

AN ABSTRACT OF THE THESIS OF

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The tadpoles of several anuran species with group-forming larvae are relatively unpalatable to many of their potential predators. To more fully understand the role of palatability and its relationship to group formation as an antipredator defense in anuran amphibian larvae, I investigated the palatabilities of the larvae of three species of anurans, the Western toad (Bufo boreas), Cascades frog (Rana cascadae) and Pacific treefrog (Hyla regilla) to several syntopic insect and salamander predators. Both B. boreas and R. cascadae tadpoles form social aggregations in nature.

Salamanders ate proportionately fewer B. boreas than R. cascadae and H. regilla tadpoles of similar size and same developmental stage. Differential predation on B. boreas, R. cascadae and H. regilla tadpoles was probably due to distasteful qualities of B. boreas larvae as opposed to differences in prey size or escape ability.

After being captured by salamanders, B. boreas tadpoles were usually released unharmed. Both newly-hatched and mid-developmental B. boreas tadpoles were unpalatable to salamanders whereas R. cascadae and H. regilla tadpoles were palatable at all developmental stages tested. The relative palatability of B. boreas, R. cascadae and H. regilla larvae to insect predators was similar regardless of whether tadpoles were newly-hatched, in mid-larval development or at metamorphic climax.

Kin selection may have played a role in the evolution of Bufo unpalatability and group formation in the ancestral population, however, it may not be important for maintaining these traits in extant B. boreas populations. Because group-forming R. cascadae tadpoles are palatable to the predators tested, they may utilize other antipredator defenses to avoid being eaten.

Unpalatability as an Antipredator Defense
in Anuran Larvae

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UNPALATABILITY AS AN ANTIPREDATOR DEFENSE IN ANURAN AMPHIBIAN LARVAE

INTRODUCTION

An enhanced ability to detect and avoid predators is one of numerous advantages an individual may gain by living in a group (e.g. Hamilton, 1971; Alexander, 1974; Bertram, 1978; Milinski, 1979; Treherne and Foster, 1982; Caraco and Pulliam, 1984). As an antipredator mechanism, group formation may be especially important during vulnerable stages of an animal's life history. For example, young animals that are particularly vulnerable to predators may be able to avoid them by being hidden within a group (Kruuk, 1972; Estes, 1976). Additional benefits to group members could be gained through an enhancement of an individual's inclusive fitness (Hamilton, 1964) if members are related and if group members cooperated with one another or warned others in the group of the presence of predators.

Because the larvae of many species of anuran amphibians aggregate in nature, tadpoles may gain many of the benefits of group living that are realized by other animals (e.g. Beiswenger, 1975; Wilbur, 1977; Waldman, 1982; Brodie and Formanowicz, 1987; Blaustein, 1988). Furthermore, mortality due to predation is often highest during the larval stages of an anuran's life cycle (Calef, 1973; Licht, 1974; Smith, 1983). In addition to

the potential antipredator function of group living, tadpoles have several mechanisms that help reduce the risk of predation including cryptic coloration (Wassersug, 1971, 1973), rapid growth to a size refuge from size-limited predators (Caldwell et al., 1980; Brodie and Formanowicz, 1983; Travis et al., 1984; Crump, 1984; Cronin and Travis, 1986) and the use of chemical cues to detect predators (Petranka et al., 1987; Kats et al., 1988). Several studies suggest that tadpole unpalatability, frequently in conjunction with group formation, are important means by which anuran amphibians avoid predation (Liem, 1961; Voris and Bacon, 1966; Wassersug, 1971; Kruse and Francis, 1977; Brodie et al., 1978; Formanowicz and Brodie, 1982; Kruse and Stone, 1984; Brodie and Formanowicz, 1987; Kats et al., 1988).

Unpalatability may be beneficial to an individual if, after being sampled by a predator (i.e. tasted), the tadpole is released unharmed. However, if tadpoles are killed or seriously injured as a result of being sampled by a predator, then the maintenance of unpalatability would likely be through kin selection (e.g. Fisher, 1930; Hamilton, 1964; Benson, 1971; Harvey and Greenwood, 1978). For example, if members of a single brood (kin) aggregate with one another and a predator that samples one or more distasteful siblings learns to avoid others in the group, then a gene for distastefulness could

increase in frequency through kin selection. Because toad (Bufo) tadpoles are conspicuously black and toad tadpoles of many species are distasteful to potential predators, it has been suggested that these larvae are aposematic and a kin selection model has been used to explain the evolution of toad tadpole group formation (Wassersug, 1973; Waldman and Adler, 1979). However, it is also possible that the conspicuous colors of toad tadpoles have evolved in a non-aposematic context (e.g. to enhance thermoregulation; see Guilford 1988 for discussion) and the larval ecology of certain species of group-forming toad larvae may preclude their association with kin (O'Hara and Blaustein, 1982; Blaustein et al., 1987; Blaustein, 1988).

To better understand the role of unpalatability and its relationship to group formation as an antipredator defense in anuran larvae, I investigated the palatabilities of the larvae of two species of anurans, the Western toad (Bufo boreas) and Cascades frog (Rana cascadae) to syntopic salamander and aquatic insect predators. Tadpoles of both species form social aggregations in nature (O'Hara, 1981; Blaustein, 1988). Larvae of the Western toad (B. boreas) are black in color and form highly conspicuous aggregations composed of numerous kin groups and hundreds of thousands of individuals (O'Hara, 1981; O'Hara and Blaustein, 1982;

Blaustein et al., 1987; Blaustein, 1988). Tadpoles of the Cascades frog (R. cascadae) are cryptically colored and form small, cohesive groups, usually less than 100 individuals (O'Hara and Blaustein, 1981; Blaustein, 1988), presumably composed primarily of kin in nature (O'Hara, 1981; O'Hara and Blaustein, 1981; Blaustein and O'Hara, 1987; Blaustein, 1988).

Because the tadpoles of many anuran species with group-forming larvae are unpalatable to their natural predators (Voris and Bacon, 1966; Wassersug, 1973; Brodie et al., 1978; Kruse and Stone, 1984; Brodie and Formanowicz, 1987), I tested the hypothesis that B. boreas and R. cascadae larvae would be distasteful to their natural vertebrate and invertebrate predators. Anuran larvae that are newly-hatched or in metamorphic climax are probably most vulnerable to predation (e.g. Wassersug and Sperry, 1978; Huey, 1980; Crump, 1984; Brodie and Formanowicz, 1987), therefore, I also tested the hypothesis that tadpoles are most unpalatable at these particularly vulnerable stages of larval development (see Brodie and Formanowicz, 1987 for discussion).

METHODS

Relative palatability of anuran larvae were evaluated using both vertebrate and invertebrate aquatic predators. Two species of salamanders, Roughskin newts (Taricha granulosa) and Northwest salamanders (Ambystoma gracile) were the vertebrate predators used while Giant water bug larvae (Lethocerus americanus) and Predaceous diving beetle larvae (Dytiscus sp.) were the invertebrates used. All of these species are important predators of anuran larvae (e.g. Calef, 1973; Licht, 1974; Brodie et al., 1978) and are frequently found in lakes and ponds with R. cascadae and B. boreas tadpoles (personal observation). With the exception of the T. granulosa used in experiments 5 and 6 (see below), all animals used in this study were collected from lakes and ponds in the Oregon Cascade Mountains and were transported to a laboratory in Corvallis for testing.

Study animals were kept at 20-22°C in a laboratory under a natural photoperiod. Tadpoles were maintained in 38-liter aquaria, with dechlorinated tapwater and an aeration stone. They were fed a diet of rabbit pellets daily and their water was changed as needed. Salamanders were held in 120 cm x 60 cm tanks filled 15 cm deep with dechlorinated tapwater. While in the laboratory salamanders were maintained on tubifex worms (Tubifex sp.). Aquatic insects were held in 10 cm x 10 cm

containers filled 6 cm deep with dechlorinated tapwater. Each container had several pieces of pond weed in it for substrate. Insects were fed Pacific treefrog (Hyla regilla) tadpoles in the middle stages (Gosner 1960) of larval development daily.

Salamander Experiments

All of the A. gracile were captured from Fay Lake in Linn County which is not used for breeding by R. cascadae or B. boreas (personal observation, R. O'Hara personal communication). Because A. gracile rarely immigrate to Fay Lake (see Taylor, 1977), it is unlikely that any A. gracile used in this study had either a previously learned avoidance or preference for R. cascadae or B. boreas tadpoles. The T. granulosa used in experiment 3 (see below) were also captured from this lake and it is possible that these individuals had previously encountered R. cascadae or B. boreas tadpoles because adults may leave the lake after breeding. However, the T. granulosa used in experiments 5 and 6 were taken from a lake in Benton County where neither R. cascadae or B. boreas occur (Nussbaum et al., 1983).

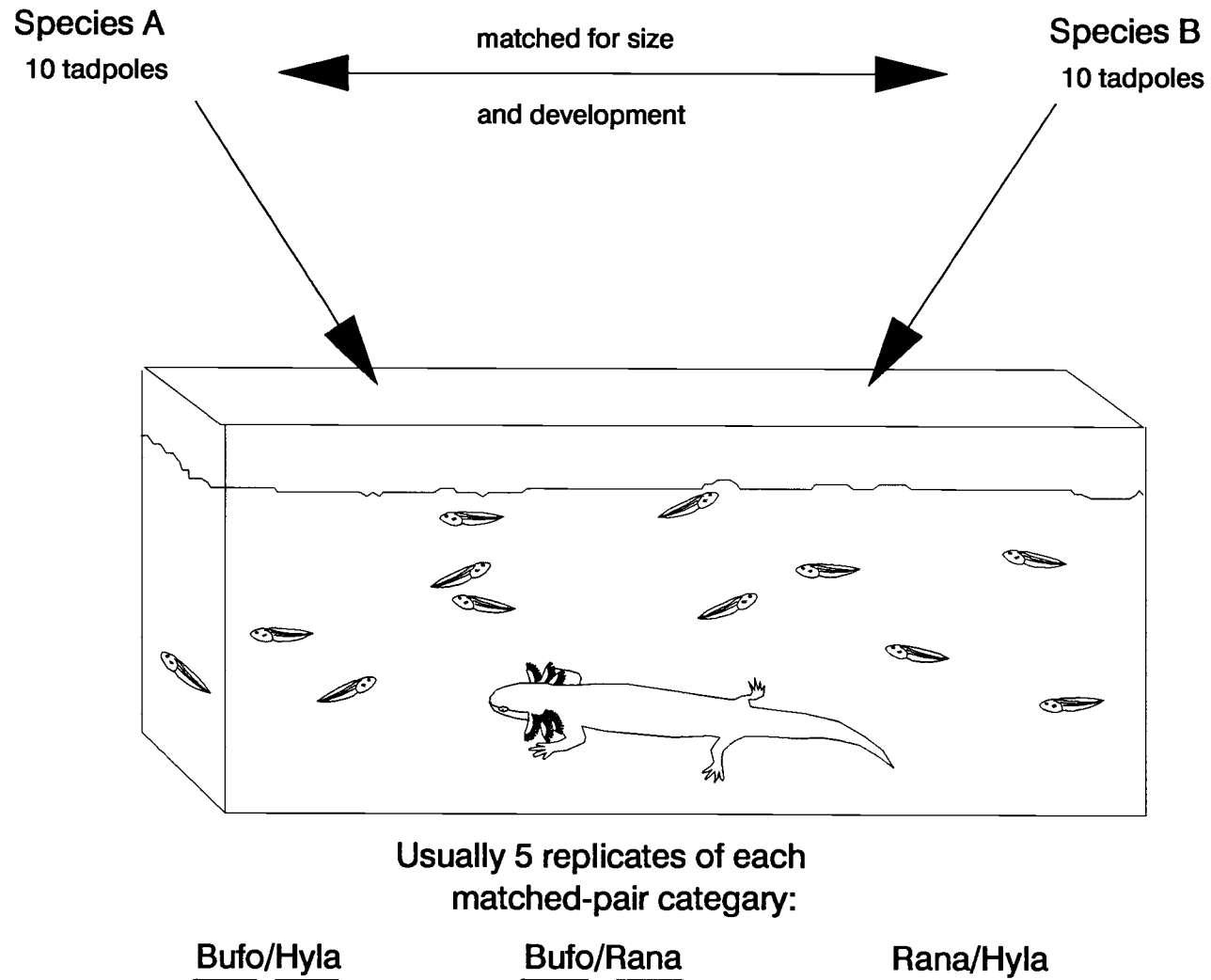
Tadpole palatability was evaluated using methods similar to those of Brodie and Formanowicz (1987). In each trial a starved salamander was offered 20 tadpoles, 10 of one species and 10 of another. In all experiments,

tadpoles were matched as close as possible for size although it is difficult to control for size and developmental stage simultaneously (see figure 1). Because tadpole palatability may change with development, and vulnerability to predation may change with growth (e.g. Brodie and Formanowicz, 1983, 1987), a series of experiments that control precisely for developmental stage and size were carried out.

In experiments 1, 2, 3, and 4 (see below), tadpoles of each species were matched for developmental stage. In experiment 5, tadpoles of each species were carefully matched for size. Most of the matched-pairs offered to salamanders consisted of tadpoles from a species suspected as being distasteful and another species thought to be palatable. However, in some initial experiments (1 and 2) I paired B. boreas (B), R. cascadae (R), and H. regilla (H) in all possible combinations (B/H, B/R, R/H) and presented these matched-pairs to A. gracile larvae to evaluate the relative palatability of each species. H. regilla tadpoles were known to be palatable to these predators at all stages in larval development (O'Hara & Blaustein, unpublished data).

Predator hunger levels were standardized by placing a salamander in a 25 cm x 15 cm plastic test container filled 5 cm deep with dechlorinated tapwater and allowing it to feed ad libitum on Tubifex worms for 24 hours.

Figure 1. Experimental design.



After this period, all food was removed, the water was changed, and predators were starved.

Trial times were predetermined such that, on the average, a trial would last until about 25% of one species had been consumed; thus, a predator's choice of prey items was never largely biased towards one species of tadpole. At the end of a trial the number of tadpoles that survived and the number that were eaten was recorded.

Prior to testing, 20-30 tadpoles of each species were randomly drawn and measured (total length in mm), their developmental stage was determined (Gosner, 1960), and larger tadpoles were gently wiped dry with absorbant paper and weighed to the nearest centigram. Wet weights of predators were determined by wiping salamanders dry and weighing them to the nearest centigram. Upon completion of the study, surviving animals were released at their sites of capture.

Experiment 1: Relative palatability of tadpoles to A. gracile was evaluated at 2 times in larval development. In this experiment, newly-hatched (stages 24-26) tadpoles were offered to larval A. gracile (n=15) that had been starved for 24 hours. Each category of matched-pair (B/R, B/H, R/H) had 5 replicates. Trials lasted for 7 hours.

Experiment 2: A. gracile (n=15) larvae were starved for 72 hours before being offered newly-hatched tadpoles (stages 25-26). A longer starvation period was used to determine whether the hunger level of a salamander has an effect on the acceptability of suspected distasteful tadpoles (Kruse & Stone, 1984). Five replicates of three categories of matched-pairs (B/R, B/H, R/H) were conducted. All trials lasted for 6 hours.

Experiment 3: Different natural predators may be more or less tolerant of tadpole chemical defenses. Therefore, newly-hatched B. boreas and H. regilla (B/H) tadpoles (stages 25-28) were offered to adults of another salamander predator, T. granulosa (n=7), that had been starved for 24 hours. Trials lasted for 24 hours.

Experiment 4: To evaluate the relative palatability of tadpoles in the middle stages of larval development (stages 34-41), large neotenic adult A. gracile (n=14) were starved for 72 hours and offered either a B/R matched-pair (n=7) or a B/H pair (n=7). The results of preliminary experiments using large A. gracile as predators of mid-developmental tadpoles suggested that R. cascadae and H. regilla tadpoles were similar in palatability, but B. boreas tadpoles may be distasteful (unpublished data). Therefore, suspected unpalatable B. boreas were paired with both R. cascadae and H. regilla tadpoles. Trials lasted 24 hours.

Experiment 5: Tadpole vulnerability to predation may change with size, therefore, in this experiment I carefully controlled for between-species differences in tadpole size. Adult T. granulosa (n=20) were starved for 52 hours and then offered B. boreas and R. cascadae (B/R) tadpoles (stages 27-31). Tadpoles of each species were weighed by gently blotting tadpoles dry with absorbant paper and then placing them in a cup of water on a balance (Mettler AC 100). Tadpoles of both species were matched for size. All tadpoles used in a trial weighed within 0.02 g of each other and tadpole weights ranged from 0.10 g to 0.15 g. Trials lasted 12 hours.

Experiment 6: Rejection of tadpoles by adult T. granulosa (n=5) was quantified by starving salamanders for 70 hours and recording the outcome of all attacks on tadpoles in a 10 minute trial. B. boreas and R. cascadae tadpoles were weighed as in experiment 5 and matched for size. All tadpoles weighed 0.10-0.12 g. I recorded all captures (i.e. salamander engulfs at least head of tadpole and holds tadpole in buccal cavity) as either "rejected" (tadpole engulfed and subsequently released) or "swallowed" (tadpole engulfed and not released). I also noted whether "rejected" tadpoles survived or died.

For experiments 1, 2, and 4, a G-test of independence with Yates' correction for continuity (Sokal and Rohlf, 1969) was used to test whether the proportion

of R. cascadae tadpoles that survived in an experiment was different from the proportion of H. regilla tadpoles that survived. If no difference was found, R. cascadae and H. regilla tadpoles were considered to be similar in palatability and were treated as "palatable control" tadpoles. A 2-tailed Wilcoxon paired-sample test (Zar, 1984) was then used to test whether the number of B. boreas eaten by salamanders was different from the number of "control" tadpoles eaten. For experiment 3, a 2-tailed Wilcoxon paired-sample test was used to test whether the number of B. boreas tadpoles (suspected as being unpalatable) that survived a trial was greater than the number of H. regilla tadpoles (known to be palatable) that survived. A paired t test (Snedecor and Cochran, 1980) was used to test whether the number of B. boreas surviving a trial differed from the number of similar sized R. cascadae tadpoles that survived in experiment 5.

Insect Experiments

Insects were captured from 3 ponds and from Fay Lake, in Linn County, Oregon. R. cascadae and H. regilla breed in the 3 ponds where insects were taken, but only H. regilla breeds in Fay Lake. I have no evidence that B. boreas breeds at any of these sites. Therefore, it is probable that insects had previously encountered R. cascadae and H. regilla tadpoles but unlikely that they had any experience with B. boreas tadpoles.

Relative palatability of anuran larvae to L. americanus was assessed at 3 times during larval development: shortly after hatching (stages 24-27), mid-development (stages 30-37) and at metamorphic climax (stages 45-46). In the lakes and ponds where R. cascadae and B. boreas breed, Dytiscus larvae usually emerge 2-4 weeks after tadpoles are hatched and L. americanus larvae are found 5-8 weeks after tadpoles hatch (personal observation). Therefore, it is unlikely that newly-hatched (ie., stages 24-26) B. boreas and R. cascadae occur with L. americanus larvae and they are probably rarely found with Dytiscus larvae. To evaluate the palatability of newly-hatched tadpoles to aquatic insect predators, tadpoles were taken from high elevation sites where breeding may occur weeks later than lower elevation sites and were tested with predators from lower elevation sites.

Palatability tests on newly-hatched and mid-developmental tadpoles (experiments 7, 8, and 11, see below) followed an experimental design identical to that described earlier. A starved insect was offered 10 tadpoles of one species and 10 of another for a predetermined period of time. Tadpoles of different species were matched as closely as possible for size and developmental stage. At the end of a trial I would count the number of individuals of each species that survived

and the number that were killed. Anuran larvae nearing metamorphic climax could not be tested using this method because many individuals could climb out of test containers and others would drown if left to swim for several hours. Instead, stage 45 and 46 anurans (metamorphic climax) were offered to predators one at a time (experiment 9) or in pairs (experiment 10, see below). Experiments 9 and 10 (see below) were conducted using clear, cylindrical, plastic containers 17 cm in diameter. All other experiments using insect predators were carried out in 25 cm x 15 cm opaque plastic containers. Test containers were filled 5 cm deep with dechlorinated tapwater. Because both species of insect predators breathe atmospheric air, 5 pieces of an aquatic plant, usually Elodea, each 10 cm long, were placed in the test containers so that insects could perch near the surface on the floating plants and breathe. Predators and tadpoles were used once.

The procedures used to standardize L. americanus hunger levels were identical to those described for salamanders except that mid-developmental H. regilla tadpoles were used as food and aquatic plants were placed in containers. Unless otherwise stated, L. americanus larvae were starved for 24 hours prior to testing. Hunger levels of Dytiscus larvae were standardized by offering each predator 4 mid-developmental H. regilla

tadpoles 24 hours prior to testing.

Before an experiment was initiated, 25-30 tadpoles of each species were randomly drawn and measured in a manner identical to that previously described. However, before experiment 10 (see below) only 15 newly-metamorphosed individuals of each species were measured.

It has been suggested that predators that pierce the skin of tadpoles with their mouthparts and suck body fluids, such as L. americanus, may not be deterred by tadpole chemical defenses (Wassersug, 1973; Heyer et al., 1975; Kruse and Stone, 1984). To evaluate the relative palatability of newly-hatched and mid-developmental B. boreas, R. cascadae, and H. regilla larvae to a piercing and sucking predator, tadpoles of each species were paired in all possible combinations (B/R, B/H, R/H) and offered to L. americanus.

Experiment 7: Newly-hatched tadpoles (stages 24-27) were offered to L. americanus larvae (n=14) for 12 hours. The B/H and R/H matched-pair categories had 5 replicates while the B/R matched-pair category had 4 replicates.

Experiment 8: The relative palatability of tadpoles in mid-larval development (stages 30-37) to a piercing and sucking invertebrate predator was assessed by offering three types of matched-pairs (B/R, B/H, R/H) to L. americanus larvae (n=15) for 12 hours. Each category

of matched-pairs had 5 replicates.

Experiment 9: Anurans in or near metamorphic climax (stages 45 and 46) could not be tested using the experimental design described above. Therefore, I evaluated the relative palatability of metamorphic climax anurans by feeding L. americanus larvae a diet of stage 45 and 46 B. boreas, R. cascadae or H. regilla for a period of 4 days. L. americanus predators that had been starved for 12 hours were offered stage 45 and 46 anurans one at a time for 8 hours each day. Insects were fed only one species of anuran. When an insect had finished consuming an anuran and dropped it, then another metamorphic climax individual of the same species would be placed in the test container. Typically 1 to 3 individuals would be consumed by each predator in an 8 hour period. Insects were again gently wiped dry with absorbant paper and weighed 18 hours after the last feeding period. Percent growth (ending wt.-begining wt./begining wt.) was determined for each insect. Each treatment (B, R, H) had 5 replicates. On a random sample of each species of anuran I measured the rate at which L. americanus predators consumed prey items (g/min.) and the percent of anurans that were consumed (ending wt.-begining wt./begining wt.). I would predict that insects maintained on a relatively unpalatable anuran species would grow less than insects maintained on a palatable

species (e.g. H. regilla), that the percent of an unpalatable anuran consumed by insects would be lower than that of a palatable species and that consumption rate of unpalatable anurans would be lower than that of palatable ones.

Experiment 10: To further evaluate the relative palatability of metamorphic climax B. boreas and R. cascadae to L. americanus larvae, insects were given an opportunity to choose between anuran species that may differ in palatability. One stage 46 R. cascadae and one stage 46 B. boreas were simultaneously placed in a test container with a L. americanus that had been starved for 12 hours. Trials lasted for 4 hours. I recorded which individuals had survived the trial and the order in which individuals were killed. At the end of the first trial I waited one hour before beginning the next trial. Each L. americanus (n=16) was used in 4 trials (2 per day).

Experiment 11: Invertebrate predators that ingest tadpole skin along with body fluids may be deterred by tadpole chemical defenses (Wassersug, 1973). Therefore, Dytiscus larvae (n=12), which may consume small portions of skin along with body fluids (Brodie et al., 1978; personal observations), were offered either newly-hatched (stages 24-26) B/R matched-pairs (n=6) or B/H matched-pairs (n=6) for 2 hours as a comparison with experiment 7.

Chi-square tests of independence with Yate's correction for continuity (Zar, 1984) were used to test whether the proportion of anurans that survived trials in experiments 7, 8 and 11 was independent of species. A 2-tailed Wilcoxon paired-sample test was used to test whether the number of stage 46 B. boreas that survived trials with L. americanus was different than the number of R. cascadae that survived (experiment 10). A Kruskal-Wallis test (Zar, 1984) was used to evaluate whether L. americanus growth was the same when maintained on three different species of metamorphic climax anurans (experiment 9). I used a one-way ANOVA to test for the effect of food type (anuran species) on the percent of a prey item consumed. A Bartlett test for homogeneity of group variances (Zar, 1984) showed that there were unequal variances in consumption rate between anuran species, therefore, this variable was log transformed and a one-way ANOVA was used to test for food type effect on insect consumption rate.

RESULTS

In all experiments, B. boreas tadpoles appeared more active than either R. cascadae or H. regilla tadpoles. B. boreas tadpoles continuously swam throughout the test container without overtly avoiding the predator whereas R. cascadae and H. regilla tadpoles tended to remain still, usually near the bottom of the container.

Salamander Experiments

There was no significant difference between the proportion of R. cascadae tadpoles eaten by both larval and neotenic adult A. gracile and the proportion of H. regilla tadpoles eaten (Table 1). The relative palatability of R. cascadae and H. regilla tadpoles to A. gracile was similar in both early and mid-larval development (Table 1).

Larval A. gracile and adult T. granulosa consumed more newly-hatched R. cascadae and H. regilla tadpoles than B. boreas larvae of similar size and developmental stage (Table 2). Furthermore, neotenic adult A. gracile consumed more mid-developmental R. cascadae and H. regilla tadpoles than B. boreas tadpoles (Table 2). In experiments 1 to 4 B. boreas tadpoles were, on the average, slightly smaller than both the R. cascadae and H. regilla tadpoles with which they were paired. However, when tadpoles were carefully matched for size

Table 1. Relative palatability of Rana cascadae and Hyla regilla tadpoles to Ambystoma gracile.

Experiment	Predator	Starve Time	Tadpoles				Adjusted G-statistic	Significance (α = 0.05)
			<u>Rana</u>		<u>Hyla</u>			
			S	E	S	E		
1	<u>A. gracile</u> larvae	24 hrs.	77	23	79	21	0.028	NS
2	<u>A. gracile</u> larvae	72 hrs.	44	56	54	46	1.622	NS
4	<u>A. gracile</u> larvae	72 hrs.	58	12	60	10	0.052	NS

S = number that survived

E = number that were eaten

Table 2. Palatability of Bufo boreas, Rana cascadae, and Hyla regilla tadpoles to Ambystoma gracile and Taricha granulosa.

Exper.	Predator	Predator ^C Weight		Tadpoles			P< (2-tailed)
				<u>Bufo</u>	Control		
					<u>Rana</u>	<u>Hyla</u>	
1 ^a	<u>A. gracile</u> larvae	3.81 ± 0.37 (n = 15)	Stages length ^d	25-26 13.9 ± 0.2 (n = 20)	25-26 16.9 ± 0.3 (n = 20)	24-25 14.2 ± 0.4 (n = 20)	0.05
			% survived	100	77	79	
2 ^a	<u>A. gracile</u> larvae	4.97 ± 0.52 (n = 15)	Stages length ^d	25-26 14.3 ± 0.1 (n = 25)	25-26 17.4 ± 0.2 (n = 25)	25-26 15.5 ± 0.2 (n = 25)	0.01
			% survived	94	44	54	
3 ^b	<u>I. granulosa</u> adults	10.70 ± 1.24 (n = 7)	Stages length ^d	25-27 15.1 ± 0.2 (n = 25)		25-28 17.9 ± 0.4 (n = 25)	0.05
			% survived	89		50	
4 ^a	<u>A. gracile</u> adults	10.72 ± 1.11 (n = 14)	Stages length ^d	35-41 38.9 ± 0.5	34-40 50.2 ± 0.7	34-40 35.8 ± 0.8	0.01
			weight ^C	0.51 ± 0.02 (n = 25)	0.90 ± 0.04 (n = 25)	0.64 ± 0.04 (n = 25)	
			% survived	99	83	86	

^aThere were no trials where more Bufo were eaten than Hyla or Rana tadpoles.

^bIn one of 7 trials more Bufo were eaten than Hyla tadpoles.

^cMean weight (g) ± standard error of the mean.

^dMean length (mm) ± standard error of the mean.

(experiment 5), adult T. granulosa consumed fewer B. boreas tadpoles than R. cascadae (Table 3). In all of the salamander experiments, regardless of whether tadpoles were newly-hatched or in mid-developmental stages, fewer B. boreas were eaten than either R. cascadae or H. regilla.

In experiment 6, T. granulosa (n=5, mean wt.=10.91 g) rejected 57% of all B. boreas tadpoles captured during the trial (23 captured, 13 rejected). Only one R. cascadae tadpole captured was later rejected (18 captured, 17 swallowed). This individual was captured simultaneously with a B. boreas tadpole and was released when the B. boreas tadpole was released. B. boreas tadpoles were usually expelled within 60 seconds of being captured, however, salamanders held some individuals in their buccal cavity for up to 3 minutes before releasing them. B. boreas tadpoles usually survived the process of being engulfed and expelled by salamanders with no apparent ill effects. In all of the salamander experiments, only two newly-hatched B. boreas individuals were found dead and uneaten.

The acceptability of newly-hatched B. boreas tadpoles relative to R. cascadae and H. regilla of similar size and developmental stage did not appreciably differ when A. gracile larvae were starved for 72 hours as opposed to 24 hours (Table 2).

Table 3. Palatability of 0.10-0.15 g Rana cascadae and Bufo boreas tadpoles to Taricha granulosa.

Experiment	Predator	Predator ^a Weight	Tadpoles		Two-tailed Paired t
			<u>Bufo</u>	<u>Rana</u>	
5	<u>I. granulosa</u> adults	10.83 ± 1.39 (n = 20)	Stages 27-31 (n = 30)	27-30 (n = 30)	t = 2.685*
			% survived	69.5	40.5 df = 19

*
P<0.025

^aMean weight (g) ± standard error of the mean.

Insect Experiments

There was no significant difference between the relative proportions of B. boreas, R. cascadae and H. regilla tadpoles consumed by L. americanus predators, regardless of whether tadpoles were newly-hatched or in mid-larval development (Table 4, see also Table 5). In experiment 10, the number of metamorphic climax B. boreas that survived trials with L. americanus larvae was not significantly different from the number of R. cascadae that survived (Wilcoxon $T^+=22$, $T^-=44$, adjusted $n=11$, see Table 5). Furthermore, the relative proportion of B. boreas and R. cascadae eaten in the first trial of a day did not differ from the proportions eaten in the second trial of a day (chi-square with Yate's correction= 1.858, $df=1$, $p=0.1728$), suggesting that insects did not learn to avoid either prey species.

Growth of L. americanus larvae over a 4 day period in experiment 9 was not affected by the species (B. boreas, R. cascadae and H. regilla) of stage 45 and 46 anurans that they were fed (Table 6). The percent of an anuran that was consumed by insects did not differ between the three species (Table 6). However, after anurans were captured, insects did not consume all three species at the same rate (Table 6). A Newman-Keuls multiple comparisons test showed that H. regilla were

consumed at a slower rate than were B. boreas, but consumption rate of R. cascadae was not significantly different from that of B. boreas or H. regilla (Table 6). Although there were no significant between-species differences in mean length of anurans offered to insects in experiment 9 (Table 6), there was a significant difference between the mean weights of metamorphic climax anurans fed to insects (Table 6). A Newman-Keuls test indicated that mean weight of metamorphic climax H. regilla was smaller than that of B. boreas.

The proportion of B. boreas killed in experiment 11 by Dytiscus larvae was not significantly different from the proportion of R. cascadae killed by these predators. However, the proportion of H. regilla killed by Dytiscus larvae was significantly smaller than the proportion of B. boreas killed (Table 4, see also Table 5). Unlike L. americanus larvae, several Dytiscus larvae left tadpoles dead but apparently uneaten. A total of 10 B. boreas, 2 H. regilla and 1 R. cascadae were apparently killed but uneaten.

Table 4. Relative palatability of Bufo boreas, Rana cascadae and Hyla regilla tadpoles to Lethocerus americanus and Dytiscus.

Experiment	Predator	Tadpoles						^a χ ²	^b p
		<u>Bufo</u>		<u>Rana</u>		<u>Hyla</u>			
		S	D	S	D	S	D		
7	<u>L. americanus</u>	38	2	33	7			0.157	NS
		26	24			26	24	0.000	NS
				19	31	23	27	0.369	NS
8	<u>L. americanus</u>	46	4	44	6			0.111	NS
		33	17			30	20	0.172	NS
				38	12	39	11	0.000	NS
11	<u>Dytiscus</u>	34	26	31	29			0.134	NS
		9	51			42	18	34.919	p<.001

^aChi-square statistic with Yates' correction.

^b $\alpha = 0.05$

S = Number that survived.

D = Number that died.

Table 5. Summary statistics of experiments using Dytiscus and Lethocerus americanus as predators of Bufo boreas, Rana cascadae and Hyla regilla larvae.

Exp.	Predator	Predator weight ^b		Tadpoles		
				<u>Bufo</u>	<u>Rana</u>	<u>Hyla</u>
7	<u>L. americanus</u>	$\bar{X} = 0.285$ SEM = 0.031 n = 14	stages length ^a (n = 30) % survived	24-26 11.2 ± 0.2 71	24-25 14.4 ± 0.4 58	24-27 14.4 ± 0.6 49
8	<u>L. americanus</u>	$\bar{X} = 0.917$ SEM = 0.169 n = 15	stages length ^a weight ^b (n = 30) % survived	30-36 32.6 ± 0.5 0.381 ± 0.013 79	31-35 40.4 ± 0.5 0.508 ± 0.018 82	32-37 32.7 ± 0.5 0.341 ± 0.018 69
10	<u>L. americanus</u>	$\bar{X} = 1.972$ SEM = 0.363 n = 16	stages length ^a weight ^b (n = 15) % survived	46 18.1 ± 0.3 0.480 ± 0.010 42	46 19.6 ± 0.9 0.652 ± 0.059 48	
11	<u>Dytiscus</u>	$\bar{X} = 0.929$ SEM = 0.074 n = 12	stages length ^a (n = 25) % survived	25-26 12.5 ± 0.2 38	25-26 18.6 ± 0.6 52	25-26 13.7 ± 0.3 70

^aMean length (mm) ± standard error of the mean.

^bMean weight (g) ± standard error of the mean.

TABLE 6. Growth of Lethocerus americanus when fed stage 46 Bufo boreas, Rana cascadae or Hyla regilla larvae (experiment 9). SEM = standard error of the mean.

		Prey Species			Statistical test
		<u>Bufo</u>	<u>Rana</u>	<u>Hyla</u>	
% Growth of <u>L. americanus</u>	\bar{X}	17.6	9.3	18.6	Kruskal-Wallis stat. = 1.82 p = 0.402
	SEM	9.7	3.0	5.5	
	n	5	5	5	
% of prey species consumed	\bar{X}	18.3	21.9	17.5	ANOVA F = 0.806 df = 54 p = 0.452
	SEM	9.7	12.0	12.2	
	n	19	19	17	
consumption rate of prey species (10^{-4} g/min)	\bar{X}	10.6	7.5	4.8	ANOVA F = 5.658 df = 54 p = 0.006
	SEM	7.4	4.9	2.5	
	n	19	19	17	
prey weight (g)	\bar{X}	0.454	0.398	0.332	ANOVA F = 6.840 df = 104 p = 0.002
	SEM	0.021	0.030	0.016	
	n	35	37	33	
prey length (mm)	\bar{X}	16.0	16.1	16.4	ANOVA F = 0.140 df = 104 p = 0.869
	SEM	0.5	0.5	0.5	
	n	35	37	33	

DISCUSSION

The hypothesis that group-forming tadpoles are unpalatable to their natural salamander predators is partially supported by the results obtained for B. boreas tadpoles which were distasteful to salamander predators. However, highly social R. cascadae tadpoles were palatable to salamanders and insects at all stages in larval development tested. Moreover, aquatic insect predators treated B. boreas, R. cascadae, and H. regilla larvae as if they were similar in palatability. The hypothesis that B. boreas and R. cascadae tadpoles display differential palatability to their salamander and insect predators at different stages of development was not supported.

Proportionately fewer B. boreas tadpoles were consumed by A. gracile and T. granulosa predators than R. cascadae and H. regilla of similar size and developmental stage, regardless of whether anuran larvae were newly-hatched or in mid-larval development. The proportion of R. cascadae tadpoles eaten by A. gracile did not differ significantly from the proportion of H. regilla tadpoles consumed.

Differential predation on B. boreas, R. cascadae, and H. regilla tadpoles by salamanders was likely due to distasteful qualities of B. boreas larvae as opposed to

differences in prey size or escape ability. When tadpoles were carefully matched for size (experiment 5), R. cascadae tadpoles were selectively preyed on over B. boreas tadpoles. In laboratory tests, Bufo and Rana tadpoles are about equal in their abilities to escape predators (Hoff, 1986). However, in these experiments, B. boreas tadpoles constantly swam and thus were frequently captured by salamanders which tended to focus their attacks on moving prey. Once B. boreas tadpoles were captured, they were usually held in the buccal cavity for several seconds and then were expelled.

After starving T. granulosa for an extended period of time (70 hrs.), I found that 57% of all B. boreas captured were released unharmed. B. boreas tadpoles usually survived the process of being captured and rejected with no apparent ill-effects. None of the tadpoles that I observed being rejected died as a result of predator handling. Both newly-hatched and mid-developmental B. boreas tadpoles were captured and rejected by salamanders (personal observations). Kruse and Stone (1984) reported that largemouth bass (Micropterus salmoides) rejected B. americanus and B. woodhousei tadpoles in a similar way and that only 0.7% of the tadpoles rejected by bass died.

Unlike salamanders, aquatic insect predators that pierce the skin and suck body fluids (L. americanus and

Dytiscus) were not deterred by the chemical defenses of B. boreas larvae. Interestingly, the proportion of newly-hatched H. regilla tadpoles killed by Dytiscus larvae was significantly lower than the proportion of B. boreas killed. It is unlikely that this difference is the result of Dytiscus larvae being repelled by chemical defenses of newly-hatched H. regilla tadpoles. Several vertebrate and invertebrate predators have been maintained on early and mid-developmental H. regilla tadpoles in our lab and there was no indication that predators found newly-hatched H. regilla tadpoles distasteful (O'Hara and Blaustein, unpublished data). Behavioral differences between the two anuran species are the likely cause of differential predation by Dytiscus larvae. As is the case with salamanders, Dytiscus larvae seemed to focus their attacks on moving prey. Many B. boreas tadpoles were killed by Dytiscus after swimming near the head region of these predators. However, H. regilla tadpoles seemed to have fewer encounters with insects because they tended to remain motionless for much of the trial, although this was not quantified.

Brodie and Formanowicz (1987) report that metamorphic climax B. americanus are unpalatable to L. americanus predators (see also Brodie et al., 1978). My study provides no evidence to suggest that B. boreas individuals at metamorphic climax are unpalatable to L.

americanus. Mean growth of insects fed a diet of only metamorphic climax B. boreas did not differ significantly from mean growth of insects fed stage 46 R. cascadae or H. regilla. After capturing stage 46 anurans, the mean percent consumed by L. americanus larvae was similar for all three species. Furthermore, when insects were offered a choice between metamorphic climax B. boreas and R. cascadae, predators did not avoid preying on suspected unpalatable B. boreas. There is no evidence to suggest that after sampling stage 46 B. boreas individuals L. americanus larvae avoided B. boreas in subsequent encounters. Interestingly, L. americanus larvae consumed metamorphic climax H. regilla individuals at a significantly lower rate than that for B. boreas. Mean weight of stage 46 B. boreas fed to insects was greater than that of H. regilla. Metamorphic climax B. boreas individuals are also more spherical in shape and seem to have proportionately less mass in extremities than H. regilla. Perhaps insect handling times were lower for B. boreas because predators consuming H. regilla had to move over more of the body and make more beak insertions to receive an equivalent amount of material.

Previous studies suggest that the degree of palatability of Bufo tadpoles to natural predators may be quite variable. Several reports show that Bufo tadpoles are distasteful to both vertebrates (humans: Wassersug,

1971; fish: Voris and Bacon, 1966; Glandt, 1984; Kruse and Stone, 1984; Kats et al., 1988; salamanders: Heusser, 1971; Cooke, 1974; Walters, 1975; Brodie and Formanowicz, 1987) and invertebrate predators (diving beetle larvae: Brodie et al., 1978; water bug nymphs: Brodie et al., 1978; Brodie and Formanowicz, 1987; and dragonfly naiads: Brodie and Formanowicz, 1987). However, many species of natural predators apparently find Bufo larvae palatable (salamanders: Heusser, 1971; Morin, 1983; diving beetle larvae: Young, 1967; Neill, 1968; Brodie et al., 1978; Kruse, 1983; water bugs: Brodie et al., 1978; Hews, 1988; dragonfly naiads: Brockelman, 1969; Heyer & Bellin, 1973; Heyer et al., 1975; Hews, 1988; birds: Beiswenger, 1981; snakes: Arnold & Wassersug, 1978).

Two general hypotheses have been proposed to explain the variable palatability of Bufo larvae. It has been suggested that Bufo tadpoles are unpalatable to vertebrate predators that masticate, bite or somehow taste tadpoles, but palatable to invertebrate predators and vertebrates with poorly developed taste receptors or those that swallow tadpoles whole (Wassersug, 1973; Heyer et al., 1975; Kruse and Stone, 1984). This hypothesis suggests that the distasteful qualities of Bufo larvae are located in the skin (Wassersug, 1971; Flier et al., 1980). Therefore, predators that pierce the skin and suck body fluids or those that swallow tadpoles without

damaging the skin are not expected to be deterred by tadpole chemical defenses.

A second hypothesis concerning Bufo tadpole palatability suggests that there are ontogenetic changes in the palatability of larvae (Brodie and Formanowicz, 1987). Newly-hatched and metamorphic climax stages may have chemical defenses that deter both vertebrate and invertebrate predators, whereas intermediate tadpole stages may be palatable. Anurans in metamorphic climax stages are more vulnerable to predation than tadpoles just prior to or immediately after this stage because emergence of the forelimbs and presence of a partial tail hinder swimming movements and, until tail reabsorption is complete, saltatory locomotion on land is also relatively poor (Wassersug and Sperry, 1977; Arnold and Wassersug, 1978; Huey, 1980; Crump, 1984). Similarly, newly-hatched tadpoles are less able to escape by swimming than are tadpoles in intermediate developmental stages. Therefore, antipredator adaptations, such as chemical defenses, may be important in these particularly vulnerable stages of an anuran's life history (Crump, 1984; Brodie and Formanowicz, 1987). Newly-hatched tadpoles may acquire distasteful qualities from the egg yolk, whereas chemical defenses in metamorphic climax stages may be associated with increased activity of the granular glands that produce toxins in adult Bufo

(Formanowicz and Brodie, 1982; Brodie and Formanowicz, 1987). Predators with a learned avoidance of the chemical defenses of hatchlings may also avoid palatable intermediate stages, thus further enhancing the survival of palatable mid-developmental Bufo tadpoles (Brodie and Formanowicz, 1987).

There is some support for each hypothesis and there are some data that are contrary to each hypothesis. For example, it has been demonstrated that newly-hatched and metamorphic climax B. americanus are unpalatable to several invertebrate predators, both those that pierce the skin with mouthparts and suck body fluids and those that chew tadpoles (Brodie et al., 1978; Brodie and Formanowicz, 1987). Furthermore, some vertebrates presumably capable of tasting their prey are not deterred by Bufo larvae (Heusser, 1971; Morin, 1983).

My results indicate that mid-developmental B. boreas larvae (stages 35 to 41) are distasteful to A. gracile. Kruse and Stone (1984) have shown that mid-developmental B. americanus and B. woodhousei tadpoles are unpalatable to largemouth bass (Micropterus salmoides). Similarly, newts (Triturus spp.) and three-spined sticklebacks (Gasterosteus aculeatus) are reported to avoid Bufo tadpoles in intermediate developmental stages (Heusser, 1971; Cooke, 1974; Glandt, 1984) and some salamanders may even die after eating mid-developmental Bufo tadpoles

(Dawson, 1982). Therefore, in several Bufo spp., chemical defenses are not limited to only early and late larval developmental stages.

Several factors may be responsible for the differences in Bufo tadpole palatability reported in the literature. First, experimental protocol used when measuring tadpole palatability differs between studies. Many early studies were not carefully controlled because the developmental stages of the tadpoles being tested were not determined, predator hunger levels were not known or predators were not offered a choice between Bufo larvae, suspected as being unpalatable, and alternative palatable prey (for a discussion see Brodie et al., 1978). In natural situations the acceptability of a prey item is probably dependent on the availability of alternative palatable prey; therefore the concept of prey palatability should be interpreted in a relative sense (Brodie and Formanowicz, 1987). Similarly, the acceptability of relatively unpalatable prey may be correlated with predator hunger levels (Kruse and Stone, 1984).

It is possible that there are significant interspecific and intraspecific differences in both Bufo tadpole chemical defenses and predator tolerance of tadpole distasteful qualities (Wassersug, 1973). For example, stage 46 B. americanus are unpalatable to larval

L. americanus (Brodie et al., 1978). However, my results suggest that L. americanus larvae are not deterred by the chemical defenses of stage 46 B. boreas. Adults of different Bufo species differ in the amount and type of toxins found in the skin (Daly and Witkop, 1971). If larval Bufo vary in a similar way, it may be expected that a given predator would find some species unpalatable, but others palatable. Furthermore, certain predators may be deterred by chemical defenses of hatchlings and metamorphs, but not by the defenses of intermediate stages, even though other predator species find all larval developmental stages unpalatable.

Unpalatability as an antipredator mechanism may be more or less beneficial to an individual depending on the type of predators it encounters in its habitat and the risk of predation. For example, fish predation may play a very important role in structuring larval anuran communities (Heyer et al., 1975; Kats et al., 1988) and unpalatability may be especially beneficial to tadpoles that live in permanent aquatic habitats that have fish (Kats et al., 1988). Small-mouthed salamander larvae (Ambystoma texanum) from populations that coexist with fish spend more time in refuges when exposed to fish chemicals than larvae from populations that do not encounter fish (Kats et al., 1988). Perhaps tadpole unpalatability varies within a species in a similar way.

Tadpoles from populations that breed in permanent waters with fish may be relatively more unpalatable than larvae from sites that have not been subjected to fish predation.

It is difficult to know for certain whether unpalatability or sociality evolved first or in conjunction with one another in B. boreas tadpoles. Both characteristics may be important antipredator mechanisms. Tadpole sociality and unpalatability are generally characteristic of the genus Bufo, therefore, it is possible that kin selection could have led to these traits in the ancestral population (Wassersug, 1973). Kin selection may be important for the maintenance of tadpole sociality and unpalatability in some extant Bufo, such as B. americanus (Wassersug, 1973; Waldman and Adler, 1979; Kruse and Stone, 1984). However, this seems less likely for B. boreas tadpoles. Although the genetic affinities of tadpoles comprising B. boreas aggregations are not known, it is likely that numerous non-kin comprise the aggregations. Individuals from numerous clutches from several areas of a lake or pond disperse widely and rapidly to form large aggregations (O'Hara, 1981; Blaustein, 1988; Olson, 1988). There are about 12,000 eggs per clutch and aggregations may include thousands or even a million individuals (O'Hara and Blaustein, 1982; Blaustein, 1988). Therefore, if

individuals died as predators learned of the distasteful qualities of tadpoles, both kin and non-kin may benefit and one may have to invoke group selection for the evolution of B. boreas unpalatability and sociality. Furthermore, individuals usually survived the process of being sampled by salamanders which is consistent with individual selection as the mechanism for the evolution of unpalatability.

Because of their general unpalatability and tendency to form conspicuous aggregations, it has been suggested that the black coloration of Bufo tadpoles may be aposematic (Wassersug, 1973; Waldman and Adler, 1979; Waldman, 1982; Kruse and Stone, 1984). However, there is a paucity of empirical evidence demonstrating that tadpole aggregations or conspicuous black coloration acts to warn experienced predators of Bufo tadpole chemical defenses. Kruse and Stone (1984) report that bass learn to avoid unpalatable Bufo larvae and presumably can use visual cues to distinguish black Bufo tadpoles from other species. To further support the contention that black coloration is aposematic, a test comparing predator preference of distasteful tadpoles that are black in color (e.g. many Bufo spp.) with distasteful tadpoles that are less conspicuous (e.g. Rana catesbeiana, Kruse and Francis, 1977) should be conducted. If predators learn to avoid unpalatable black tadpoles sooner or if

avoidance behavior persists longer than learned avoidance of unpalatable non-black tadpoles, then the hypothesis that black tadpole coloration is aposematic would be supported. An equally plausible explanation for the evolution of the conspicuous black color and massive schools of B. boreas is that it serves a thermoregulatory function (Beiswenger, 1977; O'Hara, 1981; see discussion by Guilford, 1988). Experiments by O'Hara (1981) showed that water temperatures may rise 2 to 3 C within a B. boreas aggregation due to absorption of solar radiation and metabolic activity. This rise in temperature significantly affects growth and development in B. boreas tadpoles.

R. cascadae tadpoles are cryptically colored, display an explosive escape response when potential predators approach (O'Hara, 1981; O'Hara and Blaustein, 1981), and are palatable to their natural predators. Therefore, the aposematic/unpalatability hypothesis cannot explain the maintenance of kin association in present populations of R. cascadae tadpoles. One possible explanation, among others (e.g. O'Hara and Blaustein, 1981; Blaustein and O'Hara, 1982; Blaustein, 1988), for the function of kin recognition and kin association in R. cascadae tadpoles is concerned with warning via chemical cues. Hews and Blaustein (1985) demonstrated that R. cascadae tadpoles display an escape-

like alarm response when exposed to substances released by damaged conspecifics. Because R. cascadae aggregations are, presumably, composed chiefly of siblings (O'Hara and Blaustein, 1985; Blaustein and O'Hara, 1987), individuals that release alarm substances when damaged by predators would likely warn kin. If the alarm response of R. cascadae tadpoles reduces predator capture efficiency in a way similar to the response of B. boreas tadpoles (see Hews, 1988), then kin recognition and kin association may be maintained by kin selection.

To better understand the significance of unpalatability and group formation as antipredator mechanisms, further research on interspecific and intraspecific differences in Bufo tadpole palatability, ontogenetic changes in tadpole chemical defenses, and between species differences in predator tolerance of tadpole defenses is needed. Furthermore, until there is empirical evidence indicating a warning function of Bufo tadpole aggregations and black tadpole coloration, the hypothesis that these traits are aposematic should be viewed with caution (Guilford, 1988). Clearly, strategies other than unpalatability have likely evolved as antipredator defenses in R. cascadae tadpoles.

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