

EASTERN GRAY TREEFROG PLASTICITY AS A RESULT
OF PREDATOR-INDUCED STRESS

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ABSTRACT

Tadpoles are known to change behavioral and morphological traits in response to predatory cues. However, little is known about if and how tadpoles will react to cues from predatory game fish, which may be introduced into previously fishless ponds for recreational purposes. Using Eastern gray treefrog tadpoles (*Hyla versicolor*), I studied the effects of rockbass (*Ambloplites rupestris*) and yellow perch (*Perca flavescens*) on the morphological and behavioral plasticity. I raised 90 predator-naïve tadpoles in 9 experimental tubs to simulate environments with yellow perch, rockbass, or a predator free control. Activity levels and seven morphological traits were examined.

Tadpoles in the presence of both yellow perch ($p=0.351$) and rockbass ($p=0.338$) tended to be less active in order to avoid visual fish predation. In the presence of yellow perch, tadpoles tended to grow deeper tails ($p=0.342$) and in the presence of rockbass the tadpoles tended to grow longer tails ($p=0.129$). Muscle depth and width showed no change in tadpoles exposed to rockbass. However, tadpoles exposed to yellow perch grew thinner tail muscles ($p=0.080$). No significant data was seen with regards to body size, so possessing a large body is not an effective means of avoiding fish predation.

Collectively, this experiment shows that Eastern gray treefrog tadpoles do alter their morphology and behavior in response to different predators. These conclusions should be treated cautiously, as the data collected was statistically weak.

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INTRODUCTION

Phenotypic plasticity is the ability of an organism to alter various traits in response to environmental variability. Previous studies have shown that larval anurans alter several morphological and behavioral traits in response to varying environments. Anuran tadpoles mature in a variety of aquatic environments, each of which favors different phenotypes. The presence of predators is one important variable that can determine the predominant phenotype within each habitat. For example, wood frogs (*Rana sylvatica*) develop deeper tails and shorter bodies in the presence of uncaged predators, but grow more slowly (Relyea 2002 and Relyea and Auld 2004). This trait is beneficial in the presence of predators, but slows maturation. Therefore, these traits are not expressed in predator-free environments. The ability to develop some traits as the need arises is evolutionarily desirable.

Morphological and behavioral plasticity have been observed in Eastern gray treefrog tadpoles (*Hyla versicolor*) in response to different invertebrate predators and small minnows. Relyea (2001c) found that Eastern gray treefrog tadpoles employ a specific strategy for defending themselves from a given species of predator. For example, deep-tailed and narrow-bodied prey evade dragonfly larvae (*Anax* spp.) more often. As a result, dragonfly larvae predation cues cause narrower bodies and deeper tail fins in some tadpoles (Relyea 2001a). In other cases, reducing activity levels is more effective. In the presence of the central mudminnow, the tail is still deepened, (*Umbra limi*), but the tadpoles employ a greater decrease in activity (Relyea 2001a).

Eastern gray treefrogs inhabit deciduous and mixed forests, as well as swamps in the eastern portion of the United States. Ponds, swamps, and shallow areas of lakes are

the most common breeding areas for the Eastern gray tree frog. Natural predators for the tadpoles include aquatic insects, turtles, and small fish.

The ability of Eastern gray treefrogs to alter their development in response to predatory cues from invertebrate predators has been examined very closely (Relyea 2001a). However, the morphological and behavioral responses to large fish predators have not been studied. The introduction of game fish into formerly fishless ponds has increased the frequency of this predator-prey interaction (Harding 1997), yet little is known about the effects of fish like such as rockbass (*Ambloplites rupestris*) or perch (*Perca flavescens*) on the traits of Eastern gray treefrogs. Using experimental tubs, I examined if and how Eastern gray treefrog tadpoles alter traits in response to perch and rock bass cues. It was hypothesized that Eastern gray treefrog tadpoles should react to fish predatory signals by decreasing activity. Additionally, the tadpoles should develop larger fins and smaller bodies in the presence of these predatory cues.

MATERIALS AND METHODS

On June 3 and June 4, 2004, I collected adult Eastern gray treefrogs in amplexus from a nearby pond. The resulting eggs were then raised in plastic containers. After hatching, 10 tadpoles were massed (6 mg +/- 1 mg) and then randomly assigned to one of 9 tubs. This method of tadpole collection kept the tadpoles predator-naïve. Three 75L Rubbermaid® tubs apiece were each assigned as rockbass, yellow perch, and a predator free control. Each tub was filled to a depth of 20cm with well water and supplied with 15g of Tetramin® fish flakes and a 475mL sample of pond water, to stimulate algal and plankton growth. Two rockbass and 2 yellow perch were kept in similar tubs for the

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duration of the experiment and fed 4 tadpoles every week. Every three days, a 400mL sample of water was taken from the fish tubs and added to its respective experimental tub. Well water was used in the case of the control tubs. This method has been shown to provide the necessary predator cues, and eliminates the need for cages (Petranka et al. 1987). The tubs were placed outside in a field, and covered with 70% shade cloth. The experiment lasted 3 weeks.

Tadpole activity was measured by slowly approaching the tubs and counting the number of active tadpoles (swimming or feeding). Behavioral observations were taken at 8:30 AM and 5:00 PM every day during the final week. This number was converted into a proportion of total live tadpoles. The proportion is a better approach because it accounts for fatalities. The data was tested using an ANOVA test with a post-hoc Bonferroni test in SYSTAT.

After the experiment was terminated, I weighed each tadpole and measured seven morphological traits: tail fin depth and length, tail muscle depth and width, and body length, width, and depth (Relyea 2000). To compare morphological traits, all final measurements were log-transformed and regressed against body mass to estimate overall size and remove the effects of size variance in morphology. The residuals from this regression of each tadpole were used as the specific phenotype for that tadpole and then grouped by treatment to estimate a mean phenotype and variance (Relyea 2001a). This data was tested using an ANOVA with a post-hoc Bonferroni test in SYSTAT.

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RESULTS

While there were patterns in most means, no statistically significant differences ($p < 0.05$) were seen. Both behavioral and morphological differences contained relationships approaching significance, but not actually significant.

Behavioral Traits

The percent of active tadpoles in both rockbass and yellow perch tubs was less than it was in the control tub (Figure 1). Tadpoles in the control tubs seemed to be more active than the rockbass-exposed tadpoles ($p = 0.338$) and more active than the yellow perch-exposed tadpoles ($p = 0.351$). While the mean activity levels do appear different, they are not statistically significant.

Morphological traits

Tail depth data contained notable morphological differences (Figure 2). Tadpoles in the presence of yellow perch tended to have deeper tail fins ($p = 0.342$). Tadpoles in the presence of rockbass showed no difference in mean tail depth.

Tail length also contained slight morphological differences (Figure 3). Tadpoles raised in the presence of rockbass had longer tails than control or perch-exposed tadpoles; however, this data is not statistically significant ($p = 0.129$).

There was a small difference in tail muscle width (Figure 4). Tadpoles in the presence of yellow perch had much thinner tail muscles than the control. These differences were not statistically significant at the 5% level, but were significant at the 10% level ($p = 0.080$). Rockbass-exposed tadpoles showed no effects in muscle width ($p = 1.000$).

Body size traits (width, length and depth), as well as tail muscle depth showed no significant differences (Figures 5-8)

DISCUSSION

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The anuran tadpole employs a host of strategies in different environments in order to best compete. These strategies are geared towards maturation and avoiding predation, two basic components of tadpole fitness. They are utilized selectively in order to ensure a safe, yet efficient tadpole. In this way, the tadpole is assured the greatest possibility of reaching the adult stage and reproducing. The results of this study support these claims.

Behavioral traits

One of the best defenses the tadpole can employ is to limit its activity. By decreasing activity, the tadpole decreases visibility to the predators, resulting in fewer encounters with the predator. This form of defense is especially important when dealing with fish predation. Yellow perch and rockbass are extremely quick and rely heavily on visual cues when hunting. For this reason, hiding and remaining inactive are important ways for a tadpole to avoid predation. In this study, both rockbass and yellow perch-exposed tadpoles showed decreased activity. As a result, these tadpoles would have a greater chance for survival in the presence of predators than their more active counterparts.

Morphological traits

By increasing body size, a tadpole increases its rate of maturation (Relyea 2004). As a result, in predator free environments, large-bodies tadpoles are favored. However, there is a tradeoff between body size and tail fin size (Relyea 2001c), in that a tadpole's

maturation slows as a result of devoting energy and nutrients to a larger tail. In a predatory environment, the tadpole will grow a larger and stronger tail to guard against predation, but grows at a slower as a result.

Tadpoles in the presence of yellow perch had deeper tails. These deeper tails enable the tadpole to escape predators through quickness (Relyea 2001a). By increasing speed, the tadpole has a greater chance of reaching safety or escaping predation when confronted by the yellow perch. However, tadpoles exposed to yellow perch did not grow longer tails. This could mean that a short tail is just as effective for quick bursts of speed, but rock bass cues did affect tail length. Therefore, a more likely explanation for this inconsistency is the limited scope of the study. Perhaps with greater exposure and time to develop, this trait would have seen greater effects.

Rockbass induced a longer tail fin. A longer tail fin provides more surface area, which gives a more powerful swimming stroke (Relyea 2001c). This enables the tadpole to be quicker, and avoid fish predation. Tadpoles did not show increased tail depth in the presence of rockbass. Because the perch affected this trait, this inconsistency probably would not be present if the study had allowed more time for development and exposure.

There was no difference in muscle depth or muscle width for tadpoles exposed to rockbass. One possible explanation is that building up this extra muscle to avoid predation is not worth the energy it takes. Increasing tail surface area must be a more effective strategy than increasing tail muscle size. In tadpoles exposed to yellow perch, muscle depth showed no significant difference as well. However, yellow perch-exposed tadpoles had thinner tail muscles. One possible explanation is that a low profile gives a smaller target for the fish to catch. A thinner tail could help the tadpole slip out of the

perch's mouth more easily, or the perch could miss with its strike more often. Further studies are needed on the survival of thin vs. thick tail muscle depth tadpoles from yellow perch predation in order to accept or refute this claim.

Components of body size (i.e. body depth, length and width) showed no significant changes. The reasons for this are twofold. A larger body size presents a larger target. This larger target is more susceptible to predation because it is easier to strike. Additionally, it is harder for a larger tadpole to escape a fish once it has been captured (Relyea 2001c). Secondly, a larger body size requires extra energy to produce, at a cost to an increased tail size. A tadpole with a large body and small tail should be more susceptible to fish predation than one with a small body and large tail. Because a tadpole that wastes energy building up a large body is only increasing its chances of being predated upon, body size does not differ in the presence of predators.

The ability to react to fish predators has become increasingly important. Recent decreases in swamps in some areas are forcing the Eastern gray treefrog to utilize less desirable sites to lay their eggs. This factor, combined with the introduction of these fish into ponds, has increased the predator-prey interaction between the Eastern gray treefrog and fish. This experiment has given weak evidence that Eastern gray treefrog tadpoles show plasticity in the face of fish predation. This predator-prey interaction is extremely dangerous for Eastern gray treefrog populations. Because there is no solid proof that the tadpoles alter their morphology or behavior, neither the introduction of game fish nor the destruction of swamps are advised without further studies. A combination of these two factors could devastate local Eastern gray treefrog populations.

This discussion has operated under one very important caveat: that the data, while showing trends, do not provide statistically significant results. However, the patterns in the data are there. It would be wrong to ignore these patterns because they are logical and clear.

There are two reasons for the weak data. The first and most important is time constraints. The limited timeframe in which this experiment operated constricted the data by not allowing for proper exposure to the fish predators. The limited timeframe also cut down on the tadpole's ability to differentiate morphologically. With only three weeks to mature in the presence of the predation cues, the tadpoles simply could not grow enough to produce significant differences. In future studies, a six to eight week period might yield better results. The second limitation of this study was the quantity of tadpoles used. If much higher numbers had been used, on the order of five or six hundred tadpoles, the data would have been more much powerful. Perhaps the slight morphological changes seen in the limited time span would suffice to produce significant data.

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LITERATURE CITED

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- Harding, J. H. 1997. Amphibians and Reptiles of the Great Lakes Region. University of Michigan Press, Ann Arbor.
- Petranka, J.W., L.B. Kats, and A. Sih. 1987. Predator-Prey Interactions Among Fish and Larval Amphibians: Use of Chemicals to Detect Predatory Fish. *Animal Behavior* 35:420-425.
- Relyea, R. 2000. Trait-Mediated Indirect Effects in Larval Anurans: Reversing Competition With the Threat of Predation. *Ecology* 81:2278-2289.
- Relyea, R. 2001a. The Relationship between Predation Risk and Antipredator Responses in Larval Anurans. *Ecology* 82:523-540.
- Relyea, R. 2001b. Predator-Induced Stress Makes the Pesticide Carbaryl More Deadly to Gray Treefrog Tadpoles. *PNAS* 98:2491-2496.
- Relyea, R. 2001c. Morphological and Behavioral Plasticity of Larval Anurans in Response to Different Predators. *Ecology* 82:541-554
- Relyea, R. 2002. Local Population differences in phenotypic plasticity: Predator-Induced Changes in Wood Frog Tadpoles. *Ecological Monographs* 72: 77- .93
- Relyea, R.A., and J.R. Auld (2004) Having the guts to compete: How intestinal plasticity explains costs of inducible defenses. *Ecology Letters*. In Press.

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Figure 1. Average Percent of Tadpoles Active

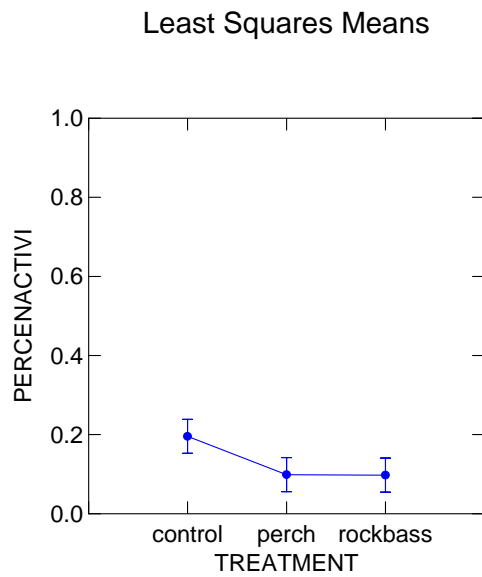


Figure 1 illustrates the difference in activity levels between predator-exposed tadpoles and the naïve control tadpoles. Yellow Perch-exposed tadpoles ($p=0.351$) and rockbass-exposed tadpoles ($p=0.338$) were less active than the controls. While neither of these results is statistically significant, the trend is clear.

Figure 2. Differences in Mean Tail Depth Residuals

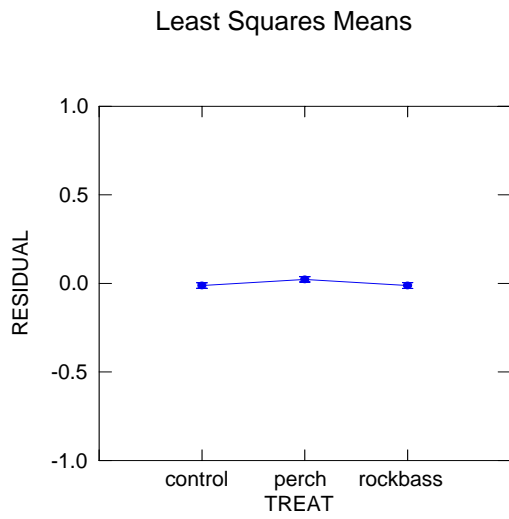


Figure 2 illustrates the differences in mean tail depth residuals. While not statistically significant, the tadpoles in the presence of perch had a higher mean tail depth.

Figure 3. Mean Tail Length Residuals

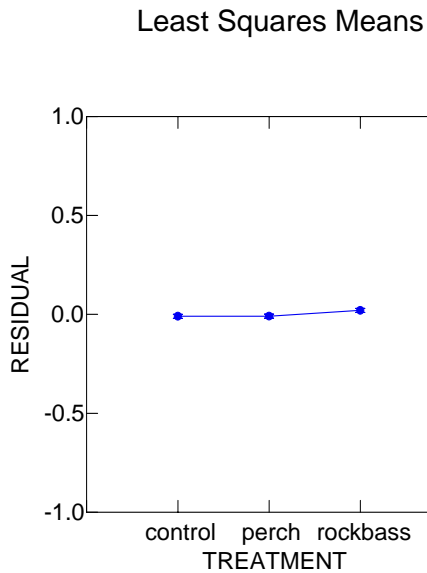


Figure 3 illustrates the difference in tail length between treatments. Tadpoles exposed to rockbass possessed longer tails. However these results were not statistically significant ($p=0.129$).

Figure 4. Mean Muscle Width Residuals

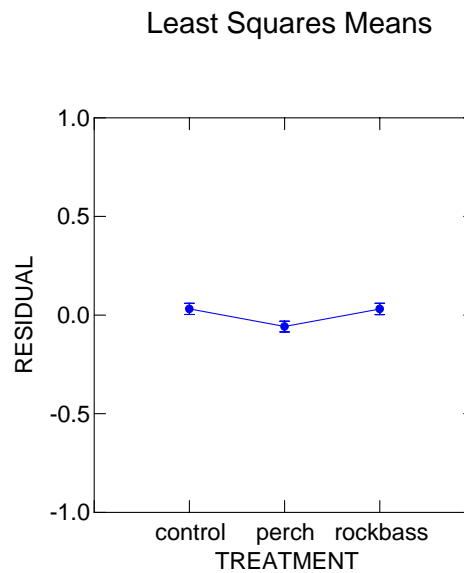


Figure 4 illustrates the difference in mean muscle width between tadpoles exposed to yellow perch and the other treatments. Yellow perch-exposed tadpoles had thinner tails and this was significant at the 10% level ($p=0.090$).

Figure 5. p-Values of Muscle Depth

	Control	Yellow Perch	Rockbass
Control	1.000		
Yellow Perch	1.000	1.000	
Rockbass	1.000	1.000	1.000

Figure 5 shows the p-values for a difference between mean muscle depths of control-yellow perch, control-rockbass, and yellow perch-rockbass. There was no statistical difference between any means.

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Figure 6. p-Values of Body Width

	Control	Yellow Perch	Rockbass
Control	1.000		
Yellow Perch	1.000	1.000	
Rockbass	1.000	1.000	1.000

Figure 6 shows the p-values for a difference between mean body widths of control-yellow perch, control-rockbass, and yellow perch-rockbass. There was no statistical difference between any means.

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Figure 7. p-Values of Body Length

	Control	Yellow Perch	Rockbass
Control	1.000		
Yellow-Perch	0.945	1.000	
Rockbass	1.000	1.000	1.000

Figure 7 shows the p-values for a difference between mean body lengths of control-yellow perch, control-rockbass, and yellow perch-rockbass. There was no statistical difference between any means.

Figure 8. p-Values of Body Depth

	Control	Yellow Perch	Rockbass
Control	1.000		
Yellow Perch	1.000	1.000	
Rockbass	1.000	1.000	1.000

Figure 8 shows the p-values for a difference between the mean body depths of control-yellow perch, control-rockbass, and yellow perch-rockbass. There was no statistical difference between any means.