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Peter B. McIntyre · Sandra Baldwin ·
Alexander S. Flecker

Effects of behavioral and morphological plasticity on risk of predation in a Neotropical tadpole

Abstract Predator-induced phenotypic plasticity is widespread among aquatic animals, however the relative contributions of behavioral and morphological shifts to reducing risk of predation remain uncertain. We tested the phenotypic plasticity of a Neotropical tadpole (*Rana palmipes*) in response to chemical cues from predatory *Belostoma* water bugs, and how phenotype affects risk of predation. Behavior, morphology, and pigmentation all were plastic, resulting in a predator-induced phenotype with lower activity, deeper tail fin and muscle, and darker pigmentation. Tadpoles in the predator cue treatment also grew more rapidly, possibly as a result of the nutrient subsidy from feeding the caged predator. For comparison to phenotypes induced in the experiment, we quantified the phenotype of tadpoles from a natural pool. Wild-caught tadpoles did not match either experimentally induced phenotype; their morphology was more similar to that produced in the control treatment, but their low swimming activity was similar to that induced by predator cues. Exposure of tadpoles from both experimental treatments and the natural pool to a free-ranging predator confirmed that predator-induced phenotypic plasticity reduces risk of predation. Risk of predation was comparable among wild-caught and predator-induced tadpoles, indicating that behavioral shifts can substantially alleviate risk in tadpoles that lack the typical suite of predator-induced morphological traits. The morphology observed in wild-caught tadpoles is associated with rapid growth and high competition in other tadpole species, suggesting that tadpoles may profitably combine a morphology suited to competition for food with behaviors that minimize risk of predation.

Introduction

The identity and abundance of predators vary widely among aquatic habitats (e.g., Gascon 1992; Wellborn et al. 1996; Van Buskirk 2002a), making it difficult for prey species to achieve a uniform level of risk throughout their range. This uncertainty has selected for plastic rather than constitutive anti-predator defenses in many taxa (Havel 1987; Tollrian and Harvell 1999). Prey often rely on chemical cues to assess the presence and activity of predators (Dodson et al. 1994; Kats and Dill 1998), and respond by altering their phenotype to reduce their exposure or susceptibility to predation.

Behavioral shifts are the most common response of aquatic animals to chemical cues from predators. These include reductions in activity, enhanced use of cover, and spatial avoidance, and are known from a wide variety of fishes, amphibians, and invertebrates (Lima and Dill 1990; Kats and Dill 1998). Morphological responses, such as changes in body dimensions and defensive structures, are also widespread (Havel 1987; Tollrian and Harvell 1999). Anuran larvae have become a model system for experimental evaluations of predator-induced phenotypic plasticity because their responses to predator cues are strong, and there are demonstrable differences in fitness among alternative phenotypes (reviewed in Anholt and Werner 1999). This paper presents a test of behavioral and morphological responses to predators in a Neotropical tadpole, and evaluates their relative effectiveness in reducing risk.

Most animals must move in order to forage, but movement increases their exposure to predators. This results in a tradeoff between growth rate and risk of

P. B. McIntyre (✉) · A. S. Flecker
Department of Ecology and Evolutionary Biology, Corson Hall,
Cornell University,
Ithaca, NY, 14853-2701, USA
e-mail: pbm3@cornell.edu
Tel.: +1-607-2544240
Fax: +1-607-2558088

S. Baldwin
US Geological Survey, Woods Hole Field Center,
384 Woods Hole Road,
Woods Hole, MA, 02543, USA

predation (Sih 1987; Ludwig and Rowe 1990). In the short term, experimental exposure to chemical cues from predators elicits reduced swimming activity in tadpoles from numerous frog families (e.g., Relyea 2001a; Van Buskirk 2002a). This decreases risk of predation (Skelly 1994), most likely by reducing visibility to predators (Feder 1983; Azevedo-Ramos et al. 1992). By responding to the concentration of chemical cues from predation events, tadpoles match their behavior to the riskiness of the environment (Van Buskirk and Arioli 2002). Some predators elicit little or no behavioral response (Relyea and Werner 1999; Schmidt and Amezquita 2001), while others have lasting effects on behavior even in the absence of fresh chemical cues (Semlitsch and Reyer 1992; Van Buskirk 2002b; Van Buskirk et al. 2002).

Morphological responses to chemical cues from predators have also been documented in numerous animal taxa (Tollrian and Harvell 1999), including tadpoles of many families from North America and Europe (e.g., Lardner 2000; Relyea 2001a; Van Buskirk 2002a). The specific pattern of plasticity depends on the predator and tadpole species tested, but most tadpoles develop reduced body dimensions and an enlarged tail fin and muscle when exposed to predator chemical cues (e.g., Relyea 2001a; Van Buskirk 2002a). Experiments indicate that selection imposed by lethal predators operates in the same direction as these inducible shifts in morphology (Van Buskirk et al. 1997; Van Buskirk and Relyea 1998; Van Buskirk and Schmidt 2000). Though the larger tail of predator-induced tadpoles has relatively small effects on swimming performance (McCollum and Leimberger 1997; Van Buskirk and McCollum 2000b), it may reduce the lethality of attacks by directing them toward the dispensable tail fin rather than the body (Doherty et al. 1998; Hoff and Wassersug 2000; Van Buskirk et al. 2003).

Despite widespread evidence of behavioral and morphological responses to predators by tadpoles, few studies have compared the effectiveness of these two types of phenotypic plasticity at reducing risk. Proportional changes in morphology are much smaller than those in behavior (Relyea 2001a; Van Buskirk 2002a, 2002b), and recent work suggests that they are evoked by somewhat different sets of cues (Van Buskirk and Arioli 2002) and serve as independent solutions to the problem of avoiding predators (Relyea 2001b; Van Buskirk 2002a). Direct tests of the relative effectiveness of behavioral and morphological plasticity are complicated by simultaneous shifts in both suites of traits in response to cues from predators. Using a combination of predator cues and feeding manipulations, Van Buskirk and McCollum (2000a, 2000b) found that swimming activity contributes to risk more than any single morphological attribute of tadpoles. However, differential risk has also been observed among groups of tadpoles that differed in morphology but not behavior (McCollum and Van Buskirk 1996; Van Buskirk and McCollum 1999), hence the relative importance of plasticity in these traits remains unresolved.

There have also been surprisingly few direct comparisons between tadpole phenotypes produced in natural environments and those generated under experimental conditions. The available studies indicate that exposure to predators (or predator cues) induces similar phenotypic changes under both experimental and natural conditions in cladocerans (see Tollrian and Dodson 1999), larval insects (Arnqvist and Johansson 1998; Dahl and Peckarsky 2002), and larval amphibians (Smith and Van Buskirk 1995; Van Buskirk and McCollum 1999; Van Buskirk and Schmidt 2000). However, such comparisons have been published for only a few of the dozens of tadpole species tested for predator-induced phenotypic plasticity. Natural environments vary greatly in other important characteristics such as competitor density and productivity, requiring phenotypes that balance diverse and conflicting pressures (Relyea 2002, 2004). Quantifying phenotypes of wild-caught organisms can be used to test whether responses to stimuli under experimental conditions match those elicited in complex natural systems (e.g. Dahl and Peckarsky 2002). In addition, experimental tests of performance that include both naturally occurring phenotypes and experimentally induced ones may offer insight into phenotype-performance relationships by virtue of broadening the range of trait combinations under consideration.

This paper addresses the relative contribution of behavioral and morphological traits to reducing risk of predation in a Neotropical tadpole, *Rana palmipes*. We begin by testing for behavioral, morphological, and pigmentation plasticity in response to chemical cues from a caged predator. To provide context for the phenotypes induced by the presence or absence of predator cues, we compared them to the phenotype of tadpoles from a natural pool. Finally, the link between phenotype and performance was tested by exposing all three groups of tadpoles to a free-ranging predator. This combination of phenotypic and performance comparisons using experimentally induced and wild-caught tadpoles offers a unique perspective on the relative contributions of behavior and morphology to reducing risk of predation.

Materials and methods

Study system

Rana palmipes (web-footed frog) is broadly distributed in the lowlands of northern South America east of the Andes (Hillis and de Sa 1988). Our study area is along Rio Las Marias, a fourth-order stream (5–12 m wide, <1 m deep during dry season) flowing through deciduous forest in the Andean piedmont near Guanare, Venezuela (9°10′N, 69°44′W). The study was conducted early in the dry season (January–February 2001) when *R. palmipes* tadpoles were found in riverside pools, tributary streams, and slow-moving parts of the river channel. These tadpoles are generalized consumers of benthic algae and detritus collected from cobblestone, tree leaf, and sand substrates (Flecker et al. 1999). Their distribution was very patchy even among seemingly suitable habitats. Where tadpoles were found, their density was usually 10–50 individuals per m^2 but a few isolated pools contained >200 individuals per m^2.

R. palmipes tadpoles are exposed to a variety of potential predators. Vertebrate predators include numerous fishes, but stomach content surveys indicate that they do not consume *R. palmipes* tadpoles (B. Daley and A. Flecker, unpublished data). In fact,

predatory fishes often swim near active tadpoles without attacking, suggesting that the tadpoles possess chemical defenses. Predatory invertebrates such as hemipterans, dragonflies, and beetles are also found in the same habitats as tadpoles, and they readily consumed *R. palmipes* in preliminary feeding trials. Belostomatid water bugs were used as predators in this study because they were common at all locations where we collected tadpoles, and they have been recognized as important predators of tadpoles at other Neotropical sites (Gascon 1992; Schmidt and Amezquita 2001).

Phenotypic plasticity experiment

To test for predator-induced phenotypic plasticity in *R. palmipes*, we exposed tadpoles to one of two chemical cue treatments in plastic wading pools. In the predator treatment, a cage (400 ml plastic cup sealed with mosquito netting) enclosing one *Belostoma malkini* and two tadpoles was placed in each pool. A cage containing only two tadpoles was used in the control treatment in order to provide chemical cues from tadpoles in the absence of predation. Caged tadpoles in both treatments were replaced every 2 days, and predators consumed every tadpole provided to yield an average feeding rate of one tadpole per day (0.3–0.4 g wet mass).

The experiment was executed outdoors in round plastic pools (44 cm diameter) arranged in complete blocks to yield seven replicates of each treatment. Treatments were assigned to alternating positions in adjacent blocks. Experimental pools contained 22 l of water from Rio Las Marias, six periphyton-coated cobbles, and ten submerged leaves (primarily *Anacardium excelsum*). This mimicked the physical structure and food sources most common where tadpoles were collected. Pools were shaded by a natural canopy, and were covered with mosquito netting to prevent colonization by non-experimental organisms.

We collected tadpoles for the experiment from two tributary streams and one riverside pool (>200 m between sites). Each population comprised >1,000 tadpoles, and the absence of tadpoles in the river between these populations suggested that movement among them was negligible. Collecting individual clutches was impossible at this site due to the sporadic timing and location of oviposition, but our method ensured representation of a variety of genetic lines in an unbiased way. Tadpoles were gently sorted by size using sieves, and 33 like-sized individuals (0.3 g wet mass) were selected randomly from each of the three populations. These 99 tadpoles were combined into a single group, from which 7 individuals were added to each experimental pool. The experiment lasted for 37 days, after which the number of tadpoles surviving in each pool was recorded.

Behavioral responses to treatments were quantified using instantaneous counts of the number of tadpoles visible and the number swimming. A single observer recorded these data once during daylight hours (1730 to 1850 hours) and once after dark (2110 to 3000 hours, using a white flashlight) on six dates during the last 2 weeks of the experiment. We observed no indications that observer presence or temporary illumination at night affected tadpole behavior.

Life history responses to treatments were assessed using wet mass, developmental stage (Gosner 1960), and survival at the end of the experiment. Changes in life history could be a direct response to predator cues, an indirect response mediated by tadpole behavior, or an indirect response mediated by algal responses to treatments. To address these alternative mechanisms, we also measured algal biomass and ammonium concentrations in each experimental pool on the last day of the experiment. Periphyton was sampled from three randomly selected cobbles per pool (19.6 cm^2 per cobble) using a brush, and a subsample was collected on a glass fiber filter, extracted for 24 h in 90% ethanol, and measured for chlorophyll *a* concentration using a fluorometer (Nusch 1980). The mean value from the three cobbles was analyzed. Ammonium concentration was quantified using a fluorometer after Holmes et al. (1999).

Morphological responses to treatments were quantified once at the end of the experiment. Tadpoles were lightly anesthetized using MS-222, and dial calipers were used to measure six linear dimensions: body length (along spinal axis from front of body to base of tail), maximum body depth (perpendicular to spinal axis), tail length (along spinal axis from base to tip), tail fin depth at the base of the tail (perpendicular to spinal axis), tail muscle depth at the base of the tail, and tail muscle width at the base of the tail. We also scored by eye two aspects of tail pigmentation: the background color of the tail fin based on a chart of degrees of grayness overlaid on a pale yellow background (plate 4, tone scale 2 in Kornerup and Wanscher 1978), and the number of black spots (>1 mm diameter) on the left side of the tail. Tadpoles were processed as quickly as possible, then grouped by treatment and revived in large plastic pools of fresh river water.

Phenotypic comparison to wild-caught tadpoles

Our second goal was to compare the morphology of experimental tadpoles to that of wild-caught tadpoles. At the conclusion of the plasticity experiment, we could find only one natural population that included tadpoles of the size range produced in the experiment. This was a shaded riverside pool (~5 m^2 in area, 0–70 cm deep), and was one of the three sites from which tadpoles for the plasticity experiment were sampled. From this pool, we caught several hundred tadpoles and haphazardly selected 48 individuals encompassing the size range of experimental tadpoles. These tadpoles were anesthetized, measured, scored for tail pigmentation, and revived as described above. While capturing tadpoles, we observed *Belostoma*, larval beetles, and libellulid dragonfly larvae in this pool, however we did not quantify predator densities.

Predation experiment

To address the effect of phenotypic characteristics on risk of predation, we measured predation by a free-ranging predator on tadpoles of the three groups described above (predator treatment of the plasticity experiment, control treatment of the plasticity experiment, or wild-caught). This experiment was conducted in 24 plastic pools (44 cm diameter) arranged in eight complete blocks containing one replicate for each tadpole group. Pools held 13 l of fresh river water, two algae-covered cobbles, and five large leaves that covered most of the substrate. Each pool received five randomly selected tadpoles from the same group. All of these tadpoles had been previously anesthetized for morphological measurements, but there was no indication of behavioral changes or physical damage due to processing.

To minimize size differences among the three groups, we used the largest individuals from the control treatment of the plasticity experiment [range 31–42 mm total length; pool mean 35.8±2.9 (SD)] and the smallest tadpoles from the predator treatment (range 33–43; mean 38.6±2.8) for comparison to the wild-caught group (range 26–43; mean 38.0±4.1). The differences among groups in mean size were small (<3 mm) compared to the range within groups (>9 mm), however control tadpoles were significantly smaller than counterparts from the other two groups. Detailed studies of size-dependent predation risk indicate that these differences among groups would have a negligible effect on risk for tadpoles of the sizes used in the experiment (Caldwell et al. 1980; Smith 1983; Travis et al. 1985; Richards and Bull 1990; Eklov and Werner 2000).

Tadpoles were allowed to acclimate to experimental pools overnight, and one free-ranging *Belostoma malkini* was added to each pool in the morning to begin the experiment. The predators [20.5±1.7 (SD) mm body length] were starved for 24 h prior to the experiment, and were randomly assigned to pools. Beginning 2 h after introducing the predator, we recorded the number of visible and swimming tadpoles in each pool five times at 2-h intervals. After each set of observations, we also counted the number of surviving tadpoles in order to standardize behavioral data. The experiment was ended after 36 h, and the final number of survivors was determined.

Statistical analyses

Treatment effects on tadpole behavior in the plasticity experiment were assessed using the proportion of surviving tadpoles that were visible and the proportion of visible tadpoles that were swimming. Day- and night-time data were averaged separately across dates for each pool, arcsine transformed, and analyzed for treatment effects using an ANOVA with time of day (day or night) as a repeated measure.

Treatment effects on tadpole life history and pigmentation were assessed using mean values of tadpoles from each experimental pool. A MANOVA tested for effects on life history (wet mass, developmental stage, and arcsine-transformed survival) and tail pigmentation (number of dark spots and background color), and univariate ANOVAs were used to evaluate patterns in each characteristic separately. To account for changes in pigmentation as a function of tadpole size, we analyzed residuals following regression against tail area (0.5× tail depth × tail length).

Treatment effects on tadpole morphology were tested after adjusting measurements to account for the overall size of the tadpole (see Van Buskirk and Relyea 1998). Original measurements were log-transformed, and a principal components analysis (PCA) was performed on the covariance matrix. The first principle component score (PC-1) reflected the overall size of a tadpole, and explained 87.5% of the variance in the data. Each log-transformed measurement was regressed against PC-1, and the mean of the residuals from tadpoles in the same experimental pool were analyzed. MANOVA was used to test the effects of treatments on the mean PC-1 and residual values, followed by univariate ANOVAs for each variable.

Periphyton chlorophyll a and ammonium concentrations violated parametric model assumptions despite transformation, so untransformed data were compared using Mann–Whitney U tests. Three of the 14 experimental pools experienced dense phytoplankton blooms just before the end of the experiment (two in the predator treatment, one in control treatment), and these pools were excluded from analyses of ammonium. Linear regression was used to test the relationship between ammonium concentration and algal biomass.

Discriminant function analysis (DFA) was used to compare the morphology of wild-caught tadpoles to individuals from the phenotypic plasticity experiment. Size-adjusted morphological variation among tadpoles from all three groups was quantified using the PCA approach outlined earlier. DFA estimated the best linear combination of morphometrics to distinguish between tadpoles from the control and predator experimental treatments. This function was applied to wild-caught tadpoles to evaluate their overall morphological similarity to experimental tadpole groups. Univariate ANOVAs were used to assess similarities among groups in specific morphological traits, however these tests are interpreted qualitatively due to differences between sample population structure of experimental (seven small replicate populations per treatment) and wild-caught tadpoles (one large, natural population). Tail pigmentation patterns were also compared among tadpole groups using MANOVA.

In the predation experiment, differences between tadpole groups in visibility, swimming activity, and survival were tested using MANOVA. Behavioral data were calculated as described earlier and averaged across observations within each pool. All three proportions were arcsine transformed.

As outlined above, multivariate analyses were conducted whenever possible. The structure of behavioral, algal, and nutrient data from the plasticity experiment required separate analysis from other data types, and the limited number of experimental units prohibited using a single MANOVA to simultaneously test effects on morphology, life history, and pigmentation. Block effects were included in all preliminary models, but were pooled in the error term because they were never significant ($\alpha=0.05$). Tukey's HSD tests were used in post hoc comparisons to maintain experimentwise error of $\alpha=0.05$.

Results

Phenotypic plasticity experiment

Tadpoles responded behaviorally to both treatment and time of day (Table 1). Swimming was curtailed in the predator treatment, and both visibility and swimming were significantly higher after dark in both treatments (Fig. 1). An interaction between treatment and time-of-day effects on tadpole visibility arose because tadpoles were more often concealed during the day in pools with predator cues, but were equally visible in both treatments after dark.

Treatments also influenced tadpole life history and pigmentation (Wilks' $\lambda=0.09$, $F_{5,8}=16.39$, $P<0.001$). Individuals exposed to predator cues achieved greater mass (mean $=0.76$ g ± 0.02 SE) than counterparts from control pools (0.51 g ± 0.02; $F_{1,12}=58.50$, $P<0.001$),
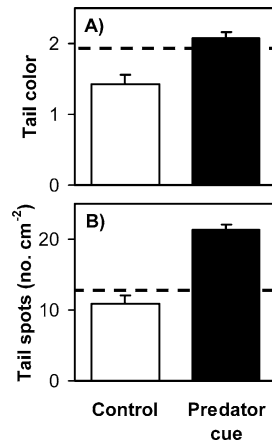

Fig. 1a,b Effects of predator chemical cues and time of day on the proportion of *R. palmipes* tadpoles **a** visible and **b** swimming in the plasticity experiment. Proportions were averaged across 6 day and 6 night observations per pool, and means (+SE) from seven pools per treatment are shown
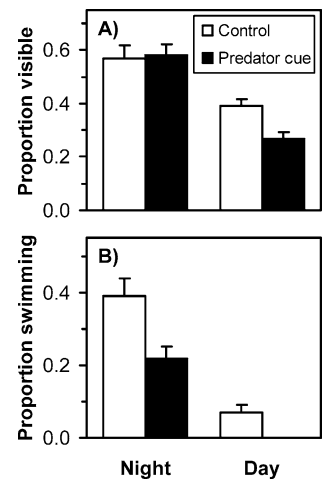
Table 1 Repeated-measures ANOVA of tadpole behavior in the phenotypic plasticity experiment. The proportion of tadpoles visible and the proportion of visible tadpoles that were swimming are compared between predator treatments, and across observation times (day vs night) within predator treatments

Response	df	MS	F	P
Visibility				
Between treatments				
Predator	1	0.026	1.69	0.218
Error	12	0.015		
Within treatments				
Time	1	0.450	90.05	0.001
Time × Predator	1	0.036	7.23	0.020
Error	12	0.005		
Swimming				
Between treatments				
Predator	1	0.323	62.75	0.001
Error	12	0.005		
Within treatments				
Time	1	1.460	81.82	0.001
Time × Predator	1	0.004	0.21	0.656
Error	12	0.018		

Fig. 3 Effects of predator chemical cues on the size-adjusted morphology of *R. palmipes* tadpoles in the plasticity experiment. Data are residuals following regression of log-transformed measurements against PC-1; see Materials and methods for details. Means (±SE) of the average residual value from seven pools per treatment are shown. *Asterisk* (*) signifies $P<0.05$
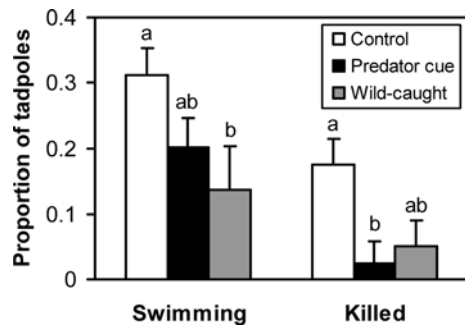
though there were no effects on developmental stage (mean =26.9 in both treatments) or survival (100% in control, 98±2% in predator treatment). Exposure to predator cues induced more black spots ($F_{1,12}=27.39$, $P<0.001$) and a marginally darker background color ($F_{1,12}=3.95$, $P=0.071$; Fig. 2).

Predator cues induced significant differences in tadpole morphology (Wilks' $\lambda=0.08$, $F_{6,7}=13.38$, $P=0.002$). Tadpoles in the predator treatment were significantly larger in overall size than control tadpoles (mean PC-1: 0.10 ± 0.01 vs -0.10 ± 0.01; $F_{1,12}=114.4$, $P<0.001$). After adjusting for overall size, tails of tadpoles exposed to predator cues were shorter ($F_{1,12}=8.09$, $P=0.015$), deeper ($F_{1,12}=8.94$, $P=0.012$), and had deeper muscles ($F_{1,12}=6.38$, $P=0.027$) than counterparts in control pools (Fig. 3). Body length was somewhat reduced in the predator treatment ($F_{1,12}=3.18$, $P=0.100$), while tail muscle width was unaffected ($P=0.376$).

Periphyton biomass, measured as chlorophyll *a*, was significantly higher in the predator treatment (median=10.6 mg/m^2, range 5.1–29.1) than in control pools (median=7.4 mg/m^2, range 1.9–10.4; Mann–Whitney $U=9.0$, $\chi^2=3.92$, $P=0.048$). Ammonium concentrations were also marginally higher in the predator treatment (median=13.6 μg N/L, range 2.1–43.5) than in controls (median=6.6 μg N/L, range 0.6–10.5; Mann–Whitney $U=5.0$, $\chi^2=3.33$, $P=0.068$). There was a positive relationship between concentrations of chlorophyll *a* and ammonium within the 11 experimental pools analyzed ($F_{1,9}=4.86$, $P=0.055$), though relatively little variance was explained ($R^2=0.35$).

Comparison to wild-caught tadpoles

Discriminant function analysis correctly assigned 37 of 48 (77%) tadpoles to the predator treatment and 35 of 49 (71%) tadpoles to the control treatment. When applied to the residuals on PC-1 from the wild-caught individuals, 40 of 48 (83%) tadpoles were assigned to the control group and the remaining eight to the predator group. Only wild-caught tadpoles in the lower third of the size range were assigned to the predator treatment; all larger tadpoles were grouped in the control treatment. ANOVAs of individual components of morphology supported this pattern of assignments. Tadpoles from the control and wild-caught groups had statistically similar body length and depth compared to those from the predator treatment, and control tadpoles were intermediate between the wild-caught and predator groups in tail length, depth, and muscle width (Table 2). The only aspect of morphology shared by wild-caught and predator-treatment tadpoles was deep tail muscles compared to the control group.

Size-adjusted tail pigmentation of wild-caught tadpoles was also intermediate between values from experimental groups (Wilks' $\lambda=0.74$, $F_{4,282}=11.69$, $P<0.001$; Fig. 2). The wild-caught and predator groups were similar in background color (pairwise comparison, $P=0.992$), and significantly darker than control tadpoles ($P<0.001$ in both cases). In contrast, the control and wild-caught tadpoles

Table 2 Comparison of morphology of wild-caught and experimental tadpole groups. Mean residual values are shown for each morphological dimension following regression against PC-1 to correct for differences in overall size. *Letters* indicate pairwise differences between tadpole groups

Population	*n*	PC-1 range	Mean residuals on PC-1					
			Body length	Body depth	Tail length	Tail depth	Muscle depth	Muscle width
Wild-caught	48	−0.398–0.245	0.002a	0.005a	0.013a	−0.016a	0.006a	−0.009a
Control	49	−0.327–0.088	0.004a	0.003a	0.002b	0.002b	−0.010b	0.000ab
Predator	48	−0.098–0.314	−0.007b	−0.008b	−0.015c	0.014c	0.004a	0.008b

Fig. 4 Effects of a free-ranging *Belostoma malkini* on behavior and mortality of *R. palmipes* tadpoles in the predation experiment. Tadpoles were from the control (*white bar*) and predator (*black bar*) treatments of the plasticity experiment, or wild-caught from a natural pool (*grey bar*). Swimming was averaged across five daytime observations per pool, and mortality was recorded at the conclusion of the experiment. Means (+SE) from eight pools per tadpole group are shown, and *letters* indicate significant differences between groups

both lacked dark spots (P=0.443) when compared to individuals from the predator treatment ($P<0.001$ in both cases).

Predation experiment

The three tadpole groups differed in behavior and survival when exposed to a free-ranging *Belostoma* (Wilks' λ=0.30, $F_{6,38}$=5.20, $P<0.001$; Fig. 4). Groups differed significantly in swimming activity ($F_{2,21}$=5.51, P=0.012) but not in visibility (P=0.321). Post hoc comparisons indicated that control tadpoles swam significantly more than wild-caught ones (P=0.029) and marginally more than the predator-treatment group (P=0.100). The wild-caught and predator groups were similar in swimming (P=0.784).

Differences between tadpole groups in survival were also significant ($F_{2,21}$=5.18, P=0.015; Fig. 4). Survival of control tadpoles (83%) was considerably lower than that of counterparts from the predator (98%; P=0.017) or wild-caught groups (95%; P=0.056). The predator and wild-caught groups had similarly low mortality (P=0.847). Based on the distinctive condition of tadpole corpses recovered at the conclusion of the experiment, all mortality could be unambiguously interpreted as predation by *Belostoma*.

Discussion

Rana palmipes tadpoles exhibited plasticity in behavior, pigmentation, and morphology in response to chemical cues from a caged predator. Each component of tadpole phenotype shifted in the direction predicted to reduce risk of predation based on studies with temperate species. For example, enhanced tail pigmentation probably directs the attention of predators away from the body, decreasing the probability that an attack will be lethal (Caldwell 1982;

Doherty et al. 1998; Hoff and Wassersug 2000; Van Buskirk et al. 2003). This study is the first to show a pigmentation response to predator cues in a ranid tadpole (Fig. 2), though *R. palmipes* do not develop as much dark tail pigmentation as hylid tadpoles (McCollum and Leimberger 1997; Van Buskirk and McCollum 2000a) or larval newts (Van Buskirk and Schmidt 2000).

Curtailed swimming by *R. palmipes* tadpoles in the presence of predator chemical cues is expected to reduce the likelihood of being detected by predators (Feder 1983; Azevedo-Ramos et al. 1992), and is a common response to predators by many tadpole taxa (Skelly 1994; Relyea 2001a; Van Buskirk 2002a). Our results also suggest that nocturnal foraging is important for *R. palmipes* (Fig. 2). As illustrated by vertical migration in zooplankton (Lampert 1989), inactivity and use of spatial refuges during the day can reduce exposure to visual predators, and night-time feeding may be sufficient to meet energetic needs. Diel periodicity in tadpole behavior has received little attention but could be widespread, particularly as a response to predators (see also Peacor and Werner 2001). It was also notable that behavioral differences between treatment groups during the plasticity experiment persisted even when all tadpoles were exposed to a free-ranging predator in the predation experiment. This lack of behavioral response to a change in predator regime contradicts the expectation of rapid matching of behavior to the concentration of chemical cues from predators (see also Semlitsch and Reyer 1992; Van Buskirk 2002b; Van Buskirk et al. 2002; Relyea 2003).

The changes in tadpole morphology elicited by predator cues are also expected to reduce risk of predation. *R. palmipes* developed a shorter, deeper tail, a deeper tail muscle, and marginally reduced body length in the presence of the caged predator (Fig. 3). Selection experiments indicate that increases in tail depth and muscle size reduce susceptibility to predatory dragonflies (Van Buskirk et al. 1997; Van Buskirk and Relyea 1998; Van Buskirk and Schmidt 2000). Similar patterns of plasticity in body and tail dimensions have been recorded in tadpoles of ten ranid frogs from North America and Europe (Relyea 2001a; Van Buskirk 2002a), though exact responses depend on the tadpole and predator species tested (Relyea 2001a). In fact, cues from *Belostoma* induced no morphological changes in bullfrog tadpoles (*R. catesbeiana*), and small tails with larger bodies in green frogs (*R. clamitans*; Relyea 2001a).

As predicted from the direction of phenotypic shifts, tadpoles with the predator-induced phenotype suffered less mortality from a free-ranging *Belostoma* than counterparts from the control treatment (Fig. 4). This survival advantage in the presence of predators is one of the basic requirements for the evolution of predator-induced plasticity (Tollrian and Harvell 1999). *Belostoma* are among the most common predators at the study site, and they clearly pose a threat to *R. palmipes* tadpoles despite having lower capture efficiency and longer handling time than some other predator taxa (Relyea 2001b). Our results indicate that these pierce-and-suck predators both induce

phenotypic shifts in *R. palmipes* and select for the induced phenotype. Further work is needed to test whether the trait shifts documented in this study match the trait-specific selection imposed by *Belostoma* (e.g., Van Buskirk et al. 1997; Van Buskirk and Relyea 1998 for dragonflies), and to determine the costs of the predator-induced phenotype in the absence of predators.

The doubling of *R. palmipes* growth when exposed to predator cues was unexpected, and is unusual in predator-cue experiments (see also Relyea and Werner 2000; Barnett and Richardson 2002). Enhanced growth despite decreased foraging rates can result when over-harvesting of resources is suppressed (Abrams 2003), as has been observed when tadpoles respond to predator cues by reducing their activity (Peacor 2002; Peacor and Werner 2000, 2001). The decreased swimming and increased biomass of tadpoles and algae in the predator treatment of the plasticity experiment may be attributable to this trait-mediated indirect pathway, however it is also possible these changes were by-products of a nutrient subsidy created by feeding the caged predator (e.g., Persson 1997; McCollum et al. 1998). Marginally higher ammonium concentrations in the predator treatment support the subsidy hypothesis. The positive relationship between algal biomass and ammonium concentration was expected in this nitrogen-limited system, and algal biomass on cobbles in the river (11.8 mg/m^2; Flecker et al. 2002) is higher than observed in the control treatment but comparable to the predator treatment.

If nutrients from feeding the predator increased algal growth rates, then tadpoles would have grown faster and swum less due simply to increased food availability (Anholt et al. 2000). Peacor (2002; Peacor and Werner 2001) found that the effects of nutrient supplementation were outweighed by predator-induced modifications of foraging behavior in 1,300 l mesocosms, however this result may depend on the rate at which predators are fed, the water volume of the system, and the nutrient-limitation status of primary producers. In our small experimental pools with nitrogen-limited algae, we suspect that the nutrient subsidy from the caged predator was at least partly responsible for enhanced tadpole growth. In any case, the effects of nutrient loading to mesocosms through caged predators merit further consideration from experimental community ecologists.

Comparing effects of behavior and morphology on risk

Though behavioral and morphological responses to predators have been documented in a wide variety of taxa (Havel 1987; Tollrian and Harvell 1999), there have been few tests of the relative importance of these two suites of traits in reducing risk of predation. Such assessments have been complicated by the fact that many components of phenotypes often shift in concert in plasticity experiments. We tried to circumvent this problem by testing the performance of both the wild-caught and experimentally induced tadpole phenotypes in the predation experiment.

Wild-caught tadpoles possessed a combination of behavioral and morphological traits that did not match either of the experimentally induced phenotypes, but instead combined elements from each. The swimming activity of wild-caught tadpoles was similar to that of tadpoles from the predator treatment, and significantly lower than that of control tadpoles (Fig. 4). In contrast, wild-caught tadpoles were more similar to control tadpoles in all morphological traits except tail muscle depth.

The morphological disparity between wild-caught and predator-induced tadpoles contrasts with previous comparisons of amphibian larvae exposed to predators under natural and experimental conditions (Van Buskirk and McCollum 1999; Van Buskirk and Schmidt 2000). We cannot resolve the specific influences on the phenotype of wild-caught tadpoles, but it is notable that their large bodies and shallow tails may facilitate rapid growth (Van Buskirk et al. 1997; Van Buskirk and Relyea 1998), and experimental increases in competitor density induce morphological shifts in these directions (Relyea 2002) and dampen responses to predator cues (Relyea 2002; Relyea and Hoverman 2003; Relyea 2004). The shaded pool from which wild-caught tadpoles were collected contained both many predators and a high density of *R. palmipes* (>1,000 tadpoles in ~5 m^2). This raises the possibility that wild-caught tadpoles combined predator-induced behaviors with a competitor-induced morphology to simultaneously maximize growth rates and minimize risk of predation.

Regardless of their causes, the phenotypic contrasts among tadpole groups offer an opportunity to examine the relative influence of morphology and behavior on risk of predation. If morphology had a stronger influence, wild-caught and control tadpoles would have been expected to suffer similarly high mortality when exposed to a lethal predator. If behavior were more important, wild-caught and predator-induced tadpoles would have shared low risk. The results of the predation experiment support the primacy of behavior; wild-caught tadpoles were killed less often than control tadpoles ($P=0.056$), and were comparable in risk to predator-treatment tadpoles (Fig. 4). This suggests that wild-caught tadpoles compensated for any risk associated with their morphology by swimming less than control tadpoles. This conclusion must be tempered by the fact that wild-caught tadpoles possessed deep tail muscles, and perhaps other unmeasured traits, like those of predator-induced tadpoles. However, the low mortality of wild-caught tadpoles would not have been predicted based on the body of experiments demonstrating that tadpoles with larger tails and smaller bodies have lower risk of predation.

Several earlier studies concluded that morphology is more important than behavior because groups of morphologically distinct tadpoles differed in risk of predation despite a lack of behavioral differences (Van Buskirk et al. 1997; Van Buskirk and McCollum 1999). However, an individual-level analysis of the association between phe-

notype and risk of predation indicated that swimming activity was a better predictor of risk than any single morphological trait, though the integrated predator-induced morphotype also enjoyed a survival advantage (Van Buskirk and McCollum 2000a). Comparing these studies to ours is complicated by differences in level of analysis and species tested. In addition, the natural cobble and leaf substrates used in our experimental arenas probably offered very different opportunities for foraging and concealment than the fully artificial conditions used in most other studies. Habitat complexity is inversely related to risk of predation in experiments with tadpoles (Babbitt and Tanner 1998), and the efficacy of particular phenotypic traits for reducing risk may be affected by experimental venue (see also Skelly and Kiesecker 2001; Skelly 2002).

Conclusions

Amphibian larvae experience a wide variety of environments in nature (Gascon 1992; Wellborn et al. 1996; Van Buskirk and McCollum 1999), and this study indicates that much remains to be learned about the causes and consequences of phenotypic variation. The plasticity experiment demonstrated that *R. palmipes* respond to predator cues by shifting their phenotype, and the predation experiment confirmed that these changes reduce risk of predation. However, wild-caught tadpoles that lacked most components of the predator-induced morphotype were no more vulnerable to a lethal predator. Together, these patterns suggest that behavioral and morphological responses to predators can be decoupled, and behavioral shifts alone may be sufficient to reduce risk in some situations.

Further field surveys of phenotypic variation among tadpole populations are needed. Such surveys will provide important context for results from mesocosm studies by quantifying the phenotypic responses of tadpoles to natural variation in the abundances of predators, competitors, and food resources. In addition, testing the performance of both naturally occurring and experimentally induced phenotypes within controlled experiments may strengthen inferences about phenotype-performance relationships by expanding the range of phenotypic variation that can be tested.

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