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The Effects of Snake Predation on Metamorphosis of Western Toads, *Bufo boreas* (Amphibia, Bufonidae)

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Abstract

For some anuran species, synchronous metamorphosis may function as an antipredator adaptation by swamping predators during the period of transformation. We examined the levels of synchrony of emergence from the water of metamorphosing western toads (*Bufo boreas*) in the presence and absence of a live snake predator, the common garter snake (*Thamnophis sirtalis*) in a laboratory experiment. To compare between the treatments, we measured the time to emergence from the water, the number of metamorphs emerging together, and the level of aggregation (before and during emergence) of the toads in each treatment. There was a difference between the treatments when all three factors were considered. We attributed these differences to a behavioral response in which *B. boreas* emerged sooner in the presence of the predator, regardless of whether individual toads had reached the point at which they were physically better suited to the terrestrial environment than the larval environment.

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Introduction

Many animals with complex life cycles undergo shifts in habitat, diet, behavior, and selective pressures. These 'ontogenetic niche shifts' are often associated with meta-morphosis, a period of extensive morphological reorganization (Werner & Gilliam 1984). Metamorphic animals that are no longer suited to their larval environment, and are incompletely suited to their new habitat, might be particularly vulnerable to predation. This is often true for amphibians, and particularly anurans, which pass through a period of decreased locomotor ability as they make the transition from an aquatic herbivore to a terrestrial carnivore (De Jongh 1968; Wassersug & Sperry 1977; Huey 1980; Dudley et al. 1991). For example, some anuran species are most vulnerable to predation by garter snakes (genus *Thamnophis*) near the climax of metamorphosis (Wassersug & Sperry 1977; Arnold & Wassersug 1978; Huey 1980; Drummond & Garcia 1989). The selective pressures associated with the hazards of anuran metamorphosis may have acted to shorten the relative length of this life stage, which may comprise only 15% of larval development (Szarski 1957; Wassersug & Sperry 1977).

Animals have many strategies for dealing with predation during vulnerable life stages. For some species with complex life histories, such strategies may include synchrony of metamorphosis and aggregation of transforming individuals to satiate predators that take advantage of the transition period. Examples include eclosion synchrony in periodical cicadas and molt synchrony in crustaceans (LLOYD & DYBAS 1966; REAKA 1976).

Metamorphic synchrony (temporal proximity in metamorphosis and/or emergence from the water) and aggregation (spatial proximity of individual animals) during emergence from the water have been suggested as possible antipredator adaptations in transforming anurans (ARNOLD & WASSERSUG 1978). Terrestrial aggregations of metamorphosing anurans (including *B. boreas*) have been observed at the shorelines of lakes and ponds during emergence (LILLYWHITE & WASSERSUG 1974; ARNOLD & WASSERSUG 1978; A.R.B., J.D., J.M.K., A.M., pers. obs.). Possible explanations for grouping behavior of anurans during metamorphosis include antipredator defense (by predator satiation and/or by the selfish herd effect) and protection from desiccation (LILLYWHITE & WASSERSUG 1974; ARNOLD & WASSERSUG 1978; HEINEN 1993).

Hypotheses to explain synchrony of anuran metamorphosis and emergence from the water include: antipredator defense (by predator satiation), and a mass exodus of tadpoles from drying temporary larval habitats (ARNOLD & WASSERSUG 1978). The proximate factors leading to synchronous transformation and emergence from the water are not well understood. The rate of development of amphibian larvae and the timing of amphibian metamorphosis are often plastic responses to many environmental cues including: changes in temperature, water level, density, food availability, and the level of predation (WILBUR & COLLINS 1973; WERNER 1986; ALFORD & HARRIS 1988; NEWMAN 1988, 1992; CRUMP 1989; SKELLY & WERNER 1990; ROWE & LUDWIG 1991). Moreover, growth and development can be influenced by the genetic relatednes of individuals (HOKIT & BLAUSTEIN 1994).

The importance of predation in shaping life histories on the evolutionary scale has long been recognized, but its effect on the life histories of many animals in ecological time has been studied only relatively recently (SIH 1987; LIMA & DILL 1990). Amphibians can respond facultatively to the presence of predators by varying the time or size at which they hatch or metamorphose (SKELLY & WERNER 1990; WILBUR & FAUTH 1990; SIH & MOORE 1993; WARKENTIN 1995).

In this study, we attempt to determine whether a connection exists between predation pressures and the degree of (1) aggregation and (2) synchrony of emergence from the water exhibited by transforming anurans. We conducted a laboratory experiment to test whether the presence of a predator (the common garter snake, *Thamnophis sirtalis*) would affect the level of aggregation of transforming western toads, *Bufo boreas*, and the degree of synchrony with which they emerged from the water.

Methods

We collected *B. boreas* tadpoles and adult *T. sirtalis* in late Jul. and early Aug., 1996 at Lost Lake, located in the Oregon Cascade Mountains (Linn County, elevation 1220 m). *T. sirtalis* feed on larval and transforming *B. boreas* during metamorphosis at this site (A.R.B., J.D., J.M.K., A.M., pers. obs.). The animals were transported to

the laboratory, where the tadpoles were kept in aquaria at $\approx 16°C$ on a 14:10 h light:dark cycle and fed alfalfa pellets ad libitum for up to two weeks.

The experiment was conducted in early Aug. at $\approx 20°C$ on a 14:10 h light:dark cycle. We divided 14 plastic tubs (80 × 40 × 14 cm) lengthwise with nylon mesh screen, filled them with ≈ 5 l dechlorinated tap water, and covered them with perforated plastic lids. The tubs were tilted at an angle ($\approx 25°$) so that half of the floor of each tub was submerged and the other half was completely dry. Five *B. boreas* tadpoles (stage 41, immediately prior to the eruption of the forelimbs; GOSNER 1960) were placed on one side of each container. In each of seven experimental containers, we placed a single *T. sirtalis* (SVL = 54–87 cm) on the stimulus side of the mesh divider. Throughout the duration of the experiment, toads in the experimental treatments were exposed to a snake for 24 h at a time, every other day. To minimize inter-snake variability, we rotated four snakes between the seven experimental tubs. In all (experimental and control) treatments, we placed one tadpole and one metamorph on the stimulus side of each tub. These toads served as food for the snakes in the experimental treatments. In the experimental treatments, feeder toads that had been eaten by the snakes were replaced during observation periods. The tubs were arranged in a randomized block design.

We recorded the positions of tadpoles (and later metamorphs) at 0800 and 2.000 h every day for the duration of the experiment. The toads were considered to have emerged from the water if they were found in the upper third (13.3 cm) of the dry half of the tub, or if they had climbed to the top of the mesh divider, above the level of the water. Toads which had emerged from the water were removed from the tub.

To compare the level of synchrony of emergence exhibited between treatments, we recorded the number of toads removed from each tub during the same observation period. From this we determined the geometric mean (the average number of toads with which an individual toad had emerged) for each toad (SOKAL & ROLF 1981). A higher average number of emerging toads per observation period would indicate a higher level of synchrony of emergence.

To compare the level of aggregation between treatments, the proportion of individuals in groups (within one body length of another toad) was recorded for each tub during each observation period. Statistical analyses were performed on the mean values of all observations for each container.

Individuals that emerged from the water were measured, and their snout–vent length and total length were recorded to compare relative tail length (tail length/snout–vent length). Mean relative tail lengths for each tub were used in statistical analyses. To assess whether differences in time to emergence between treatments could be attributed to behavioral or developmental effects, we used multiple linear regression analysis to compare the rates of tail loss between treatments. We would expect that a difference in developmental rates between treatments would correspond with a difference in the rate of tail loss. Conversely, the lack of a difference in the rate of tail loss would indicate a behavioral effect in which tadpoles would attempt to aggregate with conspecifics and/or increase synchrony of emergence by leaving the water at suboptimal stages, either by emerging before locomotion becomes more efficient on land than in the water (i.e. before the loss of the tail; HUEY 1980), or by remaining in the water even after losing the tail.

We used multivariate analysis of variance (MANOVA) to test the treatment effect on the tank means of the dependent variables, aggregation, synchrony, and time to emergence (TABACHNICK & FIDELL 1989). We then analyzed the three response variables individually using independent t-tests with pooled variances. Our Bonferroni adjusted significance level was 0.017 (ZAR 1984).

Based on the selfish herd and predator satiation hypotheses, which have been proposed as possible antipredator defenses for transforming anurans including *B. boreas* (ARNOLD & WASSERSUG 1978; LIMA & DILL 1990), we expected the toads to exhibit a higher degree of aggregation and to leave the water more synchronously in the experimental treatments. Therefore, we used a one-tailed t-test to analyze these effects.

Since the toads used in the experiment were already starting to undergo metamorphosis at the beginning of the experiment, we predicted that a higher degree of aggregation and synchrony would be achieved by a behavioral effect in which metamorphs of slightly different stages would leave the water together. Therefore, we expected to find an overall difference between the two treatments in the time taken by the toads to leave the water. This effect was analyzed using a two-tailed t-test.

Results

MANOVA revealed that the presence of the predator *T. sirtalis* had a significant overall effect on the characteristics (time to emergence, aggregation, and synchrony) of *B. boreas*

Table 1: Results of manova for overall effects of *Thamnophis sirtalis* on the time to emergence from the water, and levels of aggregation and synchrony of transforming *Bufo boreas* and independent t-tests for each response variable. Response variables are time to emergence, in h (time), number of toads found in groups (aggregation), and number of toads emerging together (synchrony)

	F	df	p	
manova	7.183	3, 10	0.006	(2-tailed)
T-tests	T	df	p	
Time	8.000	12	0.015	(2-tailed)
Aggregation	5.820	12	0.017	(1-tailed)
Synchrony	3.927	12	0.036	(1-tailed)

Significance level for independent t-tests is 0.017 (Bonferroni-adjusted for three response variables).

as they emerged from the water (p = 0.006) (Table 1). An independent two-tailed t-test showed that *B. boreas* left the water sooner in the experimental treatments than in the controls (p = 0.015) (Fig. 1). Independent one-tailed t-tests showed that the toads (as both tadpoles and juveniles) were more aggregated (p = 0.017), and that the metamorphs tended to emerge from the water more synchronously in the presence of the predator (p = 0.036) (Fig. 1).

Multiple linear regression showed a significant correlation ($r^2 = 75.29\%$) between tail length (tail/SVL) and time (tail length = 1.17 + 0.118 time); p = 0.0001. The addition of the independent variable, treatment, to the multiple linear regression model did not account for significantly more variation (p = 0.900), indicating that there was no difference in the rate of tail loss (i.e. developmental rate) between treatments. This supports the hypothesis that the earlier emergence observed for toads in experimental treatments can be attributed to a behavioral response.

Discussion

Our results demonstrate that transforming *B. boreas* show a behavioral response to the presence of a snake predator by controlling their spatial distribution and the time at which they emerge from the water. Metamorphic *B. boreas* emerged from the water sooner, showed higher levels of aggregation, and tended to emerge more synchronously in the presence of *T. sirtalis* than did control animals.

In some species, transforming anurans are captured by snakes more often than larvae or juveniles (WASSERSUG & SPERRY 1977), and evidence suggests that this is the case for *B. boreas* (ARNOLD & WASSERSUG 1978; HUEY 1980). In the field, aggregation has been observed in transforming toads of many species, including *B. boreas*, and was suggested by ARNOLD & WASSERSUG (1978) as a possible defense against predation by garter snakes. It has also been suggested that transforming anurans may aggregate to prevent desiccation
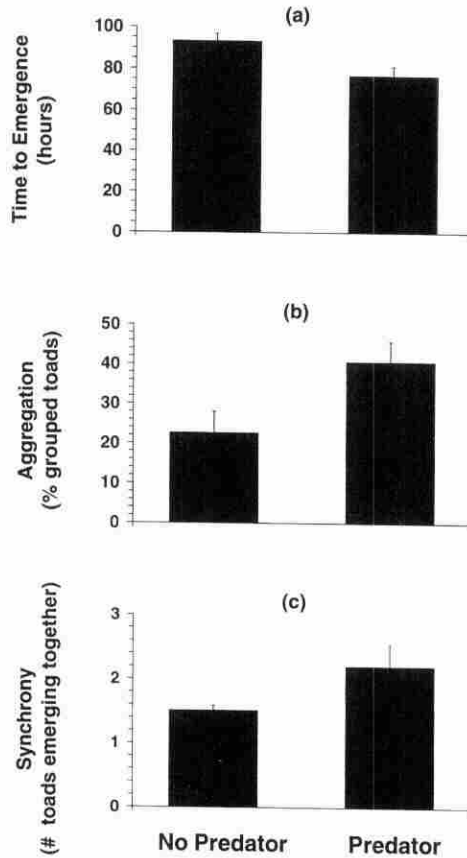
Fig. 1: (a) Mean ($\pm$ SE) time to emergence from the water (h) for *B. boreas* tadpoles, starting from stage 41 (GOSNER 1960). (b) Mean ($\pm$ SE) level of aggregation (percentage of toads in each tub found in groups). (c) Mean ($\pm$ SE) level of synchrony (number of toads in the same tub emerging during the same observation period)

(BLACK & BLACK 1969; ARNOLD & WASSERSUG 1978). HEINEN (1993) tested the predation and desiccation hypotheses for the aggregation of American toads (*B. americanus*). His results provide evidence to support the desiccation hypothesis for *B. americanus*. HEINEN (1993) also found that juvenile *B. americanus* in a completely terrestrial test arena did not aggregate in response to the presence of a snake predator confined in a glass aquarium. However, our results provide evidence that before and during emergence from the water, metamorphosing *B. boreas* aggregate in response to the presence (revealed by visual, chemical, and mechanical cues) of snake predators.

Transforming individuals in the experimental treatments may have responded to predator cues by emerging sooner in the presence of snakes regardless of whether they had reached the point in development at which anuran locomotion becomes more efficient on land than in the water. As a result, more toads in the experimental treatment were in

the same place at the same time (i.e. on land) when snakes were present. Under natural conditions, this could serve to satiate predators at the shoreline. There may be strong selective pressures on metamorphic toads to remain in the aquatic environment until the point when they can move more effectively on land. This point centers around the loss of the tail, which is the primary means of aquatic locomotion and a major hindrance in terrestrial locomotion (WASSERSUG & SPERRY 1977; HUEY 1980). However, if synchrony of emergence is used by metamorphosing anurans as an antipredator defense, there may be opposing selective pressures for an individual to emerge from the water sooner (and with as many conspecifics as possible), whether or not its tail has been lost. Toads which emerge prematurely to reduce predation risk may face alternative threats including an increased susceptibility to dehydration and a decreased ability to behaviorally thermo-regulate (CRUMP 1989; LILLYWHITE et al. 1973; POUGH et al. 1992).

Because the differential exposure to predators was imposed at the larval stage (41; GOSNER 1960) immediately preceding metamorphosis, the higher level of synchrony of emergence and the significant difference in time to emergence exhibited by *B. boreas* in response to the presence of *T. sirtalis* in this experiment could not be attributed to long-term differences in developmental rate. Instead, it appears to be a flexible behavioral response to the presence of a predator. This conclusion is supported by the fact that the linear regression model of tail length over time is not significantly improved by including treatment as an independent variable; *B. boreas* are not developing (i.e. losing their tails) faster in the presence of predators to emerge from the water sooner.

The metamorphs used in our experiment may have responded to any of the cues to which they were exposed (visual, mechanical, and chemical), but we suspect, based on past experiments in which amphibians have responded to chemical cues (e.g. KIESECKER et al. 1996; CHIVERS et al. 1997; FLOWERS & GRAVES 1997), that a laboratory test performed with only chemical cues (i.e. the scent of the snake, the injured conspecific feeder tadpoles, and the snake feces) might yield the same results as our experiment. However, in the field, metamorphosing toads are faced with a more complex set of stimuli. In a shoreline environment, which may be permeated with predator odor, the presence of several cues might be needed to elicit some antipredator behaviors.

Observed behavioral responses of newly metamorphosed toads to the presence of snake predators (on land) include the active choice of cryptic substrates followed by an overall reduction of movement, and engaging in a stereotyped 'crouching' behavior (HEINEN 1985; HAYES 1989; HEINEN 1994). HAYES (1989) also showed that newly metamorphosed *B. americanus* responded differently to different types of contact by snake predators (i.e. toads attempted to avoid detection by remaining motionless except when contacted by the head of a snake, at which time they attempted to flee).

The presence of snake predators has thus been shown to elicit several short-term behavioral responses in larval and juvenile toads, and we have shown that some of these responses may contribute to the high densities of metamorphosing toads along the shorelines of larval ponds during metamorphosis. However, there may be other (long-term) effects of snake predators on the life-history of *B. boreas*, and some of these may also contribute to synchrony of metamorphosis. For the majority of larval *B. boreas* in a single habitat to metamorphose within days of each other, the tadpoles must have developed to approximately the same stage over the course of the entire season. It is possible that

the presence of predators in the larval environment may influence behaviors affecting the long-term developmental rate of the larval population. Long-term behavioral and developmental responses to predation pressure have been documented for larval *B. americanus* (SKELLY & WERNER 1990). Morphological changes in anuran larvae have also been observed as an antipredator response (MCCOLLUM & VANBUSKIRK 1996).

One mechanism that may lead to synchrony of metamorphosis is the explosive breeding strategy of *B. boreas*, in which the majority of eggs laid in a particular habitat during any one breeding season are laid within days of each other (e.g. OLSON 1989). Another possible mechanism that may facilitate synchronous metamorphosis in the field is the tendency of *B. boreas* tadpoles to travel in large schools throughout the larval period (e.g. O'HARA & BLAUSTEIN 1982; BLAUSTEIN & WALLS 1995). The constant exposure of schooling tadpoles to similar environmental conditions (i.e. water temperature and food supply) may function to keep the developmental growth rate similar among the members of a school. The tendency of some species of toad larvae to aggregate with kin (BLAUSTEIN & WALLS 1995), and with larvae of similar size (BREDEN et al. 1982), may also lead to increased synchrony of metamorphosis for schooling tadpoles. Growth and development may also be influenced by the genetic composition of the tadpole schools (HOKIT & BLAUSTEIN 1994). The movement of larvae in the aquatic environment may also be directly influenced by snake predators; KIESECKER et al. (1996) showed that larval *B. boreas* from Lost Lake respond to *T. sirtalis* cues by reducing movement and avoiding stimulus areas.

These factors, combined with the short-term responses to predation pressure observed in this study, may allow *B. boreas* to swamp snake predators during metamorphosis. This strategy would decrease the probability of being preyed upon for each transforming toad during the period most vulnerable to predation.

Acknowledgements

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