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## Response of *Plethodon cinereus* to Chemical Cues from Different Numbers of Conspecifics

Loren M. Hurst and Geoffrey R. Smith<sup>2</sup>

### Abstract

The red-backed salamander, *Plethodon cinereus*, is a species that generates and responds to chemical cues. Such cues can affect interactions among conspecifics. We studied the response of adult male and female *P. cinereus* to cues from different numbers of conspecifics using pairwise choice experiments. Females tended to avoid substrates containing cues from five conspecifics as opposed to one or zero, whereas males made no distinction. The sex of the donor salamander had no effect on substrate choices. Our results indicate that there are differences in how males and females respond to cues about the density of conspecifics.

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The response of the red-backed salamander, *Plethodon cinereus* Green, to varying chemical signals affects interactions within this species and influences their distribution and population density within a given habitat (Tristram, 1977). *Plethodon cinereus* use chemical signals to gain information about both heterospecific and conspecific salamanders and neighboring territories (e.g., Jaeger *et al.*, 1986; Mathis, 1990; Graves, 1994; Martin *et al.*, 2005), as well as assess prey availability (Placyk and Graves, 2002; Karuzas *et al.*, 2004) and predation risk (e.g., Madison *et al.*, 1999; Sullivan *et al.*, 2002, 2003, 2004). However, it is not clear how these salamanders respond to cues from different numbers of conspecifics (i.e., whether they will prefer substrates with cues from many conspecifics or substrates with no or few cues).

We examined the response of adult male and female *P. cinereus* from a population in central Ohio to the chemical cues of different numbers of conspecifics (0, 1, or 5 individuals). Field and laboratory observations of the *P. cinereus* in this population suggest that the salamanders are not territorial. For example, we have observed up to 13 salamanders under a single cover object (G.R. Smith, unpubl. data), and adult males show no territorial behavior in staged encounters in the laboratory (A.A. Burgett and G.R. Smith, unpubl. data). We therefore predicted that there would be little if any response to the different numbers of conspecifics if territoriality is driving the choice of individual salamanders. However, if individual salamanders are choosing or avoiding marked substrates for other reasons, such as avoiding competition (e.g., prefer substrates with cues from fewer competitors), we might expect a preference for fewer conspecifics, especially among females who may have greater energetic demands due to egg production and brooding (Yurewicz and Wilbur, 2004), or who may seek to avoid harassment by courting males (e.g., Rohr *et al.*, 2005).

### Materials and Methods

Adult *P. cinereus* were collected from secondary growth forest, under natural and artificial cover objects, at the Denison University Biological Reserve, Licking Co., Ohio during October 2003, the start of the breeding season in *P. cinereus*. Salamanders were sexed by candling (Gillette and Peterson, 2001). All salamanders were adults, and no females were observed guarding nests when collected.

In the laboratory, we transferred salamanders to 12 clear, plastic boxes (30 cm x 15 cm x 12 cm) on a substrate of several pieces of damp 15cm filter paper, each piece cut in half (i.e., to



make a semi-circle). Four of the boxes contained 1 salamander each (2 boxes containing males, 2 containing females); another four boxes housed 5 salamanders (2 containing 3 males and 2 females, and 2 containing 2 males and 3 females); and the final four boxes housed no salamanders (controls). These numbers of donor salamanders were chosen because they reflect numbers of salamanders that might be expected to be commonly encountered in nature by individual *P. cinereus* in this population. Salamanders housed in the boxes served as donor salamanders and were maintained at approximately 17-19°C. Salamanders were not fed, but we misted the salamanders and filter paper with water once a day (including the control containers). Donor salamanders were housed for 5 d, and then returned to the location from which they were collected.

We performed experiments on 6, 12, and 19 October 2003. After 5 d, we removed donor salamanders from the plastic boxes. Following the removal of feces, we transferred the filter paper to 15 cm diameter petri dishes. Three choice experiments were set up using the petri dishes: 0vs1, 0vs5, and 1vs5. For each petri dish in the 0vs1 combination, we placed a piece of filter paper from a blank (control) box on half of the petri dish and filter paper from a box holding one donor salamander on the other half of the petri dish. The same procedure was used for the 0vs5 and 1vs5 combinations using filter paper from the appropriate donor boxes. We then misted the dishes with water and transferred one test salamander, collected earlier that day (at least 8 – 10 h earlier), to each dish. Donor and test animals were collected from different areas of the Denison University Biological Reserve to reduce possible familiarity among salamanders that might influence their behavioral responses (e.g., Guffey *et al.*, 1998; Jaeger and Peterson, 2002). A total of 117 salamanders was used as test salamanders over the course of the study, with 51 males and 66 females tested.

Table 1. Responses of red-backed salamanders, *Plethodon cinereus*, to the cues from varying numbers of conspecific salamanders. Numbers of salamanders choosing the area with cues from the lower and higher number of donor salamanders are given for each experimental condition (see text for explanation of experimental set-up).

	<u>0 vs 1</u>	<u>0 vs 5</u>	<u>1 vs 5</u>
All salamanders			
Lower 16	25	26	
Higher	26	13	11
Females only			
Lower	8	17	15
Higher	16	5	5
Males only			
Lower	8	8	11
Higher	10	8	6



Fifteen minutes after salamanders were introduced into the test petri dish, we began observing the salamanders, recording their position every 5 minutes for 1 hour. We recorded position according to the side of the petri dish where the majority of the salamander's body was located. If the salamander's body was evenly divided between the two sides of the petri dish, we recorded the side with the head as the chosen side. Tests were conducted from 2200h to 2400h, with shades closed and lights off (observations of salamanders were made using indirect lighting from a flashlight). *Plethodon cinereus* is nocturnal and responds to chemical cues more strongly in the evening than during the day (Madison *et al.*, 1999). Petri dishes were randomly oriented and located on a laboratory bench. Salamanders collected for testing became the new donor salamanders for the following week's test.

For statistical analysis, we first determined whether an individual salamander avoided or preferred the side with the cues from the highest number of donor salamanders. "Avoidance" was assigned if the salamander used the side with the cues from the highest number of donor salamanders < 50% of the time, and "Preference" was assigned if the salamander used the side with the cues from the highest number of donor salamanders > 50% of the time (see Madison *et al.*, 1999; Sullivan *et al.*, 2004). We then used a series of Chi-square analyses to examine the effects of the independent variables on the responses of the test salamanders. We used a sequential Bonferroni correction on the  $\chi$  value to account for multiple statistical tests on a single dataset, and all results referred to as significant were statistically significant following this correction.

### Results

The sex of the donor in the 0vs1 experiment had no effect on the behavior of males ( $\chi_1^2 = 3.54$ ,  $P = 0.060$ ) or females ( $\chi_1^2 = 0.076$ ,  $P = 0.78$ ). Likewise, the make-up of the donors in the 5 salamander groups (i.e., 2 males:3 females vs. 3 males:2 females) did not affect the behavior of males ( $\chi_1^2 = 0.022$ ,  $P = 0.88$ ) or females ( $\chi_1^2 = 3.38$ ,  $P = 0.07$ ). We therefore pooled across donor sex and donor group compositions in all subsequent analyses.

Overall, salamanders tended to prefer the side with cues from the highest number of donor salamanders in the 0vs1 experiments, but they avoided the side with cues from the highest number of donor salamanders in the 0vs5 and 1vs5 experiments (Table 1;  $\chi_2^2 = 9.99$ ,  $P = 0.0068$ ). This pattern held true when only females were analyzed (Table 1;  $\chi_2^2 = 11.77$ ,  $P = 0.0028$ ), but not when only males were analyzed (Table 1;  $\chi_2^2 = 1.52$ ,  $P = 0.47$ ).

### Discussion

*Plethodon cinereus* tended to avoid areas containing odors from five conspecifics, preferring areas marked by either one or zero conspecifics, but when given the choice between areas containing cues from 0 or 1 individual, they tended to prefer the area with the cues from a single individual. However, we found that it was only females that showed this response. Male *P. cinereus* showed no differences in preferences between the experimental conditions, which is consistent with the lack of territorial behaviour among males we have observed for this population (A.A. Burgett and G.R. Smith, unpubl. data).

Our results suggest that female *P. cinereus* in this population are able to differentiate among substrates marked by cues from varying numbers of conspecifics, and that there is a tendency to avoid substrates marked by larger numbers of conspecifics. It may be that females are simply avoiding areas with a higher concentration of social chemical cues. For example, female *N. viridescens* avoid large groups of males, presumably because they can reduce the cost of courtship that might occur in the presence of several males (Rohr *et al.*, 2005). Alternatively, the salamanders may be



avoiding areas with alarm cues that may be released during physical interactions between conspecifics (i.e., during biting or other physical interactions in the "donor" boxes). Indeed, Sullivan *et al.* (2003) found that *P. cinereus* avoid areas with cues from injured conspecifics. Male *Notophthalmus viridescens* will avoid chemical cues from injured conspecifics but this avoidance is lessened by the presence of cues from females during the breeding season (Rohr and Madison, 2001; see also Rohr *et al.*, 2002, 2003), thus a similar response in *P. cinereus* could explain the difference between males and females in our study. Finally, females may be avoiding competition for food. Females may be more "choosy" about the number of potential competitors than males because females use a great deal of energy on egg production and brooding (Yurewicz and Wilbur, 2004).

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