The Effect of Predator Chemical Cues and Conspecific Alarm Signals upon Behavior in Early and Late Developmental Stages of American Toad (Bufo americanus) Tadpoles

Carol Lynette Johnson

Eastern Illinois University

This research is a product of the graduate program in Biological Sciences at Eastern Illinois University. Find out more about the program.

Recommended Citation

https://thekeep.eiu.edu/theses/1607

This is brought to you for free and open access by the Student Theses & Publications at The Keep. It has been accepted for inclusion in Masters Theses by an authorized administrator of The Keep. For more information, please contact tabruns@eiu.edu.
THESIS/FIELD EXPERIENCE PAPER
REPRODUCTION CERTIFICATE

TO: Graduate Degree Candidates (who have written formal theses)

SUBJECT: Permission to Reproduce Theses

The University Library is receiving a number of requests from other institutions asking permission to reproduce dissertations for inclusion in their library holdings. Although no copyright laws are involved, we feel that professional courtesy demands that permission be obtained from the author before we allow these to be copied.

PLEASE SIGN ONE OF THE FOLLOWING STATEMENTS:

Booth Library of Eastern Illinois University has my permission to lend my thesis to a reputable college or university for the purpose of copying it for inclusion in that institution's library or research holdings.

[Signature]

Date

I respectfully request Booth Library of Eastern Illinois University NOT allow my thesis to be reproduced because:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

[Signature]

Date
The Effect of Predator Chemical Cues and Conspecific Alarm Signals upon Behavior in Early and Late Developmental Stages of American toad (Bufo americanus) Tadpoles.

BY

Carol Lynette Johnson

1976 -

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Masters of Zoology

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

2000

I HEREBY RECOMMEND THAT THIS THESIS BE ACCEPTED AS FULFILLING THIS PART OF THE GRADUATE DEGREE CITED ABOVE
Acknowledgements

I first wish to express gratitude to my committee members: Dr. Kipp Kruse, Dr. Robert Fischer, and Dr. Charles Pederson for their insight and support throughout the duration of my work. I also would like to thank Dr. Jeff Laursen for his invaluable direction in the collection and housing of my numerous tadpoles. Many thanks also to family, friends, and Ted Watson for all their encouragement, companionship and never-ending support throughout my time at Eastern.

All tadpoles and predatory beetles were collected by permit on the Fox Ridge State Park, whose staff was extremely helpful; bluegill were collected from the Campus Pond at Eastern. EIU's biology department provided laboratory space, equipment, and some financial support. A grant from Eastern Illinois University was instrumental in funding this thesis.
Abstract

The chemosensory capability and subsequent habitat choice of larval American toad (*Bufo americanus*) tadpoles were quantified using choice of refuge with both early and late developmental stages. Treatments were performed with two tadpole densities to ascertain the effect of social aggregations upon behavior. Bluegill (*Lepomis macrochirus*) and predaceous diving beetle larvae (*Dytiscus sp.*) predators were used to condition water. Conspecific tadpoles as well as Southern leopard frog (*Rana sphenocephala*) tadpoles were used to prepare treatment extracts.

Tadpole density (n=10 and n=20) had no significant effect upon the percentage of tadpoles seeking cover in any treatment. The percentage of toad tadpoles of early developmental stages seeking cover was significantly higher when exposed to both early and late staged conspecific alarm signals as compared to the control, however, the treatment responses were statistically indistinguishable from one another. Similarly, the presence of beetle larvae in test chambers elicited a strong fright reaction by tadpoles when compared to controls. Tadpoles responded more strongly to the presence of beetle larvae than to chemical cues of larval beetles. Likewise, the addition of bluegill cues elicited a significant antipredator response from toad tadpoles.

Late developmental stage toad tadpoles showed no significant increases in the percentage seeking cover in any treatment; these results are attributable to the high percentage of larvae seeking cover in control trials. My results suggest that tadpoles may display antipredator tactics that are stage-specific and geared toward differing suites of predators.
Introduction

Predation is a constant threat throughout the life of an amphibian; therefore behavioral responses appropriate to the developmental stage may be shaped by natural selection. The antipredator responses of amphibian larvae have been well studied; tadpoles are known to respond to predators in a variety of ways ranging from altering life history strategies (Skelly and Werner 1990, Werner and Anholt 1993) to various behavioral responses (Petranka et al. 1987, Skelly 1994). Behavioral antipredator tactics include altering activity levels (Lawler 1989, Anholt et al. 1996), seeking refuge (Babbitt and Tanner 1997), and swarming (Watt et al. 1997). A distinct evolutionary advantage would exist for tadpole species able to detect predators before contact occurs, thereby increasing the escape interval as well as the possibility of avoiding a predation episode entirely. Several species of larval amphibians, including toads (Bufonidae), use indirect signals (Petranka and Hayes 1998) to detect and respond to substances associated with predators including diet and waste metabolites (Hews 1988, Lawler 1989, Wilson and Lefcort 1993, Laurila et al. 1997, Petranka and Hayes 1998), as well as alarm signals issuing from the skin of damaged conspecifics (Hews and Blaustein 1985, Petranka and Hayes 1998, Kiesecker et al. 1999).

Larvae of the American toad, *Bufo americanus* are capable of further antipredator tactics. They exhibit aposematic coloration which alerts predators to their toxic and noxious qualities caused by a chemical called bufotoxin, which is only produced late in the larval period (Brodie et al. 1978) beginning at developmental stage 41 Gosner (1960). This production of bufotoxin by granular glands in the skin just prior
to the onset of metamorphosis causes toad tadpoles to be unpalatable or even occasionally toxic (Brodie et al. 1978) to several species of predators, including some insect predators (e.g., dytiscids) and fish (Brodie et al. 1978, Formanowicz and Brodie 1982). In earlier developmental stages, however, tadpoles may be fully palatable (Brodie et al. 1978, Semlitsch and Gavasso 1992) and consequently much more susceptible to predation.

Toad tadpoles form complex aggregations (Beiswenger 1975, Rodel and Linsenmair 1997, Watt et al. 1997), in which members both recognize and preferentially associate with siblings (Waldman and Adler 1979, Waldman 1982). This schooling behavior has several potential benefits to members, including enhanced predator vigilance and food location (Beiswenger 1975), along with increased survival due to the dilution effect (Watt et al. 1997). These groups may also lend protection to palatable individuals. Used in conjunction with aposematism and kin preference, swarming is instrumental in increasing inclusive fitness of swarm members (Brodie and Formanowicz 1981, Brodie and Formanowicz 1987) by facilitating avoidance of unpalatable larvae by predators. Social aggregations are known to be comprised of mainly early stage tadpoles, whereas later stage tadpoles (Gosner 1960 stage 41 or later) have been demonstrated to be much less social (Beiswenger 1975).

Bufonids also will modify activity levels, avoid areas containing predators (Skelly and Werner 1990, Anholt et al. 1996), and utilize cover in escaping predators (Babbitt and Jordan 1996, Babbitt and Tanner 1997). These tactics, while effective against some predators, are extremely costly in terms of growth (Kupferberg 1997). Therefore, tadpoles need to adaptively balance the risk of predation they experience against the
percentage of time spent foraging. At the time of metamorphic climax, tadpoles may be exceptionally vulnerable to predators (Arnold and Wassersug 1978, Crump 1984) as limbs develop and the tail is reabsorbed which has been shown to severely decrease swimming velocity beginning at Gosner (1960) developmental stage 42 (Huey 1980). Because tadpoles cease feeding at stage 42 (Beisenger 1975) they may have no need to engage in foraging, and therefore may appropriate more time to the avoidance of predators. Given the differing physiologies, nutritive needs, and swimming capabilities, it seems possible that toad tadpoles may exhibit antipredator tactics that are stage-specific.

To further explore the role of chemo-sensory capability in mediating behavior choice in early and late developmental stage American toad tadpoles, several questions were posed and then translated into laboratory experiments. First, what is the extent of chemo-sensory capabilities in both early and late stage tadpoles? Second, how do these chemo-sensory capabilities affect behavior of early and late stage tadpoles?

Experimental treatments were performed using chemical cues of conspecifics, heterospecifics, and two predators to condition water. Treatments were performed with two densities of tadpoles (n=10, n=20) to determine how and if social aggregations affected behavior, and choice of refuge was used to quantify behavior. Predaceous diving beetle larvae (Dytiscus sp.) were chosen because they are known to be a highly efficient predator of toad tadpoles, and have been found to kill and eat more toad tadpoles of all sizes than other predators (Brodie and Formanowicz 1983). Diving beetle larvae have also been shown to be primarily non-visual, relying instead upon tactile and chemical cues before initiating a strike (Formanowicz 1987). In contrast, bluegill sunfish, Lepomis macrochirus, are visually oriented predators that only occasionally are syntopic with toad
tadpoles. Toad tadpoles are distasteful to bluegill (Voris and Bacon 1966), although naïve predators may still engulf tadpoles. Furthermore, tadpoles are known to behaviorally respond to these fish (Lawler 1989), perhaps due to their fragility.
Methods

Collections

American toad (Bufo americanus) tadpoles, Southern leopard frog (Rana sphenoecephala) tadpoles, and predaceous diving beetle larvae (Dytiscus sp.) were dip-netted from an ephemeral pond in Coles County, Illinois, beginning early May 2000. Tadpoles were separated by species and housed in aerated plastic aquaria (39x25x10.5 cm) filled to a depth of 10 cm with tap water treated using a commercial aquarium water conditioner, and were housed in environmental chambers kept at 22°C, with 14:10 photoperiod (which approximates the natural photoperiod during this time of the year); water changes were performed every second day. Aquaria were wrapped in black plastic to reduce startling tadpoles by human presence. Tadpoles were maintained on a diet of commercial fish pellets fed ad libitum; plastic plants were provided for refuge. Tadpoles were kept in the laboratory for at least 48 hours prior to testing to allow for acclimation. Beetle larvae were housed individually in 1.5L plastic containers filled with conditioned water to a depth of 4 cm and containing plastic plants, with their diet consisting of backswimmers (notonectids). Bluegill sunfish (Lepomis macrochirus) were seined from a permanent pond on the Eastern Illinois University campus (Coles Co., IL) and were housed in aerated 40L aquaria. Fish were fed bloodworms and guppies; sufficient live food was provided to ensure continuous access to prey. All predators were kept at room temperature near windows under natural photoperiod conditions; chambers were cleaned every 48 hours. Predators also were held at least 48 hours in the laboratory before use to ensure no residual chemicals from prey captured in the wild remained in their digestive
track. Following conclusion of the study, tadpoles and predators were released at the site of capture.

**Experiments**

The experimental protocol was consistent for all treatments tested. Test chambers were of the same dimensions as holding chambers, and filled with conditioned tap water to a depth of 4 cm. All experiments were conducted under fluorescent lighting at room temperature. Forty percent (40%) of each chamber contained plant cover as one contiguous clump, consisting of 10-12 stalks approximately 20 cm long of plastic *Elodea* and *Anacharis* plants. In each experimental chamber, cover was randomly placed in either the north or south direction of each arena. Toad tadpoles were netted from aquaria and staged according to Gosner (1960). For experiments in Part I, tadpole stages 25-27 were used and designated as early stage. In Part II, tadpole stages 41-42 were used and designated as late stage. Toad tadpoles were then introduced into test arenas and allowed 10 minutes for acclimation. Each experiment lasted 15 minutes, during which period the number of tadpoles under cover was recorded immediately and then every 3 minutes thereafter (repeated measure). Each of the experimental treatments was performed with tadpole densities of 10 and 20. For each density 5 replicates were performed; no tadpoles were used twice (excluding those in experiment #7-see below) so all data are independent.

Additions of chemical and control stimuli were poured into the center of test arenas during each experimental replicate. To prepare extract, tadpoles of similar size and stage were humanely killed then homogenized for approximately 15 seconds using a blender.
and 150 ml of deionized water. From this mixture, 10 ml of liquid were collected and slowly poured into the center of the arena. Fresh extract was used for each trial to prevent potential degradation of chemical cues, and a total of 12 beetle larvae and 10 bluegill were used in both preparing extracts as well as in certain trials (see below). For treatments requiring addition of an extract to the test chamber, a control was established as follows. Five minutes into the acclimation period, 10 ml of conditioned tap water was poured into the center of the arena, which controlled for the tactile disturbance effect of adding liquids. At the conclusion of each experiment, arenas were thoroughly washed, rinsed, and refilled with conditioned water.

**Part I: Treatments**

To determine how early stage toad tadpoles respond to conspecific alarm signals, extracts of early and late stage tadpoles were prepared and added to test aquaria. To determine the response of early stage tadpoles to a heterospecific, an extract of *R. sphenoecephala* tadpoles was prepared and added to test aquaria.

Tadpole response to diving beetle larvae was ascertained using two methods. First, chemical cues were collected from a chamber holding a diving beetle larva and added to the test chamber. Prior to use, beetle larvae were transferred and held individually for a minimum of 5 hours in a clean chamber with 100 ml of conditioned water to prevent introduction of conflicting chemical cues from prey. From a chamber containing an individual beetle larva, 10 ml of water were collected and carefully added to the center of the test arena. Secondly, to approximate natural conditions more closely, a single, live beetle larva was placed in an enclosure in each test arena. Enclosures consisted of styrofoam cups that were thoroughly washed before use and then discarded after each
trial. A small washed rock was placed in the bottom of each cup to prevent it from floating. Each cup was pierced 60-70 times with a sterile wooden toothpick to allow for water flow. Enclosures were introduced prior to the introduction of tadpoles for acclimation, and randomly placed in one of 3 locations approximately 5 cm from the edge of the arena opposite the side containing plants. A control for this treatment was established using empty enclosures.

A series of experiments were designed to test tadpole response to bluegill using chemical cues. Ten bluegill were kept for 5 hours in a clean chamber with 1L of conditioned tap water prior to use. From this holding chamber, 10 ml of water were collected and introduced into the test chamber 5 minutes into the acclimation period.

Following use in the prior experiment, the response of each tadpole group to fish chemical cue conditioning was determined. Each tadpole group was removed from the test chamber and housed separately in 1.5L containers for 48 hours. Every 8 hours, 50 ml of water from fish aquaria were added. After conditioning the tadpoles to fish stimuli in this manner, they were then retested for response to fish cues. Controls were performed in which tadpoles were housed in identical groups and conditioned using conditioned tap water additions every 8 hours.

**Part II: Treatments**

Treatments in Part II were performed using protocols similar to those in Part I. Controls were established as in Part I using late stage tadpoles, and predators were randomly assigned to replicates.

Late stage tadpoles were exposed to additions of tadpole extract prepared using early stage tadpoles and late stage tadpoles. Similarly, tadpoles were exposed to predator cues
using bluegill cues collected from a 1L aquarium housing 10 bluegill, and 10 ml water samples taken from a larval diving beetle chamber.

Analysis

Data was converted to percentage of tadpoles under cover, then transformed using an arcsine transformation (Sokal and Rohlf 1981). An analysis of variance was used to compare means, with time as a repeated measure. Computer software (NWA STATPAK 1986) was used, with an alpha value set a priori at 0.05. Figures are composed of untransformed data, in the form of percentages of tadpoles seeking cover, with means + one standard error shown.
Results

A three-way ANOVA, with main effects (tadpole density and treatment) was calculated with one repeated measure (time). Results of these analyses revealed that tadpole density did not contribute a significant amount of variance to the statistical model in any experiment. Consequently, density was pooled in further analyses, resulting in a two-way ANOVA with one repeated measure (time), and treatment effect.

Part I: Early Stage

The mean percentage of toad tadpoles seeking cover varied significantly (F=5.12, P=0.0047) among treatments (Fig. 1). Significantly higher mean percentages of tadpoles exposed to early and late stage extracts were found seeking cover (mean=47.1±8.2%, mean=46.8±6.7%, respectively) as compared to tadpoles exposed to control treatments (mean=21.6±4.8%), however, the treatment means were statistically indistinguishable from one another. Similarly, the mean percentages of tadpoles in the control (mean=21.58±4.81%) and R. sphenocephala treatments (mean=28.5±7.47%) were not different. Over time, the percent of tadpoles in these treatments seeking cover increased significantly (F=8.35, P<0.0001--Fig. 2) as compared to controls.

The mean percentage of toad tadpoles seeking cover varied among Dytiscus sp. treatments. Tadpoles exposed to caged beetle larvae had a mean percent under cover (53.95±2.52%) that was significantly higher than the mean percent (43.49±3.70%) observed for tadpoles exposed to an empty cage (F=9.85, P=0.005--Fig 3). Trials with beetle larvae cues alone also differed significantly (F=7.24, P=0.014), with the mean percent of tadpoles in control water conditions under cover being 34.97±3.91%, while the
mean percent of tadpoles seeking cover during exposure to beetle larval cues was 45.52±2.97% (Fig 4). The two control treatments utilized (empty predator cage and water additions), did not differ significantly from one another (F=3.01, P>0.05). Of these two sources of beetle larvae cues, caged beetles had a significantly greater effect on cover usage (F=13.52, P=0.0017) than did the addition of water from beetle larvae chambers.

Treatments involving bluegill stimuli varied in the percentages of tadpoles seeking cover (Fig. 5). A significant difference (F=8.97, P=0.007) was found between the mean percentages of tadpoles seeking cover in water controls (mean=21.58±4.81%) and fish cue treatments (mean=38.42±3.51%-- Fig 5). A significant difference (F=22.87, P=0.0001) also resulted between tadpoles conditioned to bluegill cues (mean=25.92±2.85%), and tadpoles treated with conditioned tap water (mean=42.42±2.98%--Fig 6). Surprisingly, more tadpoles were found seeking cover in the conditioning control than in the fish treatment. Of the two control treatments used, tadpoles assigned to the conditioning regime were found under cover a significantly higher percentage of time (F=17.05, P=0.0006) than tadpoles exposed to water additions (mean = 50.3±3.0%, mean = 36.0±3.8%, respectively). Initially, 47.5% of tadpoles responded to the addition of bluegill cues by seeking cover, whereas after conditioning, tadpoles significantly reduced use of cover to 39.7%. The percentage of tadpoles seeking cover increased significantly (F=4.15, P=0.0013) with time following a 48 hour conditioning period to bluegill cues (Fig 7).

Part II: Late Stage

There were no significant differences among treatment means (Fig 8) involving late stage toad tadpoles (F=1.11, P=0.363). During exposure to extracts of both early and
late stage tadpoles, use of cover varied significantly (F=3.89, P=0.0025) over time (Fig 9). Analysis of controls for early versus late stage tadpoles revealed that a greater percentage of late stage tadpoles sought cover (F=12.47, P=0.002). The results of pairwise analysis of variance in early and late stage tadpoles are shown (Table 1).
Fig 1. Effects of water controls, early [Bufo(e)] stages (25-27) Bufo americanus, late [Bufo(l)] stages (41-42) B. americanus tadpole extract, and early stages Rana sspenocephala (Rana) extract upon the percentage of early stage B. americanus tadpoles seeking cover. Means + one standard error are shown.
Fig 2. The effects of time upon the percentage of early stages (25-27) *Bufo americanus* tadpoles seeking cover in treatments: water controls (CTRL), extracts of early stages *B. americanus* [B(e)], late stages (41-42) *B. americanus* [B(l)], and early stages Southern leopard frog (Rana). Means + one standard error are shown.
Fig 3. Effects of controls with empty predator cages (C. cage) and enclosed predaceous diving beetle larvae (Dytiscus) upon the percentage of early stage (25-27) Bufo americanus tadpoles seeking cover. Means + one standard error are shown.
Fig 4. The effects of water controls [Ctrl (w)] and diving beetle larvae (Dytiscus sp.) chemical cues [Dyts(w)] upon the percentage of early stages (25-27) *Bufo americanus* tadpoles seeking cover. Means + one standard error are shown.
Fig 5. Effects of water controls (Control) and *Lepomis macrochirus* chemical cues (Fish I) upon the percentage of early stages (25-27) *Bufo americanus* tadpoles seeking cover. Means + one standard error are shown.
Fig 6. Percentage of early stages (25-27) *Bufo americanus* tadpoles seeking cover following 48 hour conditioning treatments to controls [Ctrl(c)], and *Lepomis macrochirus* stimuli [Fish(c)]. Means + one standard error are shown.
Fig 7. Effects of time upon the percentage of late stages (41-42) *Bufo americanus* tadpoles seeking cover following a 48 hour conditioning period to water controls [Ctrl(c)], and *Lepomis macrochirous* stimuli [Fish(c)]. Means ± one standard error are shown.
% Tadpoles Under Cover

Time (minutes)

- Ctrl (c)
- Fish (c)
Fig 8. Effects of various treatments upon the percentage of late stages (41-42) Bufo americanus tadpoles seeking cover. Means + one standard error are shown.
Fig 9. Effects of time upon the percentage of late stages (41-42) *Bufo americanus* tadpoles seeking cover during exposure to controls, extract of early stages (25-27) *B. americanus* tadpoles, and extract of late stages *B. americanus* tadpoles.
Table 1. Results of pairing AMYAN at daily 167 stages (26-27) American toad, Bufo americanus, with 167 stages (21-22) American toad, Bufo americanus.
Table 1. Results of pairwise ANOVA's of early (E) stages (25-27) American toad, *Bufo americanus*, tadpoles (b) compared to late stages (41-42) toad tadpoles.

<table>
<thead>
<tr>
<th></th>
<th>0 Min</th>
<th>3 Min</th>
<th>6 Min</th>
<th>9 Min</th>
<th>12 Min</th>
<th>15 Min</th>
<th>Mean</th>
<th>Error</th>
<th>F_cal</th>
<th>P_cal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eb</strong> Early ext</td>
<td>34</td>
<td>45</td>
<td>35</td>
<td>51</td>
<td>56</td>
<td>53</td>
<td>46</td>
<td>5.22</td>
<td>0.721</td>
<td>0.401</td>
</tr>
<tr>
<td><strong>Lb</strong> Early ext</td>
<td>37</td>
<td>32</td>
<td>49</td>
<td>39</td>
<td>45</td>
<td>34</td>
<td>41</td>
<td>2.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Eb</strong> Late ext</td>
<td>40</td>
<td>35</td>
<td>52</td>
<td>53</td>
<td>48</td>
<td>55</td>
<td>47</td>
<td>4.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lb</strong> Late ext</td>
<td>22</td>
<td>39</td>
<td>41</td>
<td>40</td>
<td>42</td>
<td>47</td>
<td>39</td>
<td>3.16</td>
<td>2.256</td>
<td>0.150</td>
</tr>
<tr>
<td><strong>Eb</strong> Fish cue</td>
<td>31</td>
<td>31</td>
<td>35</td>
<td>37</td>
<td>48</td>
<td>43</td>
<td>34</td>
<td>3.51</td>
<td>0.636</td>
<td>0.426</td>
</tr>
<tr>
<td><strong>Lb</strong> Fish cue</td>
<td>24</td>
<td>34</td>
<td>42</td>
<td>37</td>
<td>32</td>
<td>37</td>
<td>34</td>
<td>3.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Eb</strong> Dyt. cue</td>
<td>38</td>
<td>31</td>
<td>34</td>
<td>36</td>
<td>39</td>
<td>30</td>
<td>35</td>
<td>2.98</td>
<td>0.633</td>
<td>0.437</td>
</tr>
<tr>
<td><strong>Lb</strong> Dyt. cue</td>
<td>42</td>
<td>34</td>
<td>29</td>
<td>42</td>
<td>37</td>
<td>38</td>
<td>37</td>
<td>3.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Lefcort (1998) found that group size affected behavior in larvae of *Bufo terrestris* under laboratory conditions. Similarly, Graves et al. (1993) with *B. cognatus*, and Hokit and Blaustein (1995) found further that larger groups of *Rana cascadae* tadpoles moved significantly more than did smaller groups in response to predator cues. Group size may be an especially important mediator of behavior in bufonids because of their complex aggregative behaviors (Beiswenger 1975, Hokit and Blaustein 1995). In contrast to these studies, however, I found that group size did not have any significant effect upon cover use by *B. americanus* tadpoles regardless of the developmental stage. The possibility exists that the numbers of tadpoles I used were too small to have a significant impact upon tadpole behavior, although Rodel and Linsenmair (1997), using *Phrynornantis microps*, found density effects beginning with a sample size of $n=10$ tadpoles; group sizes of 10 and larger preferred open waters. My findings do however support the results of Hews and Blaustein (1985) who found that alarm substances tended to break up swarms of tadpoles; it seems possible that this effect may also inhibit swarm formation in the presence of predatory cues. Waldman and Adler (1979), Hokit and Blaustein (1995), and Watt et al. (1997), demonstrated that tadpoles are more likely to form aggregations when with siblings than when with non-siblings. Since I collected tadpoles randomly it was likely that the coefficient of relatedness among them was low, therefore inhibiting the formation of swarms. After collection, tadpoles were reared with conspecifics, which has also been demonstrated to affect the intensity with which tadpoles respond to predator cues (Bridges and Gutzke 1997).
Bufonids are known to recognize conspecifics through release of alarm signals during predatory events (Hrbacek 1950, Adams and Claeson 1998), and to exhibit avoidance tactics (Petranka 1989) upon detection of those signals. This early warning system allows tadpoles to modify their behavior in advance of an encounter with a predator and has been shown to significantly reduce predator success (Hews 1988). I found that early stage toad tadpoles sought cover in significantly higher percentages when exposed to extracts of both early and late stage conspecifics, which is congruent with previous studies of other Bufonids (Petranka 1989, Petranka and Hayes 1998). Although both cue types elicited an increase in the percentage of tadpoles seeking cover, no apparent differences existed between extracts of early or late stages. This may be an adaptive generalized antipredator response of early stage tadpoles to all stages of conspecific alarm signals. These results suggest that no alterations to alarm signals occur prior to metamorphosis, however, Belden et al. (2000) found an apparent alteration in signals between larvae and juvenile *Bufo boreas*.

The rising percentages of tadpoles seeking cover over the 15 minute time period in several of the experiments might be explained by slow diffusion rates of cues through the test chamber. However, chemical cues are known to rapidly degrade, as demonstrated by Petranka (1989), who showed that *B. americanus* tadpoles failed to respond to alarm cues after 8 minutes in open water, thereby imposing a limit to experimental duration.

Though they are often syntopic, American toad tadpoles exhibited no significant response to *R. sphenocephala* larvae extract. This could be evolutionarily advantageous because the two species are subject to different predators at least during the latter
development stages, a possibility attributable to the great size of *R. sphenoecephala* larvae. *Rana sphenoecephala* larvae attain a much greater size than toad tadpoles, and owing to the fact that many tadpole predators are size-dependant (e.g. diving beetle larvae—Formanowicz 1986, Babbitt and Tanner 1998), ignoring alarm signals from heterospecifics would be adaptive if no inherent threat existed.

Exposure to beetle larvae in enclosures as well as chemical cues from water taken from larval beetle chambers resulted in increased use of available cover by toad tadpoles as compared to controls. These treatments were designed to simulate two separate situations in nature. By adding water, a situation existed similar to when a diving beetle larva inhabits and then vacates an area; the cues slowly dissipate and degrade. Interestingly, the number of tadpoles seeking cover with beetle larvae maintained in enclosures was approximately 16% greater than when just chemical cues of larval beetles were added. This may have been a response to the heightened risk of predation posed when an actual predator was present. Anuran larvae are known to perceive and respond differentially to varying levels of predation (Horat and Semlitsch 1994, Anholt et al. 1996, Laurila et al. 1997). It is possible that cues emanating from the caged larvae were more potent than the chemical cues present in the water sample, or perhaps the tadpoles may have sensed movement within the cage and responded with increased cover use.

Use of chemical cues from bluegill resulted in higher numbers of tadpoles seeking cover as compared to the control. While bufonids have been shown to be unpalatable to fish (Voris and Bacon 1966, Kruse and Stone 1984, Lefcort 1998), they may still respond to fish cues if they operate under a generalized antipredator defense system. Lawler (1989) demonstrated that refuge use increased by tadpoles in response to the presence of
fish; furthermore, Semlitsch and Gavasso (1992) also showed that tadpoles decreased swimming time in the presence of fish (but see Kats et al. 1988). This strategy may be adaptive for small, vulnerable larvae that may be injured or killed by naïve predators even if they were to be expelled, uneaten (Brodie et al. 1978). It is interesting to note that following a 48 hour conditioning period the percentage of tadpoles seeking cover decreased; this effect has been previously shown (Semlitsch and Ryer 1992). Tadpoles are also known to respond to novel stimuli (Manteifel 1995), and in a geographic range where toad tadpoles rarely encounter fish, this is also a plausible explanation. During the conditioning period, tadpoles either became habituated to the novel stimulus, or, through lack of predatory attempts, failed to respond to fish cues. Quite surprisingly and inexplicably, tadpoles that were “conditioned” with conditioned tap water sought cover at a rate similar to tadpoles assigned to the fish cue treatment. It is unlikely that this resulted from the simple addition of liquid because tadpoles conditioned with fish water actually decreased cover usage.

In Part II, late stage tadpoles showed no significant responses to predators or conspecific alarm signals as compared to the controls. It was found, however, that in treatments involving use of early and late stage B. americanus extracts, the percentages of tadpoles seeking cover increased by 8.8% over time, however, this was statistically insignificant. Late developmental stage toad tadpoles in control trials were found seeking cover in significantly higher percentages when compared to control trials with early developmental stage toad tadpoles. This increased use of cover by late stage tadpoles could explain the lack of statistical significance in further analyses of early versus late developmental stage tadpoles. Perhaps this increased use of cover is a generalized
response to predators by tadpoles at a highly vulnerable time in life. At Gosner (1960) stage 42, tadpoles cease feeding (Beiswenger 1975), and production of bufotoxin surges (Brodie et al. 1978, Formanowicz and Brodie 1982), which may then become the primary antipredator defense mechanism in this toad species.

Behavioral disparities could likely exist between early and late developmental stage toad tadpoles due to a variety of factors such as differing suites of predators, habitat, escape tactics, biochemistry, and chemical detection capability. Studies focusing on these behavioral changes between juveniles and pre-metamorphic individuals are few, but see (Bridges and Gutzke 1997, Belden et al. 2000). In this study, American toad tadpoles responded adaptively to conspecific alarm signals and predator cues, at least in early stages of development. However, this was not found to be the case with tadpoles nearing metamorphosis. This study is one of the first to demonstrate that toad tadpoles approaching metamorphic climax exhibit behaviors that are different than that of tadpoles in earlier developmental stages.
Literature Cited


