

Does the Aquatic Salamander, *Siren intermedia*, Respond to Chemical Cues from Prey?

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Early detection of prey can be an important factor in determining whether a predation event is ultimately successful (Lima and Dill, 1990). In aquatic amphibians prey detection could involve a number of sensory modalities, including vision, chemoreception (smell and taste), tactile / mechanoreception (including the lateral line), and electroreception (see Heatwole and Dawley, 1998). Most experimental studies of prey detection in amphibians have focused on use of either visual (overview in Roth et al., 1998) or chemical (overview in Dawley, 1998) cues. In general, visual cues appear to play a central role in well-lit habitats with active prey, and chemical cues are more important if prey are cryptic or if habitats have reduced visibility because they are dark, cloudy, or highly vegetated (Dodson et al., 1994; Dawley, 1998).

Siren intermedia is a paedomorphic salamander whose habitat ranges from shallow ditches and ponds to permanent swamps or sloughs (Petranka, 1998). Because sirens tend to burrow in sediments or occupy vegetated areas or underwater crayfish burrows, they can be difficult to locate and capture (Carr, 1940; Cockrum, 1941; Freeman, 1958). Despite their cryptic existence, sirens may be important as both predators and competitors in some wetland communities (Fauth and Resetarits, 1991; Resetarits and Fauth, 1998; Fauth, 1999; Snodgrass et al., 1999). Their contribution to the biomass of the community can be substantial: Gehlbach and Kennedy (1978) found siren density and standing crop biomass to be as high as 1.3 sirens/m² and 56.6 g/m², respectively, and Frese (2000) estimated siren density and biomass in southeastern Missouri to be 1.7 sirens/m² and 58 g/m².

Sirens prey on a wide range of taxa. Stomach contents have revealed snails, bivalve shells, aquatic insects, algae, and mud (Dunn, 1924; Noble and Marshall, 1932; Carr, 1940; Collette and Gehlbach, 1961). In addition to invertebrate prey, sirens rely on amphibian larvae and small fish as seasonally important food items (Altig, 1967; Sullivan, 1999). Sirens are believed to forage at night (Davis and Knapp, 1953; Asquith and Altig, 1987) by consuming organisms closely associated with the substrate (Hardin and Mount, 1978).

Because sirens have small eyes and typically are active under conditions of restricted visibility (at night

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in turbid habitats), nonvisual cues may be important for sirens in identifying and locating prey. We specifically hypothesize that sirens will be attracted to prey based solely on chemical cues. We tested this hypothesis in two laboratory experiments and one field experiment.

In the laboratory, we used two Y-maze choice experiments (e.g., Waldman, 1985; Cogalniceanu, 1994; Woody and Mathis, 1998) to determine whether sirens discriminated between chemical stimuli from prey and a blank control. We collected adult sirens on 21–22 March 1998 from the Mingo National Wildlife Refuge in Stoddard County, Missouri. With the exception of two animals that were housed together, each siren was kept in the laboratory in a separate 38-L aquarium with 7.6 L of dechlorinated tap water, aquatic vegetation (*Anacharis*), and a 25-cm section of PVC tubing for use as a burrow. Sirens were kept on a 14 L:10 D photoperiod at approximately 23 C and were fed commercial shrimp pellets four to five times per week. Individuals were not fed for 36 h prior to testing to standardize hunger levels.

The two laboratory experiments used two different food stimuli. Scroggin and Davis (1956), Altig (1967), and Sullivan (1999) reported that aquatic gastropods (snails) were abundant in siren digestive tracts. In the first experiment, water conditioned with snails was used as the stimulus. Aquatic snails (*Elimia potosiensis*) were collected from the James River, Greene County, Missouri and the stimulus solution was produced by keeping 50 snails in 1 L of dechlorinated tap water for 72 h prior to testing. The stimulus for the second experiment was a solution produced by mixing 10 g of crushed shrimp pellets (familiar laboratory diet) with 500 ml of dechlorinated water. Both solutions were filtered through filter paper immediately prior to testing to eliminate solid particles. Discoloration was evident after filtration. For each treatment, an equal volume of dechlorinated tap water was used as a control.

The testing chamber was a 38-L aquarium partitioned to form a Y-shaped maze with two stimulus arms and a neutral area. The chamber was filled with 7.6 L of dechlorinated water. A movable plexiglass barrier separated a "neutral" area from the two arms of the testing area. Small holes were drilled through the movable barrier to allow diffusion of the stimulus. Stimulus and control solutions were injected in 20-ml doses through lengths of plastic tubing attached at the back wall of the stimulus arms. Sides of the maze were randomly designated as stimulus or control, and the chamber was thoroughly cleaned between trials.

Twelve sirens were tested in random order for each experiment. Each siren was removed from its holding tank with a dip net and gently released into the "neutral" portion of the test chamber. The sirens almost immediately began to swim slowly around the neutral chamber and did not appear to be stressed by the transfer. Our experience has been that sirens that are disturbed dash around their aquaria, often hitting the glass walls of the tanks. After the siren was placed into the neutral chamber, the stimulus and control solutions were simultaneously injected into opposite arms of the Y-maze. According to separate dye trials, the stimulus reached the neutral chamber in approximately two minutes. At the end of five minutes the barrier was removed and the siren was free to move

into the arms of the Y-maze. The time spent on each half of the maze was recorded with location determined as the position of the siren's snout. Each trial lasted ten minutes.

The proportion of time spent with the prey stimulus was calculated as: (time in stimulus arm) ÷ (time in stimulus arm + time in control arm). A Wilcoxon Matched-Pairs Signed-Rank Test (Siegel, 1956) was used to determine if the proportion of time on the stimulus side deviated from the expected proportion of 0.50 for random selection ($\alpha = 0.05$).

In our field study, we used a trapping experiment to determine whether sirens would be attracted to chemical stimuli from a third prey type, tadpoles of *Rana sphenoccephala*. Similar trapping experiments have been successful in other tests of hypotheses about attraction to or avoidance of chemical stimuli (e.g., Mathis and Smith, 1993; Wisenden et al., 1994; Woody and Mathis, 1997). We established a trap grid in a large ephemeral pool located in the Mingo National Wildlife Refuge. A single wire minnow trap was placed at each of the 110 stations. Minnow traps had funnels on either end and measured approximately 43 × 23 cm. Stations were located approximately 5 m apart; we considered this distance to be sufficient for traps to be independent replicates. Total grid dimensions were approximately 55 m × 55 m, and water depth was approximately 15 cm.

Cellulose sponges (approximately 3 × 5 × 8 cm) were soaked with either experimental or control stimuli and were attached to the interior of the minnow traps with copper wire. The experimental stimulus was produced by conditioning 1 L of dechlorinated tap water with 24 *Rana sphenoccephala* tadpoles (mean total length = 45 mm) for 36 h. Dechlorinated tap water was the blank control. Each sponge contained approximately 12 ml of the stimulus solution. A total of 55 traps contained the prey stimulus and 55 traps contained the blank stimulus. Traps were randomly placed throughout the grid at approximately 1800 h on 30 April 1999 and retrieved at approximately 0800 h on the following morning. After data were recorded, sirens were released at the point of capture.

The mean proportion of time spent with the prey stimulus was not significantly different from the expected random proportion of 0.5 in either the first (Wilcoxon: $T = 29.0$, $P = 0.456$, Fig. 1A) or second laboratory experiment (Wilcoxon: $T = 55.0$, $P = 0.224$, Fig. 1B).

In the field experiment, sirens were captured in nine prey-marked traps and 10 blank traps. There was no significant difference in the proportion of traps between the two treatments ($\chi^2 = 0.95$; $P = 0.95$). There also was no significant difference between the mean number of sirens captured in traps with the prey stimulus and the blank control (Mann-Whitney U-test, $W = 3080$; $P = 0.81$; Fig. 1C).

In all three of our experiments, adults of *Siren intermedia* did not appear to use chemical cues in localization of prey. The lack of statistically significant differences among treatments does not appear to be due to lack of statistical power. Our experiments used sample sizes that were similar to those used in other studies (see below) and the P -values were relatively high (all > 0.20) (see Johnsson, 1996 for a discussion of "nonsignificant" results).

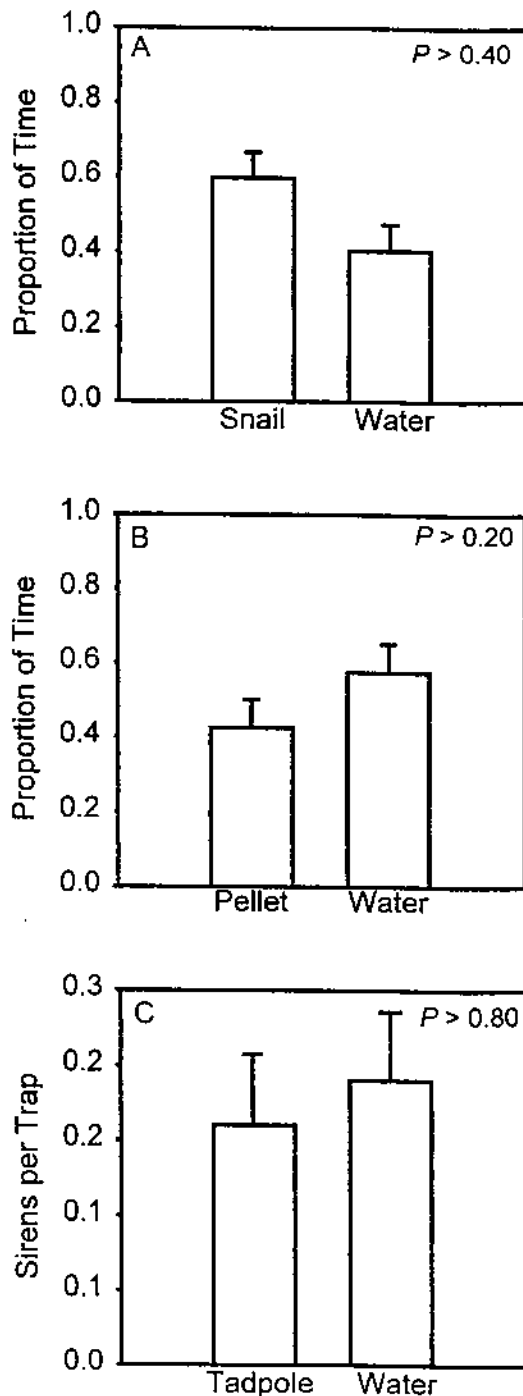


FIG. 1. Responses of sirens to chemical stimuli from prey in two laboratory experiments (A, B) and one field experiment (C). Data for the laboratory experiments are proportions of time spent on different sides of the testing chamber containing either water labeled by a food stimulus (snails of *Elimia potosiensis* or crushed shrimp pellets) or a control of dechlorinated tap water. Probability values are from a Wilcoxon Signed Rank Test and

Our results should be interpreted with some caution. First, our tests included only three types of prey. It is possible that chemical stimuli from other prey or at stronger concentrations might elicit stronger responses. A second problem is that both the Y-maze and trapping techniques lack sensitivity to responses other than attraction (Mason et al., 1998). Animals may respond to chemical stimuli from prey by increasing their activity levels (Lemly and Smith, 1987) or by increasing searching behavior (Cooper and Vitt, 1989), and these responses would not have been detected by our design. Third, even if chemical cues alone are insufficient for prey detection, they may be effective in combination with other stimuli. For example, prey detection by newts of *Notophthalmus* (Martin et al., 1974) is more efficient when both visual and olfactory cues are used. However, because sirens did not distinguish between prey and control stimuli in any of our experiments, we hypothesize that sirens do not rely heavily on chemical cues as their primary means of locating prey.

It seems unlikely that sirens are compensating for chemical cues with greater reliance on visual cues because their eyes are small, they are nocturnal, and they live in habitats where visibility is limited. Instead, we hypothesize that foraging by sirens is generally accomplished by random suctioning of material associated with benthic debris or vegetation. Sirens have ventral mouths and appear to be predominately filter feeders (Altig, 1967). Alternatively, sirens may utilize disturbances in electrical fields to locate prey using a dense network of neuromasts located on the head (reviewed in Fritzsche and Neary, 1998) or by using mechanoreceptors in the lateral line which sirens retain throughout development (Reno and Middleton, 1973).

Aquatic salamanders from some other families of salamanders have been shown to utilize chemical cues (or at least nonvisual cues) in prey detection (e.g., larval *Ambystoma*: Lannoo, 1986; *Triturus*: Joly, 1981 and Ranta et al., 1990; *Proteus*: Uiblein et al., 1992). However, permanently aquatic salamanders apparently have received little study. Because there is some evidence that the olfactory organs are generally less complex in the permanently aquatic salamanders (Jurgens, 1971), future tests of salamanders in the families Sirenidae, Cryptobranchidae, Amphiumidae, and Proteidae may be particularly interesting. Comparative studies of prey discrimination in different families of lizards has been extremely successful in generating and testing ecological and phylogenetic hypotheses (e.g., Cooper, 1994). A similar approach for studies of amphibians could prove equally productive.

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$N = 12$ in both experiments. Data from the field experiment are number of sirens caught in traps labeled with stimuli from either *Rana* tadpoles (Tadpoles) or dechlorinated tap water. Probability values are from a Mann-Whitney U-test and there were 55 traps in each treatment. All data are $\bar{x} \pm 1$ SE.

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