TOXIC PREY AND PREDATOR AVOIDANCE: RESPONSES OF TOXIC NEWTS TO CHEMICAL STIMULI FROM A PREDATOR AND INJURED CONSPECIFICS

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ABSTRACT—Predator avoidance behavior minimizes predation risk because the prey never enters the perceptual field of the predator. For toxic species, avoiding potential predators indiscriminately is unlikely to be the best predator avoidance strategy because many predators will be incapable of consuming the toxin. We investigated the role of chemical-based avoidance behavior in predator-prey interactions between a toxic prey species, the Rough-skinned Newt (*Taricha granulosa*), and its major predator, the Common Garter Snake (*Thamnophis sirtalis*). Terrestrial newts avoided predator kairomones only when the predator had recently consumed a conspecific, but did not avoid damage-released alarm cues. These results indicate newts may be able to chemically differentiate between toxin-resistant and toxin-sensitive snakes in the same population and avoid only those snakes actively preying upon their cohort.

Key words: alarm cue, antipredator, Common Garter Snake, predator avoidance, Rough-skinned Newt, *Taricha granulosa*, tetrodotoxin, *Thamnophis sirtalis*, TTX

Of all antipredator strategies, predator avoidance should provide the greatest reduction in predation risk because prey entirely avoid the perceptual field of the predator and the sequence of a predation event is never initiated (Endler 1986; Brodie and others 1991). Predator kairomones or injury-released alarm cues are common signals used by prey organisms to avoid predators before detection (Chivers and Smith 1998; Kats and Dill 1998). Optimally, these behaviors result in reduced predation risk and increased time available for other fitness enhancing activities such as foraging, mating or searching for mates, or territorial defense (Lima and Dill 1990; Chivers and Smith 1998; Kats and Dill 1998). Research indicates that responding to these cues does indeed increase the survival time of prey and should enhance the fitness of organisms expressing these behaviors (Mathis and Smith 1993; Chivers and others 2002).

While predator avoidance is clearly advantageous (failure to respond can result in death), responding to each presumed predatory threat would likely leave little time available for behaviors required for normal growth and development (Lima and Dill 1990). Prey should therefore balance the benefits and risk associated with predator avoidance and other activities to maximize lifetime fitness (Lima and Dill 1990). However, prey are likely to have more information available to them than chemical cues alone, and it would be incautious to think they would not incorporate this information in decision making. For example, a prey organism may detect chemical cues from a predator, but past experience indicates the predator is unlikely to be actively foraging at that particular time. Based on this additional information, the organism may disregard the chemical information in lieu of past experience and continue normal activity. This threat-sensitive predator avoidance hypothesis (Helfman 1989) has been demonstrated in a variety of taxa including fish (Helfman 1989; Chivers and others 2001a), amphibians (Mathis and Vincent 2000; Puttlitz and others 2001), and invertebrates (Peckarsky 1996). In addition to learned information, prey may also incorporate phenotypic components into decision making such as background matching, escape ability, or toxicity.

We investigated the role of chemical-based avoidance behavior in predator-prey interactions between the Rough-skinned Newt (*Taricha granulosa*) and its major predator, the Common Garter Snake (*Thamnophis sirtalis*). In various places across its range, *T. granulosa* has high levels of the neurotoxin tetrodotoxin (TTX) in its skin (Hanifin and others 1999). Rough-skinned Newts are believed to be the most poisonous of all salamanders, containing as much as 14 mg of toxin, or enough to kill 10 to 20 humans (Mosher and others 1964; Wakely and others 1966; Brodie and others 1974; Hanifin and others 1999). The emergence of extreme toxicity is believed to be a response to the evolution of TTX resistance among garter snakes (Brodie and Brodie 1990; Motychak and others 1999; Geffeney and others 2002; Brodie and others 2005). In the most poisonous populations where toxicity and resistance are well matched (Hanifin and others 2008), salamanders can be eaten by a relatively small number of extremely resistant snakes (Williams and others 2003). Unlike most predator-prey systems, where the prey may be victim to a large array of potential predators and constant vigilance is necessary for survival, Rough-skinned Newts may be somewhat relaxed from ''normal'' risk due to their toxicity. It is unknown how extreme toxicity or the possible relaxation of predation risk has influenced predator avoidance behaviors in Rough-skinned Newts. Using the most poisonous population of Rough-skinned Newt, we examined whether newts respond to chemical cues from a predator with varying levels of associated risk (such as snakes feeding versus not feeding on newts) and whether they possess an injury-released chemical alarm cue.

METHODS

Adult Rough-skinned Newts (hereafter newts) were collected in March 2009 and 2010 from Soap Creek pond near Corvallis, Oregon. They were transferred to Utah State University and housed in 5.7 L plastic tubs filled with 3 L of filtered tap water. Bloodworms were provided weekly. Five weeks prior to testing, the newts were transferred to individual terrestrial containers filled with a thin layer of moist sphagnum moss and fed crickets weekly.

Stimulus from snakes that had not consumed newts was obtained from 4 Common Garter Snakes collected on 6 June 2010 near Corvallis. Snakes were housed in 37-L aquaria with newspaper substrate and constant access to water. Snakes were not fed prior to stimulus collection. Although we did not specifically control the diet of these individuals prior to collection, the snakes were housed without food for 4 d prior to collection of stimulus to allow evacuation of gut contents. Moreover, they were offered and refused newts shortly after stimulus collection, but readily consumed Northern Leopard Frogs (*Lithobates pipiens*).

The 2 snakes used in the snake-fed-newt treatment were long-term captive snakes collected from 2 populations in El Dorado and Sonoma counties, California, that are extremely resistant to TTX (husbandry conditions same as above). The snakes were deprived food for 2 wk before the introduction of a newt. Three days prior to stimulus collection, each snake was fed 1 newt from Soap Creek, which they each immediately consumed. The number of snakes used in each treatment to collect chemical stimuli in this study is similar to the number used in previous studies examining the effect of predator kairomones on salamander behavior (Cupp 1994; Madison and others 1999a; Murray and Jenkins 1999), and Madison and others (1999b) demonstrated that salamander responses do not differ to cues from individual *T. sirtalis*.

Terrestrial newts were exposed to chemical stimuli from one of the following treatments: snakes that had not recently consumed a newt ($n = 18$); snakes fed a newt ($n = 19$); macerated newt skin ($n = 18$); or filtered-tap water [blank control ($n = 18$)]. Stimulus samples were collected in unused 3.7-L glass jars with 35 ml of filtered tap water. A snake was weighed and measured, placed inside the jar and the lid was taped on to prevent escape. After 24 h the snake was removed, and 5 ml/g body mass filtered tap water was added to the jar. We subtracted the original 35 ml from the total volume of water to be added to the jar to ensure that the stimulus was diluted with exactly 5ml water/g body mass. Stimulus solutions from different snakes within a treatment were combined, mixed thoroughly, and frozen at −80°C in 40 ml aliquots. Freezing does not affect the efficacy of garter snake secretions (Sullivan and others 2002). For the blank control treatment, filtered tap water was added to an empty jar that had never contained a snake, and then frozen as for stimulus solutions. For the macerated newt skin treatment, a newt was killed by freezing. The skin was removed (1.33g), macerated with a mortar and pestle, mixed with 200 ml of filtered tap water, and frozen in 40 ml aliquots.

The test chambers consisted of 9.46 L opaque buckets lined with 2 semi-circular paper towels in the bottom with the towels separated by a 1.5-cm gap to prevent mixing of the stimuli. One side of the chamber was marked with ''A'' and the other marked with ''B.'' One of the sides was randomly chosen as the treatment side and 7 ml of stimulus solution was poured onto the center of the paper towel. The towel on the other side was moistened with 7ml of the previously frozen blank filtered tap water. A 9-cm diameter by 12-cm long clear plastic cylinder was set on end in the center of the test chamber. The test chamber was misted 3 times to increase humidity and standardize the moisture on both sides of the paper towels.

Newts were assigned a number, one of these numbers was randomly selected, and that newt was placed inside the clear tube. Newts were tested in only 1 treatment and were never retested within a treatment. A piece of clear plastic wrap was placed over the bucket, followed by an opaque lip with a 9 × 9 cm hole cut for viewing. This prevented moisture levels from declining during the trials and prevented air currents from influencing odors or choice and minimized external visual stimuli. After a 20-min acclimation period the clear cylinder was removed and the trial started. The chamber was rotated 90° every 30 min to control for side bias. Experiment observers were blind to the identity and position of the treatment. The position of the newt on the paper towels was recorded every 5 min for 2 h. If the newt was located across both sides of the chamber, a score was given based on the position of its head. At the conclusion of testing, the newt was removed, weighed, and returned to its housing container. The experimental chamber was washed with hot water and air dried.

We counted the number of observations on the treatment side of the test chamber for each trial. A total of 24 observations were possible for each newt. Newts that did not move after the removal of the acclimation cylinder were removed from the analyses (3 trials). Using a Paired t-test, or a Wilcoxon Signed Rank Test for data that did not meet assumptions for parametric statistics, we then determined if newts spent significantly less time than expected from random choice on the stimulus side of the test chamber for each treatment. This or similar analyses are commonly used to evaluate the responses of terrestrial amphibians to chemical stimuli (Cupp 1994; Lutterschmidt and others 1994; Marvin and Hutchison 1995; Murray and Jenkins 1999; Chivers and others 2001b; Murray and others 2004).

RESULTS

Terrestrial newts avoided areas on a paper towel labeled with stimulus solution from *T. sirtalis* that had recently consumed a newt ($t = -2.368$, $P = 0.029$, Fig. 1). However, newts did not avoid the blank control ($Z = 0.812$, $P = 0.431$, Fig. 1), or areas on a paper towel that had been labeled with stimulus solution of *T. sirtalis* that had not consumed a newt ($Z = 3.234$, $P = 1.0$, Fig. 1) or macerated newt skin ($t = -0.872$, $P = 0.396$, Fig. 1).

DISCUSSION

Our results indicate that the Rough-skinned Newt avoids kairomones from their only major predator, the Common Garter Snake, only when that predator has recently consumed a conspecific newt. Dietary cues can be important components of predator kairomones, and predator avoidance responses are often strengthened when the predator has recently consumed conspecifics (Kats and Dill 1998). For example, the terrestrial plethodontid salamander, *Plethodon cinereus* (Eastern Red-backed Salamander), exhibited stronger avoidance responses to chemical cues from salamander-fed snakes than from worm-fed snakes (Murray and Jenkins 1999; Sullivan and others 2002). Greater intensity responses to predators whose diets have been supplemented with conspecific prey are often assumed to be due to specific recognition

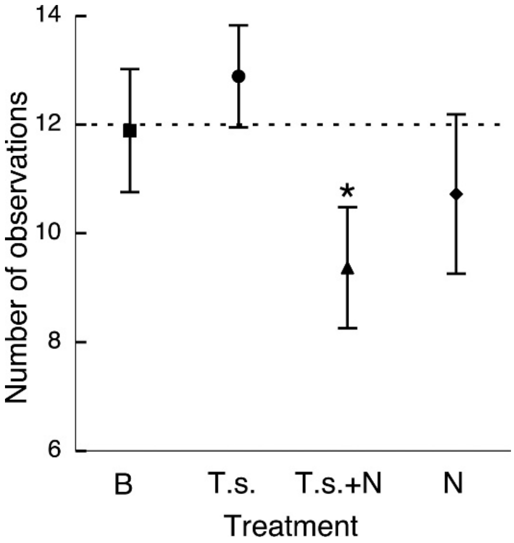

FIGURE 1. Mean (± SE) number of times terrestrial Rough-skinned Newts (*Taricha granulosa*) were observed on the stimulus side of the test container. B = filtered water control; T.s. = filtered water with chemical cues from *Thamnophis sirtalis* that had not consumed a newt; T.s.+N = filtered water with chemical cues from *Thamnophis sirtalis* that had recently consumed a newt; and N = filtered water with macerated newt skin. Dashed line indicates random distribution between sides of the test chamber. * $P < 0.05$.

of the prey's alarm cue. If this is the case, prey should also strongly avoid alarm cues from injured conspecifics in the absence of any predator kairomones. Several other salamandrids, including species of *Triturus*, *Cynops*, and *Notophthalmus*, have been documented avoiding damaged-released alarm cues of conspecifics (Margolis 1985; Marvin and Hutchison 1995; Rohr and Madison 2001). In our study, newts did not respond to macerated newt skin with predator avoidance behavior, indicating a combination of predator kairomones and dietary cues are necessary to elicit avoidance behavior. *Plethodon cinereus* also responded to predators fed conspecific salamanders but did not avoid chemical alarm cues from injured salamanders (Murray and Jenkins 1999). These authors suggested that digestion and metabolism of the prey's chemical signature might be necessary to trigger functional fright responses, and similar processes may be necessary to trigger a response from newts.

Prey that avoid only those predators actively preying upon their cohort, based on the detection of specific dietary cues in the kairomones, should minimize risk of predation without sacrificing time for foraging or other similar actions. Our results suggest that newts only avoid snakes when they have consumed another newt. This may ensure time and energy are not wasted avoiding potential predators that are, at present, not a threat.

Unlike most prey species, this particular population of *T. granulosa* is prey to only 1 predator, *T. sirtalis*, and the presence of TTX in the dorsal skin of the newts has likely modulated this outcome (Brodie and Brodie 1990; Brodie and others 2002). Coevolution between populations of toxic newts and toxin-resistant snakes has resulted is multiple populations across the western United States with disparate levels of toxicity and resistance (Brodie and others 2005; Hanifin and others 2008). Of all these populations, Soap Creek phenotypes are one of the most closely matched relative to newt toxicity and snake resistance. Consequently, most of the newts can be consumed by only the few extremely toxin-resistant snakes (Williams and others 2003). It would be maladaptive for newts from this population to avoid all snakes, regardless of diet, because only a fraction of those present are actually capable of consuming a newt. Avoiding only those snakes that are physiologically resistant to the toxin and are labeled as such by dietary cues should minimize predation risk and maximize lifetime fitness.

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