Juvenile western toads, *Bufo boreas*, avoid chemical cues of snakes fed juvenile, but not larval, conspecifics

L. K. BELDEN, E. L. WILDY, A. C. HATCH & A. R. BLAUSTEIN

Department of Zoology, Oregon State University

Previous investigations have demonstrated the importance of predator diet in chemically mediated antipredator behaviour. However, there are few data on responses to life-stage-specific predator diets, which could be important for animals like amphibians that undergo metamorphosis and must respond to different suites of predators at different life-history stages. In laboratory choice tests, we investigated the chemically mediated avoidance response of juvenile western toads, *Bufo boreas*, to four different chemical stimuli: (1) live conspecific juveniles; (2) live earthworms; (3) snakes fed juvenile conspecifics; and (4) snakes fed larval conspecifics (tadpoles). Juvenile toads avoided chemical cues from snakes that had eaten juvenile conspecifics, but did not respond to the other three stimuli, including chemical cues from snakes fed larval conspecifics. In addition, the response to cues from snakes fed juveniles differed significantly from that of snakes fed larvae. To our knowledge, this is the first study to demonstrate the importance of diet in predator avoidance of juvenile anurans and the ability of juvenile toads to distinguish between chemical cues from predators that have consumed larval versus juvenile conspecifics.

© 2000 The Association for the Study of Animal Behaviour

Responses of prey animals to chemical cues from predators have been documented in a wide variety of taxa, spanning six phyla (for review see Kats & Dill 1998). Antipredator responses documented in the class Amphibia include changes in life history (e.g. Sih & Moore 1993; Warkentin 1995; Laurila et al. 1998), morphology (e.g. McCollum & Leimberger 1997) and behaviour (e.g. Kats et al. 1988; Chivers et al. 1996a; Kiesecker et al. 1996; Flowers & Graves 1997). Although not responding to predator cues appropriately can be lethal for prey, an excessive or unnecessary response can also have detrimental effects. Animals must make decisions about foraging, reproduction and other activities based on their perceived risk of mortality from predation (Lima & Dill 1990; Lima 1998). Thus, over evolutionary time, selection should act on proper assessment of predation risk.

Responses to predation based on detection of chemical cues often rely on prey being able to associate specific regions with specific chemosensory cues. Regions can be labelled by either the predator (predator avoidance) or other prey animals (alarm signalling). In addition, the predator may label itself by consuming and digesting prey (Chivers & Smith 1998). The importance of predator diet in mediating prey response has been demonstrated in many predator–prey systems. Crowl & Covich (1990)

demonstrated a life-history shift in the freshwater snail, *Physella virgata*, exposed to chemical cues of crayfish, *Orconectes virilis*, fed conspecific snails, but not to crayfish fed only spinach. Similarly, naïve damselfly larvae *Enallagma* spp, reduced activity in the presence of cues from predatory pike, *Esox lucius*, fed damselflies and sympatric fathead minnows, *Pimephales promelas*, but did not reduce activity when pike had been fed mealworms, *Tenebrio molitor* (Chivers et al. 1996b).

In all of the amphibian studies that have incorporated a diet treatment, the 'control' has been a predator with a diet consisting of organisms from a different taxa (e.g. Wilson & Lefcort 1993; Murray & Jenkins 1999) or a starved predator (e.g. McCollum & Leimberger 1997). For other vertebrate taxa, the only exception to these two types of controls is a study on fathead minnows. Naïve fathead minnows respond with antipredator behaviour to predatory pike fed a diet of nonbreeding male fathead minnows, but do not respond when the pike have been fed breeding male minnows (Mathis & Smith 1993). This antipredator response is triggered by minnow alarm pheromone in the diet of the predator. Male minnows temporarily lose their alarm pheromone cells during breeding (Smith 1973), and therefore no antipredator behaviour is observed when breeding males are consumed.

To our knowledge, no one has investigated the response of prey to predators consuming various life-history stages of the test organism. This could be very important for amphibians, where the transition from

Correspondence: L. K. Belden, Department of Zoology, 3029 Cordley Hall, Oregon State University, Corvallis, OR 97331, U.S.A. (email: beldenl@bcc.orst.edu).

water to land during metamorphosis may require development of a response to a different suite of predators. A continued response to predators that are eating only tadpoles would not be required for a juvenile anuran that has metamorphosed. Unnecessary antipredator responses that limit feeding time, activity and growth rate could be detrimental.

Most studies of chemically mediated antipredator responses in anuran amphibians have focused on aquatic larvae (see review in Kats & Dill 1998). Results from these studies indicate that many species, including western toads, *Bufo boreas* (Hews & Blaustein 1985; Hews 1988; Kiesecker et al. 1996), are able to respond to potential predators and conspecific alarm signals. Studies of terrestrial anurans have generally focused on postmetamorphic juveniles (young of the year). Flowers & Graves (1997) found that great plains toads, *Bufo cognatus*, and southwestern toads, *B. microscaphus*, avoid chemical cues from their respective snake predators. Diet of the snakes in this study was not controlled. A similar avoidance was seen in midwife toads, *Alytes muletensis*, to a snake predator fed on a nontoad diet for 3 weeks prior to testing (Schley & Griffiths 1998). Juvenile red-legged frogs, *Rana aurora*, and western toads avoid chemical cues of injured conspecifics, while Cascades frogs, *Rana cascadae*, do not respond to conspecific alarm cues (Chivers et al. 1999). In addition, juvenile *B. boreas* avoid regions that contain cues from predatory garter snakes fed conspecific juveniles for several weeks, although no other predator diet was tested (Chivers et al. 1999).

To investigate the effect of life-stage-specific predator diet on antipredator behaviour of juvenile western toads, we tested the avoidance response to four different sources of chemical stimuli: (1) live juvenile conspecifics; (2) live earthworms; (3) garter snakes fed larval conspecifics (tadpoles); and (4) garter snakes fed juvenile conspecifics.

METHODS

Snakes

We collected a single gravid female red-spotted garter snake, *Thamnophis sirtalis concinnus*, 18 km north of Corvallis, Oregon (Benton County, elevation 100 m) in June 1998. One month later she gave birth to 26 offspring in the laboratory. At 1 week of age, we randomly selected 10 of the young and released the rest, along with the mother, at the site of collection. We divided the 10 offspring remaining in the laboratory into two groups of five snakes. We placed each group in standard 38 litre aquaria with bedding, a single piece of bark for cover, and a petri dish for water. The snakes were kept at 18°C on a 14:10 h light:dark cycle. One group of five was started on a diet of *B. boreas* larvae (tadpoles prior to Gosner stage 42; Gosner 1960) and the other five were started on a diet of *B. boreas* juveniles (post-Gosner stage 45). Prior to the beginning of these treatments, none of the snakes had eaten. Therefore, whichever diet they were started on was the only food type they ever received. One tank of snakes was given 10 tadpoles and the other tank was given 10 juvenile toads every 5–7 days. Although we do not know

that each snake ate at each feeding, all 10 tadpoles/juveniles were consumed at each feeding time, and the total mass of the snakes in each group was the same at the time of testing.

Feeder Toads

Bufo boreas to be fed to the snakes were brought into the laboratory at Gosner stages 24–26 from Lost Lake (96 km east of Albany, Oregon, Linn County, elevation 1213 m) and Frog Lake (136 km northeast of Albany, Oregon, Wasco County, elevation 1174 m) in July 1998. These were housed in standard 38-litre aquaria and fed rabbit chow ad libitum. After forelimb emergence (Gosner stage 42), they were moved to 38 litre aquaria that were slanted at an angle of 30° and filled with water to allow for a dry section of tank on the uphill side (hereafter, slant tank). This allowed metamorphosing animals to leave the water at an appropriate time in development. Metamorphic toads in the slant tanks were fed crickets ad libitum. All aquaria were kept at 18°C on an LD 14:10 h cycle. We fed the snakes a random mixture of *B. boreas* from the two populations (Lost Lake and Frog Lake). To prevent any overlap in the life stages of the toads being fed to the snakes, we defined individuals as tadpoles prior to the emergence of forelimbs (Gosner stage 42), and as juvenile toads after having reabsorbed most of their tail (Gosner stages 45–46).

Test Toads

Juvenile *B. boreas* to be tested were collected at Big Lake (104 km east of Albany, Oregon, Linn County, elevation 1415 m) on 24 August 1998 (5 days prior to testing). We collected a total of 80 juveniles at Gosner stages 45–46 and housed them 20 per 38 litre aquaria in slant tanks. Aquaria were fitted with mesh lids and kept at 18°C on an LD 14:10 h cycle. Animals were fed crickets ad libitum.

Preparation of Chemical Cues

We prepared chemical cues from live earthworms, live juvenile toads, snakes fed tadpoles and snakes fed juvenile toads. We placed the stimulus animals in tanks with water so that cues from them would accumulate in the water. We set up four slant tanks, similar to those described above, with equal stimulus biomass to dechlorinated water ratios 48 h prior to the testing. We weighed the five snakes from each group (combined weight per groups 19 g) and placed each group in a separate slant tank with 2.1 litres of water. We then weighed out 19 g of live earthworms and placed them in a separate slant tank with 2.1 litres of water. We used the earthworm cue as a nontoad, nonpredatory cue to ensure that animals were not responding to snakes simply because they were novel. The mass of live conspecific juveniles (from the feeder population) used was approximately half that of the other groups, and therefore only half as much water was placed in the slant tank. The live conspecific cue was

used to make sure juveniles would not avoid cues from other toads. The two snake groups were our experimental groups of interest, while the earthworm and live conspecifics groups served to verify our methodology.

We left all of the tanks undisturbed for 48 h. Monitoring during this 48 h period indicated that all the stimulus animals were spending time in the water portion of their tanks. On the day of testing, we removed the stimulus animals from their respective tanks. Water in each tank was then mixed by gently rocking the tank five times. After mixing, we removed 100 ml of the water (chemical cue) from each tank to be used in the choice experiments.

Choice Tests

We followed the protocol of Chivers et al. (1996a, 1999) to test avoidance responses. On the day of the testing, we lined the ends of 60 plastic boxes (32 × 18 cm and 8 cm high) with paper towels. A 2 cm space was left in the centre of the box, between the paper towels, to avoid mixing of the chemical stimuli. Fifteen boxes were assigned to each of the four stimulus treatments (earthworm, live conspecific, snake fed tadpole, snake fed juvenile). For each container, we applied 5 ml of the appropriate cue to the paper towel on one randomly selected side of the box (treatment), and 5 ml of dechlorinated water to the other side (control). We then sprayed the entire container with dechlorinated water to equally saturate both sides, thus avoiding the possibility of test animals selecting location based on moisture content of the paper towels.

After all 60 containers had the appropriate cue added, we placed a single juvenile toad in the centre of each box and fitted each box with a lid. We then assigned a random mixture of 20 boxes to each of three observers, who were blind to which treatment groups they were observing, although they did know which side of each container was considered the stimulus side. Test animals were allowed to acclimate for 10 min. The location of each individual in the containers was recorded every 6 min for 1 h. Halfway through the experiment, all containers were rotated 180°, to prevent bias due to geographical orientation of the test animals. If the animals were in the centre of the box, the position of the mouth was used to assign location.

When the trials were completed, we tallied the number of times each individual spent (out of 10) on the stimulus side of the container. We analysed the avoidance response for each of the four groups using a signed-rank test on each group to determine whether the median percentage of time spent on the stimulus side differed from 50%. We then tested whether the response in the snakes fed juveniles group differed significantly from the response to the cues from snakes fed larvae, using a Wilcoxon test.

RESULTS

A single individual in the snakes fed juveniles group climbed the wall away from the stimulus while on the stimulus side of the container. Therefore, it is unclear
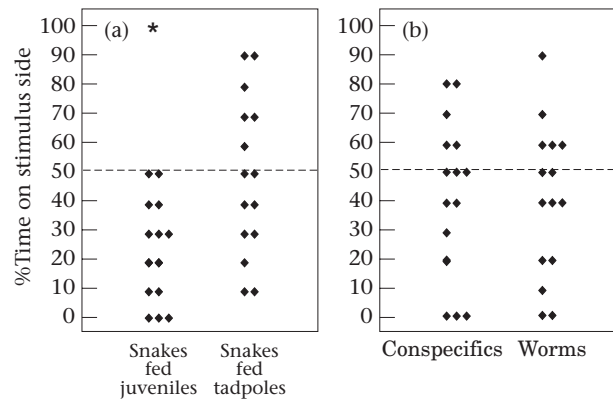
Figure 1. Percentage of time spent on stimulus side of the container for each test toad. (a) Experimental groups; (b) method verification groups. Each point represents a single test toad. *Single outlier in the snakes fed juveniles group that climbed away from the stimulus and was not included in the analyses.

whether the response was avoidance. Hence, this toad was not included in analyses.

In trials testing juvenile toad response to chemical cues from live earthworms, live conspecific juvenile toads, and snakes fed conspecific toad tadpoles, the median percentage of time spent on the stimulus side of the container did not differ significantly from 50% (signed-rank test: for worms: $S = -17.5$, $N = 15$, $P = 0.242$; for live conspecifics: $S = -12.5$, $N = 15$, $P = 0.341$; for snakes fed tadpoles: $S = -1$, $N = 15$, $P = 0.977$; Fig. 1). However, in the trial testing juvenile toad response to cues from snakes fed juvenile toads, there was a significant avoidance of the stimulus side of the container (signed-rank test: $S = -39$, $N = 14$, $P = 0.005$). In addition, there was a significant difference between the median percentage of time spent on the stimulus side for the test toads in the snakes fed juveniles and snakes fed larvae groups (two-sided exact Wilcoxon test: $P = 0.0096$).

DISCUSSION

Our results indicate that juvenile *B. boreas* are able to differentiate between predators that have been eating juvenile versus larval conspecifics. Although previous studies on amphibians have demonstrated the importance of predator diet (e.g. Wilson & Lefcort 1993; Laurila et al. 1998; Murray & Jenkins 1999), we believe this is the first study to demonstrate the importance of diet in chemically mediated predator avoidance of juvenile anurans. In addition, the ability of an anuran to differentiate between cues generated by consumption of conspecifics at different life-history stages has not, to our knowledge, been demonstrated previously.

From an evolutionary perspective, the ability to assess predation risk based on the diet of the predator has obvious selective advantages. Avoiding predators that have not consumed conspecifics may interfere with foraging or reproduction. In addition, the more specific the information is on potential risk, the more useful it may be in behavioural decision making. Therefore, there may be

a benefit to being able to distinguish not only between predators that eat conspecifics, but also for determining the specific life stage of the prey that have been consumed. For amphibians, most of which undergo a distinct metamorphosis, this ability could be especially important. Continuing to respond to chemical cues of predators that are only consuming larval conspecifics could potentially affect activity levels and growth rates of juvenile amphibians. Thus, avoiding chemical cues of insignificant predators may be costly.

The red-spotted garter snakes used in this study are predators of *B. boreas* in Oregon (Nussbaum et al. 1983; Devito et al. 1998). Whether they prefer to eat larval or juvenile toads is unknown. Larval toads would be available as prey for most of the summer, until they begin to metamorphose in the autumn. *Bufo boreas* from many sites in the Oregon Cascade Mountains tend to undergo synchronized metamorphosis (Devito et al. 1998), so that all the larvae metamorphose in a relatively short time. This would mean that snakes could obtain both juveniles and larvae for a brief period, with larvae becoming increasingly scarce. Our results indicate that during this period, toads that have metamorphosed should not respond to predators that are consuming only larvae. We do not know the general dietary habits of *T. s. concinnus*. However, these results do indicate that questions concerning *T. s. concinnus* prey preferences for various anuran life history stages are worth pursuing.

The physiological mechanisms controlling the ability of juvenile *B. boreas* to discriminate larval versus juvenile-fed predators are unknown. Either quantitative and/or qualitative differences in the chemical cue produced could be responsible. We suggest three potential explanations that should be examined further. First, that the larval and juvenile alarm pheromones are completely different chemicals. Second, that predators may metabolize larvae and juveniles differently, or there may be different concentrations of alarm pheromone in them. If there is a minimum concentration threshold of sensitivity for juveniles, they might only avoid the higher concentration of alarm pheromone. A third potential explanation is that juveniles may no longer be able to respond to larval cues due to a change in the number or type of receptors that are used in chemical communication. Because adult anurans are thought to be more visually oriented than aquatic larvae (Burghardt 1990), this type of physiological change in sensory systems might be expected during metamorphosis.

Acknowledgments

References

Burghardt, G. M. 1990. Chemically mediated predation in vertebrates: diversity, ontogeny, and information. In: *Chemical Signals in Vertebrates 5* (Ed. by D. W. Macdonald, D. Muller-Schwarze & S. E. Natynczuk), pp. 475–499. Oxford: Oxford University Press.

Chivers, D. P. & Smith, R. F. J. 1998. Chemical alarm signalling in aquatic predator–prey systems: a review and prospectus. *Ecoscience*, **5**, 338–352.

Chivers, D. P., Kiesecker, J. M., Anderson, M. T., Wildy, E. L. & Blaustein, A. R. 1996a. Avoidance response of a terrestrial salamander (*Ambystoma macrodactylum*) to chemical alarm cues. *Journal of Chemical Ecology*, **22**, 1709–1716.

Chivers, D. P., Wisenden, B. D. & Smith, J. F. 1996b. Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Animal Behaviour*, **52**, 315–320.

Chivers, D. P., Kiesecker, J. M., Wildy, E. L., Belden, L. K., Kats, L. B. & Blaustein, A. R. 1999. Avoidance response of post-metamorphic anurans to cues of injured conspecifics and predators. *Journal of Herpetology*, **33**, 472–476.

Crowl, T. A. & Covich, A. P. 1990. Predator-induced life history shifts in a freshwater snail. *Science*, **247**, 949–951.

Devito, J., Chivers, D. P., Kiesecker, J. M., Marco, A., Wildy, E. L. & Blaustein, A. R. 1998. The effects of snake predation on metamorphosis of western toads, *Bufo boreas* (Amphibia, Bufonidae). *Ethology*, **104**, 185–193.

Flowers, M. A. & Graves, B. M. 1997. Juvenile toads avoid chemical cues from snake predators. *Animal Behaviour*, **53**, 641–646.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183–190.

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. *Animal Behaviour*, **36**, 125–133.

Hews, D. K. & Blaustein, A. R. 1985. An investigation of the alarm response in *Bufo boreas* and *Rana cascadae* tadpoles. *Behavioral and Neural Biology*, **43**, 47–57.

Kats, L. B. & Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience*, **5**, 361–394.

Kats, L. B., Petranka, J. W. & Sih, A. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology*, **69**, 1865–1870.

Kiesecker, J. M., Chivers, D. P. & Blaustein, A. R. 1996. The use of chemical cues in predator recognition by western toad tadpoles. *Animal Behaviour*, **52**, 1237–1245.

Laurila, A., Kujasalo, J. & Ranta, E. 1998. Predator-induced changes in life history in two anuran tadpoles: effects of predator diet. *Oikos*, **83**, 307–317.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator–prey interactions. *Bioscience*, **48**, 25–34.

Lima, S. L. & Dill, L. M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.

McCollum, S. A. & Leimberger, J. D. 1997. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia*, **109**, 615–621.

Mathis, A. & Smith, R. J. F. 1993. Chemical labeling of northern pike (*Esox lucius*) by the alarm pheromone of fathead minnows

(*Pimephales promelas*). *Journal of Chemical Ecology*, **19**, 1967–1979.

Murray, D. L. & Jenkins, C. L. 1999. Perceived predation risk as a function of predator dietary cues in terrestrial salamanders. *Animal Behaviour*, **57**, 33–39.

Nussbaum, R. A., Brodie, E. D., Jr & Storm, R. M. 1983. *Amphibians and Reptiles of the Pacific Northwest*. Moscow, Idaho: University Press of Idaho.

Schley, L. & Griffiths, R. A. 1998. Midwife toads (*Alytes muletensis*) avoid chemical cues from snakes (*Natrix maura*). *Journal of Herpetology*, **32**, 572–574.

Sih, A. & Moore, R. D. 1993. Delayed hatching of salamander eggs in response to enhanced larval predation risk. *American Naturalist*, **142**, 947–960.

Smith, R. J. F. 1973. Testosterone eliminates alarm substance in male fathead minnows. *Canadian Journal of Zoology*, **51**, 875–876.

Warkentin, K. M. 1995. Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proceedings of the National Academy of Sciences, U.S.A.*, **92**, 3507–3510.

Wilson, D. J. & Lefcort, H. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Animal Behaviour*, **46**, 1017–1019.