastern United States has become increasingly clear, especially in the context of predator-prey interactions (Brodie, 1977, 1983; Brodie *et al.*, 1979, 1991; Ducey & Brodie, 1983; Fenster & Fenster, 1996). The red-backed salamander (*Plethodon cinereus*) may have the most substantial influence on the community dynamics of eastern woodlands because it is likely the most abundant vertebrate (in terms of biomass) in eastern forests (Burton & Likens, 1975), and functions as predator or prey to many species (Petranka, 1998; Maerz, 2000).

For these reasons, we examined how phylogenetic relatedness and known competitive and predatory relationships between red-backed salamanders and syntopic prey guild members influenced red-backed salamander responses to chemical cues from prey guild members. Adult red-backed sala-

manders were exposed to rinses (non-injured) and tissue extracts from red-backed salamanders (*P. cinereus*), Allegheny dusky salamanders (*Desmognathus ochrophaeus*), slimy salamanders (*P. glutinosus*), and earthworms (*Lumbricus sp.*) during their courtship season. Conspecifics represent competitors and potential mates, but all conspecifics should have similar chemical alarm defenses, so we predicted that red-backed salamanders would avoid extracts from macerated conspecifics but show no avoidance of, or even mild attraction to, stimuli from non-injured individuals. We predicted that red-backed salamanders would show either no response or mild avoidance of non-injured dusky salamanders because dusky salamanders are similar-sized competitors, ranging from 6-12 cm total length (Jaeger *et al.*, 1998; Petranka, 1998). However, we expected red-backed salamanders to avoid macerated dusky salamanders because they are preyed on by the same suite of predators. Unlike the dusky salamander, which is confamilial to red-backed salamanders, slimy salamanders are congeneric, and are therefore more likely to release alarm cues homologous to those of red-backed salamanders. Furthermore, the much larger slimy salamanders (11.5-20.5 cm total length) are potential predators of red-backed salamanders (Powders, 1973; Powders & Tietjen, 1974; Petranka, 1998). We therefore predicted that chemical cues from both non-injured and macerated extracts of slimy salamanders would be avoided. Lastly, we predicted that red-backed salamanders would be attracted to non-injured earthworm cues because earthworms frequently inhabit the same refuges as red-backed salamanders, and represent a potential food source (Maerz, 2000). But, we hypothesized that red-backed salamanders would avoid macerated earthworm because earthworm alarm cues (discovered by Ressler *et al.*, 1968) attract garter snakes (*Thamnophis sirtalis*) (Halpern *et al.*, 1986), a documented predator of red-backed salamanders (Hamilton, 1951).

Methods

Collection and maintenance

In May 2001, we collected 280 adult red-backed salamanders from the Binghamton University Nature Preserve (BUNP), Broome County, New York for use in behavioral trials. Each salamander was housed individually in 15 cm-diameter Petri dishes lined with moistened paper towels and maintained at approximately 15°C and a 15L : 9D photoperiod. Test salamanders were maintained in the laboratory for two days prior to the beginning of the experiment,

and were not fed in captivity. Each salamander was tested once and released at the capture site within one week of collection.

We also collected the following stimulus donor animals (equal numbers of males and females for each salamander species) from BUNP: four additional red-backed, two slimy, and four dusky salamanders, and four juvenile earthworms. Stimulus donor animals were housed together by species in plastic shoeboxes (30 × 15 × 12.5 cm).

Experimental protocol

We examined the response of red-backed salamanders to eight different treatments made up of both rinses and macerated extracts from the four donor species. Rinses from non-injured donors were tested to control for cues that might elicit avoidance unrelated to damage-release cues, such as territorial cues. These rinses were prepared by maintaining donor animals in plastic shoeboxes with 250 ml distilled water for 48 h. After this period, organisms were removed from their containers, quickly killed by decapitation, and the entire body was homogenized in a blender with 50.0 ml of distilled water per 1.0 g of tissue. We used whole body macerates (instead of skin extracts used by Chivers *et al.*, 1997) to more closely simulate a predation event where damage may occur to skin, muscle, *etc.* (Rohr & Madison, 2001; Rohr *et al.*, 2002). Rinses and extracts were filtered with a porcelain Büchner funnel to remove large particulate matter, were rapidly frozen in 50 ml conical-bottom plastic tubes using liquid nitrogen, and were then stored in a freezer (−20°C) until use. All samples were used within five days of preparation to minimize cue degradation.

We used previously established behavioral bioassay procedures to test for treatment responses (Madison *et al.*, 1999a,b; Sullivan *et al.*, 2002). In brief, we placed two filter paper semicircles on opposite sides of 15 cm Petri dishes while maintaining a 3 mm gap between each semicircle. We added 1.5 ml of treatment solution (macerate or rinse) or control water to each semicircle using a 10 ml tuberculin syringe, and randomly distributed each treatment dish on an 8 × 7 grid on the floor of our experimental room. Fifty-six different male and female salamanders were tested each night over five consecutive nights. Treatments were randomly stratified over the five nights so that seven replicates of each treatment were repeated each night for a total of 35 replicates per treatment. We transferred each salamander from its home dish to the assigned experimental dish with a cotton swab, and placed a 15 mm collar of brown paper around the dish to visually isolate each salamander within test dishes during the set-up period. After all salamanders were distributed (~15 min), the lights were turned off and the trial was recorded for 60 min in complete darkness with a video camera sensitive to infrared light. Conducting trials in total darkness forced salamanders to select substrates using only non-visual cues and eliminated potential visual biases. We began recording trials between 23.55 and 24.05 h each night.

We recorded the side occupied by each salamander every three minutes for one hour. If a salamander straddled the 3-mm gap, the side with more than half of the individual was considered the occupied side. We chose this criterion, instead of the location of the sensory organs, because it is conceivable that an individual may ‘freeze’, an anti-predator response shown by red-backed salamanders (Brodie *et al.*, 1974; Madison *et al.*, 1999a), when it detects an alarm substance on one of the filter papers. In such a scenario, the sensory receptors of red-backed salamanders may be over the alarm substrate and therefore may ‘show a preference’ for the alarm side, but the body of the individual is mostly on the control side, indicating a more accurate negative response to the alarm substance. We added the number of times each

salamander was located on the treatment substrate (macerated extract or non-injured rinse) within the Petri dish for the 21 observations.

Red-backed salamanders tend to be relatively inactive and their response tends to be bimodally distributed as described in Madison *et al.* (1999a), precluding the use of parametric analyses. We used a combination of Chi-square goodness-of-fit tests and binomial tests to determine whether the number of salamanders that avoided the treatments differed significantly from random expectation. We considered salamanders located on the treatment substrate ≤ 6 out of 21 observations as avoiding the treatment because this ratio is below an alpha value of 0.05 using a binomial test (Siegel & Castellan, 1998), and those located on the treatment side ≥ 15 out of 21 observations as being attracted to the treatment. Individuals on the treatment substrate for >6 and <15 observations were considered indifferent to the treatment, and were excluded from the analysis. We used Chi-square goodness-of-fit tests to determine whether the number of salamanders avoiding the treatment differed significantly from random expectation.

Results

Only 24 of 280 (8.6%) red-backed salamanders were indifferent to treatments based upon the criteria described above (Fig. 1a & 1b). Those red-backed salamanders that appeared to make a choice did not significantly avoid rinse from non-injured red-backed ($\chi^2 = 0.71$; $p = 0.398$) or dusky salamanders ($\chi^2 = 0.27$; $p = 0.602$), but significantly avoided extract from both species ($\chi^2 = 8.76$; $p = 0.003$, $\chi^2 = 9.32$; $p = 0.002$, respectively; Fig. 1a & 1b). They also significantly avoided slimy salamander rinse ($\chi^2 = 4.83$; $p = 0.028$) and extract ($\chi^2 = 5.83$; $p = 0.016$; Fig. 1c). Lastly, red-backed salamanders did not significantly avoid the rinse ($\chi^2 = 0.03$; $p = 0.857$) or extract from earthworms ($\chi^2 = 0.27$; $p = 0.602$; Fig. 1d).

Discussion

Our study demonstrates that red-backed salamanders avoid chemical cues from injured con- and heterospecific plethodontid salamanders, but not those from a non-salamander member of the same prey guild. Because red-backed salamanders did not avoid rinse from non-injured conspecifics or dusky salamanders, it is unlikely that the avoidance of macerated salamander extracts is due to repulsive, non-damage-release pheromones or allomones, respectively. Consequently, red-backed salamander avoidance of damage-release chemicals from the various species of plethodontid salamanders in this study

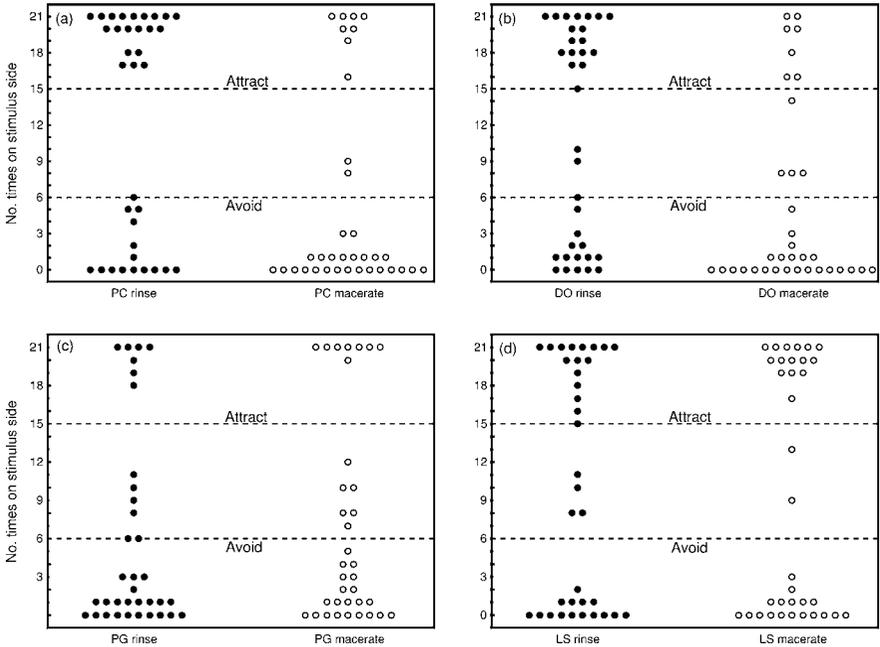


Fig. 1. Response score distribution for *Plethodon cinereus* exposed to non-injured rinses and macerated extracts from (a) *P. cinereus*, PC, (b) *Desmognathus ochrophaeus*, DO, (c) *P. glutinosus*, PG, and (d) *Lumbricus sp.*, LS. Each point represents the number of observations (0–21) that an individual was located on the treatment side (macerated extract or non-injured rinse) of the experimental dish. An individual located on the stimulus side ≤ 6 times out of a possible 21 observations significantly ‘avoided’ the treatment, and those located on the stimulus side ≥ 15 times were significantly ‘attracted’ to the treatment. See text for statistical analysis.

suggests an adaptive mechanism for evading areas of high potential predation risk.

Whether the avoidance of macerated red-backed and dusky salamanders by *P. cinereus* can be attributed to phylogenetic relatedness or membership in the same prey guild is not certain, but both seem plausible. Support for the phylogenetic relatedness hypothesis in salamanders comes from Lutterschmidt *et al.* (1994), who showed that *D. ochrophaeus* avoided chemical alarm cues from *D. ochrophaeus* and *D. brimleyorum* despite allopatry. However, Hucko & Cupp (2001) showed that *P. richmondi* avoid crushed auto-tomized tails from conspecifics, but not a sympatric congeneric salamander (*P. dorsalis*). These data suggest that damage-release alarm chemicals are

not conserved within Plethodontidae, and therefore phylogenetic relatedness alone may not explain the results of this study.

There is also evidence that heterospecific alarm cue responses can evolve when members of a prey guild learn to recognize alarm cues from syntopic, but distantly related species (*e.g.* Mathis & Smith, 1993; Chivers *et al.*, 1997; Mirza & Chivers, 2001; Mirza *et al.*, 2001). In our study, all salamander species were confamilial (Plethodontidae) and syntopic, so we can not say definitively that the *P. cinereus* alarm response to these different salamander species is due to prey guild membership. However, our unpublished work examining predator diet effects on antipredator responses of red-backed salamanders suggests that the degree of habitat overlap among prey species is correlated positively with the antipredator response of red-backed salamanders to predators. For instance, *P. cinereus* avoided predators fed syntopic prey species, but not those predators fed allotopic prey.

However, if ecological overlap alone was responsible for heterospecific alarm cue recognition, then we would have expected *P. cinereus* to avoid macerated earthworms, an ecologically related, but phylogenetically distant species that shares the same microhabitats and predators (Hamilton, 1951; Carpenter, 1952; Cunningham & Burghardt, 1999). The lack of macerated earthworm avoidance by *P. cinereus* has two likely explanations. First, the limited evolutionary history between *P. cinereus* and the recently invasive *Lumbricus sp.* may preclude the recognition of cross-taxon alarm cues. Second, it is possible that the rapid death of macerated earthworms prevented the secretion of the earthworm alarm cues as demonstrated by Jiang *et al.* (1989) who used electric shock to induce secretion. A third, less likely hypothesis suggests that the hunger state of individual salamanders compromised the avoidance response to macerated earthworms (a potential food source). This seems unlikely because in unpublished studies, we found no differences in the predator avoidance behaviors of salamanders fed *ad libitum*, and those intentionally starved for four weeks. We also would expect significant attraction to the earthworm rinse, since red-backed salamanders at our study site often cohabit cover objects with, and frequently prey on earthworms (Maerz, 2000). Although the earthworm treatments do not clearly reveal which hypothesis (ecological overlap or phylogenetic relatedness) is responsible for the evolution of heterospecific alarm cue recognition, the lack of avoidance of macerated *Lumbricus sp.* does demonstrate that the avoidance of macerated salamander cues is not the avoidance of all damaged animal tissues.

Red-backed salamander avoidance of non-injured and macerated slimy salamander treatments is somewhat difficult to interpret. The avoidance of the non-injured body rinse suggests a response to chemical cues from an interspecific competitor or predator. Slimy salamander adults are larger than adult red-backed salamanders (Petranka, 1998), and consume smaller salamanders including conspecifics (Powders, 1973; Powders & Tietjen, 1974). Consequently, it seems plausible that slimy salamanders may be a predation threat to red-backed salamanders, and thus avoidance of their rinse could be a predator avoidance response. The avoidance of macerated slimy salamander extract may be the avoidance of predator cues or chemical alarm cues.

This study shows that the avoidance of heterospecific alarm cues should not be dichotomized into two mutually exclusive hypotheses. It seems more likely that learned responses among members of a prey guild, phylogenetic relatedness, and intraguild predation each play important roles in heterospecific alarm responses. Future studies should examine salamander responses to chemical alarm cues obtained from allopatric and sympatric prey guild members. This approach would allow us to draw more definitive conclusions regarding the importance of prey guild membership in cross-species alarm responses and would help determine which factors may be the predominant forces driving their evolution.

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