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## The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs

Received: 16 September 2002 / Accepted: 27 November 2002 / Published online: 25 January 2003  
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**Abstract** Studies of phenotypic plasticity typically focus on traits in single ontogenetic stages. However, plastic responses can be induced in multiple ontogenetic stages and traits induced early in ontogeny may have lasting effects. We examined how gray treefrog larvae altered their morphology in four different larval environments and whether different larval environments affected the survival, growth, development, and morphology of juvenile frogs at metamorphosis. We then reared these juveniles in terrestrial environments under high and low intraspecific competition to determine whether the initial differences in traits at metamorphosis affected subsequent survival and growth, whether the initial phenotypic differences converged over time, and whether competition in the terrestrial environment induced further phenotypic changes. Larval and juvenile environments both affected treefrog traits. Larval predators induced relatively deep tail fins and short bodies, but there was no impact on larval development. In contrast, larval competitors induced relatively short tails and long bodies, reduced larval growth, and slowed larval development. At metamorphosis, larval predators had no effect on juvenile growth or relative morphology while larval competitors produced juveniles that were smaller and possessed relatively shorter limbs and shorter bodies. After 1 month of terrestrial competition among the juvenile frogs, the initial differences in juvenile morphology did not converge. There were no differences in growth due to larval treatment but there were differences in survival. Individuals that experienced low competition as tadpoles experienced near perfect survival as juvenile frogs but individuals that experienced high competition as tadpoles suffered an 18% decrease in survival as juvenile frogs. There were also morphological responses to juvenile competition, but these changes appear to be due, at least in part, to allometric effects. Collectively, these results

demonstrate that larval environments can have profound impacts on the traits and fitness of organisms later in ontogeny.

**Keywords** Anurans · *Hyla versicolor* · Predator-induced plasticity · Competitor-induced plasticity · Ontogeny

### Introduction

Interest in environmentally induced phenotypes has exploded in the past decade as studies have demonstrated its importance in ecology, developmental biology, and evolution (Schlichting and Pigliucci 1998). Over this time, we have documented a tremendous diversity of plastic responses exhibited by a wide variety of taxa (Bradshaw 1965; Cook and Johnson 1968; Myers and Bazely 1991; Stearns 1992; Schlichting and Pigliucci 1998). In exploring phenotypic plasticity, the primary focus has been on single developmental stages. However, phenotypic induction early in ontogeny may put individuals on different developmental trajectories that have lasting effects on phenotypes later in ontogeny (Roff 1992; Stearns 1992; Schlichting and Pigliucci 1998). Furthermore, phenotypic induction early in ontogeny may have fitness impacts that are not exhibited until later in ontogeny, thus making it difficult to detect the costs and benefits associated with possessing alternative phenotypes early in ontogeny (Via and Lande 1985, 1987; Via 1987; Gomulkiewicz and Kirkpatrick 1992; Moran 1992).

Several studies have shown that environments experienced early in ontogeny can affect individuals later in ontogeny (see review by Pechenik et al. 1998). For example, embryonic exposure to nutritional or toxic metal stress in mammals can alter behavior and growth later in life (Ravelli et al. 1976; Rice 1996a, 1996b), low food levels in young birds can affect future survival and fecundity (Haywood and Perrins 1992; Merilä and Svensson 1997), extended larval periods can cause marine invertebrates to grow slower in the juvenile stage (Pechenik and Cerulli 1991; Pechenik et al. 1993,

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1996a), and larval competition can reduce development, survival, and fecundity of post-metamorphic amphibians (Berven and Gill 1983; Smith 1983; Newman 1988; Semlitsch et al. 1988; Pfenning et al. 1991). While we have abundant evidence that early environments can affect subsequent performance (growth, fecundity, and survival), we have a poor understanding of the underlying mechanisms that are responsible. One reason for this poor understanding is that few investigators examine the behavioral, physiological, and morphological traits expressed both early and later on in ontogeny (but see Tollrian 1995; Crill et al. 1996; de Moed et al. 1997), even though these trait changes are what collectively determine performance. Thus, we need to know how early environments affect both the subsequent traits of individuals as well as the subsequent performance of individuals.

In addressing how early environments affect subsequent traits and performance, we would ideally use taxa that exhibit a wide range of plastic phenotypes early in ontogeny. Amphibians are an ideal candidate system because they exhibit a great deal of phenotypic plasticity in the larval stage. Predators typically induce low activity, relatively deep tails, and relatively small bodies in tadpoles whereas intra-specific competitors typically induce high activity, relatively shallow tails, relatively large bodies, and extended larval periods (Adolph 1931; Wilbur 1977; Sokol 1984; Lawler 1989; Smith and Van Buskirk 1995; Relyea and Werner 2000; Relyea 2001b, 2002a). Predator-induced tadpoles are more resistant to predation but less competitive whereas competitor-induced tadpoles grow faster but are less resistant to predation (Van Buskirk et al. 1997; Van Buskirk and Relyea 1998; Relyea 2002b). Thus, anurans provide an excellent opportunity to study the consequences of larval environments on the traits and performance of individuals later in ontogeny.

A previous study (Relyea 2001b) demonstrated that larval predator environments alter both the traits of larval wood frogs (tadpoles) and the traits of post-metamorphic wood frogs (juveniles). Juvenile frogs that emerged from predator environments had relatively longer limbs and narrower bodies than frogs emerging from predator-free environments. Correlations among larval traits and juvenile traits demonstrated that even though predators altered tadpole behavior and morphology, it was the predator-induced extension of the larval period that appeared to cause the change in juvenile morphology. If this is correct, then other environments that alter the behavior and morphology in different directions but still extend the larval period (e.g., competitive environments; Relyea 2002a) should also produce metamorphs with relatively longer limbs and narrower bodies. Furthermore, if this mechanism is common among anurans, we should observe the expected result in other anuran species as well as in non-anuran species that undergo metamorphosis.

When larval environments alter traits later in ontogeny, they also should affect performance later in ontogeny (i.e., growth or survival). For example, rodents which

grow slower early in ontogeny are able to compensate by growing faster later in ontogeny and arrive at the same final mass as a control group (Ernst et al. 1999). Indeed, there are numerous studies that have examined ontogenetic shifts in growth rates across a wide range of taxa (reviewed in Arendt 1997). While we know that larval environments can alter juvenile growth rates, we do not know whether these differences affect juvenile morphology and survival. If initial trait differences in juvenile frogs affect performance but converge over time, these differences might quickly become unimportant to the overall fitness of the individual.

Whereas morphological plasticity is well documented in many species of anurans in the larval stage (Smith and Van Buskirk 1995; McCollum and Van Buskirk 1996; Relyea 2001a), there appear to be no studies that have examined whether anurans are morphologically plastic in the juvenile stage. Indeed, there appears to have been little attention to multiple environmental inductions over ontogeny in any taxa. If traits such as relative morphology (i.e., shape) are phenotypically plastic in both the larval and juvenile stage, then the adult phenotypes that we observe in nature become a complex product of both larval and juvenile environments.

We addressed these issues using gray treefrog tadpoles reared under a factorial combination of intraspecific competitors and nonlethal (caged) predators to induce changes in the larval phenotypes. We then collected the metamorphs and exposed them to either high or low intraspecific competition in a terrestrial environment. Our hypotheses were the following: (1) larval competitors and predators affect the growth rate and relative morphology of tadpoles (a well-documented but necessary first step); (2) larval competitors and predators affect the growth rate to metamorphosis, time to metamorphosis (larval period), and relative morphology of juvenile frogs; (3) larval competitors and predators affect the subsequent performance (survival and growth) of juvenile frogs; (4) differences in juvenile mass and relative morphology due to different larval environments converge over time; and (5) differences in terrestrial environments can further affect the mass and relative morphology of juvenile frogs.

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## Materials and methods

### The tadpole induction experiment

We conducted the experiment in 20 pond mesocosms arrayed in 5 spatial blocks (5 replicates) on 6 June 2000 at the University of Missouri-Columbia's outdoor research facility. The mesocosms consisted of 100-L wading pools filled with tap water, 100 g leaf litter (mostly *Quercus* spp.), 5 g rabbit chow (which acts as an initial nutrient source), and an inoculation of plankton (see similar protocols in Werner and Anholt 1996; Van Buskirk and Relyea 1998; Relyea 2001b). We equipped each pool with a predator cage (Relyea 2002c) and covered the pools with 60% shade cloth to prevent other organisms from colonizing.

The experimental design was a randomized block design with each block containing a factorial combination of low and high larval competition crossed with the presence and absence of a caged predator. We collected eggs from 21 amplexing pairs of

treefrogs on 2 June 2000 from the Baskett Wildlife Area in Ashland, Mo. and allowed them to hatch in containers filled with aged tap water. On 19 June, tadpoles were haphazardly selected from a mixture of the 21 sibships. Forty tadpoles were added to pools assigned low competition while 80 tadpoles were added to pools assigned high competition (initial mean mass  $\pm 1$  SE =  $37 \pm 3$  mg). These densities (40 and 80 tadpoles/m<sup>2</sup>) compare to natural densities of up to 90 gray treefrog tadpoles/m<sup>2</sup> (Werner et al., unpublished data). A sample of 20 tadpoles was set aside to assess 24-h survivorship due to handling; 24-h survivorship was 100%.

In pools assigned the caged-predator treatments, cages contained a single larval dragonfly (*Anax* sp.) collected from a nearby pond. Predators were fed one treefrog tadpole (approximately 300 mg) three times per week. In pools assigned the no-predator treatments, cages were empty but lifted three times per week to equalize the disturbance among treatments.

To quantify tadpole morphology, we removed a random sample of ten individuals from each pool on 5 July and euthanized and preserved them in 10% formalin. The preserved tadpoles were subsequently weighed to determine growth rates [(final tadpole mass – initial tadpole mass)  $\div$  33 days] and measured using the BioScan Optimas image analysis system (Optimas, Bothell, Wash.). The measurements were the following: tail length and depth; muscle depth and width; body length, depth, and width; and mouth width (see Relyea 2000 for a photograph of tadpole dimensions).

The tadpoles began to metamorphose on 7 July. Metamorphs were removed from their pools at Gosner stage 42 (the appearance of forelimbs, Gosner 1960) and placed into 1-L plastic containers until they completed metamorphosis by resorbing their tail (typically 2–4 days later). During this time, anurans undergo a massive reconstruction of their mouth and digestive system; thus, they do not eat. After metamorphosis (Gosner stage 46), we weighed and measured each frog using digital calipers. The seven metamorph measurements were the following: forelimb length and width; hindlimb length and width; body length and width; and mouth width (see Fig. 1 in Relyea 2001a). There are two differences between our measurements and those made by Relyea (2001a): (1) head width was not measured in our paper because of its similarity with mouth width and (2) forelimb length was measured from the end of the longest digit to the body. Growth rate for each metamorph was defined as [(mass at tail resorption – initial tadpole mass)  $\div$  (number of days from start of experiment to tail resorption)]. Measured frogs lived in 40-l aquaria with moist sphagnum moss until there were enough metamorphs from all four larval treatments to begin the juvenile performance trial 26 days later. Aquaria were housed in the Missouri laboratory at 23°C and on a 14:10 h light regime. Metamorphs were fed *Drosophila* every 2 days at a ration of 10 flies per frog. This food ration allowed the juveniles to increase in weight by between 40 and 100 mg before the start of the juvenile performance trial.

#### The juvenile performance trial

The juvenile performance trial tested whether the juvenile frogs from the four larval environments differed in subsequent growth, whether initial differences in juvenile growth and relative morphology converged over time, whether terrestrial environments could induce further changes in juvenile growth and relative morphology, and whether terrestrial environments could affect juvenile survival. Because differences in post-metamorphic growth might be more likely to appear when resources are limiting and when competing against alternative phenotypes, we raised the four juvenile groups either separately, using 7 juveniles from each treatment (i.e., four low-competition treatments replicated five times), or together in a fifth treatment that combined 7 juveniles from each of the four groups into a single, high-competition treatment (28 juveniles, also replicated five times). Therefore, the juvenile performance trial consisted of 25 experimental units (five treatments within each of five blocks). The trial began on 2 August and juveniles were kept in their original block assignments by using a randomized block design

in a laboratory in Pennsylvania. To identify the original larval environment when the four juvenile groups were combined, juvenile frogs were toe-clipped using an outer phalange. The specific toe that was clipped differed among blocks to control for any bias that the loss of a particular toe might cause. A daily food ration of 360 mg “pinhead” crickets was given to each aquarium (around 100 crickets). This ration was chosen because it satiated the aquaria containing 7 frogs (uneaten crickets remained 1 day after feeding) but caused limited food in the aquaria containing 28 frogs. After 1 month, frogs were euthanized and preserved in 70% ethanol. One month has been shown to be sufficient time to allow the effects of competition to affect growth rates in several anurans (Blouin and Loeb 1991; Goater 1994). We weighed the preserved frogs to quantify growth rate [(mass at end of performance trial – mass at beginning of performance trial)  $\div$  29 days] and measured frog morphology as described above. By the end of the experiment, the largest juveniles had grown to about half the size of an adult gray treefrog. Preserving specimens in 70% ethanol can be problematic because lipids are soluble in ethanol. However, in this study, preserving the juveniles in ethanol would conservatively bias our results. For example, juveniles in low competition treatments should possess greater lipid stores relative to juveniles in high competition treatments. Once preserved, lipid solubility would have caused a greater mass reduction in low-competition individuals than high-competition individuals. Hence, ethanol preservation biases our results against finding significant growth differences.

#### Statistical analyses

There were three analyses conducted on the experiment using multivariate analyses of variance (MANOVAs): (1) the effect of larval environments on the growth rate and relative morphology of larvae; (2) the effect of larval environments on the growth rate, relative morphology, larval period, and survival of juvenile frogs; and (3) the effect of larval environments and juvenile environments on the growth rate, and relative morphology of juvenile frogs after 1 month in the performance trial. Survival data in the third analysis was heteroscedastic, so we conducted a separate non-parametric analysis by ranking the data and conducting a two-way analysis of variance. We had to pool the survival data across the terrestrial competition treatments to satisfy the analysis assumptions, but this was of little concern because the means across the terrestrial competition treatments were nearly identical. For all analyses, we used the pool or aquarium means as our response variables.

Critical to all of these analyses was developing a measure of relative (size-independent) morphology. To accomplish this, we regressed the linear measurements of all individuals against their mass (all variables were log-transformed to improve the linearity of their relationship) and we saved the residuals. The mean residuals were calculated for each experimental unit (wading pool/aquarium) and these served as our response variables. This technique has been successfully used in past experiments (Relyea 2002a, 2002b, 2002c, 2002d). In all three analyses, when a multivariate effect was significant ( $P < 0.05$ ), univariate tests were examined. Block interactions were never significant; thus, the degrees of freedom and sums-of-squares were pooled with the error term.

## Results

In the tadpole induction experiment, competitors, predators, and their interaction had significant multivariate effects on gray treefrog tadpoles (Tables 1, 2, Fig. 1). Tadpole growth was reduced by 39% with competitors but was unaffected by predators. Both competitors and predators affected tadpole morphology; competitors induced relatively shorter tail fins and deeper bodies while predators induced relatively deeper tails, shorter bodies,

**Table 1** Results of a MANOVA on the effects of larval predator and competitor treatments on the growth rate and relative morphology of *Hyla versicolor* tadpoles

Multivariate tests	<i>df</i>	<i>F</i>	<i>P</i>
Block	36,16	2.4	0.029
Larval competition	9,4	31.3	0.002
Larval predation	9,4	18.1	0.007
Larval competition * larval predation	9,4	11.3	0.016

**Table 2** Univariate tests (*P*-values) of the significant main effects of larval competition, larval predation, and the interaction, for the data in Table 1

Univariate tests	Larval competition	Larval predation	Competition * predation
Tail length	0.015	0.436	0.535
Tail depth	0.442	<0.0001	0.013
Muscle depth	0.102	0.274	0.350
Muscle width	0.295	0.277	0.571
Body length	0.133	0.004	0.090
Body depth	0.049	0.313	0.107
Body width	0.162	0.999	0.099
Mouth width	0.792	0.002	0.444
Growth rate	<0.0001	0.823	0.984

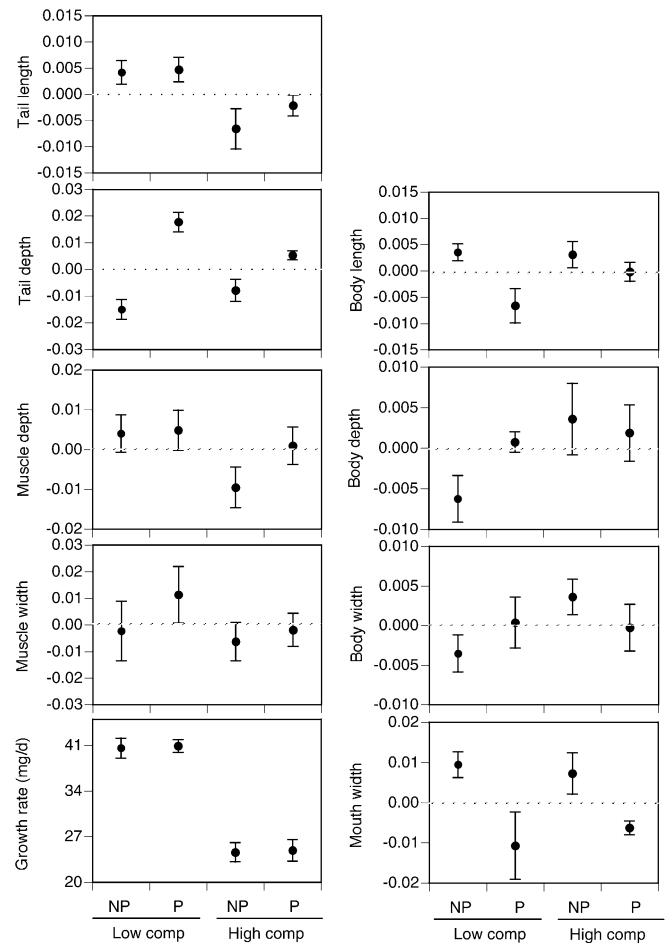
**Table 3** Results of a MANOVA on the effects of larval predator and competitor environments on the growth rate, survival, and relative morphology of newly-metamorphosed *Hyla versicolor* juveniles

Multivariate tests	<i>df</i>	<i>F</i>	<i>P</i>
Larval competition	10,7	118.9	<0.00001
Larval predation	10,7	2.1	0.166
Larval competition * larval predation	10,7	1.2	0.404

**Table 4** Univariate tests of the significant main effect of larval competition for the data in Table 3

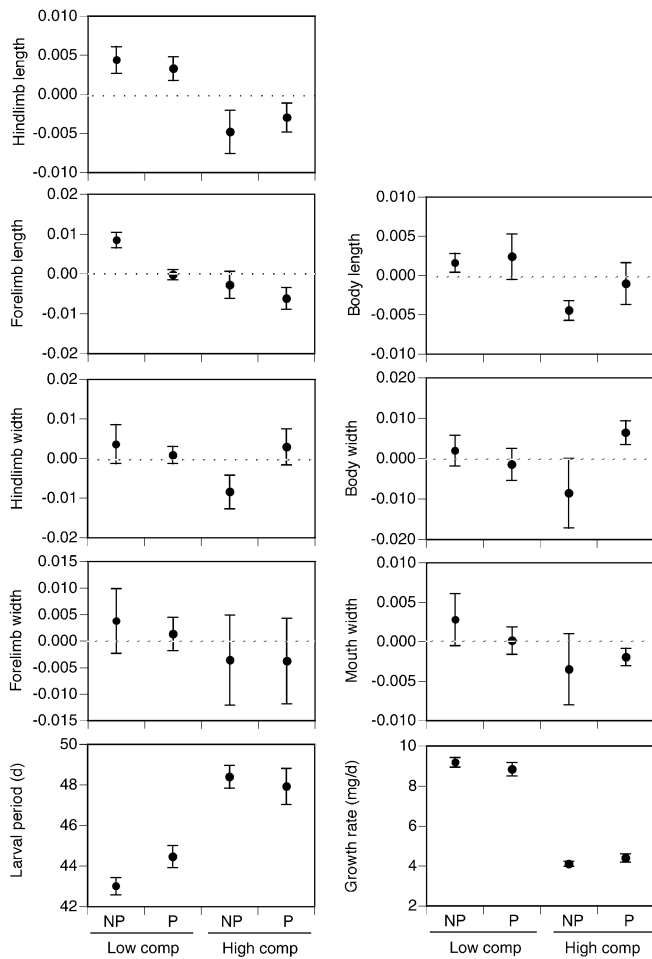
Univariate tests	<i>F</i>	<i>P</i>
Mouth width	2.0	0.179
Body width	0.1	0.805
Body length	4.9	0.042
Forelimb length	12.3	0.003
Forelimb width	0.8	0.371
Hindlimb length	14.5	0.002
Hindlimb width	1.5	0.241
Larval period	49.7	<0.00001
Growth rate	401.9	<0.00001
Survival	3.2	0.092

and narrower mouths. The interaction of competitors and caged predators existed for tail depth ( $P = 0.013$ ) and marginally existed for the three body dimensions ( $P \leq 0.1$ ). The interactions occurred because predators induced deeper tails ( $P < 0.001$ ) and wider but shorter bodies with low competition ( $P = 0.053$  and  $P = 0.039$ , respectively). In contrast, predators had marginal effects on tail depth ( $P = 0.084$ ) and no effect on body dimensions ( $P > 0.25$ ) with high competition.

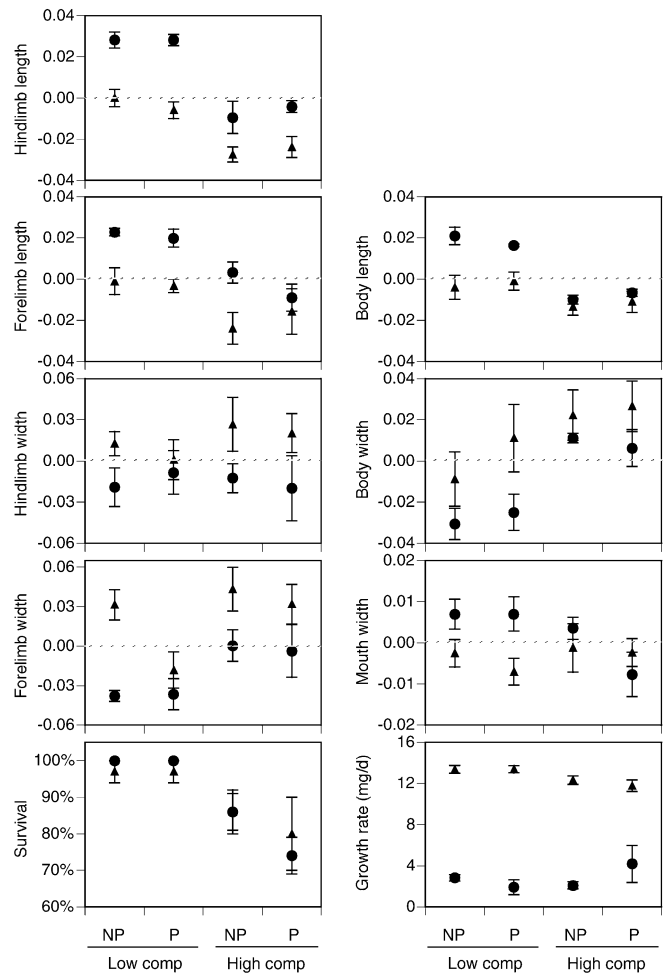


**Fig. 1** Growth rate and relative morphology of gray treefrog tadpoles (mean  $\pm 1$  SE) reared under four larval environments. Within each larval competition level (low and high), there are two predator treatments coded as NP no predator, and P predator. Relative morphology was calculated by regressing the log-transformed dimensions of all individuals against their log-transformed mass and saving the mean residuals from each experimental unit

The larval environments also had significant multivariate effects on the traits of the newly-emerged metamorphs (Tables 3, 4, Fig. 2). Mean survivorship through the larval period was 91% and did not differ among treatments. Larval predators had no effect on metamorphic traits but larval competitors had widespread effects. Frogs from high-competition larval environments grew 22% slower (thus they were 22% smaller in size) and took 4 days longer to metamorphose than frogs from low-competition larval environments. High larval competition also caused metamorphs to develop relatively shorter bodies, shorter forelimbs, and shorter hindlimbs. Using the regression coefficients that resulted from the regression of log (mass) against log (morphological dimensions), we estimated the absolute size of the metamorphic changes for frogs emerging from high-competition environments compared to low-competition environments; forelimbs were 3% shorter, hindlimbs were 2% shorter, and bodies were 1% shorter.



**Fig. 2** Growth rate, larval period, and relative morphology of gray treefrog juveniles (mean  $\pm$ 1 SE) after emerging from one of four larval environments. Within each larval competition level (low and high), there are two predator treatments coded as NP no predator, and P predator. Relative morphology was calculated by regressing the log-transformed dimensions of all individuals against their log-transformed mass and saving the mean residuals from each experimental unit



**Fig. 3** Growth rate, mass, and relative morphology of gray treefrog juveniles (mean  $\pm$ 1 SE) that were initially exposed to one of four larval environments and then reared either alone (*triangles*) or in competition with the metamorphs from the other three larval environments (*circles*). Within each larval competition level (low and high), there are two predator treatments coded as NP no predator, and P predator

**Table 5** Results of a MANOVA examining the impact of larval competition, larval predation, and metamorphic competition on the growth rate and relative morphology of *Hyla versicolor* after 1 month of juvenile growth

Multivariate tests	df	F	P value
Block	32,75	1.9	0.012
Larval competition	8,20	12.1	<0.00001
Larval predation	8,20	0.8	0.587
Juvenile competition	8,20	47.9	<0.00001
Larval competition * larval predation	8,20	0.7	0.670
Juvenile competition * larval predation	8,20	0.8	0.621
Juvenile competition * larval competition	8,20	1.6	0.193
Juvenile competition * larval predation * larval competition	8,20	1.3	0.314

**Table 6** Univariate tests of the significant main effects of larval competition and metamorph competition for the data in Table 5

Univariate tests	Larval competition		Juvenile competition	
	F	P	F	P
Mouth width	1.3	0.261	4.6	0.041
Body width	18.3	0.0002	10.4	0.003
Body length	41.0	<0.00001	19.4	0.0002
Forelimb length	22.6	0.00006	21.0	0.00009
Forelimb width	17.5	0.0003	27.7	0.00002
Hindlimb length	87.8	<0.00001	65.9	<0.00001
Hindlimb width	0.5	0.501	11.5	0.002
Growth rate	0.2	0.661	286.5	<0.00001

The third analysis examined the survival, growth, and relative morphology of juvenile frogs after being reared in high- and low-juvenile competition for 1 month (Tables 5, 6, Fig. 3). Larval predators had no effect on juvenile survival ( $P=0.487$ ) but larval competitors had a substantial impact, reducing juvenile survival by 18% ( $P<0.00001$ ; the interaction was not significant  $P=0.487$ ). In the multivariate analysis of growth and the morphological traits, only larval competition and juvenile competition had multivariate effects; there was no effect of larval predators and no significant interactions. Larval competition had no effect on juvenile growth rate but juvenile competition caused a 78% reduction in juvenile growth. Larval competition continued to affect juvenile morphology (i.e., there was no trait convergence over time); after 1 month, frogs from high-competition larval environments had relatively shorter and wider bodies, shorter hindlimbs, and shorter and wider forelimbs compared to frogs from low-competition larval environments. Frogs experiencing high juvenile competition also developed relatively longer and thinner limbs, narrower and shorter bodies, and wider mouths than frogs experiencing low juvenile competition. We estimated the absolute size of the differences between frogs from high-competition terrestrial environments compared to low-competition terrestrial environments. For a frog of average mass, forelimbs were 5% longer and 10% narrower, hindlimbs were 6% longer and 7% narrower, bodies were 3% longer and 5% narrower, and mouths were 1% wider.

## Discussion

This study demonstrates that environmental conditions experienced both early and later in life can affect the morphology of an individual and have long-term effects on performance. Early in ontogeny, treefrog larvae responded to larval predators by developing relatively deep tails, short bodies, and small mouths. These morphological responses to larval predators have been documented in numerous studies and they appear to be adaptive; they increase the probability of a tadpole surviving predation, but at the cost of slower growth (Smith and Van Buskirk 1995; McCollum and Van Buskirk 1996; Relyea 2000, 2001a, 2002a; Van Buskirk and Saxer 2001). Predator-induced changes in morphology are not unique to tadpoles; the phenomenon is widespread across many taxa (Havel 1987; Sih 1987; Karban and Myers 1989; Harvell 1990; Lima and Dill 1990; Kats and Dill 1998; Tollrian and Harvell 1999). However, most studies have failed to appreciate that predator-induced responses that occur early in ontogeny may impact traits later in ontogeny.

Larval competitors caused tadpoles to experience slower growth and development and exhibit relatively short tails and deep bodies. Slower growth and development are common responses to competition (Connell 1983; Schoener 1983) and have been linked to decreased

adult fitness in amphibians (Adolph 1931; Smith-Gill and Berven 1979; Smith 1987; Semlitsch et al. 1988; Berven 1990). Competitor-induced morphology, however, is only a recently described phenomenon in larval anurans and appears to be adaptive (Relyea 2002a, 2002d; excluding the cannibalistic responses described by Pfennig 1992a, 1992b). Tadpoles with competitor-induced morphology grow faster (Relyea 2002a) but at the cost of increased vulnerability to predation (Peacor and Werner 1997; Relyea 2001c). Competitor-induced morphology is being found in an increasing number of other taxa, illustrating its possible widespread occurrence in nature (Collins 1980; Harvell 1990; Reilly et al. 1997) and its potential for causing persistent phenotypic effects in a wide variety of organisms.

Based on an earlier study using wood frogs (Relyea 2001b), we hypothesized that changes in juvenile frog morphology, driven by environmentally-induced extensions of the larval period, should produce metamorphs with relatively longer legs and narrower bodies. In this study, we had two ways of altering the larval period: the addition of larval predators and the addition of larval competitors. Larval predators had no effect on larval period and no effect on juvenile morphology, supporting our hypothesis that the larval period has to be extended to cause limbs to become relatively longer. In contrast, Van Buskirk and Saxer (2001) found that predators induced longer larval periods in European water frogs (*Rana ridibunda*) but the resulting metamorphs had relatively shorter and wider limbs and no changes in body width. Therefore, while it is clear that larval predator environments can affect the relative morphology of juvenile frogs, it is not clear that changes in the larval period are the driving mechanism in all anurans.

Manipulating larval periods via competition offered a second method of testing our hypothesis. Under high competition, larval periods were extended and the frogs developed relatively shorter limbs and shorter bodies. These results were opposite from our hypothesis. Longer larval periods are associated with relatively shorter hindlimbs in other frogs, including *Rana sphenoccephala* and *Hyla crucifer* (Emerson 1986; Emerson et al. 1988), but not in *Hyla cinerea* (Blouin and Loeb 1991). However, these studies calculate morphology relative to body length (i.e., snout-vent length, which itself is a plastic trait; Relyea 2001c, 2002a) producing results that are difficult to interpret. For example, Emerson (1986) found that increased larval competition produces frogs with hindlimbs that were shorter relative to body length but longer relative to mass (for a discussion of ratio use problems, see Packard and Boardman 1988). In conclusion, there appears to be a link between larval period and juvenile morphology, but the direction of the response may be species-specific.

The differences in morphology between frogs from high- versus low-competition larval environments had a major impact on juvenile performance. Individuals exposed to greater competition suffered low mortality as tadpoles but much higher mortality as juveniles. This

higher mortality rate was not simply due to a smaller final mass. For example, frogs with high larval competition but low terrestrial competition were among the most massive frogs (Fig. 3), yet these frogs still experienced reduced survival. The higher mortality rate also does not appear to be connected to the amount of resources available in the terrestrial environment for two reasons. First, we found the same pattern of decreased survival when the juvenile frogs were reared under high and low food conditions in the terrestrial environment. Second, following metamorphosis, frogs from high-competition larval environments grew at the same rate as frogs from low-competition larval environments, suggesting that the cause of the higher mortality was not an inability to forage and convert food into frog biomass. Therefore, it is unlikely that the morphological differences in this treatment played a role in the increased mortality. It is more likely that the frogs from high-competition larval environments metamorphosed in poor condition and this made them more susceptible to a variety of mortality factors (e.g., disease). Studies on natural populations of chorus frogs (*Pseudacris triseriata*) and wood frogs have also demonstrated that frogs metamorphosing later and of smaller mass experience lower survival in the wild, although the cause of this lower survival rate is not clear (Smith 1983; Berven 1990). Studies on other taxa have shown that phenotypic plasticity exhibited early in ontogeny can affect traits (Taylor 1988; Milbrath et al. 1993; Crill et al. 1996; de Moed et al. 1997) and performance (Ravelli et al. 1976; Woollacott et al. 1989; Haywood and Perrins 1992; Merilä and Svensson 1997; Pechenik et al. 1996b) later in ontogeny, but rarely do we observe such dramatic and rapid impacts of larval environments on juvenile survival.

Differences in juvenile morphology did not affect juvenile growth because the differences in morphology (e.g., 2% change in absolute limb length) were probably too small to have any effect. Differences in juvenile morphology could potentially affect juvenile growth via changes in jumping ability. However, hindlimb lengths must change by 10% before differences in jumping performance can be detected (Zug 1972; Stokely and Berberian 1953; Emerson 1978, 1986). While we did not test whether the differences in morphology might provide anti-predator advantages, the existing evidence suggests that the frogs induced by high-competition larval environments would not have an improved jumping ability and thus not possess an increased ability to escape predators. The morphological changes in the juvenile frogs appear to be nonadaptive by-products of adaptive changes in the larval stage.

The differences in mass and relative morphology present at metamorphosis did not converge over time. The lack of morphological convergence may have occurred because there appear to be no fitness costs of possessing small differences in juvenile morphology. The original mass differences occurring between high- and low-competition tadpoles were carried over into metamorphosis and persisted after metamorphosis (although the

relative difference declined). Larval competition reduced tadpole mass by 36% (after 16 days), metamorphic mass by 22%, and post-metamorphic mass by 18%. This supports the work of Smith (1987), Goater (1994), and Morey and Reznick (2001) who found that initial differences in larval mass can be maintained for up to 2 years in chorus frogs, toads (*Bufo bufo*), and spadefoot toads (*Scaphiopus hammondi*). This reduced mass likely has negative effects on fecundity and time to sexual maturation (Davies and Halliday 1977; Berven 1982, 1990; Smith 1987; Semlitsch et al. 1988). The lack of mass convergence in amphibians is in contrast to the pattern observed in rodents in which individuals that grow slow early in ontogeny are able to compensate later in ontogeny by having accelerated growth rates (Ernst et al. 1999).

Surprisingly, there were two traits (forelimb width and body width) that were not significantly affected by larval competition at metamorphosis but were significantly affected by larval competition after a month of post-metamorphic growth. This suggests that substantial lag times not only occur between early environments and future survival, but also between early environments and morphological traits.

Juvenile competition also induced morphological changes in the frogs. While we have numerous studies documenting the morphological plasticity of tadpoles (Smith and Van Buskirk 1995; McCollum and Van Buskirk 1996; Relyea and Werner 2000), this appears to be the first study to document that frogs can alter their relative shape in the juvenile stage. An intriguing question is whether these plastic responses represent adaptive plasticity, that is, do frogs reared with terrestrial competitors alter their morphology to become more competitive in the terrestrial environment? Our study was not designed to test this question, but our discovery does pave the way for future studies to determine costs and benefits of alternative morphological phenotypes, the extent of morphological plasticity in juvenile and adult frogs, and the range of environments that can induce the plastic responses.

An important concern in any investigation of phenotypically plastic morphology is that morphological differences could be due to allometric growth. Because shape often is related to size (mass or developmental stage), an environment may alter relative morphology by simply retarding or accelerating the organism's growth. In tadpoles, we have addressed this issue and found that the shape differences are not due to allometry (Relyea and Werner 2000; Relyea 2002a). In juvenile frogs, we can address the allometry issue by regressing mass against relative hindlimb length, forelimb length, or body length (under low terrestrial competition). We found a significantly negative relationship ( $P \leq 0.006$ ), indicating that larger frogs inherently possess relatively shorter limbs and bodies. Low larval competition produced metamorphs that were more massive, but with relatively longer limbs and bodies. Therefore, the morphological differences between juveniles from low- and high-competition larval

environments were not due to allometry. In contrast, frogs reared for a month of low juvenile competition were more massive and possessed relatively shorter limbs and bodies than those reared with high juvenile competition. These morphological changes appear to be due, at least in part, to the allometric effect of high juvenile competition reducing frog mass.

## Conclusions

In the past decade, we have grown to appreciate that environments experienced early in ontogeny can have profound effects on an individual's traits and performance later in ontogeny (Pechenik et al. 1998). These carry-over effects on the phenotype might be adaptive if the early environmental conditions are good indicators of future environmental conditions. However, in many cases, conditions in the two environments are unrelated (e.g., an aquatic larval environment and a terrestrial adult environment). In such cases, any carry-over effects are likely non-adaptive (Relyea 2001b). In our study, we found that larval predator environments had no carry-over effects while larval competitor environments had small carry-over effects on morphology and substantial carry-over effects on growth and survival. Thus, early and late environments can interact to shape an individual's phenotype. At the same time, producing alternative phenotypes can have immediate costs and benefits during the larval stage as well as delayed costs and benefits later in life. Future studies should elucidate how traits are developmentally connected through ontogeny to determine how (mechanistically) these environments determine subsequent performance.

**Acknowledgements** We thank Christine Glaude for assistance with the experiments, Hillary Metz for care of the frogs, and Raymond Semlitsch for the use of field and laboratory facilities. Rachel Collins and Kerry Yurewicz provided valuable reviews of the manuscript. This work was supported by NSF grant 99-03761.

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