PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Innes-Gold AA, Zuczek NY, Touchon JC. 2019 Right phenotype, wrong place: predator-induced plasticity is costly in a mismatched environment. *Proc. R. Soc. B* **286**: 20192347.
http://dx.doi.org/10.1098/rspb.2019.2347

Received: 7 October 2019
Accepted: 8 November 2019

Subject Category:
Ecology

Subject Areas:
developmental biology, ecology, evolution

Keywords:
phenotypic plasticity, anura, antipredator responses, development, adaptive, trade-off

Author for correspondence:
Justin C. Touchon
e-mail: jutouchon@vassar.edu

THE ROYAL SOCIETY
PUBLISHING

Right phenotype, wrong place: predator-induced plasticity is costly in a mismatched environment

Anne A. Innes-Gold, Nicholas Y. Zuczek and Justin C. Touchon

Biology Department, Vassar College, 124 Raymond Ave, Poughkeepsie, NY 12604, USA

NYZ, 0000-0001-5243-7544; JCT, 0000-0002-1643-348X

Like many animals, tadpoles often produce different, predator-specific phenotypes when exposed to risk of predation. It is generally assumed that such plasticity enhances survival in the presence of the predator and is costly elsewhere, but evidence remains surprisingly scarce. We measured (1) the survival trade-off of opposing phenotypes developed by *Dendropsophus ebraccatus* tadpoles when exposed to different predators and (2) which specific aspects of morphology drive any potential survival benefit or cost. Tadpoles developed predator-specific phenotypes after being reared with caged fish or dragonfly predators for two weeks. In 24 h predation trials with either a fish or a dragonfly, survival was highest in the groups with their matched predator, and lowest among with those the mismatched predator, with predator-naive controls being relatively intermediate. Then, using a large group of phenotypically variable predator-naive tadpoles, we found that increased survival rates are directly related to the morphological changes that are induced by each predator. This demonstrates that induced phenotypes are indeed adaptive and the product of natural selection. Furthermore, our data provide clear evidence of an environmental cost for phenotypic plasticity in a heterogeneous environment. Such costs are fundamental for understanding the evolution and maintenance of inducible phenotypes.

1. Introduction

Many organisms have evolved to produce different phenotypes in response to varying environmental conditions, a phenomenon known as phenotypic plasticity [1,2]. These changes occur during the lifespan of a single organism and therefore require no genetic change to occur. This capacity to produce multiple phenotypes has long been of interest to ecologists and evolutionary biologists because of the theoretical fitness enhancement it allows [3]. Phenotypes that are adaptive increase survival and thus may alter the population of individuals that survive to procreate; as such, plasticity has been hypothesized to influence the course of evolution [4]. The ability to flexibly produce adaptive phenotypes is widespread in plants [5,6], as well as both invertebrate [7,8] and vertebrate [9–11] animals.

Although many types of abiotic and biotic environmental cues can induce adaptive developmental changes in animals, those originating from predators are among the most well studied. Predation cues may induce behavioural or morphological defenses, as well as shifts in the timing and magnitude of life-history events [7,12–14]. In aquatic systems, induced morphological defenses are often elicited by waterborne chemical cues released from predators during or after predation events [9,15,16]. These chemical cues are complex in nature, and prey are generally sensitive to both the magnitude and timing associated with different predators and predation events [17,18]. While many studies of predator-induced morphology have focused on invertebrates, which may develop spines, keels, neckteeth and helmets to defend themselves from predators [19,20], vertebrates also demonstrate plastic morphological responses to predators. For example, the crucian carp, *Carassius carassius*, develops a deeper body when reared with chemical cues from the piscivorous pike, *Esox lucius* [21].

Much of the theory about the evolution and maintenance of phenotypic plasticity relies on the idea that induced responses should be costly in an alternative environmental scenario. In other words, a response to one predator should be harmful in the presence of a different predator. However, despite the wealth of research on inducible phenotypes, such examples are rare. The freshwater cladocerans *Daphnia* spp., one of the classic model systems for understanding plasticity, are theorized to display this trade-off in response fish and midge larvae predators which select for different phenotypic responses [20,22], but this does not appear to have been demonstrated empirically. Plastic responses to a predator can make *Daphnia* susceptible to other risks, such as parasites [23] and populations evolve in directions suggestive of trade-offs [24], but demonstrating actual environmental costs is still paramount for understanding what maintains phenotypic plasticity.

Amphibians have become a model system for studying plastic behavioural and morphological responses to predators [9,25]. Tadpoles generally alter their behaviour by reducing activity levels in response to chemical cues from predators, which often confers survival advantages in the presence of dragonfly larvae [26–28]. For example, reduced movement can result in reduced access to food for prey, leading to slower growth rates [29], although this is not always the case [27]. Tadpoles also demonstrate plastic morphological responses when raised in the presence of predators, including changes in body size, shape and colour [30–32]. There is no single defensive morphology for all predators and some tadpoles develop different phenotypes depending on the predator present [33,34]. In response to insect predators, tadpoles generally develop deeper, more colourful tails and smaller bodies, while fish predators induce shallow achromatic tails [31,34].

While many studies have demonstrated the occurrence of these predator-induced phenotypes, actual evidence that they are adaptive is limited to select examples. *Rana pirica* tadpoles develop predator-specific phenotypes, and when placed in direct contact with a predator, survival rates were highest among groups of tadpoles with the predator-specific phenotype [33]. Similar results were seen in *Pseudacris regilla* tadpoles, where predator-induced morphologies increased the time to predation when in direct contact with the corresponding predator, and mortality was the highest among groups paired with a mismatched predator [28]. However, outside of these two examples little research has demonstrated the actual adaptiveness of inducible plasticity, which, when compared with the relative abundance of examples of plasticity, is rather striking. This suggests that either plasticity does not always help (e.g. [35]) or that it is difficult to experimentally demonstrate the adaptive nature of plasticity. Either way, the fact that tadpoles—and prey in general—often respond to different predators in unique ways suggests that there is no single adaptive response to predation, and that there may be costs and benefits to producing different phenotypes which warrant measure.

The pantless treefrog, *Dendropsophus ebraccatus*, is a Neotropical species common throughout Central America and northern South America [36]. Like most other amphibian larvae, *D. ebraccatus* tadpoles face potential predation from a great number of sources which vary spatially and temporally [37]. In response, *D. ebraccatus* appears to have two generalized responses to invertebrate or vertebrate predators; tadpoles develop large red and black tails in the presence of dragonfly larvae or giant water bugs and shallow colourless tails when reared with fish, whereas controls are generally intermediate [34,38]. The ability to produce these phenotypes is both highly variable and heritable [39]. Hypotheses for the functions of these different morphologies and colours relate to the hunting strategy of the predators. Fish are often thought of as efficient swimmers that pursue and repeatedly attack tadpoles, while aquatic invertebrates such as dragonfly nymphs or giant water bugs generally hunt alone, using a sit-and-wait strike method. The shallow tail developed in response to fish may allow for faster swimming than the deeper tail morphology [40], and the lack of colour may reduce unwanted attention [41]. The red and black tail spot seen in dragonfly-induced tadpoles may function as a lure to draw the predator's attack away from the tadpole's head [42,43]. The intermediate phenotype of the control treatment may represent a phenotype meant to balance the costs and benefits of the two extremes. In an environment where multiple species of predators are often, but not always, present and predator presence may change over time [37], these phenotypes would seem to represent adaptive, evolved responses to predator-specific risks. However, this remains untested.

Here we test if the phenotypes that *D. ebraccatus* develop when exposed to different predators are adaptive and attempt to determine the specific aspects of each phenotype which contribute to their relative success. In two separate experiments, we first measure survival trade-offs of predator-induced phenotypes and then secondly we test if differences in survival are attributable to certain morphological features. We hypothesized that survival rates would be highest for groups placed with their matched, inducing predator and conversely, would be lowest for groups placed with a mismatched predator, with controls being intermediate. Furthermore, we hypothesized that survival would be driven by the types of morphological changes induced by each predator, particularly those related to tail coloration and size.

2. Methods

The two experiments described here were conducted from September to December 2017 and September to December 2018, respectively, using a research colony of *D. ebraccatus* at Vassar College in Poughkeepsie, New York. In both experiments, adult male and female frogs were bred to obtain eggs, and thus tadpoles, for experiments. Frogs were placed into a 50 × 50 × 90 cm rain chamber containing artificial pond water (reverse osmosis water plus Kent Marine R/O Right and Kent Marine pH Stable) and several plants at approximately noon on the day of breeding. Frogs were 'rained' on for approximately 6 h to stimulate breeding activity. Pairs were either placed into a closed plastic aquarium with approximately 4 cm of artificial pond water or a one-gallon Ziploc bag and allowed to oviposit eggs overnight. We removed eggs from the side of the aquarium or plastic bag with forceps and transferred them to a plastic cup where they were misted four times daily and allowed to develop for 3 days. On the third day, the cup was flooded with artificial pond water to help stimulate hatching. After hatching, the tadpoles were allowed to develop for an additional 2 days to complete yolk resorption and development before beginning experiments. Ten pairs of frogs were bred for experiment 1 and 14 pairs of frogs for experiment 2.

(a) Experiment 1: measuring environmental costs and benefits of plasticity

After hatching, 66 tadpoles per family were haphazardly selected and digitally photographed with a ruler for scale. Extra tadpoles were used as feeders for predators during the experiment. The initial size of tadpoles was 6.69 ± 0.57 mm (mean ± s.d.). Twenty-two tadpoles were placed into each predator treatment (two more than necessary for the predation trials, in case of mortality). We placed the tadpoles in an 18 × 12 × 12 cm plastic aquarium with 2 l of artificial pond water and a mesh divider that split the tank into approximately two-thirds and one-third sections. The tadpoles were placed in the larger compartment and a predator, either the fish *Gambusia affinis* or the larval dragonfly *Aeshna* sp., was placed in the smaller compartment with a stick and a small section of 1-inch diameter PVC pipe. The third treatment was a predator-free control, which also contained the stick and the PVC pipe. Each day, the tadpoles were given one Sera micron-coated pellet of rabbit chow, and the predator was given three feeder tadpoles (raised and housed separate from the experimental tadpoles). If a predator did not eat at least two tadpoles for two consecutive days, it was swapped out for a predator of similar size. Dragonfly nymphs ate 2.73 ± 0.60 tadpoles per day, while fish consumed 2.46 ± 1.04 tadpoles per day (mean ± s.d.). Each day, tadpole faeces and any rabbit chow remaining from the previous day were removed. On alternating days, 1 l of water (half the volume of the tank) was poured out and fresh artificial pond water was added. Tadpoles developed in these treatments for 14 days.

After this two-week period, we haphazardly split the tadpoles from each tank into two groups of 10 tadpoles. If all 22 tadpoles survived, we haphazardly selected 20 for predation trials. If fewer than 20 survived, we split them evenly in two groups. All tadpoles in each group were lightly anaesthetized in MS-222 for approximately 5 min and then photographed laterally with a ruler and black colour plate to be used for colour correction. After photographing, we placed tadpoles into a plastic cup of artificial pond water from their original rearing tank to recover for 24 h while still being exposed to the predation cue. Thus, all tadpoles used in the experiment were equally exposed to anesthesia and were given ample time to recover, regardless of rearing environment or predation trial destination. After photography, a single fish or dragonfly larva was placed in a 50 × 25 × 30 cm glass aquarium containing 9 l of artificial pond water (approx. 9 cm deep) with four pieces of 1 inch diameter plastic PVC pipe and four artificial plants. Predators were left overnight to acclimate to the predation arena. The outside of each tank was covered in white paper, to prevent predators from seeing into neighbouring tanks. The predators used in these trials had been fed tadpoles throughout the weeks before the predation trials, but were not fed during the 2 days before the trial to ensure they would be hungry.

The next morning between 07.00 and 09.00, each set of 10 tadpoles was placed into an aquarium with either a fish or a dragonfly larva. Thus, tadpoles that had been raised with predators were placed with either their matched predator (the same species they had been reared with) or a mismatched predator (the species of predator they were not reared with). Predator-naive control tadpoles were set up in predation trials in the same manner. We set up a total of 56 predation trials ($n = 9$–10 trials per rearing treatment × predator combination).

Tadpoles were added as carefully as possible to the side of the tank opposite the predator. Predation trials ran for 24 h. The next morning, the predators were removed from the tank and the surviving tadpoles were anaesthetized and photographed as described above. For approximately half of trials ($n = 27$), a Kodak PixPro camera recorded the first several hours of the trial from directly above. We analysed the first hour of each camera's video footage to describe the predator's behaviour and number of successful and unsuccessful predation attempts. Predatory behaviour was categorized into either a strike (a singular motion towards the tadpole in an attempt to consume it) or a chase (the active pursuit of a tadpole). We recorded the time at which the action occurred, as well as the duration for chases, and if strikes or chases were successful (i.e. the target tadpole was consumed).

(b) Experiment 2: estimating predator selection on tadpole phenotypes

Fourteen pairs of frogs were bred in four separate cohorts of three to four pairs each. In each case, eggs were allowed to develop as above and tadpoles were allowed to mature for an additional 2 days to complete development after hatching, at which point they were combined in a single container to explicitly remove any family level effects. The goal of this second experiment was to create genetically variable co-housed individuals, resulting in phenotypically diverse animals which had identical rearing conditions. Tadpoles were raised in the same types of containers and were fed and cared for identically as in experiment 1, aside from the absence of a predator. In each of the first three cohorts, we set up five replicated groups of tadpoles and in the fourth cohort we set up four groups of tadpoles. All tadpoles were raised in groups of 25 in predator-free conditions for 14 days which were then split into two groups for predation trials with either fish or dragonfly larvae. As above, if more than 20 tadpoles survived, only 20 were haphazardly selected for predation trials. If fewer than 20 survived, the two groups were split evenly. On two occasions only 10 tadpoles survived and we only set up a single predation trial, each time with a fish. In total, we ran 17 predation trials with dragonfly larvae and 19 predation trials with fish. Tadpoles were anesthetized and photographed before and after predation trials as above, and predation trials were set up identically to above.

(c) Data analysis

Tadpole photographs were analysed in ImageJ v. 1.50i [44]. For each tadpole, five linear measurements were taken: total length, body length, tail length, maximum tail depth and tail muscle depth (see [34] for a description of measurements). The area of the tail spot was calculated by tracing the outline of the tail spot using the freehand tool, and coloration of the tail spot was measured in terms of hue, saturation and brightness values on a scale of 0–255. Hue indicates the shade of colour, with lower values indicating longer wavelength colours (i.e. reds) and increasing values moving up the colour spectrum. Saturation denotes the purity of a colour, with larger values indicating purer, more vibrant colours. Brightness denotes the amount of light reflected from the tail spot; since tadpoles were photographed on a white background, more transparent tails have higher brightness values and colourful tails have lower values.

Statistical analyses were conducted in R v. 3.6.1 [45]. In experiment 1, we used linear mixed effects models (LMMs) in the package lme4 [46] to test for effects of rearing treatment on morphology, and generalized linear mixed effects models (GLMMs) with a binomial error distribution to test for interacting effects of rearing treatment and predator treatment on survival. Binomial GLMMs were coded such that the response variable was a two-column table containing the number of

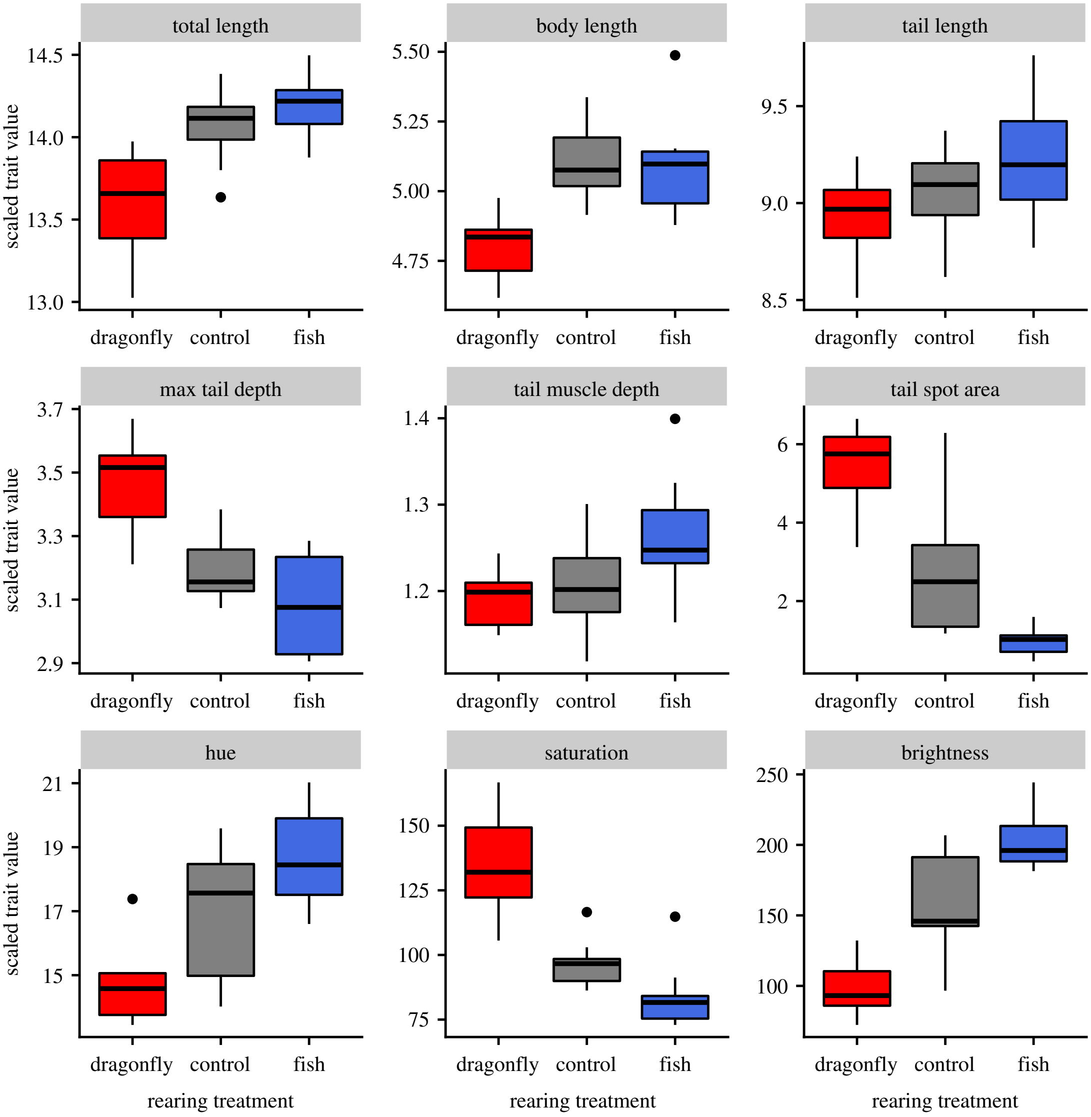

Figure 1. Morphology and coloration of *Dendropsophus ebraccatus* tadpoles varied substantially after being raised with dragonfly larvae, with fish or as predator-free controls for 14 days. Shown are six morphological measures and three tail spot coloration measures. Values shown are calculated from the estimated marginal means for each rearing tank, thereby accounting for variation in overall body size (as determined via principal components analysis) or size of the tail spot (for coloration measures). *Y*-axis units for morphological measures are millimetre for linear measures or square millimetre for tail spot area. Values for hue, saturation and brightness range from 0 to 255 with higher values indicating shorter wavelength colours, more pure colours, or brighter colours, respectively ($n = 57$ total trials). Box-and-whisker plots show the median (thick horizontal line), interquartile range (top and bottom of the coloured box) and either the most extreme values (ends of the whiskers) or 1.5 times the interquartile range and outliers (ends of the whiskers followed by points). (Online version in colour.)

surviving tadpoles and the number of tadpoles that were eaten ($n = 9$–10 trials per rearing treatment × predator combination). Family and rearing tank nested within family were included as random effects in all models to account for variation between families and the common rearing environment of tadpoles split into each pair of predation trials. Model fit was always checked by inspecting Q-Q plots and significance of predictors was assessed with nested likelihood ratio tests. Analyses of morphological plasticity included a size covariate in models, which was the first component of a principal components analysis (using the prcomp function) of all linear morphological measurements and tail spot area (e.g. [47]). Tail spot hue was log-transformed and tail spot area was square-root transformed to improve model fit. Analyses of hue, saturation and brightness included the square root of tail spot area as a covariate instead of PC1 [39]. In analyses of survival, we were most interested to know if the survival of tadpoles reared with each predator differed in each predation trial; we thus conducted post hoc analyses within each predator treatment comparing just the survival of fish- and dragonfly-reared tadpoles. Predator behaviours (i.e. attempted strikes and chases) were analysed with χ^2 goodness-of-fit tests. The presence of tail damage following predation trials was analysed with Fisher's exact test. Predator tanks were completely dismantled and tanks were cleaned in between rounds of predation trials, and trails were set up in a haphazard manner.

In experiment 2, our goal was to see if the phenotypes of groups of tadpoles differed before and after predation, which would provide evidence of selection. We used LMMs, including predator species and before/after predation as fixed effects. Before/after predation was also included as a random slope to account for the fact that tadpoles were measured twice and rearing tank and breeding cohort were included as separate (i.e. crossed) random intercepts. We excluded nine trials where predators did not eat any tadpoles as these provided no data for estimating predator selection ($n =$ six with dragonfly larvae and three with fish). Model fit and predictor significance were assessed as in experiment 1. Predator tanks were completely dismantled and cleaned in between rounds of predation trials as above.

Table 1. Results of linear mixed effects models examining the effect of rearing environment on different aspects of *Dendropsophus ebraccatus* tadpole morphology and coloration. See methods for details of random effects in models. In all analyses, the effect of rearing treatment accounts for variation in the covariate (PC1 or TSA), and vice versa. TSA was square-root transformed and hue was log-transformed to improve model fit. Significant effects are shown in italics.

response	predictor	χ^2	*p*-value
total length	rearing treatment	*26.07*	*<0.00001*
	PC1	*1218.75*	*<0.00001*
	rearing treatment : PC1	*22.71*	*0.00001*
body length	rearing treatment	*25.71*	*<0.00001*
	PC1	*987.54*	*<0.00001*
	rearing treatment : PC1	*18.59*	*0.00009*
tail length	rearing treatment	*19.47*	*0.00006*
	PC1	*1015.44*	*<0.00001*
	rearing treatment : PC1	*10.14*	*0.006*
max tail depth	rearing treatment	*30.33*	*<0.00001*
	PC1	*716.77*	*<0.00001*
	rearing treatment : PC1	5.79	0.055
tail muscle depth	rearing treatment	*22.72*	*0.00001*
	PC1	*602.23*	*<0.00001*
	rearing treatment : PC1	*8.86*	*0.011*
tail spot area	rearing treatment	*37.85*	*<0.00001*
	PC1	*261.04*	*<0.00001*
	rearing treatment : PC1	*123.1*	*<0.00001*
hue	rearing treatment	0.03	0.98
	TSA	*14.11*	*0.00017*
	rearing treatment : TSA	*6.75*	*0.034*
saturation	rearing treatment	*27.36*	*<0.00001*
	TSA	*103.2*	*<0.00001*
	rearing treatment : TSA	*11.64*	*0.003*
brightness	rearing treatment	*37.19*	*<0.00001*
	TSA	*119.62*	*<0.00001*
	rearing treatment : TSA	*51.34*	*<0.00001*

3. Results

(a) Experiment 1: measuring environmental costs and benefits of plasticity

As expected based on previous research, rearing treatment caused tadpoles to develop significantly different morphologies and coloration (figure 1). In general, dragonfly nymphs induced smaller bodies, deeper relative tails, and larger and more colourful tail spots, whereas fish induced the largest relative tail muscles and the smallest and least colourful tail spots (table 1 and figure 1). Control tadpoles were relatively intermediate, having long bodies and tails like fish-induced tadpoles, but somewhat deeper tails and smaller tail muscles like dragonfly-induced tadpoles. Control tadpoles also had intermediately coloured tail spots.

When tadpoles were exposed to predators, their survival was enhanced if they were with the matched predator that they had been reared with and reduced if they were with the mismatched predator (figure 2). Rearing treatment alone had no significant effect on survival ($\chi^2 = 1.14$, $p = 0.57$), whereas the efficacy of the predators differed considerably ($\chi^2 = 107.91$, $p < 0.0001$). Most importantly, there was a significant interaction between rearing treatment and predator, indicating the effect of the rearing environment differed when tadpoles were paired with each predator ($\chi^2 = 11.23$, $p = 0.004$). Specifically, survival of fish-raised tadpoles was nearly double that of dragonfly-raised tadpoles when they were exposed to fish predators (55.9% versus 29.0%, respectively; post hoc comparison: $p = 0.0002$; figure 2). The same pattern was visible for tadpoles exposed to dragonflies; dragonfly-raised tadpole survival was significantly higher than that of fish-raised tadpoles when exposed to dragonfly predators, although the difference was rather small (92.8% versus 84.6%; post hoc comparison: $p = 0.05$; figure 2). Predator-naive control tadpoles had intermediate survival with fish predators and were indistinguishable from dragonfly-reared tadpoles with dragonfly predators.

Dragonfly nymphs were more likely than fish to leave tadpoles injured with tail damage. In dragonfly predation

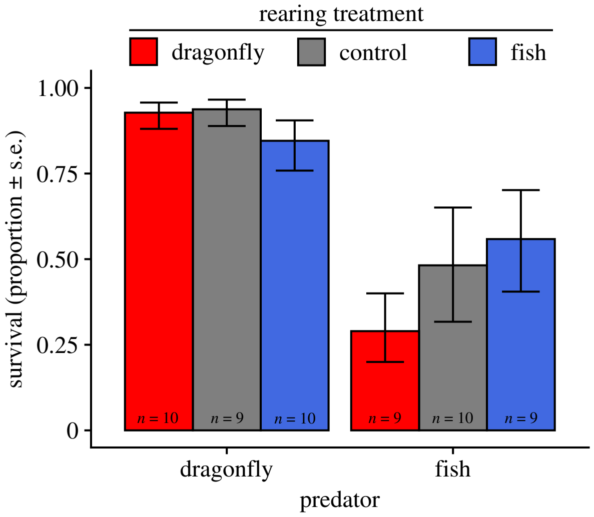

Figure 2. Tadpole survival was dependent on tadpoles being paired with their matched or mismatched predator. Tadpoles in predation trials with mismatched predators had lower survival than tadpoles with their matched predator. With fish predators, predator-naive controls were intermediate, whereas they were similar to matched tadpoles with dragonflies. Bars show the mean proportion survival ± standard error after 24 h in a structurally complex predation arena. $n = 57$ total trials. Sample sizes for each rearing treatment × predator combination are indicated in each bar. (Online version in colour.)

trials, nearly 8% of tadpoles ($n = 19$) survived with damaged tails, while only 3.2% of tadpoles ($n = 8$) survived fish predation attempts with tail damage (Fisher's exact test: $p = 0.028$).

The video footage of the predation trials showed that fish were much more active predators than dragonfly nymphs, which corroborates the finding that many more tadpoles were consumed in the fish predation trials. Fish attempted to strike the tadpoles over six times as often as dragonfly nymphs (table 2). Dragonfly nymphs, however, had a significantly higher success rate (10.3%) than fish, which only successfully consumed tadpoles 2.4% of the time (chi-square test: $\chi^2 = 5.16$, $p = 0.049$). In addition, fish were observed to chase and repeatedly strike at tadpoles, while this was rarely done by dragonflies (chi-square test: $\chi^2 = 3.73$, $p = 0.055$).

(b) Experiment 2: estimating predator selection on tadpole phenotypes

Predation was considerably higher in our second experiment, with dragonfly larvae consuming 47.4% of tadpoles and fish consuming 70% of tadpoles. Following predation trials, the phenotypic distribution of these predator–naive tadpoles was markedly different, indicating that predators had consumed particular individuals from the range of phenotypes present (table 3 and figure 3). Importantly, fish and dragonfly larvae generally had different effects on tadpole phenotypes, as indicated by significant interactions between predator type and the phenotypes of tadpole groups before and after predation (table 3). For example, small tadpoles (in terms of total length, body length and tail length) were disproportionately consumed by dragonfly larvae, leaving a population of larger tadpoles after predation events, whereas fish appeared to have little effect on the size distribution of tadpoles. Both predators selected for tail fin sizes, but in opposing directions: dragonfly larvae consumed tadpoles with shallower tail fins, whereas fish consumed tadpoles with deeper tail fins (figure 3). Fish also strongly selected against colourful tails, dramatically changing the distribution of tail spot saturation and brightness after predation. Tadpoles with highly saturated red and black tails were predated, shifting the distribution to tails with lower saturation and increased brightness (i.e. more transparent on the white background).

Table 2. The number of attempted and successful strikes and chases of *Dendropsophus ebraccatus* tadpoles, as observed in the video footage for each predator during the first hour of 24 h predation trials.

predator	attempted strikes	successful strikes	chases
dragonfly	39	4	2
fish	240	6	47

4. Discussion

Predator-induced phenotypic plasticity exists in countless prey organisms and many animals produce specific responses to different predators. However, despite abundant examples of predator-specific plasticity, relatively few studies have shown if these changes in phenotype provide actual survival benefits and if there are trade-offs associated with poor environmental matching. Moreover, the unique ways that prey response to different predators are even less studied, despite most likely being the norm instead of the exception. Here, we demonstrate that fish and dragonfly nymphs induced opposing phenotypes in *D. ebraccatus* tadpoles and that those phenotypes impacted the survival of tadpoles facing each type of predator. We also demonstrate that the morphological features that are induced by predators are in fact the phenotypic aspects which those predators select for.

Several clear examples of survival trade-offs have been seen in freshwater invertebrates. For example, giant water bug and crayfish predators each induce different responses in *Planorbella* snails; each response is advantageous with the matched predator but costly in the presence of the mismatched predator [48]. Similar trade-offs are hypothesized in numerous species of cladocerans [20]. In our experiment, *D. ebraccatus* tadpoles developed smaller bodies and deeper tails with large red and black tail spots if raised with dragonfly larvae, whereas in the presence of fish they developed shallower, clearer tails. These findings were consistent with previous literature focusing on phenotypic responses of tadpoles to different predators [27,33,34,49] but we are only aware of two studies which have demonstrated that phenotypes induced by one predator are not only advantageous with that predator, but costly with a different predator [28,33].

Our first experiment provides clear evidence that the phenotypes that *D. ebraccatus* tadpoles develop in response to fish and dragonfly larvae are adaptive yet potentially costly. Both phenotypes improved survival with the matched predators in relation to the mismatched predator (figure 2). This was most noticeable with fish, which were much more active, voracious predators than the dragonfly larvae. Although dragonflies

Table 3. Results of linear mixed effects models examining the effect of predation by fish or dragonfly larvae on different aspects of *Dendropsophus ebraccatus* tadpole morphology and coloration. See methods for details of random effects in models. TSA was square-root transformed and hue was log-transformed to improve model fit. Significant effects are shown in italics.

response	predictor	χ^2	*p*
total length	predator	*17.93*	*0.00002*
	before/after	2.26	0.133
	predator : before/after	*4.1*	*0.043*
body length	predator	*27.4*	*<0.00001*
	before/after	0.36	0.55
	predator : before/after	1.34	0.25
tail length	predator	*15.23*	*0.0001*
	before/after	3.38	0.066
	predator : before/after	*5.16*	*0.023*
max tail depth	predator	*12.04*	*0.0005*
	before/after	0.03	0.86
	predator : before/after	*5.53*	*0.019*
tail muscle depth	predator	*24.31*	*<0.00001*
	before/after	0.21	0.65
	predator : before/after	0.15	0.70
tail spot area	predator	*5.23*	*0.022*
	before/after	0.001	0.97
	predator : before/after	2.47	0.12
hue	predator	0.67	0.41
	before/after	2.25	0.13
	predator : before/after	0.77	0.38
saturation	predator	0.21	0.65
	before/after	2.82	0.09
	predator : before/after	*9.72*	*0.002*
brightness	predator	1.27	0.26
	before/after	*6.01*	*0.014*
	predator : before/after	*9.52*	*0.002*

consumed fewer tadpoles, dragonfly-reared tadpoles still fared better than those raised with fish.

While fish consumed more tadpoles than dragonflies in both experiments, they did not change the distribution of tadpole sizes present in experiment 2. This indicates that fish are less gape-limited than dragonfly larvae, able to consume relatively small or large tadpoles equally. Furthermore, we demonstrated that mosquitofish are more active predators and will chase tadpoles in an effort to consume them, which dragonflies did rarely (table 2). Tadpoles that were raised with fish in the first experiment did not reduce body size, which likely reflects two things. First, if fish are not gape-limited, there is no selection for smaller bodies in tadpole prey. Second, since *D. ebraccatus* does not divert resources towards increasing tail size and coloration in response to fish, there is no obvious physiological reason (an allocation cost *sensu* [50]) that they would not be able to maximize growth.

Fish did, however, exert strong selection against colourful tails, consuming tadpoles with highly saturated and darkly coloured tails (figure 3). The depigmentation of the tail spot is one of the most remarkable aspects of the defensive phenotype of *D. ebraccatus*, wherein tadpoles develop almost completely transparent shallow tail fins [34,39]. Tadpole tail coloration has most frequently been studied in relation to dragonfly nymph predation (e.g. [30,43,51,52]) and those studies that have addressed fish predation have not examined changes in tail coloration [27,28,53], perhaps because the species studied do not alter pigmentation in response to predation cues or because researchers did not measure it. Either way, we provide strong evidence that reducing the coloration of the tail spot contributes to the observed survival benefit we measured in *D. ebraccatus* (figure 2).

One of the more confusing results of our second experiment is that dragonfly larvae did not alter the distribution of tail spot coloration or tail spot area. Given that the colourful tail spot is the most dramatic aspect of the induced response to dragonflies, one would logically expect that tadpoles with less colourful tails would be more easily consumed if the tail spot were indeed adaptive. While adult dragonflies can have more ommatidia than any other insect [54] and have an incredible diversity of opsin genes

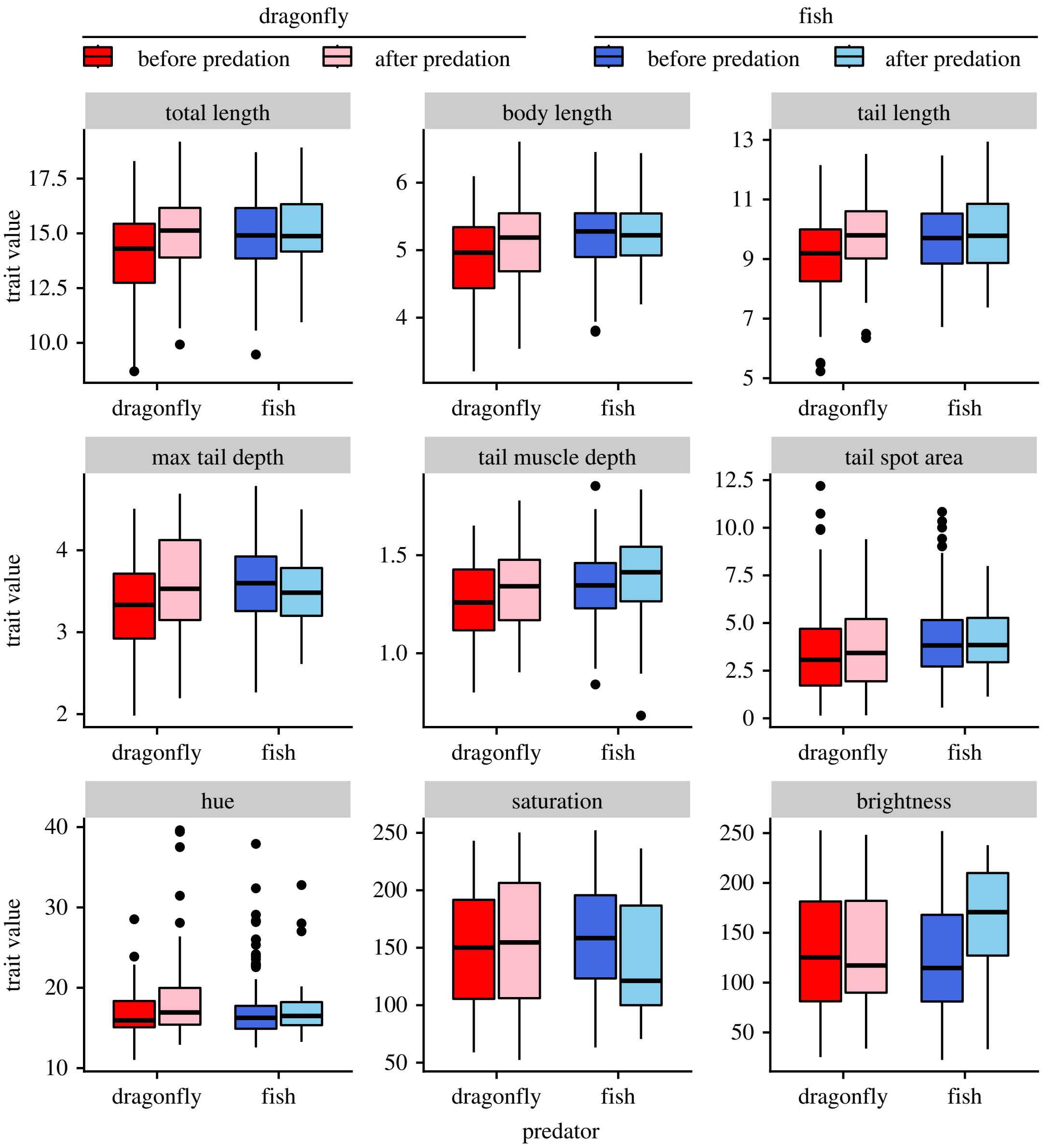

Figure 3. Fish or dragonfly larvae predators selected for certain phenotypic traits in groups of variable, predator-naive control tadpoles. Fish selected against large, dark and colourful tail spots, whereas dragonflies selected against small tadpoles and smaller tail spots. Predation trials were conducted for 24 h in structurally complex predation arenas ($n = 27$ total trials, 11 with dragonflies and 16 with fish). y-axis units for morphological measures are millimetre for linear measures or square millimetre for tail spot area. Values for hue, saturation, and brightness range from 0 to 255 with higher values indicating shorter wavelength colours, more pure colours or brighter colours, respectively. Box-and-whisker plots show the median (thick horizontal line), interquartile range (top and bottom of the coloured box) and either the most extreme values (ends of the whiskers) or 1.5 times the interquartile range and outliers (ends of the whiskers followed by points). (Online version in colour.)

expressed in the eye [55], the majority of these are not expressed in the larvae. Dragonfly larvae have long been known to use shape to identify and target prey [56], but it is not known to what extent they actually see colour. The most likely function of the colourful tail spot is as a lure to draw the strike away from the body of the tadpole [42,43] and the white background of our predation arenas may have removed the efficacy of the tail spot in this function. It is not difficult to imagine how a brown or green background in a pond might increase the contrast of the tail spot and make it stand out as compared to the body. Dragonflies did prey on tadpoles with shallower tail fins, suggesting that deeper tail fins aided in evading these predators, which has long been suggested by kinematic studies [40,57]. Future experiments should examine selection on tadpoles across a range of background colours to explore if tail spot coloration is adaptive under different environmental conditions.

Variation in survival with each predator almost certainly results from the morphological changes induced during development, although behavioural plasticity likely plays a role as well. The two predators, fish and dragonfly nymphs, demonstrated different hunting styles, potentially somewhat in response to induced changes in behaviour. *Dendropsophus ebraccatus* tadpoles raised with fish increase swimming activity while tadpoles raised with dragonflies reduce activity [58]. Fish in our experiment attempted strikes much more often than dragonfly nymphs and were much more active, repeatedly following and striking at tadpoles. Dragonfly nymphs more often remained stationary and waited for a tadpole to swim nearby before striking (table 2). Although fish were more voracious, the success rate of dragonfly nymphs was much higher, suggesting that their hunting tactics were more efficient.

The predator-free controls were surprisingly successful in both predation treatments, performing as well as dragonfly-reared tadpoles with dragonfly predators and nearly as well as fish-reared tadpoles with fish predators. Control tadpoles represent an interesting intermediate between the two induced phenotypes. They are morphologically most similar to the fish-induced morph, producing relatively long bodies and tails, with the exception of tail muscles that are most similar to the dragonfly morph. The depth of the tail fin and size and colour of the tail spot however are clearly intermediate between the two predator-induced morphs. Given the high cost of being mismatched for *D. ebraccatus* tadpoles, having an intermediate phenotype in the absence of any reliable cues of risk may be one strategy for maximizing fitness in a complex environment with multiple predators [37]. The relative success of the control phenotype may reflect balancing selection in nature. *Dendropsophus ebraccatus* tadpoles commonly co-occur with many different types of invertebrate and vertebrate predators simultaneously, and it is currently not known how they develop in the presence of cues of both fish and dragonfly nymphs. In five ponds surveyed over 2 years in central Panama, tadpoles always co-occurred with dragonfly larvae and with fish about one-third of the time [37]. This creates an interesting scenario to consider, as dragonflies therefore exhibit consistent but weaker selection than fish, which are infrequent both temporally and spatially but exhibit very strong selection when present. Future ecological research in this and other systems needs to explore the ramifications of this imbalance on phenotypic evolution and developmental plasticity.

Our second experiment examining selection by predators on the phenotypes of *D. ebraccatus* used only predator–naive control tadpoles, as opposed to measuring selection on the induced phenotypes themselves. This is an important aspect of the study and used the inherent variation in baseline phenotypes present in *D. ebraccatus* [39], allowing us to focus on variation in just morphology, removing potential influences of behavioural plasticity. In comparison with control tadpoles, *D. ebraccatus* tadpoles reared with fish increase swimming activity and those raised with dragonflies reduce activity [58]. Thus, by using control animals we strengthened our ability to say that the selection we saw in experiment 2 was due solely to variation in morphology.

While adaptive plasticity has been exhibited across many species, there have been surprisingly few demonstrations of trade-offs with multiple predators, particularly in the anuran tadpoles which have become a model system for studying predator-induced phenotypes. The clear trade-off found here demonstrates that changing morphology and coloration in response to predator cues can be advantageous but being mismatched with the surrounding environment can decrease survival. In heterogeneous natural environments, there are almost always going to be different phenotypic optima that could be occupied and plasticity allows organisms to get closer to these adaptive peaks. However, if the environment changes rapidly, organisms that might have previously occupied a fitness peak may find themselves suddenly dropped into a valley. The phenotypes shown here, and the ability to be plastic, are highly heritable [39], and thus our research provides insight into the costs of plasticity that have helped shape the evolution of, and work to maintain, adaptive plasticity.

Ethics. This research was conducted under Vassar College IACUC protocol no. 14-22B.

Data accessibility. The data used is available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.1vhhmgqnw [59].

Authors' contributions. A.A.I.-G., N.Y.Z. and J.C.T. conceived of the experiments, analysed data and wrote the paper.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by Vassar College.

Acknowledgements. Thanks to M. Coughlan, S. Gollerkeri, M. Hayman, P. Kaur and A. Reilinger for assistance with experiments, and J. Williams, Y. Yuan and C. Sanfuentes for maintenance of the research colony.

References

1. DeWitt TJ, Sih A, Wilson DS. 1998 Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77–81. (doi:10.1016/S0169-5347(97)01274-3)
2. West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. New York, NY: Oxford University Press.
3. Baldwin JM. 1896 A new factor in evolution. *Am. Nat.* **30**, 536–553. (doi:10.1086/276428)
4. West-Eberhard MJ. 2005 Developmental plasticity and the origin of species differences. *Proc. Natl Acad. Sci. USA* **102**, 6543–6549. (doi:10.1073/pnas.0501844102)
5. Karban R, Baldwin IT. 1997 *Induced responses to herbivory*, 319p. Chicago, IL: University of Chicago Press.
6. Palacio-López K, Beckage B, Scheiner S, Molofsky J. 2015 The ubiquity of phenotypic plasticity in plants: a synthesis. *Ecol. Evol.* **5**, 3389–3400. (doi:10.1002/ece3.1603)
7. Stibor H. 1992 Predator induced life-history shifts in a freshwater cladoceran. *Oecologia* **92**, 162–165. (doi:10.1007/BF00317358)
8. Harvell CD. 1986 The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs, and consequences. *Am. Nat.* **128**, 810–823. (doi:10.1086/284607)
9. Benard MF. 2004 Predator-induced phenotypic plasticity in organisms with complex life histories. *Annu. Rev. Ecol. Evol. Syst.* **35**, 651–673. (doi:10.1146/annurev.ecolsys.35.021004.112426)
10. Gotthard K, Nylin S. 1995 Adaptive plasticity and plasticity as an adaptation—a selective review of plasticity in animal morphology and life-history. *Oikos* **74**, 3–17. (doi:10.2307/3545669)
11. Doughty P, Reznick DN. 2004 Patterns and analysis of adaptive plasticity in animals. In *Phenotypic plasticity: functional and conceptual approaches* (eds TJ DeWitt, SM Scheiner), pp. 126–150. Oxford, UK: Oxford University Press.
12. Stenson JAE. 1987 Variation in capsule size of *Holopedium gibberum* (Zaddach): a response to invertebrate predation. *Ecology* **68**, 928–934. (doi:10.2307/1938364)
13. Stemberger RS, Gilbert JJ. 1987 Multiple-species induction of morphological defenses in the rotifer *Keratella testudo*. *Ecology* **68**, 370–378. (doi:10.2307/1939268)
14. Warkentin KM. 1995 Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proc. Natl Acad. Sci. USA* **92**, 3507–3510. (doi:10.1073/pnas.92.8.3507)
15. Harvell CD. 1990 The ecology and evolution of inducible defenses. *Q. Rev. Biol.* **65**, 323–340. (doi:10.1086/416841)
16. Kats LB, Dill LM. 1998 The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**, 361–394. (doi:10.1080/11956860.1998.11682468)

17. Chivers DP, Smith RJF. 1998 Chemical alarm signalling in aquatic predator–prey systems: a review and prospectus. *Ecoscience* **5**, 338–352. (doi:10.1080/11956860.1998.11682471)
18. McCoy MW, Touchon JC, Landberg T, Warkentin KM, Vonesh JR. 2012 Prey responses to predator chemical cues: disentangling the importance of the number and biomass of prey consumed. *PLoS ONE* **7**, e47495. (doi:10.1371/journal.pone.0047495)
19. Appleton RD, Palmer AR. 1988 Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proc. Natl Acad. Sci. USA* **85**, 4387–4391. (doi:10.1073/pnas.85.12.4387)
20. Tollrian R, Dodson SI. 1999 Inducible defenses in Cladocera: constraints, costs, and multipredator environments. In *The ecology and evolution of inducible defenses* (eds R Tollrian, CD Harvell), pp. 177–202. Princeton, NJ: Princeton University Press.
21. Brönmark C, Miner JG. 1992 Predator-induced phenotypical change in body morphology in crucian carp. *Science* **258**, 1348–1350. (doi:10.1126/science.258.5086.1348)
22. Taylor BE, Gabriel W. 1992 To grow or not to grow: optimal resource allocation for *Daphnia*. *Am. Nat.* **139**, 248–266. (doi:10.1086/285326)
23. Beckerman AP, de Roij J, Dennis SR, Little TJ. 2013 A shared mechanism of defense against predators and parasites: chitin regulation and its implications for life-history theory. *Ecol. Evol.* **3**, 5119–5126. (doi:10.1002/ece3.766)
24. Dennis SR, Carter MJ, Hentley WT, Beckerman AP. 2010 Phenotypic convergence along a gradient of predation risk. *Proc. R. Soc. B* **278**, 1687–1696. (doi:10.1098/rspb.2010.1989)
25. Hossie T, Landolt K, Murray DL. 2017 Determinants and co-expression of anti-predator responses in amphibian tadpoles: a meta-analysis. *Oikos* **126**, 173–184. (doi:10.1111/oik.03305)
26. Skelly DK. 1994 Activity level and the susceptibility of anuran larvae to predation. *Anim. Behav.* **47**, 465–468. (doi:10.1006/anbe.1994.1063)
27. Relyea RA. 2001 Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* **82**, 523–540. (doi:10.1890/0012-9658(2001)082[0523:MABPOL]2.0.CO;2)
28. Benard MF. 2006 Survival trade-offs between two predator-induced phenotypes in pacific treefrogs. *Ecology* **87**, 340–346. (doi:10.1890/05-0381)
29. Skelly DK. 1992 Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology* **73**, 704–708. (doi:10.2307/1940779)
30. McCollum SA, Leimberger JD. 1997 Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia* **109**, 615–621. (doi:10.1007/s004420050124)
31. Lardner B. 1998 Plasticity or fixed adaptive traits? Strategies for predation avoidance in *Rana arvalis* tadpoles. *Oecologia* **117**, 119–126. (doi:10.1007/s004420050639)
32. Kishida O, Nishimura K. 2004 Bulgy tadpoles: inducible defense morph. *Oecologia* **140**, 414–421. (doi:10.1007/s00442-004-1609-0)
33. Kishida O, Nishimura K. 2005 Multiple inducible defences against multiple predators in the anuran tadpole, *Rana pirica*. *Evol. Ecol. Res.* **7**, 619–631.
34. Touchon JC, Warkentin KM. 2008 Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. *Oikos* **117**, 634–640. (doi:10.1111/j.0030-1299.2008.16354.x)
35. Sosa JA, Ryan MJ, Schlaepfer MA. 2009 Induced morphological plasticity in lowland leopard frog larvae (*Rana yavapaiensis*) does not confer a survival advantage against green sunfish (*Lepomis cyanellus*). *J. Herpetol.* **43**, 460–468. (doi:10.1670/08-081r1.1)
36. Duellman WE. 2001 *The hylid frogs of middle America*. Ithaca, NY: Society for the Study of Amphibians and Reptiles.
37. Touchon JC, Vonesh JR. 2016 Variation in abundance and efficacy of tadpole predators in a Neotropical pond community. *J. Herpetol.* **50**, 113–119. (doi:10.1670/14-111)
38. Touchon JC, Warkentin KM. 2011 Thermally contingent plasticity: temperature alters expression of predator-induced colour and morphology in a Neotropical treefrog tadpole. *J. Anim. Ecol.* **80**, 79–88. (doi:10.1111/j.1365-2656.2010.01765.x)
39. Touchon JC, Robertson JM. 2018 You cannot have it all: Heritability and constraints of predator-induced developmental plasticity in a Neotropical treefrog. *Evolution* **72**, 2758–2772. (doi:10.1111/evo.13632)
40. Wassersug RJ, Hoff K. 1985 The kinematics of swimming in anuran larvae. *J. Exp. Biol.* **119**, 1–30.
41. Wilson RS, Kraft PG, Van Damme R. 2005 Predator-specific changes in the morphology and swimming performance of larval *Rana lessonae*. *Funct. Ecol.* **19**, 238–244. (doi:10.1111/j.1365-2435.2005.00958.x)
42. Van Buskirk J, Anderwald P, Lupold S, Reinhardt L, Schuler H. 2003 The lure effect, tadpole tail shape, and the target of dragonfly strikes. *J. Herpetol.* **37**, 420–424. (doi:10.1670/0022-1511(2003)037[0420:TLETTS]2.0.CO;2)
43. Van Buskirk J, Aschwanden J, Buckelmuller I, Reolon S, Ruttiman S. 2004 Bold tail coloration protects tadpoles from dragonfly strikes. *Copeia* **2004**, 599–602. (doi:10.1643/CE-03-283R)
44. Rasband W. 2012 *ImageJ. 1.45s ed*. Bethesda, MD: National Institutes of Health.
45. R Development Core Team. 2019 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
46. Bates D, Maechler M, Bolker B, Walker S. 2013 lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5.
47. Van Buskirk J, McCollum SA. 2000 Influence of tail shape on tadpole swimming performance. *J. Exp. Biol.* **203**, 2149–2158.
48. Hoverman JT, Relyea RA. 2009 Survival trade-offs associated with inducible defences in snails: the roles of multiple predators and developmental plasticity. *Funct. Ecol.* **23**, 1179–1188. (doi:10.1111/j.1365-2435.2009.01586.x)
49. Relyea RA. 2004 Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* **85**, 172–179. (doi:10.1890/03-0169)
50. Tollrian R, Harvell CD. 1999 The evolution of inducible defences: current ideas. In *The ecology and evolution of inducible defenses* (eds R Tollrian, CD Harvell), pp. 306–321. Princeton, NJ: Princeton University Press.
51. Caldwell JP. 1982 Disruptive selection: a tail color polymorphism in *Acris* tadpoles in response to differential predation. *Can. J. Zool.* **60**, 2818–2827. (doi:10.1139/z82-361)
52. LaFiandra EM, Babbitt KJ. 2004 Predator induced phenotypic plasticity in the pinewoods tree frog, *Hyla femoralis*: necessary cues and the cost of development. *Oecologia* **138**, 350–359. (doi:10.1007/s00442-003-1412-3)
53. Teplitsky C, Plenet S, Joly P. 2004 Hierarchical responses of tadpoles to multiple predators. *Ecology* **85**, 2888–2894. (doi:10.1890/03-3043)
54. Bybee SM, Johnson KK, Gering EJ, Whiting MF, Crandall KA. 2012 All the better to see you with: a review of odonate color vision with transcriptomic insight into the odonate eye. *Org. Divers. Evol.* **12**, 241–250. (doi:10.1007/s13127-012-0090-6).
55. Futahashi R, Kawahara-Miki R, Kinoshita M, Yoshitake K, Yajima S, Arikawa K, Fukatsu T. 2015 Extraordinary diversity of visual opsin genes in dragonflies. *Proc. Natl Acad. Sci. USA* **112**, E1247–E1256. (doi:10.1073/pnas.1424670112)
56. Pritchard G. 1965 Prey capture by dragonfly larvae (Odonata; Anisoptera). *Can. J. Zool.* **43**, 271–289. (doi:10.1139/z65-026)
57. Hoff KV, Wassersug RJ. 2000 Tadpole locomotion: axial movement and tail functions in a largely vertebrateless vertebrate. *Am. Zool.* **40**, 62–76. (doi:10.1668/0003-1569(2000)040[0062:TLAMAT]2.0.CO;2)
58. Reuben PL, Touchon JC. Submitted. Nothing is as it seems: behavioral and morphological plasticity appear correlated but are not in a Neotropical tadpole.
59. Innes-Gold AA, Zuczek NY, Touchon JC. 2019 Data from: Right phenotype, wrong place: predator-induced plasticity is costly in a mismatched environment. Dryad Digital Repository. (doi:10.5061/dryad.1vhhmgqnw)