

**Avoidance of Areas Labeled with  
Chemical Stimuli from Damaged  
Conspecifics by Adult Newts,  
*Notophthalmus viridescens*, in a  
Natural Habitat**

DAVID R. WOODY AND ALICIA MATHIS,<sup>1</sup> Department of  
Biology, Southwest Missouri State University, Springfield,  
Missouri 65804-0095, USA. E-mail: sam477@oma.smsu.edu

Chemical communication appears to be an important means of avoiding predation in aquatic environments, where murky, dark, highly structured habitats are common (Dodson et al., 1994). Chemical alarm signals play an important role in risk assessment and predator avoidance for a number of aquatic vertebrates (e.g., Hews and Blaustein, 1985; Hews, 1988; Petranka,

1989; Smith, 1992; Mathis and Smith, 1993). In many of these cases, the chemical alarm signals are released following mechanical damage to the skin. Therefore, damage-released chemical alarm signals are good indicators that a predation event has occurred recently in the vicinity, and these stimuli often elicit an avoidance response by conspecifics (e.g., Mathis and Smith, 1992; Chivers and Smith, 1994).

Newts are well-protected from some predators by their toxicity (e.g., Brodie, 1968; Webster, 1960), but may be at least somewhat vulnerable to predation by others (Brodie, 1968; Hurlbert, 1970; Shure et al., 1989). Behavioral antipredator responses to predators or to simulated predator attacks have been described for adult newts (Brodie, 1977; Ducey and Dulkiewicz, 1994), but the extent to which chemical signals influence predation avoidance for this species is not known. In a laboratory experiment, Marvin and Hutchison (1995) demonstrated an avoidance response by adult red-spotted newts (*Notophthalmus viridescens*) to terrestrial substrates labeled with chemical stimuli from injured conspecifics. Similar responses also have been reported for central newts (*N. u. louisianensis*) tested in the laboratory in an aquatic environment (Woody, 1996). We tested the hypothesis that adult newts avoid areas in a natural aquatic environment that are labeled with conspecific skin extract.

We prepared skin extracts from both *N. viridescens* and *Plethodon serratus* to serve as test stimuli. We used skin extracts from *P. serratus* as a control for general responses to damaged salamander tissue. Because *P. serratus* occupies a terrestrial habitat, we felt that it was unlikely that *N. viridescens* would have any innate/learned responses to alarm stimuli from *P. serratus* skin (Mathis and Lancaster, unpubl. data), as has been observed for some pairs of microsympatric heterogeners (e.g., Mathis and Smith, 1993; Chivers and Smith, 1994). *Plethodon serratus* extracts were also used by Marvin and Hutchison (1995) as a control in their laboratory study of red-spotted newts. We decapitated three newts (SVL: 42 mm, 40 mm, and 39 mm) and removed the skin from the head, neck, torso, and tail. This tissue consisted mostly of skin, but included some muscle. We homogenized 0.8 g of newt tissue with 100 ml of dechlorinated water, using an Omni tissue homogenizer. The mixture was then filtered through glass wool and its volume was increased to 533 ml by the addition of dechlorinated water. We prepared the *Plethodon* skin extract (PSE) in the same manner, but eight *P. serratus* (SVL mean = 40.9 mm, range 37-45 mm) were required to equal the concentration (0.8 g of tissue in 533 ml of dechlorinated water) of the newt skin extract (NSE). We divided both stimulus solutions into 10-ml portions in plastic snap-cap tubes and immediately placed them in a freezer at -20 C. Dechlorinated water was similarly frozen. All stimuli were thawed at room temperature on the morning of the trial.

Two experiments were conducted. The first experiment quantified numbers of newts caught in traps labeled with either NSE or dechlorinated tap water (H<sub>2</sub>O) and the second quantified captures of newts in traps labeled with either PSE or H<sub>2</sub>O. For both experiments, cellulose sponges (1 × 2.5 × 4 cm) were soaked with 5 ml of stimuli (NSE or H<sub>2</sub>O for experi-

<sup>1</sup> Corresponding author.

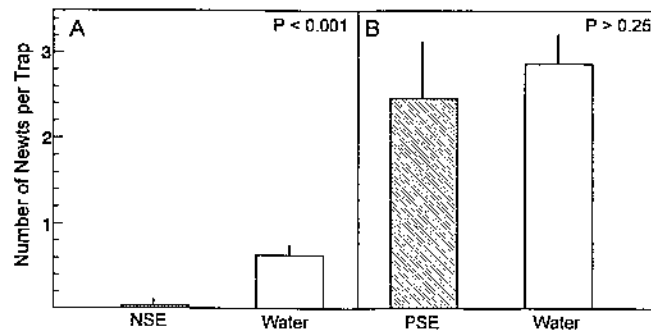


FIG. 1. Mean ( $\pm 1$  SE) number of newts caught in traps labeled with either salamander skin extracts or with dechlorinated tap water. Separate experiments (A and B) were conducted using skin extracts from newts (NSE) and skin extracts from the terrestrial salamander, *Plethodon serratus* (PSE). Probability values are from two-tailed Mann-Whitney U-tests.

ment 1, PSE or H<sub>2</sub>O for experiment 2) and immediately placed on separate trays in a freezer at  $-20^{\circ}\text{C}$ . After the sponges were frozen, we placed them into separate sealed plastic bags, one for each stimulus. The sponges were kept in the freezer or in a cooler on ice until the trials began. One sponge was wired into the middle of each minnow trap (Gee's improved minnow traps: cylinders of  $5 \times 5$  mm wire mesh, 43 cm long and 22 cm in diameter, with inverted cone entrances at each end) at the field site.

The two experiments were conducted in three ponds in March 1996. All trials were performed after dark between 1930 and 2230 h. We placed traps into the water by throwing them 1 to 3 m from shore; the traps sank to depths of less than 1 m. Traps were spaced 1 m apart in random order and were left in the pond, undisturbed, for 2 h. Two ponds were used for experiment 1 (NSE trials), one in Stone County, Missouri (oval farm pond,  $15 \times 12 \times <1$  m deep) and one in Webster County, Missouri (rectangular farm pond,  $30 \times 40 \times <2$  m deep). A total of 30 NSE traps and 30 H<sub>2</sub>O traps were used, with both approximately equally represented in each pond. Experiment 2 (PSE trials) was conducted in one pond in Webster County, Missouri (rectangular wooded pond,  $12 \times 4 \times <1$  m deep) and 15 PSE and 15 H<sub>2</sub>O traps were used. Fewer traps were used for experiment 2, because a higher density of newts in this pond resulted in greater trapping success.

After two hours, we collected the traps and recorded the number of newts in each trap. The newts were placed into a holding bucket until all the traps were checked and were then released. Newts were the only vertebrates caught in the traps.

We analyzed the data using two different statistical tests. We compared the mean number of newts caught in the two types of traps (extract versus H<sub>2</sub>O) using Mann-Whitney U-tests (Siegel, 1956). It is not known whether newts are attracted to each other, but social facilitation might amplify any differences between treatment groups. Therefore, we also compared the proportion of traps containing newts for the two treatments using Chi-square tests (Siegel, 1956). All statistical tests were two-tailed.

In the NSE trials (experiment 1), we caught significantly more newts in traps labeled with control water

(19 individuals in 30 traps) than in traps labeled with NSE (one individual in 30 traps) (Mann-Whitney U-test,  $Z = 3.56$ ,  $P < 0.001$ ; Fig. 1A). Over half (17 out of 30) of the control traps contained newts (range = 0–2 newts/trap) compared to one NSE-labeled trap ( $\chi^2 = 17.86$ ,  $df = 1$ ,  $P < 0.001$ ). In contrast, in the PSE trials (experiment 2), we caught 43 newts in 15 control traps and 37 newts in 15 PSE-labeled traps, with no significant difference between the two treatments ( $Z = 1.14$ ,  $P = 0.254$ ; Fig. 1B). Similarly, the proportion of PSE-labeled traps containing newts (14 out of 15 traps; range = 0–10 newts/trap) did not differ from the proportion of control traps containing newts (13 out of 15 traps; range = 0–5 newts per trap) ( $\chi^2 = 0$ ,  $df = 1$ ,  $P > 0.99$ ).

The results of our study indicate that adult newts in a natural habitat avoid areas labeled with stimuli from damaged conspecifics. Therefore, the results of previous studies of newts demonstrating avoidance in laboratory tests (Marvin and Hutchison, 1995; Woody, 1996) are also applicable under more natural conditions. For salamanders, as for vertebrates in general, there have been few studies of chemical communication under field conditions, largely due to methodological difficulties (Mathis et al., 1995). The methods used in this study may be broadly applicable to field studies of attraction/avoidance responses of aquatic salamanders. More detailed behavioral studies are needed to determine if newts also exhibit more subtle antipredator responses to conspecific alarm signals. In addition to avoidance, responses by fishes are known to include increased shoaling, darting behavior, hiding, and reduced foraging (Smith, 1992).

Although we, like Marvin and Hutchison (1995), interpret the avoidance of chemical stimuli from damaged conspecifics as an alarm response, an alternative explanation is that the newts were simply avoiding the scent of conspecifics. There is evidence that newts of this species occupy relatively small home ranges (Bellis, 1968) and behaviorally distinguish between familiar and unfamiliar conspecifics (Wise et al., 1993), but it is not known whether they are territorial. However, our anecdotal evidence is not consistent with the conspecific-avoidance hypothesis as up to 10 newts in this study were found in a single trap.

This study demonstrates that central newts avoid

areas in natural habitats that are labeled with a conspecific skin extract. By avoiding these areas, newts may increase their probability of avoiding predation. However, further studies are needed to clarify the fitness consequences of this behavior.

*Acknowledgments.*—We thank Dan Beckman and Robert Wilkinson for their helpful suggestions concerning the experiments and Nathan Mills for his assistance in collecting animals and locating field sites. Helpful comments on the manuscript were made by James Petranksa, Glenn Marvin, and an anonymous reviewer. Financial support was provided by the Department of Biology at Southwest Missouri State University. Special thanks go to Shari Woody for her assistance in the field and for her encouragement throughout this study.

## LITERATURE CITED

- BELLIS, E. D. 1968. Summer movement of red-spotted newts in a small pond. *J. Herpetol.* 1:86–91.
- BRODIE, E. D., JR. 1968. Investigations on the skin toxin of the red-spotted newt, *Notophthalmus viridescens viridescens*. *Amer. Midl. Natur.* 80:276–280.
- . 1977. Salamander antipredator postures. *Copeia* 1977:523–535.
- CHIVERS, D. P., AND R. J. F. SMITH. 1994. Intra- and interspecific avoidance of areas marked with skin extract from brook sticklebacks (*Culaea inconstans*) in a natural habitat. *J. Chem. Ecol.* 20:1517–1524.
- DODSON, S. I., T. A. CROWL, D. L. PECKARSKY, L. B. KATS, A. P. COVICH, AND J. M. CUIP. 1994. Non-visual communication in freshwater benthos: an overview. *J. N. Am. Benthol. Soc.* 13:268–282.
- DUCEY, P. K., AND J. DULKIEWICZ. 1994. Ontogenetic variation in antipredator behavior of the newt *Notophthalmus viridescens*: comparisons of terrestrial adults and efts in field and laboratory tests. *J. Herpetol.* 28:530–533.
- HEWS, D. K. 1988. Alarm response in larval western toads, *Bufo boreas*: release of larval chemicals by a natural predator and its effect on predator capture efficiency. *Anim. Behav.* 36:125–133.
- , AND A. R. BLAUSTEIN. 1985. An investigation of the alarm response in *Bufo boreas* and *Rana cascadae* tadpoles. *Behav. Neural Biol.* 43:47–57.
- HURLBERT, S. H. 1970. Predator responses to the vermilion-spotted newt (*Notophthalmus viridescens*). *J. Herpetol.* 4:47–55.
- MARVIN, G. A., AND V. H. HUTCHISON. 1995. Avoidance response by adult newts (*Cynops pyrrhogaster* and *Notophthalmus viridescens*) to chemical alarm cues. *Behaviour* 132:95–105.
- MATTIS, A., R. G. JAEGER, W. H. KEEN, P. K. DUCEY, S. C. WALLS, AND B. W. BUCHANAN. 1995. Aggression and territoriality by salamanders and a comparison with the territorial behaviour of frogs. In H. Heatwole and B. K. Sullivan (eds.), *Amphibian Biology*, vol. 2, *Social Behaviour*, pp. 663–676. Chipping Norton, NSW, Australia.
- , AND R. J. F. SMITH. 1992. Avoidance of areas marked with a chemical alarm substance by fathead minnows (*Pimephales promelas*) in a natural habitat. *Can. J. Zool.* 70:1473–1476.
- , AND ———. 1993. Intraspecific and cross-species responses to chemical alarm signals by brook stickleback. *Ecology* 74:2395–2404.
- PETRANKA, J. W. 1989. Response of toad tadpoles to conflicting chemical stimuli: predator avoidance versus “optimal” foraging. *Herpetologica* 45:283–292.
- SHURE, D. J., L. A. WILSON, AND C. HOCHWENDER. 1989. Predation on aposomatic efts of *Notophthalmus viridescens*. *J. Herpetol.* 23:437–439.
- SIEGEL, S. 1956. *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York.
- SMITH, R. J. F. 1992. Alarm signals in fishes. *Rev. Fish Biol. Fish.* 2:33–63.
- WEBSTER, D. A. 1960. Toxicity of the spotted newt, *Notophthalmus viridescens*, to trout. *Copeia* 1960:74–75.
- WISE, S. E., K. S. SIEK, K. M. BROWN, AND R. G. JAEGER. 1993. Recognition influences social interactions in red-spotted newts. *J. Herpetol.* 27:149–153.
- WOODY, D. R. 1996. The role of alarm pheromones in predation avoidance by adult central newts, *Notophthalmus viridescens*. Unpubl. Master's Thesis, Southwest Missouri State Univ, Springfield.

Accepted: 29 January 1997.