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Morphological Plasticity in Four Larval Anurans Distributed along an Environmental Gradient

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We investigated morphological plasticity to the presence of predators in the tadpoles of four ranid frog species distributed along a pond hydroperiod gradient in southeast Michigan. We first reared all four species (Wood Frog, *Rana sylvatica*; Leopard Frog *R. pipiens*; Green Frog, *R. clamitans*; and Bullfrog, *R. catesbeiana*) under identical laboratory conditions in the presence and absence of caged larval dragonflies (*Anax* spp.). We then reared wood frog and leopard frog in outdoor mesocosms to examine the predator-induced responses during ontogeny. Finally, we reared leopard frog with predators fed either leopard frog or wood frog larvae to determine whether prey responses depended upon predators consuming conspecific prey. All four ranids exhibited some degree of morphological change in the presence of *Anax*; these differences were species specific and fairly robust to different experimental conditions. The responses over ontogeny indicated that the changes were direct responses to the predator's presence and not an indirect result of the predator slowing anuran growth or development. Finally, larval leopard frog responded similarly to predators feeding on conspecifics and congenerics. Taken together, these results suggest that morphological responses to predators may be relatively common in larval anurans. Further, because many of the responses are known to be adaptive antipredator strategies, predator-induced morphological plasticity has important evolutionary and ecological implications.

MANY organisms have evolved the ability to alter their phenotypes in response to environmental change, and this plasticity of phenotypes can manifest itself in a wide variety of ways. Environmental changes in abiotic conditions, food availability, and the presence of predators can induce organisms to alter their morphology, behavior, life history, and physiology (Bradshaw, 1965: Schlichting, 1986: West-Eberhard, 1989). Predators, in particular, have been shown to induce a variety of behavioral, morphological, and life-history changes in many taxa (for reviews, see Lima and Dill, 1990; Schlichting and Pigliucci, 1998; Tollrain and Harvell, 1999).

Among anuran larvae, behavioral responses to predators are ubiquitous (Petranka et al., 1987; Kats et al., 1988; Lawler, 1989), but reports of morphological responses to predators are limited to three species in the family Hylidae: Spring Peeper (*Pseudacris crucifer*), Chorus Frog (*P. triseriata*; Smith and Van Buskirk, 1995; Van Buskirk et al., 1997) and Cope's Gray Treefrog (*Hyla chrysoscelis*; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997). Chorus frog and gray treefrog larvae respond to the presence of odonate larvae by developing a deeper tail fin and smaller body, and individuals with this predator-induced phenotype experience a lower risk of predation than those with the uninduced phenotype (McCollum and Van Buskirk, 1996). It is presently unclear whether these morphological responses to predators are limited to hylids or whether they are common among larval anurans.

Our objective was to determine whether species in the family Ranidae also exhibit predator-induced changes in larval morphology and how these changes are exhibited during ontogeny. We compared the morphological responses of four species of ranids to the presence of a larval odonate predator. The four species are distributed, with considerable overlap, along a gradient of aquatic habitats varying from temporary ponds to permanent ponds and lakes. Wood Frog (*Rana sylvatica*) are found in the most temporary environments, followed by Leopard Frog (*R. pipiens*), Green Frog (*R. clamitans*), and finally Bullfrog (*R. catesbeiana*), which occurs only in permanent waters (Collins and Wilbur, 1979; Dale et al., 1985; Werner and McPeek, 1994). Pond hydroperiod determines the predators that larval anurans face; for example, permanent ponds frequently contain fishes that consume many species of larval anurans (but not bullfrog) and also remove many of the large invertebrate predators (Crowley and Johnson, 1982; Crowder and Cooper, 1982; Werner and McPeek, 1994). Temporary ponds often contain predatory insects and salamander larvae (Smith, 1983; Woodward, 1983; Van Buskirk, 1993). Predators can be very patchily distributed, especially in temporary ponds due to colonization and extinction dynamics associated with drying (E. E. Werner, D. K. Skelly, K. L. Yurewicz, and R. A. Relyea, unpubl. data). Thus, because these four ranids potentially experience very different predator communities, we might expect them to differ in both morphology and morphological plasticity, reflecting interspecific differences in antipredator strategies.

MATERIALS AND METHODS

We collected wood frog, leopard frog, and green frog egg masses from natural and artificial ponds on the University of Michigan's E. S. George Reserve (ESGR), near Pinckney, Michigan. Bullfrog egg masses were collected from the Michigan Department of Natural Resources' pond facility near Saline, Michigan, 34 km southeast of the ESGR. All species' cultures were initiated with multiple (> 10) egg masses. Eggs were hatched and tadpoles reared in 400-liter, plastic wading pools in the absence of predators until experiments were initiated. Larvae were fed Purina rabbit chow ad libitum until used in the experiments. All experiments used newly hatched tadpoles (1–2 weeks posthatch, approximately Gosner stage 25; Gosner, 1960). Because wood frog and leopard frog breed in the spring (March–April) and bullfrog and green frog breed in the summer (June–Aug.), our experiments could not be randomized in time with respect to species. Previous work has demonstrated that tadpoles respond to a waterborne chemical cue that is emitted when predators consume tadpoles (Petranka et al., 1987; Kats et al., 1988) but whether the cue comes from the tadpole or the predator (or both) remains unknown.

Experiment 1.—The first experiment was conducted in the laboratory. For each species, 10 tadpoles were placed into plastic tubs (26 × 38 × 14 cm) containing 7 liters of aged well water (mean mass ± 1 SE: wood frog, 19.5 ± 0.6 mg; leopard frog, 30.2 ± 1.0 mg; green frog, 21.0 ± 1.3 mg; bullfrog, 18.6 ± 1.0 mg). At one end of the tub, we placed a predator cage (43 × 39 cm) constructed of 1 mm mesh mosquito netting. Tubs were randomly assigned one of two treatments: the presence or absence of caged, final instar, dragonfly larvae (*Anax junius* or *A. longipes*; subsequent experiments have demonstrated that different species of aeshnid dragonflies cause similar induction; RAR, unpubl. data). All treatments were replicated four times. Tadpoles were fed a 3:1 mixture of ground rabbit chow and Tetramin fish flakes three times per week at a rate of 6% of mean mass per tadpole per day. We weighed the tadpoles weekly while changing the water in the tubs and based the food ration for the subsequent week on mean mass across treatments. *Anax* individuals were fed three times per week by dropping approximately 100 mg of tadpoles into the predator cages. With one exception, tadpoles fed to predators were conspecifics. Leopard frog larvae were in short supply and leopard frog predators were fed wood frog larvae. Tubs were placed on shelves under a bank of fluorescent lights on timers to provide a 14:10 day:night cycle. After five weeks of exposure to the treatments, tadpoles were weighed, staged, and preserved for morphometric analysis. The mean (± SE) number of preserved animals per tub by species were as follows: bullfrog = 7.8 ± 0.5; green frog = 7.4 ± 0.6; leopard frog = 7.3 ± 0.9; and wood frog = 8.9 ± 0.7.

Experiment 2.—A second experiment was conducted to document the ontogeny of the morphological changes and to assess the generality of our conclusions from the laboratory experiment under more natural conditions for wood frog and leopard frog. Because of logistical constraints, the ontogeny of green frog and bullfrog morphology could not be examined. For this experiment, we used 1300-liter cattle watering tanks (2.6 m^2) as pond mesocosms that contained aged well water, 300 g of leaf litter (primarily oaks; *Quercus* spp.), 25 g of rabbit chow, and an innoculum of plankton from a nearby pond. Thus, the tanks included many components of a natural pond that were lacking in the laboratory experiment. Tanks were outfitted with four predator cages constructed of 10 × 11 cm pieces of slotted plastic drain pipe with 2-mm fiberglass screening covering each end; a small piece of polystyrene was enclosed so that the cages floated.

We applied two treatments to the tanks. In tanks designated as predator treatments, a single *Anax* specimen was placed in each of the four cages. Tanks designated as no-predator treatments contained four empty cages. Each caged *Anax* specimen was fed three times per week (200–300 mg total tadpole mass per feeding) by removing the cage from the tank, adding tadpoles to the cage, and returning the cage to the tank. To equalize disturbance between treatments, cages in no-predator tanks were also removed and replaced each time the *Anax* were fed. All tanks were covered with 60% shade cloth to prevent invasions by other amphibians and predatory insects.

Two hundred tadpoles (71/m^2) of wood frog or leopard frog were added to each tank and the predator treatments were replicated six times for each species. Initial masses were 26.7 ± 0.9 mg for wood frog and 10.6 ± 0.3 mg for leopard frog. Because leopard frog larvae were in limited supply, *Anax* specimens in both experiments again were fed wood frog larvae. Twice during the leopard frog experiment (days 44 and 53), an additional 25 g of rabbit chow was added to all tanks to increase tadpole growth that had slowed during a period of very warm weather. On each of three sample dates, we removed 20 larvae and preserved them in 10% formalin for later measurement of morphological changes over ontogeny. Final densities (± 1 SE) were 64 ± 5 and 61 ± 11 for wood frog and 40 ± 4 and 41 ± 3 tadpoles/tank for leopard frog in the absence and presence of *Anax*, respectively.

Experiment 3.—The third experiment was conducted to determine whether the responses of leopard frog in the two previous experiments were affected by feeding *Anax* specimens wood frog tadpoles rather than leopard frog tadpoles. The precise origin of the predator cue is unknown and may be emitted by the predator or by attacked prey. The experimental units were 80-liter wading pools containing well water inoculated with zooplankton and phytoplankton from a nearby pond. We also added 100 g of dry leaf litter (primarily oaks) and 5 g of rabbit chow to the pools. Each pool was outfitted with two predator cages as described in Experiment 2. Fifty leopard frog hatchlings were added to each of 15 pools.

The experimental design was a completely randomized block design because nine of the 15 pools were blue (Block A), whereas the other six were yellow (Block B). Within each of the two blocks, three treatments were randomly assigned: caged *Anax* specimens fed larval leopard frog, caged *Anax* specimens fed larval wood frog, or empty cages. The predators were fed approximately 300 mg of prey three times per week. For pools containing empty cages, we lifted the cages out of the water to equalize disturbance among treatments. The pools were covered with mosquito netting to prevent the invasion of predators and other amphibians. After 24 d, all tadpoles were removed from the pools and preserved. Ten randomly selected tadpoles were measured from each pool.

Morphological measurements and statistical analysis.—We measured larval morphology by tracing a video image of each preserved tadpole on a computer screen using the BioScan Optimas image analysis system (Optimas Corp., Bothell, WA). From a lateral view, we traced the outline of the tail fin, tail muscle, and body; from a dorsal view, we traced the outline of the tail muscle and body (Fig. 1). When tadpoles were on their side, the tail was elevated on a glass slide so that a flat, undistorted side view was available. From these tracings, we obtained seven linear dimensions: (1) from the lateral view—maximum tail fin depth, maximum tail length, maximum tail muscle depth, maximum body depth, maximum body length; and (2) from the dorsal view—maximum tail muscle width, and maximum body width.

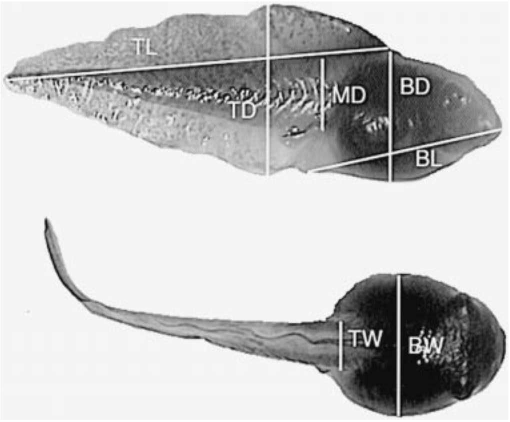


Fig. 1. Lateral and dorsal views of a wood frog tadpole showing the seven linear measures used in the analyses of morphological plasticity (BD = body depth, BL = body length, BW = body width, TD = tail depth, TL = tail length, MD = muscle depth, and MW = muscle width).

Analyses of differences in relative morphology required that we first adjust for the effects of differences in overall tadpole size. We conducted principal components analyses (PCA, using a correlation matrix) on the five tail fin and body dimensions (log-transformed); the score from the first PC axis (PC-1) for each individual was used as a measure of overall size (and all loadings were > 0.91 on all five axes). All seven morphological measures then were regressed against the first principal component scores ("shearing" in morphometric analyses; Bookstein, 1991), and the residuals were saved. Given that we adjusted for tadpole size, an increase in one dimension is necessarily accompanied by a decrease in some other dimension.

Multivariate analyses (MANOVAs) were conducted on the three experiments. For Experiment 1, we first conducted a MANOVA comparing the four species' growth (final-initial mass) and developmental stage (Gosner, 1960)

TABLE 1. RESULTS OF THE MANOVA ON FOUR RANID SPECIES' MORPHOLOGICAL RESPONSES TO THE PRESENCE OF CAGED *Anax* IN THE LABORATORY EXPERIMENT. Because of the significant interaction between predator presence and ranid species, separate univariate tests were conducted for each species.

		Multivariate Wilks' Lambda	Univariate responses							
			Overall size (PC-1)	Tail fin depth	Tail fin length	Tail muscle depth	Tail muscle width	Body depth	Body length	Body width
Predator presence	($F_{8,17}$)	0.003								
Ranid species	($F_{24,49}$)	<0.001								
Predator*Ranid	($F_{24,49}$)	0.013								
Wood frog	($F_{1,6}$)		0.029	0.024	0.194	0.088	0.159	0.237	0.360	0.510
Leopard frog	($F_{1,6}$)		0.887	0.371	0.466	0.124	0.507	0.083	0.217	0.165
Green frog	($F_{1,6}$)		0.468	0.203	<0.001	0.171	0.389	0.069	0.560	0.004
Bullfrog	($F_{1,6}$)		0.008	0.562	0.176	0.265	0.270	0.971	0.088	0.045

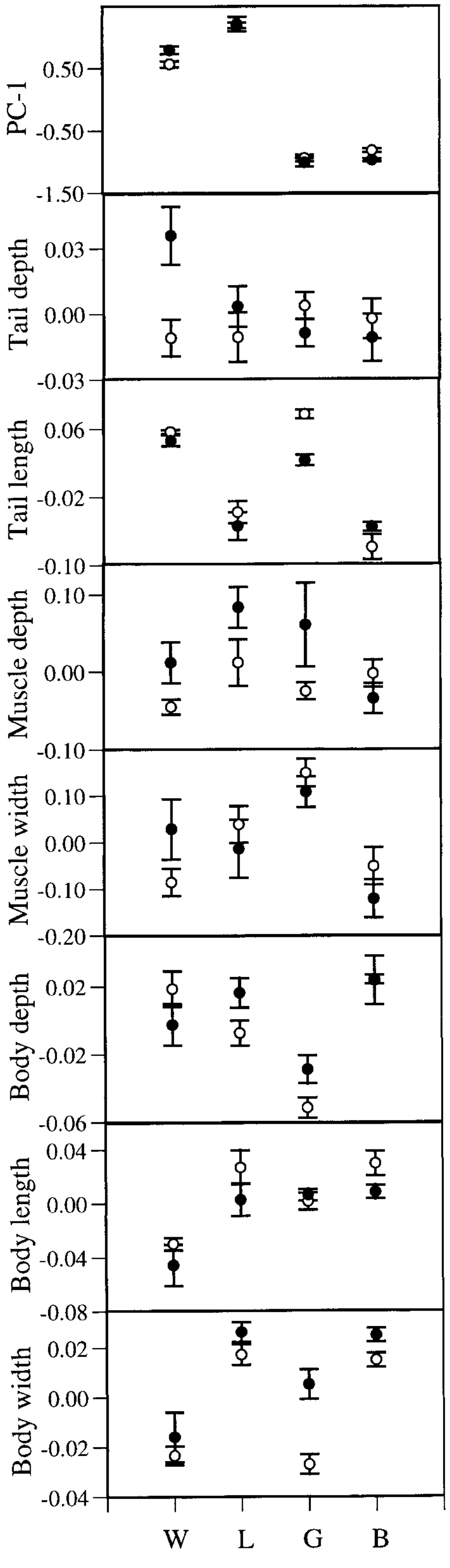
in the presence and absence of *Anax* when reared under identical laboratory conditions (although Gosner stage is a discontinuous response variable for an individual, it is a continuous variable when the mean Gosner stage of 10 tadpoles in a replicate is used as a response). We also conducted a second MANOVA comparing the four species' overall size and relative morphology in the presence and absence of *Anax.*, a split-plot MANOVA of wood frog and leopard frog responses to the presence and absence of *Anax* throughout ontogeny (Experiment 2), and a MANOVA of leopard frog responses to no predators, to *Anax* fed wood frog, and to *Anax* fed leopard frog (Experiment 3). The analysis of ontogeny was a split-plot analysis because we removed individuals at each sample period. A repeated measures MANOVA would not be appropriate for this analysis because it requires that we repeatedly sampled the same individuals each period or returned the sampled individuals back to the tanks. All MANOVAs used the Wilks' lambda multivariate test statistic and all mean comparisons were conducted using Fisher's LSD test. Block effects and their interactions were nonsignificant and dropped from the analysis.

RESULTS

Experiment 1.—In the laboratory experiment, we first examined the effects of predator presence and prey species on growth and developmental stage (Gosner, 1960), and then we examined the effects on size-adjusted morphology. The presence of the predator had no effect on tadpole growth or developmental stage (Wilks' Lambda $F_{2,23} = 0.001$, $P = 0.992$). There were differences in growth and stage among frog species (Wilks' Lambda $F_{6,46} = 92.6$, $P < 0.001$; growth, $F_{3,24} = 164.0$, $P < 0.001$; stage, $F_{3,24} = 92.8$, $P < 0.001$). Green frog was the slowest growing species (mean ± SE; 52 ± 2 mg) followed by bullfrog (77 ± 6 mg), wood frog (158 ± 9 mg), and leopard frog (346 ± 16 mg), respectively. Green frog were also the slowest developing species (Gosner stage = 25.01 ± 0.01), followed by bullfrog (25.26 ± 0.04), leopard frog (26.94 ± 0.11), and wood frog (28.65 ± 0.35), respectively. In contrast to the growth result, which was based on live mass, there were small effects of predators in the composite measure of overall size (PC-1); overall size was greater for wood frog in the presence of *Anax*, whereas bullfrog size was smaller (Table 1, Fig. 2).

Wood frog reared in the presence of caged *Anax* developed significantly deeper tail fins and marginally ($0.05 < P < 0.10$) significantly deeper tail muscles than in the absence of *Anax* (Fig. 2, Table 1). Leopard frog showed the same pattern, but the difference was not significant. There was a marginally significant effect of predators inducing deeper bodies for leopard frog. Green frog larvae exhibited a decrease in tail fin length, an increase in body width and a marginal increase in body depth in the presence of *Anax* specimens. Bullfrog larvae reared with *Anax* specimens developed a significantly wider and marginally shorter body.

Experiment 2, wood frog.—The three samples preserved over the course of the wood frog and leopard frog tank experiments allowed us to examine morphological responses to predators in a more natural environment and describe the ontogeny of morphology in the presence and absence of caged *Anax*. For wood frog, there was no significant main effect of predator because of a predator-by-sample interaction for many of the traits (Fig. 3, Table 2). Wood frog size (PC-1) increased over time and exhibited a nearly significant interaction with the predator



treatment ($P = 0.071$). On the first sample, tadpoles tended to be smaller in the presence of *Anax* but, in subsequent samples, tadpoles tended to be larger in the presence of *Anax*; however, none of these predator effects was significant within a sample date ($P > 0.16$).

The tail fin of wood frog changed during ontogeny and were affected by the presence of predators. Tail fins were deeper in the presence of *Anax* and the relative difference changed over time (i.e., there was a significant interaction term). Tail fins became shallower during ontogeny in the absence of *Anax* but initially increased and then decreased in the presence of *Anax*. In contrast, tail fin length was relatively shorter in the presence of *Anax* and this effect of predator was constant throughout ontogeny.

Relative tail muscle depth and width of wood frog also changed over ontogeny; both initially decreased and then increased. Tail muscle depth was greater in the presence of the predator. The interaction between predator and sample was marginally significant; the predator effect was nearly significant on the first sample period ($P = 0.057$), significant on the second period ($P = 0.001$), and not significant on the third period ($P = 0.873$). Tail muscle width was unaffected by the presence of the predator, but it changed during ontogeny by slightly decreasing and then sharply increasing.

The relative body shape of wood frog exhibited ontogenetic changes as well. Body depth declined and there was no overall effect of *Anax*; however, body depth was greater on the first sample date in the presence of *Anax* ($P = 0.018$). Body length increased over time but was smaller in the presence of *Anax*. Body width was consistently smaller in the presence of *Anax* and did not change in relative size through time.

Experiment 2, leopard frog.—Leopard frog also exhibited morphological responses to predators that interacted significantly over time (Fig. 4, Table 3). Leopard frog larvae increased in size over time, but they were smaller in the presence of *Anax* on the first sample date ($P = 0.001$), equal in size on the second sample date ($P =$

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Fig. 2. Relative morphological changes (mean residuals ±1 SE) in seven linear measures of four ranid larvae (W = wood frog, L = leopard frog, G = green frog, B = bullfrog) in the presence (filled circles) and absence (open circles) of caged *Anax* in the lab experiment. Effects of overall size were removed for by regressing all individuals against the first principle components scores.

0.664), and nearly significantly larger on the third sample date ($P = 0.063$).

Mean tail depth in leopard frog reared with *Anax* was significantly greater and did not change in relative size over time. Tail length increased during ontogeny in the absence of *Anax*. However, in the presence of *Anax*, the tail was longer on the first sampling date ($P = 0.014$) but decreased by the second sample to be similar to tail length in the absence of *Anax*.

The two tail muscle dimensions exhibited similar ontogeny. The relative depth and width of the muscle were initially large, then decreased, followed by an increase. The presence of *Anax* induced deeper tail muscles but muscle width was unaffected.

Relative body dimensions of leopard frog also changed over time and by treatment. Relative body depth decreased during ontogeny and was unaffected by *Anax*. Relative body width was similar across time intervals but significantly narrower in leopard frog reared with *Anax*. Relative body length was consistently reduced in the presence of *Anax* and exhibited an initial increase followed by a subsequent decrease over ontogeny.

Experiment 3.—Leopard frog reared in the presence of *Anax* fed wood frog and leopard frog altered their morphology similarly (Fig. 5, Table 4); the species of tadpole fed to the *Anax* made no apparent difference. In the presence of predators, leopard frog tadpoles did not differ in overall size, but they developed relatively deeper tails and shorter bodies than tadpoles reared in the absence of predators. Relative tail muscle depth tended to be greater in the presence of *Anax*, whereas relative body depth and width tended to be smaller in the presence of *Anax*; all three traits were marginally significant.

DISCUSSION

Predator-induced morphological plasticity.—Our results indicate that predator-induced, morphological plasticity may be quite common in larval ranids and perhaps among larval anurans in general. All four ranids in this study exhibited

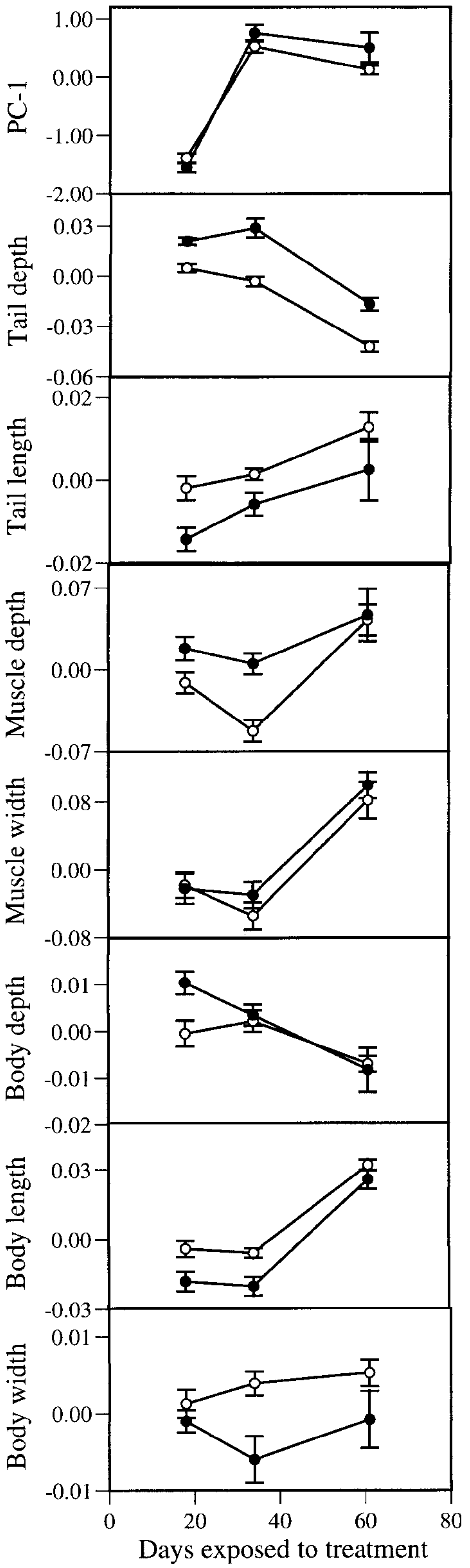

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Fig. 3. Change in overall size (PC-1) and relative morphology (mean residuals ± 1 SE) of wood frog larvae over time in the presence (filled circles) and absence (open circles) of *Anax* in the tank experiment. Differences in overall size were removed by regressing all individuals against the first principle components scores (PC-1).

TABLE 2. RESULTS OF THE MANOVA ON MORPHOLOGICAL CHANGES IN WOOD FROG BY SAMPLE DATE IN EXPERIMENT 2 TO TEST FOR ONTOGENETIC CHANGES IN MORPHOLOGY IN THE PRESENCE OR ABSENCE OF PREDATORS. *P*-values are listed for both the multivariate and univariate responses.

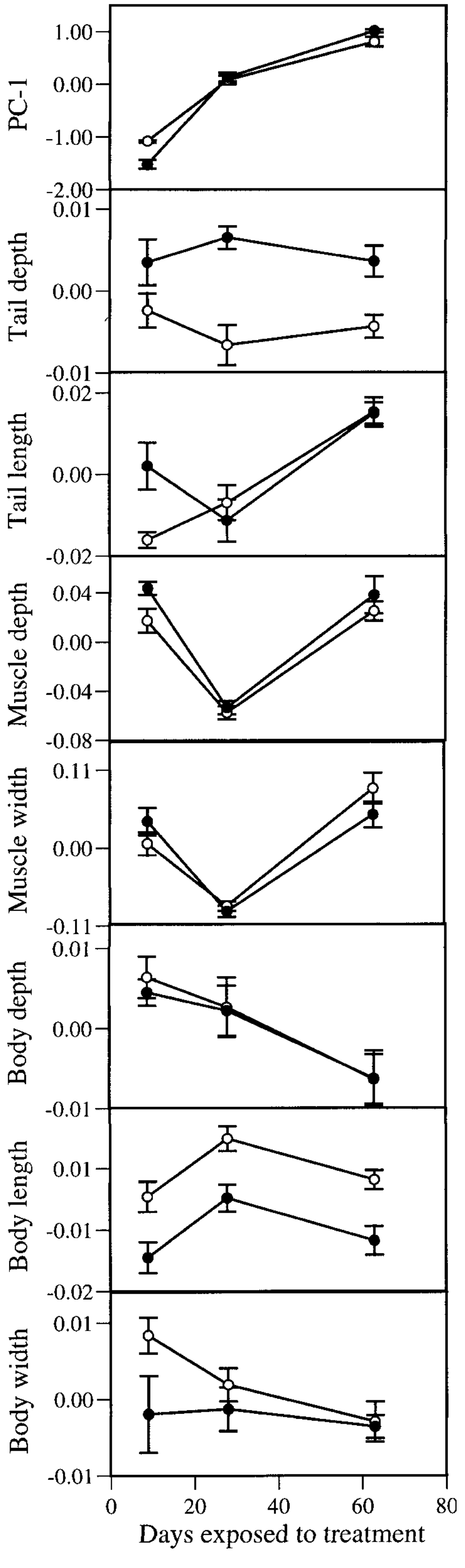
		Multivariate Wilks' Lambda	Univariate responses								
				PC-1	Tail fin depth	Tail fin length	Tail muscle depth	Tail muscle width	Body depth	Body length	Body width
Predator	($F_{8,3}$)	0.136	($F_{1,10}$)	0.342	<0.001	0.016	0.048	0.243	0.202	0.010	0.014
Sample date	($F_{16,24}$)	<0.001	($F_{2,19}$)	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	0.399	<0.001
Predator*Sample date	($F_{16,24}$)	0.017	($F_{2,19}$)	0.071	0.019	0.808	0.075	0.799	0.082	0.375	0.251

some degree of morphological change in the presence of *Anax* specimens, and these differences were fairly robust to different experimental conditions. Below we first synthesize our results across experiments for each species and then discuss the general implications of our results.

The four ranids all altered their morphology in the presence of predators, but the traits that changed were species specific. Wood frog in both the laboratory and tank experiments developed deeper tail fins and tail muscles in the presence of the predator. Body dimensions did not change in the laboratory experiment, but body length and width were significantly smaller with the predator in the tank experiment. In the three leopard frog experiments, *Anax* induced a deeper tail fin, a deeper tail muscle, and a shorter body in the more natural tank and pool mesocosm experiments (2 and 3) but not in the laboratory experiment (1). Green frog larvae developed shorter tails and wider and deeper bodies in the presence of *Anax.* Bullfrog bodies became shorter and wider when reared with *Anax.*

Other experiments on larval ranids support the results of this study. In an experiment in which 24 sibships of wood frog were reared separately in tanks in the presence or absence of caged *Anax*, we observed that tail fins became deeper, and all three body dimensions became smaller in the presence of the predator (Van Buskirk and Relyea, 1998). In a second experiment, both wood frog and leopard frog were reared together in the presence and absence of caged *Anax* in pens placed in ponds. Both species developed relatively deeper tails and shorter bodies when reared with predators (Relyea, 1998). Thus, the preponderance of the evidence is that wood frog and leopard frog develop deeper tail fins and smaller bodies in the presence of predators, particularly under more natural conditions. We have no information on how bullfrog and green frog alter their morphology in mesocosm conditions.

The patterns of predator-induced morphology in wood frog and leopard frog are similar to responses exhibited by larval hylids. In chorus frog and Cope's gray treefrog, tail fins became deeper and bodies became smaller in the presence of predators (Smith and Van Buskirk, 1995; McCollum and Van Buskirk, 1996; Van Buskirk et al., 1997). In addition, chorus frogs increased tail muscle depth in the presence of the predator. In subsequent studies of a third hylid species, the Gray Tree Frog (*Hyla versicolor*, a tetraploid relative of Cope's gray tree frog), developed a deeper tail and smaller body in the



presence of caged odonate larvae (Relyea, 1998). In both families, however, some species such as spring peeper, green frog, and bullfrog do not alter tail fin depth (this study; Smith and Van Buskirk, 1995), suggesting that morphological responses to predators might not be phylogenetically constrained or determined.

The morphological responses we observed are direct responses to the presence of the predator and not an indirect consequence of allometry caused by predator-induced reduction in growth and development. In Experiments 1 and 3, the presence of the predator had no significant effect on tadpole growth and development, but it did alter tadpole morphology. Further, in Experiment 2, there was no difference in overall size of either wood frog or leopard frog at the second sample date, but morphology differed between treatments. Thus, the morphological differences cannot be explained by trait allometry due to differences in tadpole size. Further, although the body width and body depth dimensions can be affected by the fullness of the gut, the data suggest that the differences in these dimensions were not due to differences in foraging. For example, in Experiment 1, both predator treatments were given the same food ration, and there were no differences in growth between the predator and no-predator treatments. Thus, we must conclude that tadpoles in both treatments were not consuming different amounts of food, yet their body dimensions still differed between predator treatments. In Experiment 2, wood frog in the two predator treatments did not differ in size on the second sample date, again suggesting that they must be consuming the same amount of food, yet they differed in body width. In Experiment 3, there are again no differences in size between treatments, yet there are differences in body width. Thus, although the fullness of the gut can affect these body dimensions, our results suggest that it did not play a role in our observations.

The third experiment suggested that responses of leopard frog to predators do not depend on the predator consuming conspecific prey. For the two significant responses to *Anax*, leopard frog exhibited no difference between envi-

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Fig. 4. Change in overall size (PC-1) and relative morphology (mean residuals ± 1 SE) of leopard frog larvae over time in the presence (filled circles) and absence (open circles) of *Anax* in the tank experiment. Differences in overall size were removed by regressing all individuals against the first principle components scores (PC-1).

TABLE 3. RESULTS OF MANOVA ON MORPHOLOGICAL CHANGES IN LEOPARD FROG BY SAMPLE DATE IN EXPERIMENT 2 TO TEST FOR ONTOGENETIC CHANGES IN MORPHOLOGY IN THE PRESENCE OR ABSENCE OF PREDATORS. *P*-values are listed for both the multivariate and univariate responses.

		Multivariate Wilks' Lambda	Univariate responses								
				PC-1	Tail fin depth	Tail fin length	Tail muscle depth	Tail muscle width	Body depth	Body length	Body width
Predator	($F_{8,3}$)	0.022	($F_{1,10}$)	0.380	<0.001	0.136	0.015	0.730	0.758	0.001	0.045
Sample date	($F_{16,26}$)	<0.001	($F_{2,19}$)	<0.001	0.920	<0.001	<0.001	<0.001	0.003	<0.001	0.147
Predator*Sample date	($F_{16,26}$)	0.003	($F_{2,19}$)	<0.001	0.272	0.045	0.541	0.138	0.947	0.998	0.305

ronments with predators fed conspecifics versus predators fed congenerics. This indicates that leopard frog, and perhaps many other amphibians, do not have to rely on conspecifics being killed as a stimulus for morphological response. Chemical cues are common in aquatic predators (Kuhlmann and Heckmann, 1985; Appleton and Palmer, 1988; Havel, 1987), and the most recent work suggests that prey respond most strongly to predators consuming species that are closely related to the prey (Laurila et al., 1998).

Adaptive significance of morphological plasticity.—It is likely that at least some of the morphological changes we observed are adaptive responses to the presence of predators. For example, increased tail fin depth has been associated with higher survivorship in the presence of uncaged predators in Cope's gray tree frog (McCollum and Van Buskirk, 1996). Furthermore, recent selection experiments have demonstrated that *Anax* disproportionately killed tadpoles possessing shallower tail fins in both chorus frog (Van Buskirk et al., 1997) and wood frog (Van Buskirk and Relyea, 1998). Deeper tail fins are correlated with greater swimming speeds (McCollum and Leimberger, 1997) and improved ability to escape predator strikes (Relyea, 1998) and, thus, should reduce risk of predation (Feder, 1983; Jung and Jagoe, 1995; Watkins, 1996). The anterior half of the tail produces thrust for swimming, and the tapered posterior half serves to reduce turbulence (Wassersug and Hoff, 1985). The increase in tail fin depth that we observed in wood frog and leopard frog occurred in the anterior half of the tail fin and, thus, should result in increased thrust and acceleration and reduced predation risk. In addition, Doherty et al. (1998) have recently demonstrated that the tail fin can be easily torn by predators, suggesting that a larger tail fin also may provide a larger nonlethal target for predator.

Theory predicts that, although induced responses may provide benefits in the appropriate environment, they incur costs in other environments (West-Eberhard, 1989; Dudley and Schmitt, 1996). Thus, there is selection for alternative phenotypes in different environments; this selection maintains the plastic response. Indeed, studies by Van Buskirk et al. (1997) and Van Buskirk and Relyea (1998) have shown the predator-induced morph (tadpoles with deeper tail fins and smaller bodies) grow more slowly than the noninduced morph in a predator-free environment. Growth in amphibians is positively related to traits presumed to confer higher fitness, for example, age of first reproduction and survivorship in the terrestrial stage, and reduces the susceptibility to pond drying in the larval stage (Berven and Gill, 1983; Newman, 1988; Semlitsch et al., 1988). Similarly, predator-induced morphologies among invertebrates are often associated with lower fecundity (Ketola and Vuorinen, 1989; Black and Dodson, 1990) and slower development (Riessen and Sprules, 1990).

Although green frog and bullfrog did not increase tail fin depth in the presence of *Anax*, they did exhibit morphological plasticity. This plasticity involved very different dimensions and directions of response, including a predator-induced shortening of the tail fin in green frog and a change in body shape in both species. We have no information on whether such responses are adaptive. One adaptive hypothesis is that these morphological responses are associated with an alternative antipredator strategy of lowering foraging activity to avoid detection by predators rather than developing deeper tails to enhance escape ability. Larval bullfrog and green frog exhibit much lower foraging activity (and thus lower growth) in the presence of *Anax* than leopard frog and wood frog (Relyea, 1998). Perhaps to compensate for this very low activity, green frog and bullfrog alter body shape to increase efficiency of food collection and digestion. This would not be a viable strategy for temporary pond species because low activity and slow growth could prevent achieving metamorphic size before ponds dried. Alternatively, these differences in response among ranids may reflect differences in the heterogeneity of predators that each species experiences along the pond hydroperiod gradient. For example, wood frog inhabit the most temporary ponds, and these ponds experience dynamic changes in predator populations that are associated with pond drying. Thus, the large adaptive changes in wood frog tail depth may be related to the high degree of predator heterogeneity that wood frog populations experience. These hypotheses clearly need empirical tests.

The ontogeny of morphological plasticity.—The changes in larval morphology during ontogeny

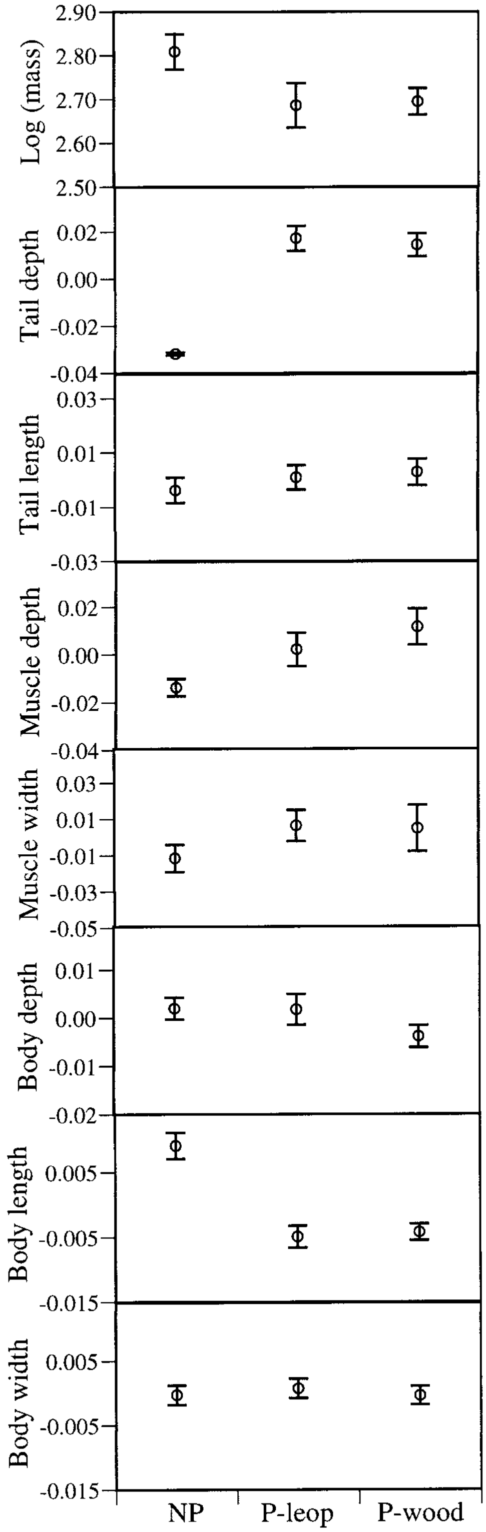

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Fig. 5. Mass and relative morphology of leopard frog reared without predators (NP), with predators fed larval leopard frog (P-leop), and predators fed larval wood frog (P-wood). Differences in body sizes were removed prior to analysis; thus all morphological differences in tadpole shape are relative.

TABLE 4. RESULTS OF THE MANOVA ON LARVAL LEOPARD FROG MORPHOLOGICAL RESPONSES UNDER THREE DIFFERENT PREDATOR ENVIRONMENTS: THE PRESENCE OF NO PREDATORS (NP), PREDATORS FED LARVAL LEOPARD FROG (P-LEOPARD), AND PREDATORS FED WOOD FROG (P-WOOD). Mean comparisons were conducted for significant treatment effects on univariate responses.

	Multivariate and univariate test statistics		Mean comparisons		
			NP vs P-Leopard	NP vs P-Wood	P-leopard vs P-Wood
Multivariate test	$F_{16,10} = 14.92$	$P < 0.001$			
Univariate tests:					
PC-1	$F_{2,12} = 1.80$	$P = 0.207$	—	—	—
Body depth	$F_{2,12} = 3.35$	$P = 0.070$	—	—	—
Body length	$F_{2,12} = 39.76$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.477$
Body width	$F_{2,12} = 3.00$	$P = 0.088$	—	—	—
Tail fin depth	$F_{2,12} = 46.64$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.751$
Tail fin length	$F_{2,12} = 0.18$	$P = 0.841$	—	—	—
Muscle depth	$F_{2,12} = 3.00$	$P = 0.088$	—	—	—
Muscle width	$F_{2,12} = 0.31$	$P = 0.737$	—	—	—

may have important functional ramifications. Brown and Taylor (1995) found that wood frog larvae captured in the wild exhibit changes in swimming performance during ontogeny. Larvae in early stages of development (stage 26) exhibited low swimming speed and endurance but high maneuverability (frequency of turning and sharpness of turning angle). In contrast, more developed tadpoles (stages 31–37) swam faster and farther but made fewer turns and exhibited shallower turning angles. Tadpoles approaching metamorphosis (stage 43) again exhibited reduced swimming speed and endurance but increased maneuverability. Tadpoles in the three samples of wood frog from our study (corresponding to average Gosner stages of 28.5, 31.7, and 37.6, respectively; Fig. 3) exhibited higher, lower, and higher relative tail muscle depth and width over ontogeny. Thus, it is possible that changes in relative muscle morphology observed in our study were related to the functional differences observed by Brown and Taylor (1995). These changes further suggest that larval wood frog may avoid predators by out-maneuvering them early in development when they are small and incapable of great speed and outrunning them later in life. Near metamorphosis, swimming speed should increase further, but it becomes compromised by the drag caused by the newly formed limbs (Wassersug and Sperry, 1977); perhaps maneuverability is again at a premium. At this early stage of the research, it is unclear whether a relatively large tail muscle is more important for speed or for maneuverability, but the correlations between our ontogeny of tail muscle and the observations by Brown and Taylor (1995) are intriguing and deserve empirical testing.

We expected reduced tadpole growth in the presence of *Anax* because all four ranids are known to reduce activity (both swimming and foraging) in the presence of *Anax* (Relyea, 1998). However, we did not witness a significant reduction in tadpole growth with predator presence in Experiments 1 and 3. A number of studies have shown that the presence of predators reduces the foraging activity of tadpoles resulting in reduced growth rates, but these are often cases where interspecific competitors are present to harvest the surplus resources left by the less active tadpole (Wilbur and Fauth, 1990; Werner and Anholt, 1996; Peacor and Werner, 1997). In our study, the tadpoles were reared in the absence of interspecific competitors which may explain our result. In Experiment 2, the tadpoles tended to be smaller in the presence of the predator on the first sampling date, equivalent in size by the second date, and larger by the third sample date. There was no effect of the caged predator on survivorship of wood frog or leopard frog ($P = 0.810$ and $P = 0.895$, respectively); thus, the increased growth that we observed with *Anax* late in development was not due to density differences between treatments. There are several potential explanations for these results. First, the amount of food available may be greater in tanks containing *Anax* because nutrients were added via the digestion of tadpoles fed to *Anax* in the cages. Second, predators, by reducing activity of the consumers, may allow resources to achieve higher standing crops that could be exploited by the tadpoles later in development when they are less vulnerable and respond less strongly to the presence of predators. A related third possibility is that the less active tadpoles imposed a moderate level of grazing on the periphyton that stimulated primary production relative to the higher grazing pressure of the more active tadpoles in the no-predator tanks. Less active tadpoles could then enjoy higher intake and growth rates (Abrams, 1992).

Conclusions.—Investigations of predator-induced morphological plasticity in larval anurans have identified a syndrome of morphological changes in response to odonate larvae. Five species encompassing three genera and two families exhibit similar responses to aeshnid dragonflies (deeper tail fins and smaller bodies). We are developing evidence that these responses are adaptive and that there is divergent selection on these features in predator and no-predator environments (Van Buskirk et al., 1997; Van Buskirk and Relyea, 1998). Other species in these genera, however, display either no response to dragonfly larvae (spring peeper, Smith and Van Buskirk, 1995) or respond in different ways (bullfrog and green frog in our study). The next step is to begin to associate these different responses to differences in the environments that species inhabit or determine whether these different responses represent alternative antipredator strategies.

The anuran system appears to be an excellent system for investigating phenotypic plasticity. It would be useful to have further studies documenting the patterns in phenotypic plasticity in additional amphibian taxa and relating these patterns to predator occurrence in natural ponds. It also would be useful to investigate these responses in multiple predator environments to obtain a more realistic picture of the directions and magnitudes of phenotypic responses. In addition, anuran larvae exhibit considerable behavioral plasticity in response to predators; thus, anurans would be an excellent system to determine how morphological and behavioral plasticity are integrated. Such studies should enable us to use the anuran system as a model for addressing general questions about the evolution of phenotypic plasticity.

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