# Characteristic behaviour and body distinction in anuran tadpole species in determining predatory avoidance techniques

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## Introduction

It is important for anuran tadpoles to adopt the appropriate behaviour to promote its survival (Teplitsky *et al.* 2004). The most common anuran predators are sit-and-wait predators (insects) and pursuing predators (fish) (Teplitsky *et al.* 2005). Predatory avoidance techniques are detrimental to the survival of anuran species through all life stages, especially tadpoles (Teplitsky *et al.* 2005). These types of techniques can vary from species to species with regards to their morphological traits and behavioural responses, as well as their environmental habitat (Teplitsky *et al.* 2005).

Highly active and muscular tadpoles are common in open, flowing current habitats (lotic) where the dominant predators are fish (Teplitsky *et al.* 2005). Tadpoles must have strong tail muscles that can move fast in order to avoid being consumed by fast swimming fish predators (Teplitsky *et al.* 2005). On the other hand, insect-dominated predators promote less active tadpoles with larger tail depths that are most commonly found in sheltered/structured, still-watered habitats (lentic) (Teplitsky *et al.* 2005). This less active type of behaviour supports the survival of anuran tadpoles among the sit and wait predators (Teplitsky *et al.* 2005). Since low activity tadpoles are less detected by predators, muscular tails and large bodies are not ideal (Spieler and Linsenmair 1999). At times of detection by the predator, low active tadpoles are able to escape harm with larger tail depths, which can act as a distraction for the predator from the small, vulnerable tadpole body (Spieler and Linsenmair 1999). Low active swimmers are more accustomed to larger habitat routes due to barriers created by the sheltered environment (increasing swim distance ability) (Spieler and Linsenmair 1999).

Experimental studies of tadpole characteristics and behaviour are detrimental in assessing predation risks to the survival of tadpoles (Smith and Doupnik 2005). In the present study, tadpole behaviour in the presence of a predator is artificially stimulated, alike to previous tadpole behaviour experiments (Kopp *et al.* 2006, Spieler and Linsenmair 1999). Using three different anuran species with varying morphological traits: *Rana sylvatica* (Wood Frog), *Rana catesbeiana* (American Bullfrog) and *Bufo americanus* (American Toad) (Fig.1 and Table 1) and their individual behaviour to an artificial predator stimulus, the endurance times and total distances swam are quantified.

(a) (b) (c)

**Fig.1** Images of three anuran tadpole species being tested: (a) *Rana sylvatica*, (b) *Rana catesbeiana*, and (c) *Bufo americanus* (Altig *et al.* 2006).

It is predicted that tadpoles exhibiting a low endurance to swim (or less active ability) and high swimming distances will have larger tail depths and smaller bodies, indicating a species that is predisposed to structured/forested water habitats (lenthic) (Altig *et al.* 2006). Based on the characteristics in Table 1, the predator avoidance behaviour of each species can help indicate the optimal living conditions for anuran tadpoles, the ideal predator avoidant behaviour for the individual species and thereby help predict successful sustaining environments for anuran populations.

**Table 1** General Characteristics for the three anuran tadpole species being tested (Altig *et al.* 2006)

| | | TADPOLE CHARACTERISTICS | | | |
|---|---|---|---|---|---|
| | | **Body Types** | **Habitat** | **Tail Features** | **Behavioural Traits** |
| **SPECIES** | *Rana sylvatica* | Small, globular | Lentic sites | Large tail depth, high dorsal fin, dark fins | Cryptic coloring |
| | *Rana catesbeiana* | Long, not strongly depressed | Lotic and lentic sites | Moderate tail depth, dorsal fin with notable arch | Brightly colored |
| | *Bufo americanus* | Thick, globular | Shallow temporary pools (from summer rains) of permanent lentic sites | Small tail depth, bi-colored tail muscle | Grouping, cryptic coloring |

## Methods

Tadpole collections, measured tests and swim endurance were performed by J. Richardson, Brock University, St. Catharines, Ontario.

### Tadpole Collection

Three different species (*Rana sylvatica*, *Rana catesbeiana*, and *Bufo americanus*) of anuran tadpoles were collected. *R. sylvatica* were collected as eggs from Lake Sasajewun, Bat Lake and Lost Ray Lake in Algonquin Provincial Park and hatched in a 12°C incubator (then transferred to fresh pond water). *R. catesbeiana* and *B. americanus* were collected as tadpoles from the Niagara region. Conditioned tap water was added to the room temperature pond water that each species was separately kept in and later transferred to 38-L Rubbermaid tubs while being fed crushed Wardley's Spirulina discs and Tetramin fish flakes. J. Richardson performed the animal collection and treatment with approval from Brock University's Animal Care and Use Committee (ACUC).

## Measured Tests (Velocity and Distance)

A rectangular container sized 23.8x15.5x5 cm was used for the speed test. Individuals from each anuran species was dropped in 2.5 cm-deep waters, at room temperature and stimulated with a dorsal sweep by a spatula to instigate a predator presence. The container was illuminated from above along with a camera to record the activity of the tadpoles. Three long/straight swims were documented for each individual. The tadpoles were returned to conditioned water in round containers, separated by species. The speed measurements were assessed using ImageJ software, AVI Splitter and Manual Tracking. Velocity and distance measurements were obtained for all individuals tested.

## Testing Swim Endurance

Using a glass tube connected to an electric pump, the swim endurance of each tadpole was tested 24 hours directly after the initial swim speed stimulus. Water flow in the swim tube exited into a bucket that was slowly adjusted for inserted tadpoles (from minimal, 1.5 L/min, to exhausting, 7.2 L/min speeds). In low flow circumstances, some tadpoles traveled through the swim tube to the exit bucket. Time to exhaustion of each tadpole was quantified as the total time endured.

## Statistical Tests

For the three anuran species, the total time endured and total distance traveled for each tadpole was applied to a statistical simulation using SAS software. Normality among endurance times and total distances swam were tested using the UNIVARIATE procedure. Endurance time indicated rejection for normality ($P < 0.0001$) and was log-transformed ($P = 0.1514$). Total distance swam showed normality ($P = 0.4559$).

Homogeneity was tested using Bartlett's Test for each variable among the three-anuran species. Both endurance time (log-transformed) and total distance swam satisfied equal variances among the experimental data (P = 0.2631 and P = 0.4731, respectively). GLM procedures were separately performed for each variable: endurance times (log-transformed) and total distance swam. Each GLM procedure tested the null hypothesis of no significant variation among and within each anuran species for each variable, using ANOVA and Tukey's studentized test.

## Results

From the statistical tests using SAS and the data output from J. Richardson's measurements, the ANOVA tests (Tables 2 and 3) for both variables (log-transformed total endurance time and total distance swam) resulted in low p-values (<0.0001). These low p-values indicate a rejection for the null hypothesis, suggesting that there is significant variation within and between at least one of the species from the others.

**Table 2** ANOVA for variation within and between the three discrete tadpole species for the continuous log-transformed total endurance time variable using SAS. The p-value (<0.0001) allows for rejection of the null hypothesis. R-squared = 0.2458 (24.6 % of the variation in the log-transformed time endured is explained by the anuran species)

| **Source of Variation** | **Sum of Squares** | **df** | **Mean Squares** | **F-value** | **P value** |
|---|---|---|---|---|---|
| Model | 7.5844 | 2 | 3.7922 | 14.99 | <0.0001 |
| Error | 23.2692 | 92 | 0.2529 | | |
| Corrected Total | 30.8536 | 94 | | | |

**Table 3** ANOVA for variation within and between the three discrete tadpole species for the continuous distance swam variable using SAS. The p-value (<0.0001) allows for rejection of the null hypothesis. R-squared = 0.2517 (25.2 % of the variation in the log-transformed time endured is explained by the anuran species)

| Source of Variation | Sum of Squares | df | Mean Squares | F-value | P value |
|---|---|---|---|---|---|
| Model | 282.1997 | 2 | 141.0999 | 15.47 | <0.0001 |
| Error | 838.9102 | 92 | 9.1186 | | |
| Corrected Total | 1121.1099 | 94 | | | |

A Tukey test was performed for each variable for the three anuran tadpole species indicating a significance (with 95% confidence) between the tadpoles tested for both variables: total time endured (log-transformed) and total distance swam.

Referring to Fig. 2 and 3, the mean values (± SE) for each tadpole species can be compared for both variables. Consistent with the Tukey test result, both variables in Fig. 2 and 3 indicate a difference between *R. sylvatica* (Rs) and the two other tadpole species. The *R. catesbeiana* (Rcat) and *B. americanus* (Ba) tadpoles had similar values for both endurance and distance tests.

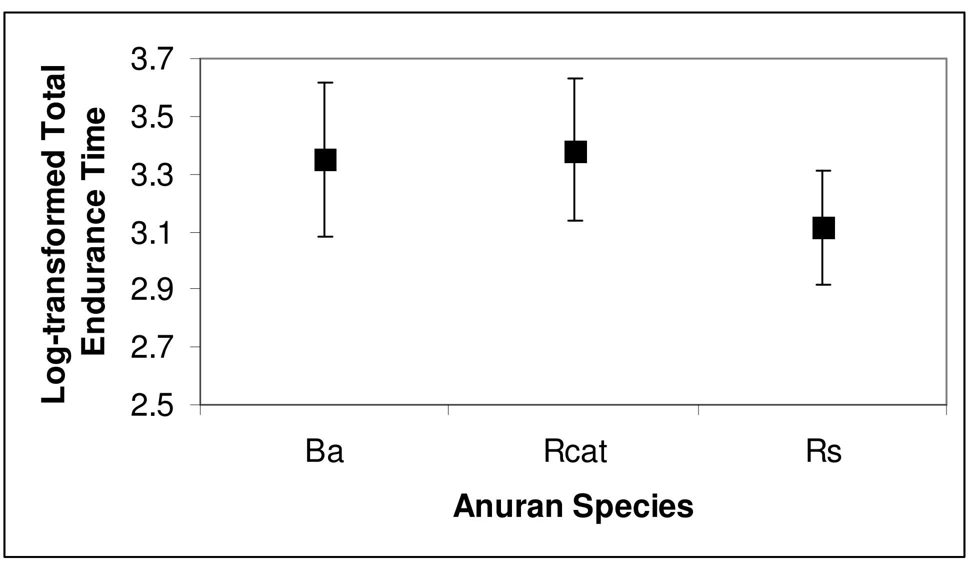


**Fig.2** Mean total endurance time (log-transformed) for all three tadpole species ± SE (in Excel). *R. sylvatica* (Rs), *R. catesbeiana* (Rcat) and *B. amercianus* (Ba).

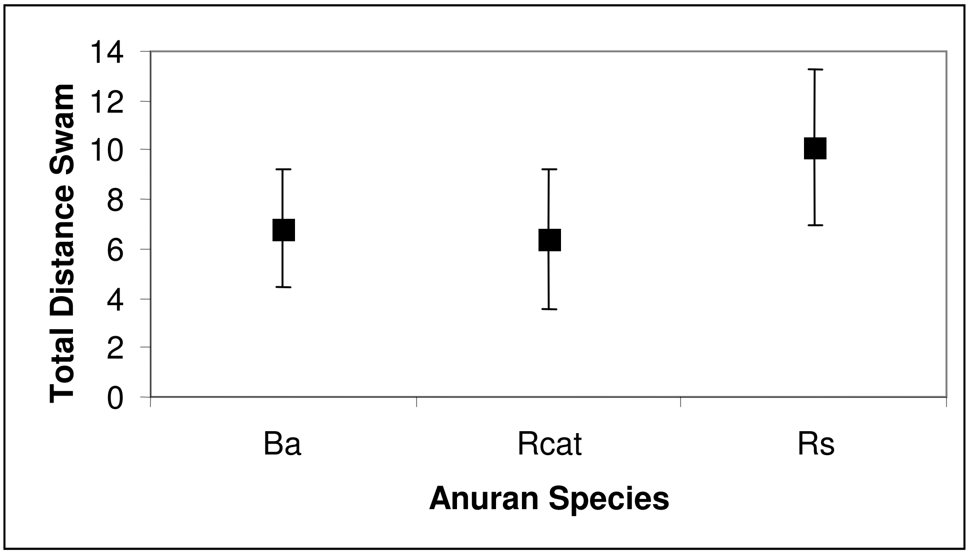


**Fig.3** Mean total distance swam for all three tadpole species ± SE (in Excel). *R.* sylvatica (Rs), *R.* catesbeiana (Rcat) and *B.* americanus (Ba).

## Discussion

The observation of three anuran tadpole species and their behavioural responses to an artificial predator indicated a correlation between total endurance time (log-transformed) and total distance swam. The results in Fig. 2 and 3 for *R. sylvatica* suggests a significance ($P < 0.0001$) in variation of endurance and distance swam (separately) compared to the other tadpole species (*R. catesbeiana* and *B. americanus*). The absence of a lenthic-type habitat among the *R. sylvatica* tadpoles may explain the difference in endurance time and distance swam variation compared to the behaviour portrayed by the other species in the presence of a predator stimulus, especially when considering body types. Anuran species more accustomed to open and flowing water currents (*R. catesbeiana* and *B. americanus*) displayed similar behavioural responses in the presence of a predator (Fig. 2 and 3).

As suggested by Smith and Doupnik (2005) regarding tadpole activity levels, behavioural responses of the same species of tadpoles were observed to be distinctly different even though accustomed to identical habitats. The standard deviations among the means in Fig. 2 and 3 may suggest that individuals of the same species may not have responded alike to the predator stimulus. The study by Smith and Doupnik (2005) also suggested that individuals of the same species may have been undergoing natural selective pressures and would have been identifiably distinct from others of the same species. In light of this new theory by Smith and Doupnik (2005), further investigation of phenotypic relationships among the species or microsatellite analysis would be useful to indicate if there is natural selection acting on certain individuals of the population as well as determining if this selective pressure would have adverse effects on behavioural studies.

The present study suggests a significant difference of predator avoidance behaviour of *R. sylvatica* in comparison to the other tadpole species tested. The lenthic natural habitat of *R. sylvatica* requires shorter swimming endurance and longer distances swam (Altig *et al.* 2006). Therefore, species that exhibit lenthic habitat qualities (ability to swim far and stay very still) may not succeed in other types of environments, such as lotic habitats where predators are faster and larger in size (Altig *et al.* 2006). Future studies should be implemented with regards to the overall community ecology of these lenthic and lotic water habitats, while observing the population dynamics and behavioural relationships of prey and predators within.

**Acknowledgements**

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## Literature Cited

Altig R., McDiarmid R.W., K. A. Nichols, and P. C. Ustach. 2006. Tadpoles of the United States and Canada: A Tutorial and Key. US Geological Survey **2007**:4.

Kopp K., M. Wachlevski, and P. C. Eterovick. 2006. Environmental complexity reduces tadpole predation by water bugs. Canadian Journal of Zoology **84**:136-140.

Smith G. R., B. L. Doupnik. 2005. Habitat use and activity level of large American bullfrog tadpoles: Choices and repeatability. Amphibia-Reptillia **26**:549-552.

Spieler M., K. E. Linsenmair. 1999. Aggregation Behaviour of *Bufo maculatus* Tadpoles as an Anti-predator Mechanism. Ethology **105**:665-686.

Teplitsky C., S. Plenet, and J. P. Joly. 2004. Hierarchical Responses of Tadpoles to Multiple Predators. Ecology **85**:2888-2894.

Teplitsky C., S. Plenet, J. P. Lena, N. Mermet, E. Malet, and P. Joly. 2006. Escape behaviour and ultimate causes of specific induced defenses in an anuran tadpole. Journal of Evolutionary Biology **18**:180-190.