Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*

Alicia Mathis

Department of Biology, Southwest Missouri State University, Springfield, MO 65804, USA

Summary. Detection of heterospecific predators and prey via chemical cues is well known, but only a few studies have examined the potential for such discrimination in cannibalistic systems. In newts, *Notophthalmus viridescens*, adults are opportunistic predators of conspecific larvae. I used a laboratory bioassay to determine whether larvae and adults distinguish between chemical stimuli from members of the different age classes. Larvae distinguished between chemical stimuli from larvae and adults by decreasing their activity only following exposure to stimuli from adults. Decreased activity is consistent with an antipredator response in many prey species, including larval newts. In contrast, adults increased their activity and increased time spent in open areas in response to stimuli from larvae, but not to stimuli from adults. Increased activity is consistent with a feeding response; adults also showed increased activity and increased time in open areas in response to chemical stimuli from familiar heterospecific prey (brine shrimp). The proximate cue that allowed the newts to distinguish between the different age classes is not associated with short-term dietary differences because all stimulus animals were fed the same prey. Stimulus "strength" was controlled by diluting the stimulus solutions according to the volume of the stimulus animal. Therefore, there appear to be intrinsic differences in the chemical signatures of larval and adult newts.

Key words. Cannibalism – intraspecific predation – prey detection – predator detection – newts – Amphibia

Introduction

In aquatic vertebrates, cannibalism appears to be common in species where large size asymmetries exist among co-occurring individuals (Kusano *et al.* 1985; Smith & Reay 1991; Nilsson & Brönmark 1999; Wildy *et al.* 2001). Some unusual species are polymorphic, with cannibalistic individuals exhibiting morphological characteristics such as enlarged jaws and teeth (Walls *et al.* 1993). In most species, however, cannibalism is opportunistic, with small individuals potentially in danger of predation from any sufficiently larger conspecific in the vicinity.

Many species that exhibit cannibalism occur in relatively dense populations (Polis 1981; Smith and Reay 1991). Because individuals frequently encounter conspecifics, small individuals attempting to avoid all conspecifics would spend a great deal of time in avoidance behavior and subsequently may experience an excessive number of missed foraging opportunities. Individuals should therefore benefit by avoiding only conspecifics that offer a high level of threat. Visual assessment of size differences can be effective, but requires individuals to be relatively close together, which could be dangerous for small individuals. Moreover, visual cues are not reliable when visibility is limited due to murky or vegetated habitats, when light levels are low, or with cryptic or ambush predators. Therefore, individuals that can use chemical cues to assess size of nearby conspecifics should experience earlier and more reliable information about the level of potential danger than individuals that rely solely on visual cues. Similarly, large individuals would benefit by using chemical information to distinguish potential prey from other conspecifics.

Newts, *Notophthalmus viridescens*, offer a good opportunity to study assessment of conspecifics through chemical cues. There are large size differences between adults and larvae, and adults are opportunistically cannibalistic on larvae (Gabor 1996). Adults and larvae often occur together in large numbers in small ponds where visibility often is limited due to high levels of sedimentation and/or vegetation (Harris *et al.* 1988). Chemical cues are important to larvae for detection of heterospecific salamanders (Mathis & Vincent 2000), and to adults for recognition of heterospecific prey (Martin *et al.* 1974). The goal of my study was to determine whether: (1) larvae distinguish between chemical stimuli from conspecific larvae and adults by showing larger decreases in activity (a fright response: Mathis & Vincent 2000) to stimuli from adults, and (2) adults distinguish between chemical stimuli from conspecific larvae and adults by showing larger increases in activity (a feeding response: Martin *et al.* 1974) to stimuli from larvae.

Materials and methods

Collection and maintenance

I collected adult ($\bar{X}$ ± 1 SD snout-vent length = 8.2 ± 0.76 cm) and larval (total length = 3.9 ± 0.47 cm) central newts

Correspondence to: Alicia Mathis, e-mail: sam477f@smsu.edu

(*N. v. louisianensis*) in Webster County, Missouri in July of 1995. Individuals were kept in separate plastic boxes (10.5 × 10.5 × 8.5 cm) in about 500 ml of dechlorinated tap water and were fed frozen brine shrimp (*Artemia* sp.) daily. The laboratory was on a 14L:10D light cycle and temperatures were 20 C ± 2.

Behavior of larvae

The purpose of this experiment was to determine whether larvae responded differently to chemical stimuli from conspecific adults and larvae. To collect the stimulus solutions, I transferred the stimulus animals to clean water 24 h prior to testing. I used volumetric displacement to determine that the volume of an average-sized larva was about 0.5 ml and that of an average-sized adult was about 2.5 ml. Therefore, to equilibrate the strength of the stimulus solutions, I kept individual larvae in 100 ml of water and individual adults in 500 ml of water.

I tested the larvae ($\bar{X} \pm 1$ SD total length = 3.9 ± 0.48 cm) in plastic containers (21 × 15 × 8.5 cm) in 1000 ml of dechlorinated tap water. I attached a polypropylene tube to the center of one of the lengthwise sides of the chamber so that the end of the tube was about 1 cm above the surface of the water. At the beginning of each trial, I introduced one randomly selected larva into the center of the testing chamber. After a 20-min habituation period, I injected 50 ml of clean dechlorinated tap water into the chamber through the tube at a rate of about 1 ml/s and then recorded the time that the larva spent active for 10 min. This observation period established baseline activity following a neutral disturbance. Activity was defined as any movement with the exception of gill flicks. At the end of the pre-stimulus period, I injected 50 ml of a stimulus solution (either water from another larva's chamber or water from an adult's chamber) into the testing chamber through the tube and recorded activity for an additional 10 min. To verify that the 10-min time frame was adequate for assessment of responses to the stimulus solution, I injected food coloring into the testing chamber; the dye reached all corners of the chamber within 30 s. Testing order was determined randomly and treatments were randomly assigned to each larva. Individual larvae were tested only once. An activity response index was calculated as post-stimulus activity - baseline activity, with positive numbers indicating increased activity and negative numbers indicating decreased activity. Decreased activity in the presence of predators has been observed for many taxa, including larval *Notophthalmus viridescens* (Mathis & Vincent 2000). Because of the well-supported predicted directions of differences, I used a one-tailed Mann-Whitney U-test (Siegel 1956) to analyze the data.

Behavior of adults: heterospecific prey

Adults were predicted to respond to chemical stimuli from larvae with a feeding response. The purpose of this experiment is to confirm Martin *et al.*'s (1974) observation that adult newts respond to chemical stimuli from prey with increased activity. Brine shrimp were chosen as the familiar prey because the adults had been maintained in the laboratory for several weeks on a diet of brine shrimp. For a familiar neutral stimulus, I used tadpoles (*Rana* sp.); these tadpoles, which were common in the pond where the newts were collected, were too large (SVL range = 3.3 -3.6 cm) to be prey for the newts.

Stimulus solutions were collected as described for the larval tests except that stimulus animals were either two tadpoles or one 2.0-g cube of frozen brine shrimp in 400 ml of water. Two tadpoles and a cube of brine shrimp cube were approximately the same volume (about 3 ml). Brine shrimp were added to the stimulus water only about 30 min prior to testing to reduce decomposition. Testing chambers were 8-l glass aquaria (30 × 23 × 15 cm) with a polypropylene tube for stimulus injection (as described above). I placed an airstone in the water about 2-cm from the stimulus-injection tube to more rapidly disperse the stimulus. Dye trials indicated that the dye was evenly dispersed within 20 s of injection. Trials and statistical analyses were conducted as described for the larval tests. I recorded two response variables: (1) Time active:
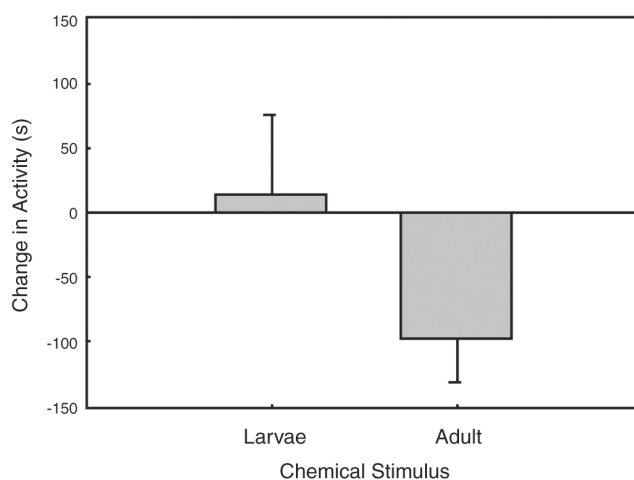

Fig. 1 Change in activity of larval newts following exposure to chemical stimuli from other larvae and from adult newts. Change in activity is the difference between the response of the larvae to a water control (baseline) and the experimental stimulus (water from tanks with larvae or adults). Data are presented as $\bar{X} \pm 1$ SE and responses were significantly different according to a Mann-Whitney U-test

searching for prey might result in increased time swimming (e.g., Lemly & Smith 1985; Kardong *et al.* 1997). (2) Percent of active time spent in the open water: adult newts spent a large proportion of the trials swimming with their snouts pressed against the wall of the glass; searching for prey might result in more time spent active in the open water. Because Martin *et al.* (1974) reported increased activity in response to prey, I used a one-tailed Mann-Whitney U-test to compare responses to prey and nonprey.

Behavior of adults: conspecific stimuli

The purpose of this experiment was to determine whether adult newts distinguished between chemical stimuli from adult and larval newts. I predicted that adults should respond to chemical stimuli from larvae, but not other adults, with a feeding response. A feeding response would be interpreted as a qualitatively similar response to that in the brine shrimp trials described above, so statistical tests were one-tailed. Stimuli were collected as described for the larval trials. The experimental protocol was the same as in the brine shrimp trials.

Results

Behavior of larvae

Larvae were significantly less active in response to chemical stimuli from conspecific adults than to chemical stimuli from other larvae (U = 11, N = 7, P < 0.05; Fig. 1). Because decreased activity is a typical response to predatory stimuli for larval amphibians, I also conducted post-hoc comparisons of pre-stimulus (baseline) versus post-stimulus activity within each treatment. The larvae did not significantly change their activity following exposure to chemical stimuli from other larvae (Wilcoxon T = 10, N = 7; P = 0.248), but significantly decreased their activity following exposure to chemical stimuli from adults (T = 2, N = 7, P < 0.025).
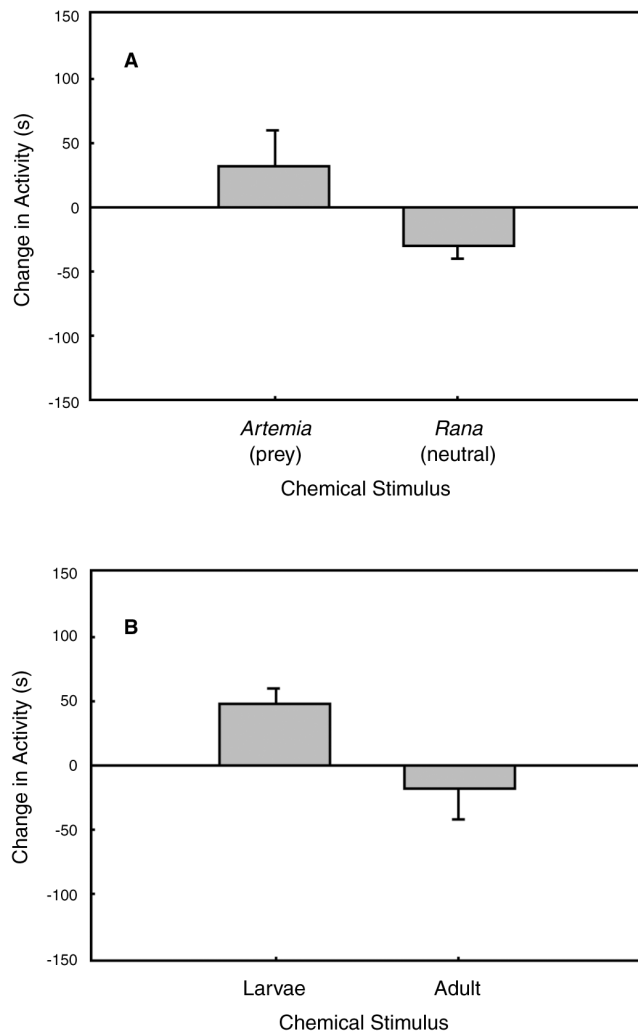
Fig. 2 Change in activity by adult newts following exposure to chemical stimuli from (A) *Artemia* (brine shrimp: familiar prey) and *Rana* tadpoles (neutral stimulus) and (B) larvae and other adult newts. Change in activity is the difference between the response of the larvae to a water control (baseline) and the experimental stimulus. Data are presented as $\bar{X} \pm 1$ SE and responses were significantly different according to a Mann-Whitney U-test
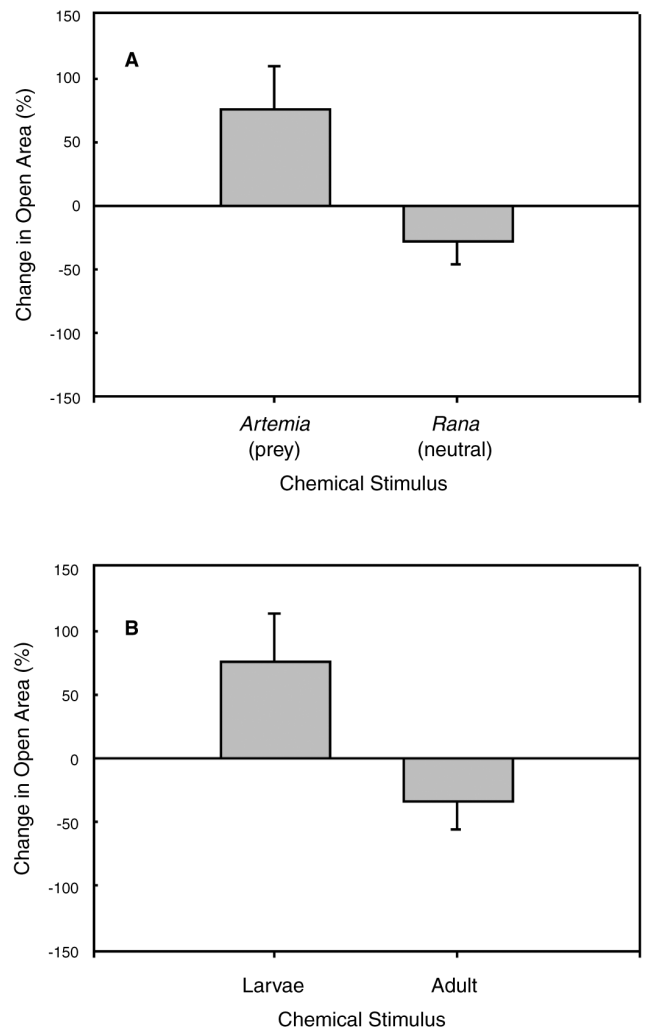
Fig. 3 Change in % time spent in the open by adult newts following exposure to chemical stimuli from (A) *Artemia* (brine shrimp: familiar prey) and *Rana* tadpoles (neutral stimulus), and (B) larvae and other adult newts. Change in activity is the difference between the response of the larvae to a water control (baseline) and the experimental stimulus. Data are presented as $\bar{X} \pm 1$ SE and responses were significantly different according to a Mann-Whitney U-test

Behavior of adults

Adults distinguished between stimuli from familiar prey and neutral stimuli. Activity was significantly greater following exposure to the brine shrimp stimulus in comparison to the *Rana* stimulus (U = 19, N = 10, P = 0.01; Fig. 2A). In addition, focal newts spent more time in the open water following exposure to the brine shrimp stimulus than to the *Rana* stimulus (U = 8, N = 10, P < 0.001; Fig. 3A).

Adults also distinguished between chemical stimuli from larvae and other adults, with the response to larvae similar to their response to heterospecific prey. Activity was significantly greater following exposure to chemical stimuli from conspecific larvae in comparison to stimuli from other adults (U = 25, N = 10, P < 0.05; Fig. 2B). More

time was spent in the open water following exposure to larval stimulus than to the adult stimulus (U = 19, N = 10, P < 0.0125; Fig. 3B).

Discussion

Both larvae and adult newts were able to distinguish between age classes of conspecifics based solely on chemical cues. The response of larvae to chemical stimuli from adults was consistent with a fright response (lower activity) whereas the response of adults to chemical stimuli from larvae was consistent with a feeding response (higher activity). Because all test animals were field-caught, it is impossible to

know whether the ability to discriminate between age classes is innate or requires experience.

Larvae in some other species of salamanders also have been shown to be able to recognize potentially cannibalistic conspecifics as dangerous based on nonvisual cues. Young (small) larval California newts (*Taricha torosa*) showed increased use of shelter in the presence of chemical cues from adult newts in comparison to a blank control (Elliot *et al.* 1993; Kats *et al.* 1994). These studies did not test whether larvae could distinguish between age (size) classes of conspecifics (adults versus larvae). In eastern long-toed salamanders (*Ambystoma macrodactylum*), some larvae are cannibalistic and have distinctive morphology (large head and teeth) associated with cannibalism. Larvae in this species were able to distinguish between cannibalistic and noncannibalistic conspecific larvae based on nonvisual cues (either chemical or mechanical) (Chivers *et al.* 1997).

In this study I interpreted reduced activity as an antipredator response for larval newts because larval newts in another study showed decreased activity in response to heterospecific predators (larval tiger salamanders, *Ambystoma tigrinum*) (Mathis & Vincent 2000). Decreased activity in response to predatory stimuli is a common antipredator response for amphibians (Stauffer & Semlitsch 1993) and other vertebrates (Downs 2002; Mirza & Chivers 2003). Although my study was a laboratory bioassay, decreased activity by larval salamanders in response to predatory stimuli also has been reported in natural habitats (Mathis *et al.* 2003). Decreased activity in response to predatory stimuli can lower the risk of predation by decreasing the probability of detection (Azevedo-Ramos *et al.* 1992; Skelly 1994).

Adult newts responded to chemical stimuli from larval newts with increased activity, which was similar to their response to familiar prey (brine shrimp). Martin *et al.* (1974) also reported increased activity by newts as a response to invertebrate prey. Increased activity in response to chemical stimuli from prey occurs in a number of taxa (Lemly & Smith 1985; Kardong *et al.* 1997) and should function to increase the probability of the predator coming in contact with the prey. Adult newts apparently use both chemical and visual cues in foraging, with chemical cues most useful in initial location of prey and visual cues (prey movement) eliciting the attack (Martin *et al.* 1974).

I do not know the proximate mechanism by which discrimination between larval and adult salamanders is made. If cues emanate from the skin, adult salamanders have more surface area and should produce more stimuli. However, I controlled for the difference in surface area by diluting the stimulus water based on salamander volume, so the "strength" of the stimulus water should have been relatively equal for adult and larval stimulus animals. Predator diet can affect recognition by prey (reviewed by Chivers & Mirza 2001), but I controlled potential short-term dietary cues by feeding adult and juvenile stimulus animals the same diet. Post-metamorphic individuals have glands that are lacking in larvae (Mathis & Vincent 2000), and it is possible that glandular secretions contribute to different chemical signatures of larvae and adults.

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