

Induced Morphological Plasticity in Lowland Leopard Frog Larvae (*Rana yavapaiensis*) Does Not Confer a Survival Advantage against Green Sunfish (*Lepomis cyanellus*)

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ABSTRACT.—Tadpoles can generally increase their probability of survival in the presence of known predators by reducing their foraging activities or modifying their tail shape to increase swimming speed or lure attacks away from the head. However, it is unknown to what extent tadpoles can induce such behavioral and morphological plasticity in response to introduced predators. Lowland Leopard Frogs (*Rana yavapaiensis*) are native to Arizona and are currently declining because of a variety of factors including introduced predators such as the Green Sunfish (*Lepomis cyanellus*). We reared Lowland Leopard Frog tadpoles in the presence of tadpole-fed Green Sunfish or in control tanks and tested whether Lowland Leopard Frog tadpoles alter their behaviors or body shape in response to the visual and chemical cues of this predator. We found that tadpoles reared in the presence of Green Sunfish were 90% less active and had significantly different body shapes (including 5% deeper tail fins, 3% larger tail muscle height, and 3% smaller tail muscle area) than tadpoles reared in control tanks. In a subsequent survival experiment with sunfish predators, however, the survival rates did not differ between the two groups of tadpoles. Thus, our results suggest that Lowland Leopard Frog tadpoles perceive Green Sunfish as potential predators, but the induced morphological changes and the experience of prior exposure do not confer a survival advantage.

Tadpoles of many species face a low probability of reaching metamorphosis because of competition for food resources and high predation, both of which can be highly variable and unpredictable (McDiarmid and Altig, 1999). To cope with this uncertainty and the conflicting demands of foraging and predator avoidance, tadpoles have evolved a suite of behavioral and morphological traits—called inducible defenses—that are expressed in the presence of known predators (Harvell, 1990). Behaviorally, tadpoles will decrease their swimming activity levels and spend more time in refuges to minimize the chances of fatal encounters with predators (Petranka et al., 1987; Kats et al., 1988; Kats and Dill, 1998; Hoff et al., 1999). Tadpoles of some species will also alter their tail and body morphology when raised in the presence of predators. The induced morphological changes are likely species- and predator-specific, but a common response observed in many families including Ranidae (Van Buskirk, 2002b; LaFiandra and Babbitt, 2004; McIntyre et al., 2004; Relyea, 2004; Steiner, 2007), Hylidae (Van

Buskirk et al., 1997; Lardner, 2000), and Myobatrachidae (Kraft et al., 2005) is an increase in tail fin depth, which may provide greater propulsion surfaces and increased burst speed (Van Buskirk and Relyea, 1998; although see Van Buskirk and McCollum, 2000; Dayton et al., 2005). In addition, larger and more brightly colored tails (Caldwell, 1982) may serve to deflect attacks away from the head toward the tail region (Van Buskirk et al., 2003; Johnson et al., 2008). Collectively, this suite of morphological and behavioral changes can confer a survival advantage when faced with a known predator (e.g., Lima and Dill, 1990; McCollum and Van Buskirk, 1996; Van Buskirk and Relyea, 1998; Alvarez and Nicieza, 2006).

However, the mechanisms and specificity underlying predator-recognition in tadpoles remain poorly elucidated (Relyea, 2004; Smith et al., 2008b). As a result, it is difficult to predict how a species will react to the presence of a nonnative predator during initial encounters. In some instances, prey may be naïve to the danger represented by an introduced predator (Cox and Lima, 2006; Salo et al., 2007; Smith et al., 2008a). For example, Red-Legged Frog (*Rana aurora*) tadpoles experienced 56% mortality in experimental enclosures with introduced American Bullfrogs (*Rana catesbeiana*)—versus 4% mortality in control enclosures without the predator—when they came from populations that had historically never been exposed to

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these predators (Kiesecker and Blaustein, 1997). In other instances, prey may have an innate avoidance mechanism that allows them to detect novel predators, possibly because of phylogenetic or phenotypic similarity to known predators. Finally, given sufficient time, tadpoles may learn (Mandrillon and Saglio, 2005; Gonzalo et al., 2007) or evolve (Kiesecker and Blaustein, 1997; Griffiths et al., 1998) to recognize nonnative predators. For example, Red-Legged Frog tadpoles from populations that have been sympatric with American Bullfrogs for 60 years survived at a rate (90.7%) that was statistically indistinguishable from tadpoles held in control enclosures without American Bullfrogs. The contrasting survival rates of sympatric and allopatric populations of Red-Legged Frog tadpoles suggest that a predator-avoidance mechanism may have evolved in the intervening period (Kiesecker and Blaustein, 1997).

The Lowland Leopard Frog (*Rana yavapaiensis*) is endemic to the southwest United States and is declining throughout its native range because of a variety of factors, including predation by the introduced Green Sunfish (*Lepomis cyanellus*) (Rosen and Schwalbe, 2002), which is native to east-central North America (Lee et al., 1983). Green Sunfish were first recorded in Arizona in 1913 (La Paz County). By the 1960s, Green Sunfish were being regularly collected in several counties throughout Arizona (Minkely SONFISH database, Unmack, 2002). We assume that Green Sunfish represent a predatory selective agent on Lowland Leopard Frogs for two reasons. First, the timing of disappearance of Lowland Leopard Frogs from several river basins (e.g., Colorado, Gila and Salt Rivers) correlates roughly with the establishment of Green Sunfish there (Clarkson and Rorabaugh, 1989; Rosen and Schwalbe, 2002; Sredl, 2005). Furthermore, Green Sunfish will readily feed upon Lowland Leopard Frog tadpoles in captivity (P. Rosen, pers. comm. and this study). However, we have no direct measure of the selective strength of Green Sunfish predation in the field.

Here, we ask whether the proximate mechanisms that many ranid tadpoles employ for eluding predators (modification of activity levels and body shape) are also induced by Green Sunfish, and whether these inducible defenses translate into a relative survival benefit. In the first part of our experiment, we raised Lowland Leopard Frog tadpoles in the presence of Green Sunfish and in control tanks to quantify differences in swimming activity levels and body shape. Subsequently, we exposed tadpoles from both groups to an untethered predator to test whether the combi-

nation of prior exposure to sunfish predation and predator-induced changes in body shape resulted in greater survival.

MATERIALS AND METHODS

Study Organisms.—Lowland Leopard Frog larvae used in this study came from a semi-captive, sunfish-free population in Tucson, Arizona, of mixed genetic stock. This hybrid population likely embodies regional genetic differences and, thus, serves as a good representation of the species' overall characteristics. Concerns about possible population-specific effects of founder individuals (caused by different historical exposures to sunfish) were addressed by an independent experiment, which revealed no significant interaction between population of origin and sunfish treatment (A. M. Lea and M. A. Schlaepfer, unpubl. data). Five partial egg clutches (approximately 100 eggs each) were collected on 29 August 2006 and shipped to Austin, Texas, the following day. Eggs and larvae from different clutches were maintained separately throughout the experiment to control for clutch-specific effects. Tadpoles were fed algae (*Spirulina*) flakes ad libitum five times weekly throughout experimentation.

Green Sunfish were collected from Arizona (Yavapai County, $N = 3$) and Texas (Travis County, $N = 6$) (mean total length: 10.5 cm, range 8–14 cm). Although all sunfish looked like prototypical Green Sunfish, we cannot rule out the possibility of some genetic introgression from other *Lepomis* species (e.g., Bluegill, *Lepomis macrochirus*) with which Green Sunfish will sometimes hybridize. All sunfish were treated prophylactically with an antibiotic treatment and maintained on a live cricket diet for several weeks before the beginning of the experiments.

Experimental Tanks for Behavior and Morphometrics.—Our experiment consisted of two treatments (sunfish predator or control), which were applied to 40-liter glass aquaria (tanks) in the laboratory. There were a total of 18 tanks grouped into nine blocks, and treatment was randomly assigned within block. Each tank contained seven tadpoles, and the 14 tadpoles within each block were from the same clutch. Thus, blocks controlled for spatial arrangement within the laboratory, genetic background, and tadpole age.

Each 40-liter tank was divided into halves using a vertical perforated, transparent sheet of plastic, which allowed for the transmission of visual and chemical cues throughout the tank. Focal tadpoles were placed on one side of the divider, and a single Green Sunfish (or nothing in the case of the control tanks) was placed on

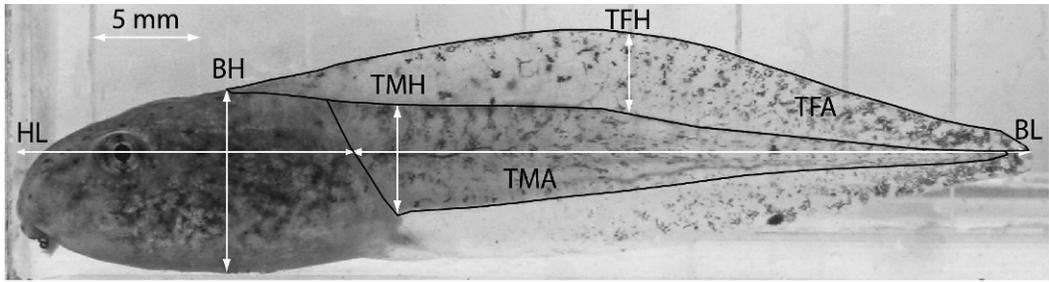


FIG. 1. Morphological traits measured on Lowland Leopard Frog (*Rana yarwapiensis*) larvae. HL (head length) horizontal distance from tip of head to insertion point of tail muscle into body; TL (tail length) horizontal distance from insertion point of tail muscle into body to tip of tail; TMH (tail muscle height) vertical height of tail muscle at lower insertion point into body; BH (body height) vertical body height where dorsal tail fin intersects with body; DTFH (dorsal tail fin height) height of dorsal tail fin at midpoint of tail fin length, which is defined from tip of tail to top point of BH; DTFA (dorsal tail fin area) area of the dorsal tail fin; TMA (tail muscle area) area of the total tail muscle.

the other. Each tank contained 30 liters of dechlorinated water, one centimeter of sand, and was wrapped in white paper to prevent focal organisms from viewing others outside their tanks.

Green Sunfish were fed three Lowland Leopard Frog tadpoles from stock populations each week (weekly mean: 2.53 g of tadpoles; range 1.17–3.45 g) during the experiment. In addition, sunfish diets were supplemented with crickets twice weekly (0.8–0.9 g weekly). Half of the water in all tanks was changed every 5–7 days, and tanks were kept at 12 : 12 light : dark photoperiod, at approximately 20°C.

The behavioral and morphological experiments began on 20 September 2006, when tadpoles were 15 days old and Gosner stage 25 (Gosner, 1960). The experiment ended 54 days later on 13 November 2006. Two of the initial 126 focal tadpoles died during the experiment (one from each treatment) for unknown reasons.

Behavioral Measurements.—On 29 different occasions over a 48-day period, starting on the fifth day of the experiment and until the 53rd day, swimming activity was scored for each tadpole by visually inspecting each tank during daylight hours. Tadpoles that were swimming or floating in the water column were scored as “active,” whereas tadpoles that were stationary on the gravel were scored as “inactive.” A nonparametric paired-sample test (Wilcoxon matched pairs signed-rank test) was used to test for differences in swimming activity levels across treatments.

Body Size, Shape, and Development.—Tadpole morphometrics were measured on 13 November 2006, when tadpoles were 71 days old and when the first tadpole reached Gosner stage 40 (emergence of foot tubercles and presence of vent tube; Gosner, 1960). The mass of each

tadpole was weighed to the nearest 0.01 g using an electric scale (Whatman®, model EK-120A). Each tadpole was then photographed twice (at a 5–7-min interval to ensure independence of photographs) in a plexiglass photo box (7.5 × 2.5 × 2.5 cm) using a macro lens and 6 megapixel digital Canon SLR camera from a standardized distance. We used Image J (<http://rsb.info.nih.gov/ij/>) to measure seven morphological traits (head length, tail length, muscle height, tail fin height, body height at fin, dorsal tail fin area, and tail muscle area) to the nearest 0.001 mm (Fig. 1). The entire tail area was not measured because the ventral fringe of the tail fin could not be discerned with confidence on each photograph. Each photograph was measured twice. The means of the four measurements for each tadpole trait (two measurements of two photos) were used in the statistical analyses.

Tadpole size was defined as the score of the first principle component axis (PC1) using all seven log-transformed morphological traits. (We repeated all analyses using log-transformed mass as a measure for size rather than PC1 and these yielded virtually identical results, which are not presented here.) Each log-transformed morphological trait was regressed against PC1 and the residuals were used as size-corrected morphological trait values (i.e., shape), which could then be compared between groups. This commonly used approach (e.g., Van Buskirk, 2002b; McIntyre et al., 2004) implicitly assumes that traits follow identical allometric patterns within each group or treatment being compared (i.e., homogeneity of slopes across groups or treatments when trait is plotted vs. size; McCoy et al., 2006). Therefore, we first tested for a significant treatment × PC1 interaction in a regression analysis and restricted our subsequent analysis to traits with

indistinguishable allometries (i.e., nonsignificant interaction terms). Nonallometric traits are described qualitatively.

Morphological response variables were analyzed with a General Linear Model with treatment (fixed) and block (random) as factors. Because tadpoles within a tank are not independent of one another, our experimental unit was the mean trait value within a tank. Therefore, treatment effects on morphological traits were tested over their interaction with block (Hurlbert, 1984). Effect sizes were obtained by obtaining mean main effects from a GLM on each log-transformed variable, with treatment as a fixed factor and PC1 as a covariate. Mean main effects were back-transformed to original units to obtain percent-differences between treatments. Analyses were conducted in Minitab 15.

Survival Experiment.—Survival experiments were conducted upon completion of the behavioral and morphometric experiments in four outdoor concrete tanks at the University of Texas' Brackenridge Field Laboratory (Travis County) from 13–31 December 2006. Two of the four concrete tanks measured $86 \times 117 \times 34$ cm (width \times length \times depth), whereas the remaining two measured $86 \times 85 \times 34$ cm. Each concrete tank contained two hollow plastic boxes ($18 \times 18 \times 1.5$ cm) and a thin layer of leaves as potential refuges from predation. Tadpoles fed on preexisting algae that coated the tank walls. Each concrete tank was fitted with wire mesh lids to prevent colonization by predatory insects, and the possibility of tadpole metamorph escapes, although no tadpole metamorphs were observed during censuses.

Tadpoles used in the survival experiment (Gosner stages 25–40; median 28) were the same as those used in the behavioral and morphological experiments. Total sample sizes for control and sunfish treatments ($N = 51$ each) were slightly reduced because some individuals had already reached metamorphosis. Each of the four concrete tanks contained 24 or 26 tadpoles, with equal numbers (12 or 13) from each treatment. Tadpoles were anesthetized for 1 min in a dilute solution (0.4 g/l) of benzocaine (ethyl 4-aminobenzoate) (Fellers et al., 1994) and marked with a single yellow or red visible implant elastomer tag (Northwest Marine Technologies, Shaw Island, WA) in the dorsal tail fin to indicate whether it had previously been reared in a control or sunfish tank. The color associated with each treatment was alternated between tanks to reduce any potential bias. Tadpoles were allowed to recuperate for 48 h before being introduced into the tanks, where they were given an additional 10 min to acclimate. One untethered adult

sunfish was then introduced into each concrete tank. Tadpoles were censused daily the first week (and then two weeks later) by temporarily removing the sunfish and capturing all surviving tadpoles. The ratio of tadpoles from each treatment that survived did not vary across tanks (see Results); thus, tadpoles were pooled across tanks and survival curves between treatments were statistically compared with a log-rank survival test, which is a modified chi-square analysis (Tabachnick and Fidell, 2001).

RESULTS

Behavior.—Tadpoles in control tanks were significantly more active than tadpoles in sunfish tanks both overall (Wilcoxon matched-pairs signed-rank test; $W_+ = 0$, $W_- = 435$, $N = 29$, $P < 0.001$) and on every sampling occasion (Fig. 2). During the first week, for example, 75% of tadpoles in control tanks were active, on average, versus 35% of tadpoles in sunfish tanks. Differences between treatments persisted despite an overall decrease in swimming activity starting week 2 (Fig. 2).

Body Size, Shape, and Development.—Tadpoles raised in the presence of sunfish had significantly taller dorsal tail fins, taller tail muscles, marginally significantly larger dorsal tail fin areas, and significantly smaller tail muscle areas relative to tadpoles reared in control tanks (Table 1). The magnitude of differences between treatments was less than 5% for all traits. Body height did not differ statistically between treatments. Two traits, head length, and tail length did not vary allometrically (treatment \times PC1 terms were significant). Indeed, small individuals raised in the presence of sunfish had long heads relative to control tadpoles, whereas large individuals raised in the presence of sunfish had short head lengths relative to controls. Conversely, small individuals raised in the presence of sunfish had short tails relative to control tadpoles, and large individuals raised in the presence of sunfish had long tails relative to controls.

There was no significant difference in log-transformed mass or size (PC1) between sunfish and control treatments (One-way ANOVA, $F_{1,122} < 1.24$, and $P > 0.27$ for both variables). However, the mass and size (PC1) of the tadpoles in the sunfish treatment were significantly more variable than that of tadpoles from control tanks (Levene's test for homogeneity of variance, test statistic > 11.6 , $P < 0.001$ for both log-mass and PC1). There was no significant difference in developmental (Gosner) stage between control (median 28; range 25–40) and sunfish (median 28; range 25–40) treatments

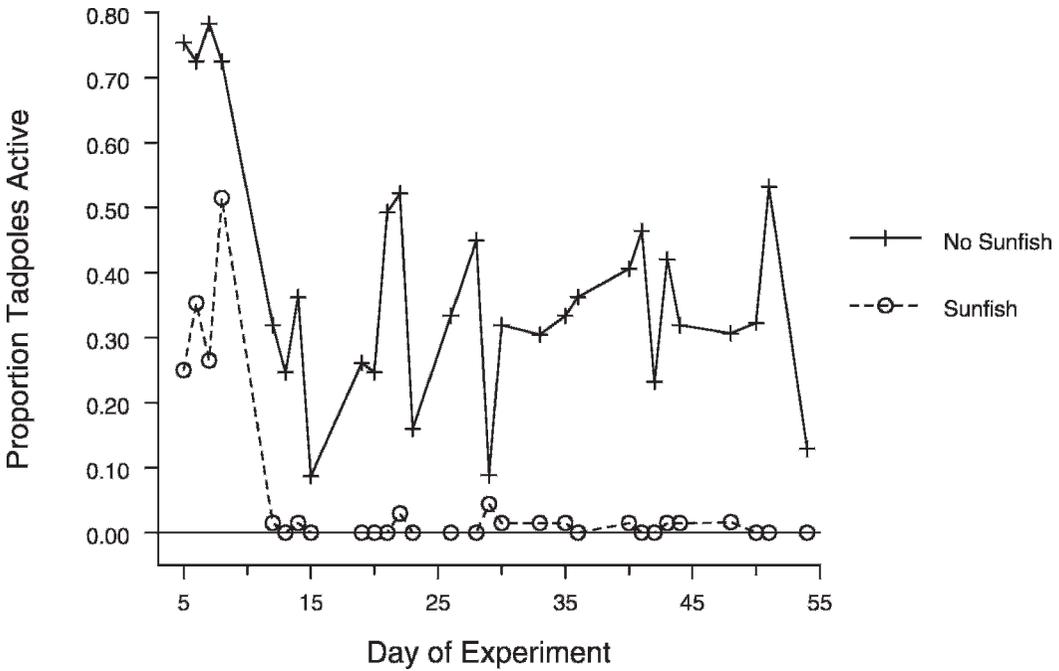


FIG. 2. Percentage of tadpoles active (defined as swimming, floating in water column or feeding) during visual surveys. Tadpoles in control tanks ($N = 63-64$) were significantly more active than tadpoles in the presence of Green Sunfish ($N = 63-64$).

(Kruskal-Wallis one-way analysis of variance, $U = 1801, P = 0.54$).

Survival.—Sunfish depleted 62% of tadpoles during the three-week survival experiment. Not one tadpole reached metamorphosis during this period, likely as a result of the cool water temperatures at this time of year. The ratio of

surviving tadpoles from control and sunfish treatments did not differ across concrete tanks. For example, after one week of exposure to the sunfish predator, the ratios of surviving control:sunfish treatment tadpoles in the four tanks were 7:7, 4:5, 12:10, and 8:8. In the pooled analysis (across tanks) there was no significant

TABLE 1. General linear model of size-corrected morphological variables (residuals of log-transformed variable against PC1, a measure of body size) of Lowland Leopard Frog tadpoles, *Rana yavapaiensis*, subjected to perceived predation risk of Green Sunfish, *Lepomis cyanellus* or held in control tanks, and associated effect size ($(\text{Sunfish} - \text{Control}) / ([\text{Sunfish} + \text{Control}] / 2)$).

Trait	Source	df	MS	F	P	Effect size (% difference)
Dorsal tail fin height	Treatment	1	18.10	11.29	0.010	4.7
	Treatment × Block	8	1.60			
Tail muscle height	Treatment	1	19.08	13.07	0.007	3.0
	Treatment × Block	8	1.46			
Dorsal tail fin area	Treatment	1	9.32	4.39	0.069	2.9
	Treatment × Block	8	2.1218			
Tail muscle area	Treatment	1	18.10	11.29	0.010	-3.0
	Treatment × Block	8	1.6028			
Body height	Treatment	1	0.21	0.09	0.77	-0.4
	Treatment × Block	8	2.26			
Dorsal tail area (dorsal tailfin area + half tail muscle area)	Treatment	1	0.0010	0.90	0.37	-0.6
	Treatment × Block	8	0.0012			

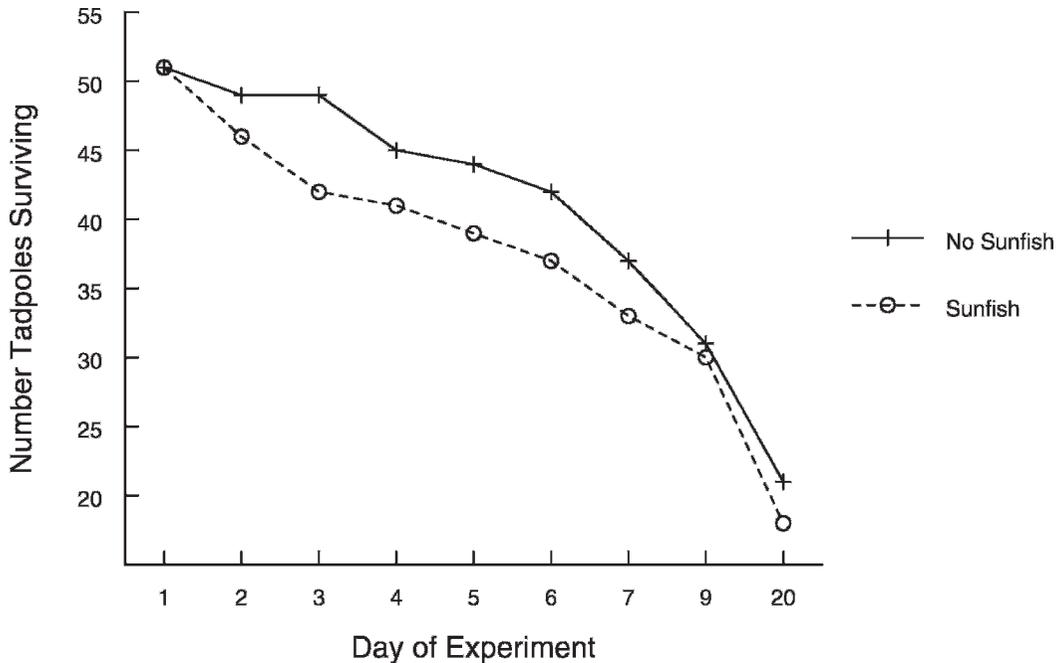


FIG. 3. Counts of Lowland Leopard Frog (*Rana yavapaiensis*) tadpoles surviving Green Sunfish predation (*Lepomis cyanellus*), by experimental treatment (raised in control tanks [$N = 51$] or in the presence of Sunfish [$N = 51$]).

difference in survivorship between tadpoles that had been raised in control tanks relative to tadpoles from sunfish tanks ($\chi^2 = 0.577$, $df = 1$, $P = 0.44$). Furthermore, at no point in time during the survival experiment were there more sunfish-treatment tadpoles surviving than control tadpoles (Fig. 3).

DISCUSSION

Our study has three principle findings: (1) we show that Lowland Leopard Frog tadpoles modify their behavior and morphology in response to Green Sunfish in a laboratory setting; (2) the direction of these changes is consistent with an adaptive response to a perceived increase in predation risk; and (3) the inducible morphological changes and the prior exposure to sunfish during development do not appear to confer an increase in survival in the presence of Green Sunfish predators.

Our behavioral observations suggest Lowland Leopard Frog tadpoles were capable of rapidly identifying sunfish as potential predators. Indeed, tadpoles in sunfish tanks were 63% less active than tadpoles in control tanks on the first day of behavioral observations, which took place on day five, after the sunfish had been fed tadpoles three times. The tadpoles may have

decreased their activity in response to alarm cues and kairomones in the water (Schoeppner and Relyea, 2005) or as an innate response to any large-bodied fish. We are unsure why tadpoles from both treatments reduced their overall activity levels around the 10th day of the experiment (when tadpoles were approximately 25 days old), but we speculate that it may correspond to an ontogenetic development of the tadpole.

Relative to tadpoles in control tanks, Lowland Leopard Frog tadpoles raised in the presence of sunfish invested greater resources into deeper tail fins, and deeper tail muscles, possibly at the expense of a shorter head and smaller overall tail muscle area (Table 1). Such morphological changes have been shown to be correlated with faster burst speeds (Dayton et al., 2005; Wilson et al., 2005; although see Van Buskirk and McCollum, 2000) and higher survival in other tadpole species facing natural predators (McCollum and Van Buskirk, 1996; Van Buskirk and Relyea, 1998; Alvarez and Nieceza, 2006; Benard, 2006; although see Johnson et al., 2008). The size effects of morphological differences between control and sunfish tadpoles in our study were relatively small (0–5%, Table 1) although comparable in magnitude to other studies on inducible tail morphologies in the

presence of known predators (e.g., Van Buskirk, 2002a; Van Buskirk, 2002b; Kraft et al., 2006). However, dorsal tail area (dorsal fin area + half tail muscle area) was not affected by treatment. One possible explanation for the smaller tail muscle area may be that tadpoles were less active in the presence of sunfish (Fig. 2), which may have led to atrophy of the tail muscle.

The absence of differences in size or mass between treatments suggests that tadpoles received equal amounts of food and that any observed differences were caused by differential allocation of resources. We also observed a greater variance in tadpole mass and size in the presence of Green Sunfish relative to controls. Peacor et al. (2007) have suggested that the presence of a nonlethal predator can increase cohort size-dependent variation (at least under high growth conditions) because smaller individuals respond more strongly than larger individuals to predation pressure. The larger variation in cohort mass in sunfish treatments relative to controls in our experiment is consistent with an increase in perceived predation pressure in the presence of Green Sunfish.

We found no difference in survival curves of tadpoles reared in the presence of sunfish and control tanks (Fig. 3). Tadpoles reared in the presence of sunfish visual and chemical cues in the first part of our study could have conceivably increased their survival thanks to (1) their induced morphological differences (e.g., greater tail fin height), or (2) learned differences in behavior from having witnessed sunfish predators throughout their development. Because we did not monitor the behavior of tadpoles during the survival experiments, we are unable to assess whether behavioral differences observed in the lab (Fig. 1) carried over into the concrete tanks. If both groups of tadpoles behaved similarly in the concrete survival tanks (because, for example, behavioral responses to sunfish are largely innate, or if tadpoles rapidly learn to associate sunfish with a source of predation), then we would conclude that the induced morphological differences alone did not confer a survival advantage. If tadpoles reared in the presence of sunfish continued to be less active than tadpoles reared in control tanks, then we would conclude that the combined morphological and behavioral differences did not confer a survival advantage. Of course, it is also conceivable that differences in tail morphology or prior experiences confer an actual survival advantage, but only at earlier Gosner stages or small sizes. In sum, we conclude that neither the induced morphological changes nor the experience gained from prior exposure to Green Sunfish during devel-

opment appear to confer a significant survival advantage to Lowland Leopard Frog tadpoles.

This experiment was not designed to test whether tadpoles exhibit different responses to native and nonnative predators. Rather, our goal was to test whether the induced response to Green Sunfish translated into a measurable increase in survival. Our results indicate that although Lowland Leopard Frog tadpoles recognize introduced Green Sunfish as potential predators, their induced responses appear ineffective against this particular predator and may explain why Lowland Leopard Frogs have been extirpated from some parts of their former range where Green Sunfish are now present.

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