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Predators Induce Morphological Changes in Tadpoles of *Hyla andersonii*

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Predators can affect the development, fitness, and behavior of prey species in myriad ways. In response to the threat of predation, tadpoles can alter growth rate, morphology, and foraging behavior. Changes to tadpole development have the potential to alter life history characteristics and are therefore of interest in species of conservation concern. Using experimental mesocosms, we explored how non-lethal predators affected the larval development of the Pine Barrens Treefrog, *Hyla andersonii*, a near-threatened species in the United States. We found that caged dragonflies (*Anax junius)* induced darker tail coloration and deeper tail fins in tadpoles of *H. andersonii*, but the dragonflies did not affect tadpole behavior, survival, or size at metamorphosis. Non-lethal predator presence also induced greater within population variation in the tail color trait compared to populations without predators. This result suggests that there may be underlying genetic variation in the ability to express phenotypically plastic traits, a concept that should be explored further because it has implications for the evolution of inducible defenses. These findings support the existence of an adaptive syndrome among hylid tadpoles, where tadpoles develop conspicuous tail morphology in response to larval dragonfly predators.

PHENOTYPIC plasticity, the ability of an organism to develop different phenotypes based on external stimuli, is a strategy that can benefit organisms living in heterogeneous environments because different phenotypes may be favored under different environmental conditions (Relyea, 2004). For prey species, the flexibility to express a defensive phenotype may be advantageous when predatory risk is variable. Inducible defenses are traits that are expressed in predator presence that can reduce prey susceptibility to predation (Harvell, 1990). These traits may be plastic rather than fixed because of fitness trade-offs associated with behavioral, morphological, or physiological changes that can occur in the presence of predators (Van Buskirk and Schmidt, 2000; Wilson et al., 2005).

Tadpoles have been used as a model system for studying inducible defenses, predator avoidance, and fitness trade-offs for decades. Past research suggests that predator presence can greatly influence tadpole behavior, growth, and morphology (McCollum and Van Buskirk, 1996; Van Buskirk, 2000; Relyea, 2001). In addition to predator presence causing decreased activity levels in tadpoles, which can lead to decreased growth rates (Werner and Anholt, 1993; Relyea, 2002), there can be morphological differences in tadpoles raised in predator-free ponds compared to those raised in ponds with predators (McCollum and Van Buskirk, 1996; Relyea, 2001). The defensive phenotype expressed by prey can differ based on the species of predator present (Relyea, 2001; Hoverman et al., 2005). For example, tadpoles of *Lithobates pipiens* had deeper tail fins in the presence of fish predators but not in the presence of larval odonate predators, suggesting that tadpoles can distinguish among predator species (Relyea, 2001). The ability to accurately respond to different predators is likely advantageous when a phenotype is protective in the presence of one species of predator but not another. For example, Kishida and Nishimura (2005) demonstrated that *Rana pirica* develop different morphologies in response to different predators. Tadpoles that had a phenotype matching the predator that induced it had higher survival compared to tadpoles whose phenotype did not match the predator present (Kishida and Nishimura, 2005). This supports the notion that predator-specific phenotypic responses are likely advantageous.

Although an inducible defense may provide an immediate benefit to the prey species (i.e., predator avoidance), potential trade-offs may ultimately lead to fitness costs for the prey (DeWitt et al., 1998; Van Buskirk, 2000). For example, some tadpoles produce conspicuously pigmented tails in response to predators, including larval dragonflies (Smith and Van Buskirk, 1995; McCollum and Van Buskirk, 1996). Plastic phenotypes that involve significant tail coloration may result in trade-offs because of high energetic costs associated with producing pigmentation (Grether et al., 2001). Despite theory predicting that inducible defenses must be associated with fitness costs or else traits would be fixed rather than plastic, some studies have found no evidence of reduced fitness among tadpoles as a result of predator-induced changes during development (Van Buskirk and Saxer, 2001; Relyea and Hoverman, 2003). Furthermore, tadpoles of *Acris blanchardi* still express their tail spot in the absence of predatory aeshnid larvae (Carfagno et al., 2011), suggesting the allocation cost of this phenotype is negligible under some conditions. However, other costs, such as increased vulnerability to a non-focal predator, may also explain why a trait is not constitutively expressed (Carfagno et al., 2011).

Predator-induced phenotypic plasticity has been demonstrated in tadpoles of many hylid species. Tadpoles of *Hyla versicolor* (Van Buskirk and McCollum, 2000a) and *Hyla chrysoscelis* (McCollum and Van Buskirk, 1996) develop morphological changes in tail color and shape that reduce attacks by predators. Furthermore, tadpoles of *A. blanchardi* with dark tail tips co-occur more frequently in ponds with high densities of aeshnid larvae (Caldwell, 1982), suggesting that although this trait seems to be polymorphic, its induction may depend on environmental variation in predator presence. It is clear that phenotypes consisting of conspicuous tail markings or coloration are a common response of hylid tadpoles to larval odonate predators (e.g., McCollum and Van Buskirk, 1996; Van Buskirk and McCollum, 2000a; LaFiandra and Babbitt, 2004). However, closely

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related hylid species may differ in whether or not they manifest inducible defenses (Smith and Van Buskirk, 1995) such as morphologically conspicuous tails. Conspicuous tadpole tails may direct predatory dragonfly attacks toward the tail, which can be a non-lethal injury, as opposed to a potentially lethal attack on the vulnerable tadpole body (McCollum and Van Buskirk, 1996; Van Buskirk et al., 2003).

The Pine Barrens Treefrog (*Hyla andersonii)* is a habitat specialist with an unusual relictual geographic distribution, with populations in southern New Jersey, the Carolinas, Alabama, and the Florida Panhandle. Despite this disjunct range, recent genetic evidence suggests that the taxon should still be considered a single species (Warwick et al., 2015). Due to its limited range and relative rarity, knowledge of the ecology of *H. andersonii* remains rather incomplete. Adults breed primarily in acidic, fish-free temporary water in shrub bog habitats in eastern USA. They are an IUCN Red List near-threatened species and are considered to be at risk of decline because of habitat loss (IUCN, 2016). *Hyla andersonii* is also listed as a threatened species in New Jersey (NJDEP, 2019), a species in need of management in South Carolina (Bennett and Buhlmann, 2015), and is considered imperiled in Florida (Florida Fish and Wildlife Conservation Commission, 2018). Adult *H. andersonii* breed asynchronously from mid-April to mid-July in New Jersey (Morin et al., 1990), and populations in New Jersey may be larger than populations in the rest of the species' range (Warwick et al., 2015). Tadpoles of *H. andersonii* are known to potentially compete with other aquatic organisms including herbivorous insects and other anuran larvae (Morin et al., 1990; Pehek, 1995). Tadpoles of *H. andersonii* are also readily eliminated from pond communities by predatory fish and salamanders (Kurzava and Morin, 1998). Larval odonates co-occur in ponds across the range of *H. andersonii* and are known to predate tadpoles. In a previous study of *H. andersonii* (Morin et al., 1990), we noticed that tadpoles sometimes manifested conspicuous darkly pigmented tails when they co-occurred with aeshnid dragonfly larvae in natural and artificial ponds.

Here we experimentally tested whether tadpole behavior, morphology, or development in *H. andersonii* changed in response to the non-lethal presence of an important predator, larvae of the odonate *Anax junius*. Tadpoles were reared in mesocosms with or without caged larvae of *A. junius* to determine whether *H. andersonii* displayed phenotypically plastic traits. We hypothesized that that tadpoles raised in ponds with odonate predators would display changes consistent with an inducible defense including the development of a conspicuous tail and slower growth compared to conspecifics reared in predator-free ponds.

MATERIALS AND METHODS

Artificial ponds.—We constructed 12 artificial ponds/mesocosms using 359-liter cylindrical polyethylene stock tanks that were housed at the Hutcheson Memorial Forest of Rutgers University (Somerset County, New Jersey). The tanks were filled with well water on 27 April 2018. Each tank received 200 g of dry grassy plant litter raked from the surrounding area to add habitat complexity, plus 15 g of Purina® Rabbit Chow as an added source of nutrients. We added 700 ml of a mixture of phytoplankton and zooplankton collected from a single pond in the New Jersey Pine Barrens to each tank on 2 May 2018 to provide an inoculum for food web development and subsequently added another 400 ml of plankton from the source pond where breeding frogs were collected to each tank on 3 May 2018. Each artificial pond was covered with a square lid constructed from wood and fiberglass screening to prevent colonization of ovipositing insects. In each tank, we provided three "ladders," pieces of fiberglass screening draped over the side of the tank, so froglets had a path for emergence from the water once metamorphosis began.

Experimental design.—Our experimental design included a predator treatment ($n = 6$ artificial ponds) and a predator-free control ($n = 6$ artificial ponds). Treatments were spatially randomized among artificial ponds using a block design. Our predator treatment included three individually caged larval odonates (late instar *Anax junius*), and the predator-free mesocosms contained three empty cages to control for possible effects of the cages alone. Odonate larvae were individually caged to prevent cannibalism. Our field sampling indicated that larvae of *A. junius* can be abundant in pond-edge vegetation, and although not quantified here, the density of *A. junius* used in mesocosms likely reflects natural conditions. Cages were made of fiberglass screening with plastic tubing at both ends to create a cylinder that was approximately 50 centimeters in length and 15 cm in diameter. These cages allowed larval dragonflies to move vertically in the water column and were permeable to zooplankton, which served as a food source. Cages were suspended at mid-depth at equidistant points around the circumference of the mesocosms by a strand of monofilament fishing line. We checked cages every other day for dead or metamorphosed dragonflies and replaced individuals as needed (approximately three individuals were replaced per tank over the course of the experiment). Caged predators have also been used in previous studies of other anuran species (Van Buskirk, 2001; Relyea and Hoverman, 2003), although these studies used conspecific tadpoles as a food source rather than zooplankton.

A caged-predator design allowed us to analyze the effect of non-lethal predator presence on tadpole development without confounding effects of reduced survival or density that might result from direct dragonfly predation. Furthermore, withholding feeding of conspecific tadpoles allowed us to determine if predator presence alone was strong enough to elicit phenotypic responses among prey. Larval odonates were collected from ponds in Bass River State Forest (Ocean County, NJ) and Somerset County, New Jersey. *Anax junius* were added to mesocosms on 9 May 2018.

We collected six breeding pairs of *Hyla andersonii* on 3 May 2018 from a single pond in the New Jersey Pine Barrens as a source of hatchling tadpoles. The frogs were placed in covered plastic containers where they deposited eggs overnight. After oviposition, frogs were returned to the site of capture (<48 hours later). The eggs subsequently hatched on 10 May 2018 (Day 0), and hatchlings were counted for addition to the tanks on 11 May 2018. Each mesocosm received a total of 270 hatchling tadpoles pooled from the six clutches of eggs. Mesocosms received supplemental additions of 10 g of rabbit chow on 29 May, 25 June, 15 July, and 7 August 2018 to provide continued support for the pond food webs. We returned all froglets to the field site where their parents were collected after they completed metamorphosis.

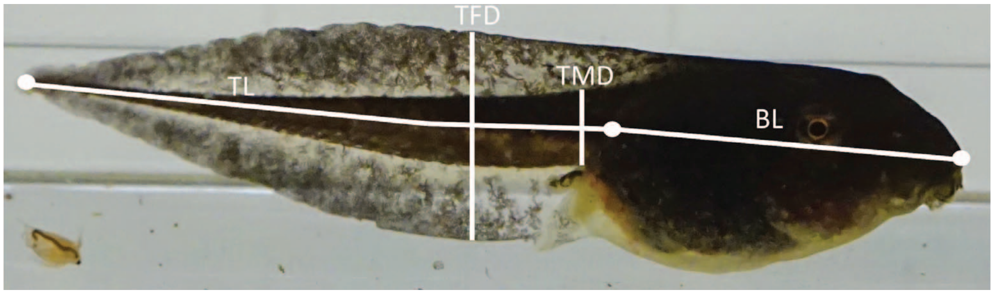

Fig. 1. Photograph of *H. andersonii* depicting the locations of tail measurements. BL = Body length, TL = tail length, TMD = tail muscle depth, TFD = tail fin depth. Body length was measured from tadpole snout to start of tail muscle. Tail length was measured along the tail muscle from start of tail muscle to tail tip. Tail muscle depth was measured at the base of the tail, and tail fin depth was measured from fin top to bottom at the deepest location along tail length. Total tadpole length (TTL) was determined by adding TL to BL.

Morphological responses.—We measured tadpole morphology at three time points during development: 32, 39, and 46 days after tadpoles hatched. These days were picked to 1) allow ample time for morphological changes to occur in the presence of the predator, 2) allow tadpoles to be large enough that they could be safely sampled with replacement, and 3) to see how morphology may differ across time until tadpoles metamorphosed. On each sampling day, we haphazardly collected (using hand nets) 25 tadpoles from each pond to photograph their phenotype for subsequent analysis. The only exception to this sampling effort occurred on Day 46 for ponds two ($n = 2$ tadpoles, –predator treatment) and three ($n = 15$ tadpoles, –predator treatment), when the onset of tadpole metamorphosis made it impossible to collect 25 tadpoles from those ponds. Tadpoles were sampled with replacement on each sampling day. Tadpoles were individually placed into a narrow aquarium with a standard grid in the background and were photographed using a Sony alpha a6000 digital camera. All photos were taken with F/7.1 aperture, a 1/320$^{\text{th}}$ second exposure time, and ISO 3200. Artificial light conditions were held constant across individuals and the three sampling dates.

We analyzed tadpole tail color and morphology using ImageJ 1.51p (Rasband, 2012). Tail color was analyzed by outlining the tadpole tail using the polygon tool and measuring the mean gray value of the photos' pixels. This unitless value is an indicator of how light or dark an image is, with lower values indicating there are more dark pixels present in the photo, and higher values indicating there are more light pixels in the photo (range: 0–255). Tadpole tail length (TL), body length (BL), tail muscle depth at the base of the tail (TMD), maximum tail fin depth (TFD), and total tadpole length (TTL) were measured after setting the scale within each photo based on the background grid (Fig. 1). Measurements of tadpole tail length, body length, tail muscle depth, and tail fin depth were divided by total tadpole length to determine what proportion of total tadpole length each response variable accounted for. These standardized measures relative to total tadpole length were used as response variables in all analyses.

Behavioral responses.—Tadpole visibility was measured at four time points on days 28, 32, 40, and 42 of larval development. This measurement evaluates the relative proportion of tadpoles that are visible to those that are hiding in the leaf litter. Before counting, all mesocosm lids were removed and tadpoles were allowed to acclimate after this disturbance for approximately five minutes. Artificial ponds were approached slowly and from a direction that ensured no shadows were cast across the water surface. We counted the number of tadpoles visible (i.e., those not hiding in leaf litter) for a total of two counts per mesocosm for each sampling day. The mean number of tadpoles visible on each day was standardized by dividing by the number of individuals that survived to metamorphosis in each mesocosm to correct for possible differences in tadpole counts due to survival differences among ponds.

Life history responses.—Artificial ponds were checked daily for metamorphosing froglets starting on day 41. Froglets that had emerged from the water were collected and housed in plastic containers where they completed metamorphosis, as determined by complete tail resorption. We measured wet weight and snout–urostyle length of each froglet after tails had been fully resorbed. We calculated larval period as days from tadpole hatching (day 0) to when tail resorption was complete. Overall survival was calculated as the percent of individuals surviving to metamorphosis in each pond. Growth index was calculated as wet mass at metamorphosis divided by larval period.

Statistics.—All statistical analyses were conducted in R version 3.3.2 (R Core Team, 2016). We used population (i.e., mesocosm) means for analyzing response variables to retain independence when testing for the effect of predator presence. We used linear mixed effects models (LMMs) with normal error distribution using the lme4 package (Bates et al., 2014) to test for differences in tadpole morphology. When testing for changes across time, repeated measurements of mesocosms nested within the spatial blocks were used as random effects to account for non-independence (Pinheiro and Bates, 2000). Predator presence and sampling date were used as fixed effects. Block was included as a random factor in the model, and this term was excluded if it did not improve model fit as assessed by likelihood ratio tests. LMMs were also used to test for differences in within pond tail color variance by using mean within pond variance as a response variable. We used the "Anova" function in the car package (Fox et al., 2016) to run Wald chi-square tests to determine confidence in model estimates. To test for differences in tadpole visibility between treatment groups and over time, we used a generalized linear mixed effects model (GLMM). Repeated measurements of mesocosms were treated as random effects.

To determine overall predator effects, we used MANOVAs on tadpole morphological measurements and metamorphosis measurements. Block interaction effects were not signif-

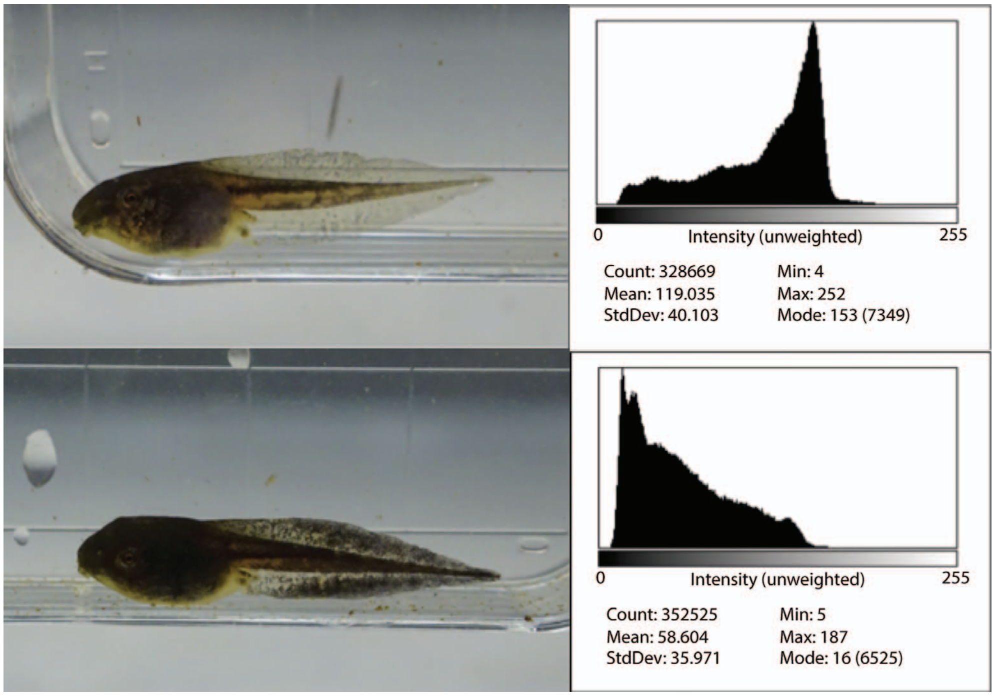

Fig. 2. Representative photos of tadpoles of *H. andersonii* reared without predators (top) and with predators (bottom) and their corresponding ImageJ pixel color histograms. Lower values indicate darker pixels, and higher values indicate lighter pixels in the image. The y-axis of the histograms indicates the frequency of pixels for each darkness score.

icant in either model, so these degrees of freedom were pooled with the error term. We then conducted univariate ANOVAs to determine which response variables contributed to the multivariate responses. We used a principal component analysis (PCA) for different tail morphological measurements (tail color, tadpole length, and standardized measures of body length, tail length, muscle depth, and fin depth) and a separate PCA for metamorphosis measurements (mass and length at metamorphosis, survival to metamorphosis, and length of larval period). PCAs provided a way to concisely visualize the multivariate responses of the tadpoles to the predator treatments, while identifying which variables exhibited correlated responses to the treatments. We used Pearson correlation matrices to compare principal component scores to original variables to interpret the principal components. We also used a Pearson correlation matrix to determine how tadpole development traits were related to one another. We applied a Bonferroni correction to adjust for multiple comparisons when comparing trait correlations.

RESULTS

Tail morphology.—Tadpoles in ponds with predators had significantly darker tails (Fig. 2) on all three sampling days (LMM: $\chi^2 = 16.2$, df = 1, $P < 0.001$; Fig. 3). There was no effect of sampling date ($\chi^2 = 2.34$, df = 2, $P = 0.31$) or treatment by date interaction ($\chi^2 = 0.25$, df = 2, $P = 0.88$) on tail color. Within population variance in tail color was significantly greater in predator treatments (LMM: $\chi^2 = 5.7$, df = 1, $P = 0.017$; Fig. 4), and there was no effect of sampling date ($\chi^2 = 4.01$, df = 2, $P = 0.13$) or treatment by date interaction ($\chi^2 = 4.15$, df = 2, $P = 0.13$).

Tadpoles in mesocosms containing predators developed significantly greater standardized tail fin depths (LMM: $\chi^2 = 10.8$, df = 1, $P < 0.001$; Fig. 5). Standardized tail fin depth also significantly decreased over time as tadpoles grew ($\chi^2 = 38.4$, df = 2, $P < 0.001$), but there was no treatment by date interaction ($\chi^2 = 2.04$, df = 2, $P = 0.36$). Predators did not affect tadpole body length, tail length, tail muscle depth, or total tadpole length, but these variables did change significantly over time as tadpoles grew and developed (Fig. 5; Table S1; see Data Accessibility).

Patterns in multivariate analysis of tadpole morphological data were consistent across sampling days, so Day 32 results are presented for simplicity. The first two principal components accounted for 80.5% of the variation in tadpole morphology based on Day 32 measurements (Table 1), and the principal components plot shows that populations clustered in ordination space based on the presence or absence of predators (Fig. 6). A MANOVA on tadpole morphological traits from Day 32 confirms a significant effect of predators on the morphology of *H. andersonii* that

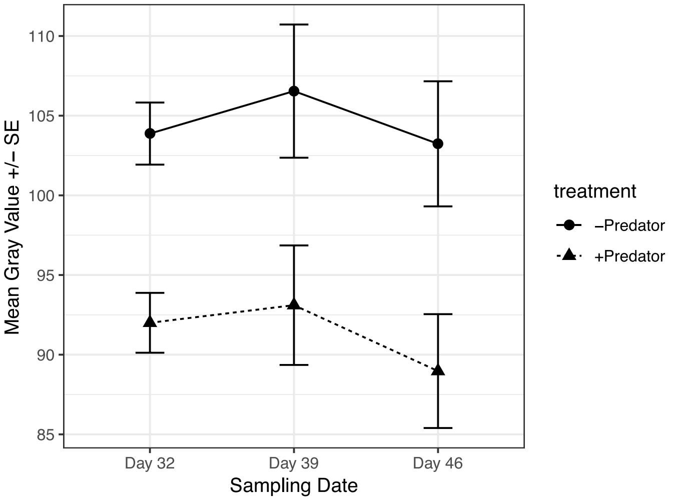

Fig. 3. Tadpole tail color (mean gray value) was significantly lower (=darker tails) in predator treatments compared to non-predator controls (LMM: $\chi^2 = 16.2$, df = 1, $P < 0.001$). Sampling date had no effect on tail color ($\chi^2 = 2.34$, df = 2, $P = 0.31$).

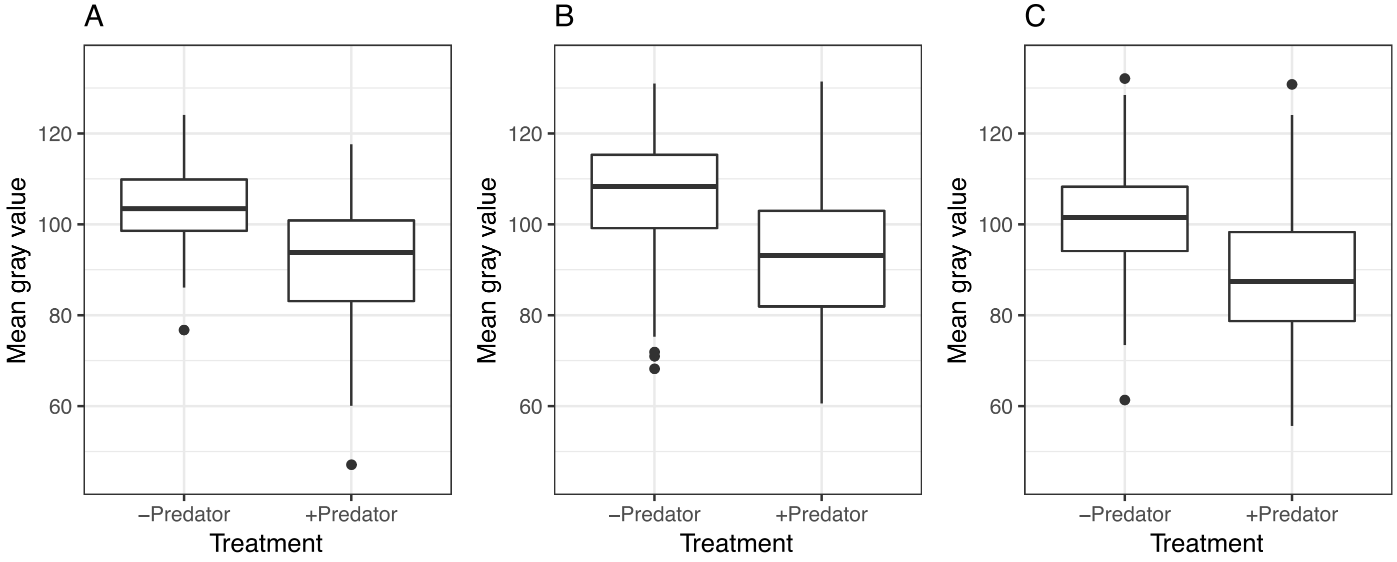

Fig. 4. Box and whisker plots showing range of tadpole tail mean gray values (a measure of tail darkness). Lower mean gray values indicate darker tail coloration. (A) Day 32 (+predator treatment mean gray value±SD = 92±12.82; −predator control = 103.88±8.54), (B) Day 39 (+predator = 93.11±15.22; −predator = 106.54±12.95), and (C) Day 46 (+predator = 88.97±14.46; −predator = 100.68±12.63). Individuals were pooled across ponds within treatments on each sampling date. Variance was significantly greater in predator treatments compared to non-predator controls (LMM: $\chi^2 = 5.7$, df = 1, $P = 0.017$).

was driven by effects on tail coloration and standardized tail fin depth (Table 2).

Tadpole behavior.—Predators had no detectable effect on tadpole visibility (GLMM: $\chi^2 = 0.82$, df = 1, $P = 0.36$), but tadpole visibility significantly decreased over time ($\chi^2 = 526.4$, df = 3, $P < 0.001$).

Life history measurements.—Tadpoles metamorphosed between days 41 and 166, with 73% of tadpoles across ponds metamorphosing within 70 days and 90% of tadpoles metamorphosing within 100 days. A total of 1,923 froglets emerged across all 12 mesocosms, making mean survival to metamorphosis among artificial ponds 59.4% (range: 17.8–77.8%; Table 3). There was a significant overall multivariate effect of predators and block on metamorphosis measurements, but there were no significant predator effects in any of the univariate tests for mass and length at metamorphosis, larval period, and survival to metamorphosis (Table 4). The first two principal components accounted for 97.6% of the variation in the metamorphosis measurements PCA (Table S2; see Data Accessibility). Population density (i.e., the number of survivors per mesocosm) was significantly negatively correlated with size at metamorphosis (Table 5).

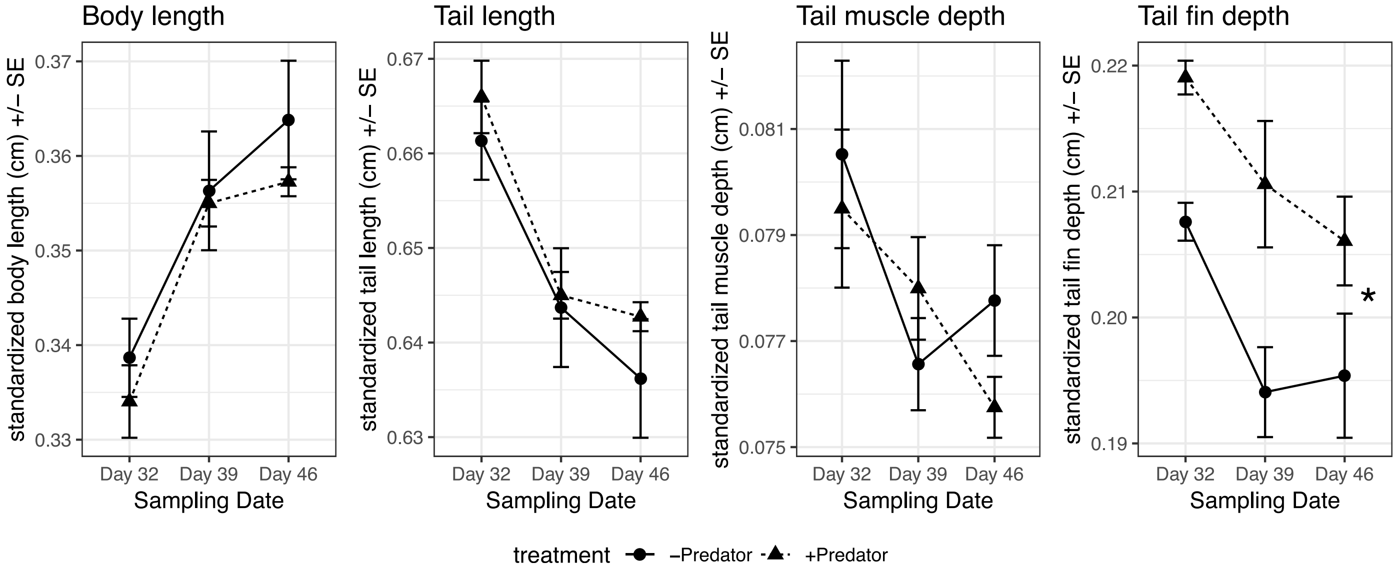

Fig. 5. Comparison of relative tadpole size across sampling dates and predator treatments. All measurements were standardized against total tadpole length to account for size differences among individual tadpoles. *$P < 0.05$ between treatments.

Table 1. Principal component analysis of Day 32 morphological traits of tadpoles of *H. andersonii* reared with or without predators. PC1 and PC2 indicate the first two principal components, and they accounted for 80.5% of the variance between treatments. The values in the table are the correlation coefficients of each morphological trait with the principal component scores.

Response variable	PC1	PC2
Tail color Day 32	−0.24	−0.78
Standardized tail fin depth	0.43	0.82
Standardized tail muscle depth	−0.65	−0.39
Standardized body length	0.76	−0.59
Standardized tail length	−0.76	0.59
Total tadpole length	−0.93	−0.12
% of variance explained	44.8%	35.7%

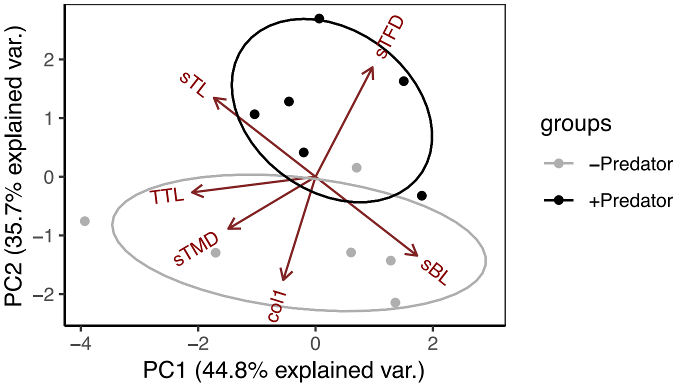

Fig. 6. Principal component analysis of the tail morphological traits total tadpole length (TTL), tail color (col1), and standardized body length (sBL), standardized tail muscle depth (sTMD), standardized tail length (sTL), and standardized tail fin depth (sTFD) sampled on Day 32.

DISCUSSION

Our findings document an important feature of the poorly known ecology of *Hyla andersonii* and contribute additional support for the existence of a probable adaptive syndrome in hylid tadpoles that represents convergent phenotypically plastic responses (conspicuous tails) to a specific class of aquatic predators. Although we did not specifically test whether or not the induced tail phenotype increased likelihood of survival after an attack, the morphological response observed in *H. andersonii* closely resembles that of other anuran species to aeshnid dragonflies, which has been previously found to be adaptive (e.g., McCollum and Van Buskirk, 1996; Van Buskirk and McCollum, 2000a). In response to larval aeshnid dragonflies, *Hyla chrysoscelis* (McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997; Richardson, 2006), *Hyla versicolor* (Van Buskirk and McCollum, 2000a), and *Hyla femoralis* (LaFiandra and Babbitt, 2004) develop conspicuous tail coloration. Tadpoles of *Acris blanchardi* lose their tail spots that are expressed both in the presence of *Anax* sp. and in no-predator controls when exposed to fishes (Carfagno et al., 2011), indicating that the conspicuous tail morphology is not beneficial against all aquatic predators. A strategy such as unpalatability might be expected in tadpoles that live in permanent ponds and encounter different classes of predators, which seems to be the case in bullfrog tadpoles and fish predators (Kats et al., 1988; Werner and McPeek, 1994).

In this study, tadpoles exposed to predators also had deeper tail fins, a response to larval dragonflies observed in other larval amphibians (Van Buskirk and Relyea, 1998; Van Buskirk and Saxer, 2001). *Pseudacris triseriata* also develop larger tail fins and greater tail muscle depth when exposed to odonates (Smith and Van Buskirk, 1995). While deeper tail fins are hypothesized to improve tadpole swimming performance and thus facilitate escape from predators, experimental evidence demonstrating this benefit is lacking (Van Buskirk and McCollum, 2000b). Rather, deeper and more conspicuously pigmented tails may increase survival by encouraging non-lethal predator attacks of the tail (Van Buskirk et al., 2003). The deeper- and darker-tailed tadpoles observed in predator treatments are consistent with the "lure effect," where changes in tadpole morphology in the presence of dragonfly larvae lure predator attacks toward the tadpole tail and away from the vulnerable tadpole head (Van Buskirk et al., 2003). These changes may facilitate the ability of tadpoles to tear away from a predator's grasp (Hoff and Wassersug, 2000) and survive with ripped tails that can be regrown.

Inducible defenses can be generated from visual, mechanical, and/or chemical cues produced in the presence of the predator. Chemical cues seem to be particularly important for predator detection in aquatic animals (Stauffer and Semlitsch, 1993; Eklöv, 2000). Chemical cues can arise from predator odor (Eklöv, 2000), the cues produced from conspecific digestion (Stabell et al., 2003; LaFiandra and Babbitt, 2004), or alarm pheromones generated by attacked conspecifics (Hews, 1988; Schoeppner and Relyea, 2005). For example, *H. chrysoscelis* exposed to caged dragonfly larvae that are fed tadpole conspecifics differ in tail color and shape from those reared in the presence of starved predators (McCollum and Leimberger, 1997). We found that tadpoles of *H. andersonii* exposed to caged dragonflies on a planktivorous diet developed conspicuously pigmented tails, suggesting that predator consumption of tadpoles is not necessary to elicit this response. If zooplankton were producing alarm cues during predation, it is probable that *H. andersonii* would be unable to recognize these cues given previous research suggesting that prey only respond to alarm cues produced by conspecifics or closely related heterospecifics (Schoeppner and Relyea, 2009). However, it is possible that general predator digestion cues, which are absent when predators are starved, are adequate to trigger a phenotypic response. Taken together, these results suggest that non-contact cues are sufficient to induce changes in tail morphology among hylids. More research is needed to identify the specific metabolites that might trigger tadpoles to display an inducible defense and determine if the metabolites that elicit the defense vary across species.

Despite changes in morphology during the development of *H. andersonii*, we did not observe any allocation costs in terms of reduced survival or size at metamorphosis in tadpoles reared in the presence of predators. Theory predicts that there should be some cost associated with expressing an inducible defense, otherwise the trait would be expected to be expressed constitutively (McCollum and Leimberger, 1997; Relyea, 2002). There is some empirical evidence of allocation costs of inducible defenses in anurans (McCollum and Van Buskirk, 1996; Van Buskirk, 2000). However, other studies on tadpoles have found allocation costs associated with inducible defenses to be difficult to document (Van

Table 2. Summary of MANOVA and univariate ANOVAs analyzing the effect of predator treatment and block on morphological measures, total tadpole length (TTL), standardized tail muscle depth (sTMD), standardized tail fin depth (sTFD), and tail color (col1) of *H. andersonii* on Day 32. Block and treatment interactions were not significant, so the degrees of freedom were pooled with the error term. Bolded values indicate statistical significance at the alpha = 0.05 level.

	Wilk's λ	F	DF	*P*
MANOVA				
Predator treatment	0.11	8.15	1, 7	**0.03**
Block	0.26	0.6	3, 7	0.8
Univariate ANOVAs				
TTL treatment		0.64	1, 7	0.45
Block		1.95	3, 7	0.21
sTMD treatment		0.18	1, 7	0.69
Block		0.68	3, 7	0.59
sTFD treatment		30.0	1, 7	**<0.001**
Block		0.76	3, 7	0.55
col1 treatment		16.35	1, 7	**0.005**
Block		0.50	3, 7	0.70

Table 4. Results of overall MANOVA and univariate ANOVAs analyzing the effect of predators on mass and length at metamorphosis, larval period, and survival to metamorphosis. Block and treatment interactions were not significant, so the degrees of freedom were pooled with the error term. Bolded values indicate statistical significance at the alpha = 0.05 level.

	Wilk's λ	F	DF	*P*
MANOVA				
Predator treatment	0.10	8.98	1, 7	**0.028**
Block	0.008	4.55	3, 7	**0.009**
Univariate ANOVAs				
Mass treatment		0.62	1, 7	0.46
Block		1.86	3, 7	0.22
Length treatment		1.22	1, 7	0.30
Block		3.5	3, 7	0.08
Period treatment		1.99	1, 7	0.20
Block		11.18	3, 7	**0.005**
Survival treatment		2.99	1, 7	0.13
Block		1.2	3, 7	0.38

Buskirk and Saxer, 2001; Benard, 2004). Our results are consistent with previous studies showing that caged predators rarely elicit smaller size or shorter time to metamorphosis in their tadpole prey (Relyea, 2007; Relyea and Rosenberger, 2018), suggesting that allocation costs of inducible defenses are uncommon (Benard, 2004). Because costs of defenses are likely greatest when food is scarce and limited resources are allocated to the defensive trait instead of growth (Van Buskirk, 2000), allocation costs may be unlikely in artificial settings where resources are often abundant. It is also possible that allocation costs associated with the expression of a plastic trait during larval development may not materialize until later life stages, such as in adult survival or performance (Van Buskirk and Saxer, 2001; Benard and Fordyce, 2003; Relyea and Hoverman, 2003).

Another potential explanation is that the cost of the induced phenotype is not observable within the range of conditions imposed by our study design. For example, allocation costs involving reduced growth might be undetectable in an experimental setting if increased resource levels offset them. Peacor (2002) found that predator-induced behavioral changes among tadpoles facilitated algal growth, which indirectly caused an increase in tadpole growth. However, this scenario seems unlikely to explain the results of our study because we found no effect of predators on tadpole behavior. Lastly, alternative costs could exist in a larger community context if an induced phenotype resulted in increased vulnerability to other predators that employ feeding mechanisms that differ from those used by odonates, such as fishes (Carfagno et al., 2011). Despite the lack of observable predator effects on tadpole growth and survival, tadpole density (i.e., number of survivors) in each pond was significantly negatively correlated with froglet size at metamorphosis. This is consistent with previous work suggesting that frog body size at metamorphosis can be influenced by tadpole density (Wilbur and Collins, 1973).

Spatial orientation of the artificial ponds in the field (i.e., blocks) influenced larval development. Specifically, mesocosms closer to the forest edge of the field experienced more shade, which could have contributed to differences in productivity across mesocosms (Morin et al., 1990). While we did not observe differences in the number of tadpoles visible between predator and predator-free treatments, overall tadpole visibility decreased over time in all ponds. We suggest that this decrease in tadpole visibility could have been due to a shift in resources in the pond. At the start of the experiment, tadpoles spent time grazing on the side of the tank closer to the surface. Eventually, the periphyton resources on the side of the tank could have become depleted, and consequently tadpoles may have shifted to acquiring resources at the bottom of tank, making them less visible during the census.

Another pattern that emerged was that the overall within population variation in the expression of tail pigmentation was greater when predators were present. Tadpoles in each mesocosm were a mixture of six different sibships, so it is possible that the observed phenotypic variation within populations represents genetic variation in levels of plasticity. Relyea (2005) found that plasticity in some tadpole traits had patterns of high heritability among *Lithobates sylvaticus,* suggesting a genetic basis for predator-induced traits. Moderate levels of plasticity, which may manifest as variability in plastic expression of a trait, may therefore have the ability to

Table 3. Summary statistics for survival, larval period, and size at metamorphosis for *H. andersonii* reared either in the absence (–predator) or presence (+predator) of dragonfly larvae. Growth index is calculated as wet mass at metamorphosis divided by larval period.

		Survival %	Mass (mg)	Larval period (days)	Growth index (mg/days)
Predator treatment	*n*	Mean±1 standard deviation			
–Predator	6	52.4±17.9	206.1±55.3	67.7±20.5	3.5±1.8
+Predator	6	66.3±9.5	189.6±15.6	61.6±6.1	3.1±0.5

Table 5. Pearson correlation matrix showing relationships between response variables in *H. andersonii*. Population density refers to the number of individuals surviving to metamorphosis in each mesocosm. Growth index is calculated as mass at metamorphosis divided by larval period. Bolded values indicate correlation coefficients that were significant after applying a Bonferroni correction adjusting for multiple comparisons.

	Tail color Day 32	Mass at metamorphosis	Length at metamorphosis	Larval period	Growth index
Mass at metamorphosis	0.14				
Length at metamorphosis	0.21	**0.97**			
Larval period	0.19	–0.63	–0.70		
Growth index	0.08	**0.97**	**0.96**	–0.78	
Population density	–0.26	**–0.89**	**–0.85**	0.37	**–0.82**

promote genetic evolution (Price et al., 2003), given that individuals expressing a trait are more likely to survive and reproduce and plasticity has some genetic basis. Variance in the ability to express a trait may be highest in communities that have temporal or spatial variability in predator presence, since there may be performance trade-offs of predator-induced traits in different environments (Van Buskirk et al., 1997). Such communities may be most likely to experience plasticity-first evolution, which occurs when initially plastic traits displayed due to environmental variation lead to evolutionary adaptation because selection acts on phenotypes that have some genetic basis (West-Eberhard, 2005). Depending on the reliability of environmental cues, selection could lead to genetic polymorphism or genetic assimilation (Semlitsch et al., 1990). Theoretical work has shown that phenotypic plasticity can affect heritable trait variation and subsequent evolution (Draghi and Whitlock, 2012), and recent empirical evidence supporting plasticity-first evolution (Levis and Pfennig, 2016; Levis et al., 2018) suggests this topic deserves further evaluation in natural systems.

Our study documents the presence of a probable inducible defense in *Hyla andersonii*, a species of conservation concern throughout its fragmented range. Phenotypically plastic tail morphologies appear to be a common feature in hylid tadpoles exposed to odonates, suggesting the presence of an adaptive syndrome in this group. More research is needed to determine if increased trait variation seen in the presence of predators represents genetic variation in the expression of the inducible trait. A study evaluating the responses of tadpoles of different sibships to predator presence would provide valuable insight to the role of genetic variation in inducible defenses in this system. Future work should also continue to explore if the conspicuous-tail adaptive syndrome is a general feature of hylid tadpoles that regularly encounter odonate predators in fish-free ponds.

DATA ACCESSIBILITY

All data generated during the present study are available from the corresponding author upon request. Supplemental information is available at https://www.copeiajournal.org/ce-19-241.

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