

Costs and benefits of defences induced by predators differing in dangerousness

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Abstract

While theoretical studies predict that inducible defences should be fine-tuned according to the qualities of the predator, very few studies have investigated how dangerousness of predators, i.e. the rate at which predators kill prey individuals, affects the strength of phenotypic responses and resulting benefits and costs of induced defences. We performed a comprehensive study on fitness consequences of predator-induced responses by involving four predators (leech, water scorpion, dragonfly larva and newt), evaluating costs and benefits of responses, testing differences in dangerousness between predators and measuring responses in several life history traits of prey. We raised *Rana dalmatina* tadpoles in the presence of free-ranging predators, in the presence of caged predators, and exposed naive and experienced tadpoles to free-ranging predators. Tadpoles adjusted the intensities of their behavioural and morphological defences to predator dangerousness. Survival was lower in the nonlethal presence of the most dangerous predator, while we could not detect costs of induced defences at or after metamorphosis. When exposed to free-ranging predators, small, but not large, tadpoles benefited from exhibiting an induced phenotype in terms of elevated survival when compared to naive tadpoles, but we did not observe higher survival either in tadpoles exhibiting more extreme phenotypes or in tadpoles exposed to the type of predator they were raised with. These results indicate that while predator-induced defences can mirror dangerousness of predators, costs and benefits do not necessarily scale to the magnitude of plastic responses.

Introduction

Predator-induced responses are, within the limits of plasticity, predicted to be carefully fine-tuned to the environment and to the intrinsic state of the organism to maximize effectiveness of defences and minimize arising costs (Werner, 1986; DeWitt *et al.*, 1998; Lima & Bednekoff, 1999; Urban, 2007a). Induced defences have been shown to depend on the abundance of predators (Van Buskirk & Arioli, 2002), on the temporal and spatial

vicinity of predators (Turner & Montgomery, 2003) on the amount and quality of prey eaten by the predators (Laurila *et al.*, 1998; Schoeppner & Relyea, 2005), or on the size of the predators (Kusch *et al.*, 2004), but also on the size (Fraker, 2008a), energetic state (Hoverman *et al.*, 2005; Fraker, 2008b) or experience (Turner *et al.*, 2006) of prey individuals. The type of predators present in the environment may be among the most important factors influencing defences, as predator species can differ in dangerousness, foraging mode or microhabitat use, and thus, appropriate responses should vary. Empirical studies have indeed delivered many examples of predator-specific responses (e.g. Relyea, 2001a; Bourdeau, 2009; Freeman *et al.*, 2009). The strength of responses has also been related to the dangerousness of predators, that is

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to the rate at which predators kill prey individuals (e.g. Kusch, 1995; Peckarsky, 1996; Teplitsky *et al.*, 2004), but this relationship has often remained speculative, either because dangerousness of predators was not estimated or because only two predators were used. Nonetheless, while some studies have evaluated if benefits are related to the magnitude of phenotypic responses induced by predators differing in dangerousness (e.g. Relyea, 2001b), we know of no similar study testing for a relationship between predator dangerousness and costs of responses.

Predator-induced plastic defences are predicted to evolve when induced defences enhance survival in the presence of enemies and defences are costly to develop or maintain (Harvell, 1990). There is a relatively large number of reports on survival benefits of induced defences (Tollrian & Harvell, 1999). Some investigations have observed generalized responses that lowered mortality in the presence of various predators (McCollum & Van Buskirk, 1996; Laurila *et al.*, 2006; Freeman, 2007), but predator-specific responses enhancing survival probabilities mostly in the presence of the predator that has induced the phenotype have also been documented (Kishida & Nishimura, 2005; Freeman, 2007; Hoverman & Relyea, 2009). Costs, such as a decreased growth, development rate or fecundity (Van Buskirk, 2000; Hoverman *et al.*, 2005; Steiner, 2007), have been proposed to arise from lowered activity (Lima, 1998), from allocation to morphological traits providing protection or enhancing escape ability (Tollrian, 1993; Johnson *et al.*, 2008), or the deleterious effects of the responses to the threat (Slos & Stoks, 2008). However, costs arising from the expression of inducible defences tend to be weak (Steiner, 2007). Also, costs do not necessarily appear in the measured traits, in all environments and simultaneously with the induced defence (Scheiner & Berrigan, 1998; Van Buskirk & Saxer, 2001). Consequently, costs of induced defences have often remained elusive, and detecting them can turn out to be a difficult task (Tollrian & Harvell, 1999). It is, however, important to note that costs may also disappear over evolutionary time, so that not finding a cost does not necessarily mean a contradiction between theory and empirical data (DeWitt *et al.*, 1998).

In this study, our aim was to relate costs and benefits of predator-induced defences to the dangerousness of different types of predators and to the magnitude of the plastic response. Studies using constrained predators are ideal for examining induced phenotypes, but they often do not test for survival benefits of induced defences. On the other hand, studies on the effects of free-ranging predators, where phenotypic changes and survival can be measured under more natural conditions, do not allow discerning between phenotypic effects of induction, thinning and selection by the predator (Van Buskirk & Yurewicz, 1998; Relyea, 2002). To clearly demonstrate survival benefits and costs of induced defences, it is necessary to integrate the two method-

ological approaches and use a combination of constrained and free-ranging predators. Consequently, we subjected anuran tadpoles, popular models of studies on predator-induced defences (Relyea, 2007), to three experiments: (i) By raising agile frog (*Rana dalmatina*) tadpoles in the presence of free-ranging predators, we estimated the relative dangerousness of the predator species used. (ii) By raising tadpoles in the nonlethal presence of caged predators, we tested the hypotheses that prey respond to different predator species with qualitatively or quantitatively varying induced defences. This experiment also allowed us to test the hypothesis that costs arising from the expression of plastic antipredator responses scale to the magnitude of the responses. By relating tadpole phenotypes developed in the presence of caged predators to relative predator dangerousness, we also tested the hypothesis that the magnitude of phenotypic responses and the costs arising from the expression of the responses scale to predator dangerousness. (iii) By exposing naive tadpoles and tadpoles exhibiting predator-induced phenotypes to free-ranging predators, we tested the hypotheses that the expression of antipredator responses results in benefits in the form of lowered probability of being captured by free-ranging predators and that induced defences are equally effective against all predators as opposed to prey showing the highest survival when facing the predator they were raised with. To provide a full picture on induced defences and, thus, to enhance the probability of observing the most important traits (Relyea, 2003), we examined a relatively large number of tadpole and metamorph characteristics.

Methods

Rana dalmatina breeds in a variety of water bodies, ranging from small ephemeral puddles to large permanent ponds and lakes, varying widely in predator regimes. In early April 2007, we collected 25 freshly laid egg clutches from a breeding site (280 m above sea level, 47°42' N, 19°02' E) located in the Pilis-Mountains, 30 km N of Budapest. We further captured at the same locality and at surrounding ponds the following predators: horse leech (*Haemopsis sanguisuga*), water scorpion (*Nepa* sp., Hemiptera, Insecta), smooth newt (*Triturus vulgaris*) and dragonfly larva (*Aeshna cyanea*). Leeches feed on anuran eggs and young or injured tadpoles, water scorpions and dragonfly larvae mainly on tadpoles and newts on both eggs and tadpoles (Laurila *et al.*, 2002; Orizaola & Braña, 2003; A. Hettyey, personal observation). These predators are all present in the breeding pond where the eggs were taken from.

We performed two mesocosm experiments. We used 30 small and 30 large rotund tubs placed outdoors in an open field on the outskirts of Budapest belonging to the Plant Protection Institute of the Hungarian Academy of Sciences. Ten days before the start of the experiment,

small and large tubs were filled with tap water (small tubs: diameter = 74 cm, height = 30 cm, 100 L of water; large tubs: diameter = 83 cm, height = 71 cm, 250 L of water). Two days later, we added 7 g rabbit pellets, 150 g dried beech (*Fagus sylvatica*) leaves and 3 L pond water to small tubs and 10 g rabbit pellets, 200 g beech leaves and 3.5 L pond water to large tubs to provide nutrients and cover for tadpoles. We covered experimental units with mosquito nets to prevent colonization of artificial ponds by predators. Large tubs further received four 0.75 L transparent cups with mosquito net bottoms and covers as predator cages and four plastic egg-holding dishes (15 × 15 cm) with mosquito net bottom. Predator cages were hung into tubs. Egg-holding dishes were put afloat with the help of wooden sticks. Both types of subcompartments were partially submerged under water. This design allowed visual and chemical contact between anuran embryos and larvae and the predators. Two days before the start of the experiment, we assigned each tub to one of five treatments in a randomized spatial block design and added ad libitum fed predators to the tubs, whereas in the control treatment we left the predator cages empty. We started experiments on April 4 (day 0) by placing eggs into the tubs. For a timeline of the experiments, see Fig. 1.

Experiment 1 – free-ranging predators

This experiment was designed to estimate natural mortality rates and the relative dangerousness of predators. Small tubs received 20 eggs from each of 15 egg clutches (resulting in 300 eggs per tub) and contained two free-ranging predators of the same species at the start of the experiment. Initial densities were chosen to be high in this experiment to ensure that some tadpoles survive until the end in all treatments. Changing starting conditions (i.e. density) would probably have resulted in slightly different estimates of dangerousness, but we consider the obtained estimates useful for comparing predator dangerousness, especially as densities were within the range of densities readily observable in nature (A. Hettyey, personal observation). Treatments were replicated six times. We monitored survival by counting tadpoles on three intermediate

occasions (18, 25 and 33 days after start of the experiment) and at termination (44 days after start), when the first tadpoles were approaching metamorphosis.

Experiment 2 – caged predators

With this experiment, we aimed to determine effects of predators on body size, shape, behaviour and survival of tadpoles, time until and mass at metamorphosis and escape ability of metamorphs, while controlling for density-dependent effects by keeping tadpole numbers constantly low. We placed three eggs from each of ten egg clutches into the four egg-holding dishes of each large tub (resulting in 120 eggs per tub) and one predator into each cage (resulting in four predators of the same species per tub). Treatments were replicated six times. Tubs holding empty cages served as controls. Caged predators were fed two *R. dalmatina* eggs and two tadpoles every other day during the first half of the experiment and two *R. dalmatina* tadpoles during the second half. Seven days after start of the experiment, when more than 90% of hatchlings had left the egg jelly in each tub, we released eleven haphazardly selected healthy tadpoles from each egg-holding dish (resulting in 44 tadpoles per tub) and removed the dishes. Tadpoles could swim around and forage in tubs, and predators could not reach them. Twelve and 27 days after hatching, we removed 11 tadpoles from each tub of each treatment and used them in the predation trials (experiment 3). Surviving tadpoles were not placed back into the large tubs they had been taken from. Thus, the decrease in density occurred simultaneously and at the same extent in all experimental populations of experiment 2 and mirrored decrease in density because of predation or pathogens under natural conditions.

We evaluated behaviour of tadpoles 16, 23, 30 and 37 days after hatching. In each tub, we counted the number of tadpoles swimming in the water column or feeding on the exposed surface of tub walls. Adding these counts together, we obtained the number of tadpoles visible. Thirty-three days after hatching, we caught a random sample of 10 tadpoles per tub, brought them to the laboratory, anaesthetized them with 0.02 g mL⁻¹

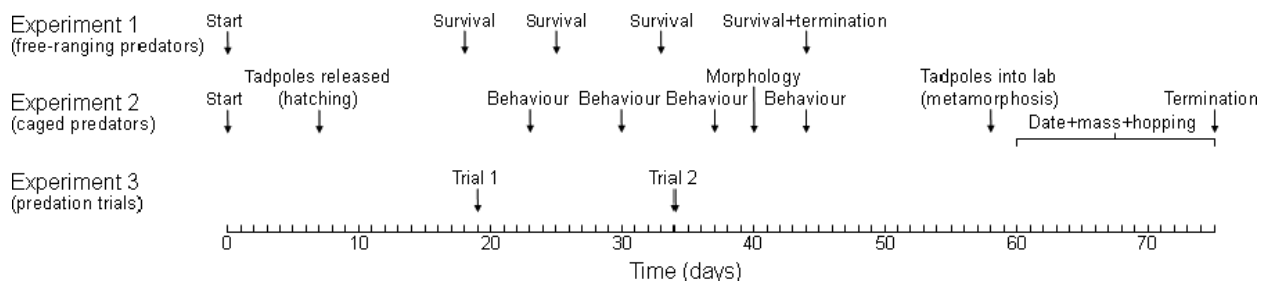


Fig. 1 Timeline of the three experiments.

MS-222 (tricaine, Sigma-Aldrich), photographed them and, after recovery, placed them back into tubs again. Recovery rates were very high (mortality was less than 1%). We later measured body length, tail length, maximum tail muscle depth and maximum tail fin depth using IMAGE TOOL 3.0 (UTHSCSA, San Antonio, Texas, USA). These parameters have previously been shown to be phenotypically plastic, and they have been suggested to be of importance for escaping ability (e.g. McCollum & Van Buskirk, 1996; Van Buskirk & Relyea, 1998).

On June 1 (51 days after hatching), when the first tadpoles approached metamorphosis (development stage 41, Gosner, 1960), we emptied outdoor tubs and transported all tadpoles to the laboratory. This was necessary as metamorphosing individuals may have been attacked by less developed tadpoles or may have drowned as they had no possibility for moving out of the water in the rearing tubs, but also because it was logistically not feasible to monitor tubs and transport metamorphosing individuals every day from the outdoor setting to the laboratory. In the laboratory, we maintained tadpoles individually in 0.5-L plastic boxes containing 0.2 L reconstituted soft water and fed them ad libitum with chopped and slightly boiled spinach. Tadpoles were kept at a 10 : 14 dark : light cycle and at a constant 21 °C. We checked metamorphosing individuals every day. As soon as forelimbs of a metamorph emerged (stage 42, Gosner, 1960), we noted the date of metamorphosis and measured mass (to the nearest mg) using an analytical balance (Mettler Toledo PL 303). After measurements, we placed individuals back into their boxes.

When an individual completed tail resorption (stage 45, Gosner, 1960), we measured its hopping ability using the method of Van Buskirk & Saxer (2001) to investigate whether the predator-induced phenotype developing during the tadpole stage affected hopping ability of froglets. Jumping performance was tested in a 50 × 100 × 40 cm arena under laboratory conditions (21 °C). We placed froglets into the middle of the arena and recorded their movements from above using a Sony CyberShot DSC W-50 digital camera. We waited until froglets made at least eight hops. Usually, they hopped spontaneously, but when they did not, we touched them with a brush to induce an escape reaction. We later measured the three longest hops with MB-RULER 4.0 (Iffezheim, Germany) and used the length of the longest hop in the analyses. When 99% of the tadpoles completed metamorphosis (day 75), we terminated the experiment. Froglets, predators and tadpoles were transported back to the Pilis-Mountains.

In one replicate an escaped dragonfly larva and in two replicates colonizing backswimmers (*Notonecta glauca*) decimated tadpoles. In one further replicate, we observed high tadpole mortality (only five metamorphs) for unknown reasons. These replicates were excluded from analyses that resulted in five replicates for the treatments containing predators and six replicates for the control.

Experiment 3 – predation trials

To assess how the predator-induced phenotypes affect survival, we performed predation trials. Twelve and 27 days after hatching, we caught 11 randomly selected tadpoles from each large tub in Experiment 2. We anaesthetized them with MS-222 and marked them according to the treatment they were taken from with a small incision on the tail fin. This method is used for marking tadpoles and does not largely affect swimming ability (Anholt *et al.*, 1998). To control for potential biases because of the placement of the incision, we varied its location between trials and treatments. Once tadpoles recovered, four marked individuals originating from each of the five treatments were placed into the small tubs used in Experiment 1 (resulting in 20 tadpoles in each tub). Tadpoles reared in the small tubs in Experiment 1 had been moved into 30-L plastic boxes the day before the predation trials and were placed back as soon as these were terminated 1 day later. Free-ranging predators remained in the small tubs and served as predators in the predation trials. On the first occasion (12 days after hatching), we used water scorpions, newts and dragonfly larvae as predators, but not leeches as we assumed that these do not prey on tadpoles (Laurila *et al.*, 2002). In the trials performed 27 days after hatching, we further excluded water scorpions as by that time tadpoles were so large that they could not be caught by these predators. Trials with each predator type were replicated six times. Predation trials were run for 24 h; remaining tadpoles were then removed from small tubs and stored in 10% formalin. We later determined number and origin of survivors using a stereomicroscope.

Statistical analyses

In Experiment 1, data on survival of embryos and tadpoles were not normally distributed and were strongly right-censored. Consequently, we compared survival among treatments using a Cox proportional hazards model (Cox regression) and handled ties using the Breslow method. We entered the four sampling dates as the time variable, event of death for each individual as the status variable, and tub identity and treatment as categorical covariates. We used simple contrasts and entered control treatments as the reference category.

In Experiment 2, where tadpoles were reared in the presence of caged predators, we first investigated if phenotypes systematically varied between treatments. We tested for treatment-dependent differences in behaviour of tadpoles with a multivariate repeated-measures general linear model (GLM), where we entered the ratio of swimming tadpoles and the ratio of tadpoles visible on the four sampling occasions as the dependent variables and treatment as a fixed factor. We calculated the ratios of swimming tadpoles as # swimming/# live tadpoles and ratios of tadpoles visible as # visible/# live tadpoles. We

estimated the number of live tadpoles using linear interpolation between initial and final tadpole numbers for each tub at each occasion. We analysed total tadpole length (body length + tail length) with a linear mixed-effects model (LMM) by entering total tadpole length as the dependent variable, treatment as a fixed factor and tub identity as a random factor. We analysed tadpole shape with a multivariate GLM by entering body length, tail length, maximum tail muscle depth and maximum tail fin depth as dependent variables and treatment as a fixed factor. We used tub averages in this analysis because data based on individuals could not be transformed to yield homogeneous variances. We present results from analyses not correcting for body size as size did not vary significantly between treatments (see Results). Entering body size into the model as a covariate, however, yielded qualitatively similar results. We analysed time until metamorphosis using a GLM with treatment as a fixed factor. We used tub averages in this analysis because data based on individuals could not be transformed to yield normally distributed model residuals. To relate the magnitude of phenotypic responses of tadpoles to predator dangerousness, we entered the average ratio of tadpoles that died in each treatment of Experiment 1 as a covariate and ratio of tadpoles visible, ratio of tadpoles swimming, body length, tail length, tail muscle depth, tail fin depth and time until metamorphosis as dependent variables into a multivariate GLM. This analysis was based on tub averages of measures taken at the morphology sampling (33 days after hatching) and at the third observation on behaviour (30 days after hatching) in Experiment 2.

Second, we searched for potential costs of induced defences in terms of lowered survival, smaller metamorph mass or lowered jumping performance. To investigate whether survival of tadpoles differed among treatments, we used generalized linear modeling (GZLM) procedures with binomial error distribution and logit-link function. Survival (dead or alive) was entered as the dependent variable, treatment as a categorical factor. We also entered tub identity nested within treatment as a categorical variable to control for the nonindependence of data on tadpoles in the same tub. To test for treatment-dependent differences in metamorph mass, we built a LMM with treatment as a fixed factor and tub identity as a random factor. As distances covered by the first three longest hops were strongly correlated within individuals (all pairwise Spearman's $R > 0.77$), we investigated jumping performance of metamorphs by entering the distance covered by the longest hop as the dependent variable, treatment as a fixed factor, tub identity as a random factor and metamorph mass as a covariate into a LMM. To relate dangerousness of predators estimated in Experiment 1 to survival, metamorph mass and jumping performance of metamorphs measured in Experiment 2, we entered survival, metamorph mass and jumping performance as dependent variables and dangerousness

of predators as a covariate into a multivariate GLM. This and the following analyses were based on tub averages.

Third, to detect potential effects of phenotypic changes in tadpoles on their survival and on mass and jumping performance of metamorphs, we used a multivariate GLM with survival, metamorph mass and jumping performance as dependent variables, and ratio of tadpoles visible, ratio of tadpoles swimming, tail fin depth and time until metamorphosis as covariates. The covariates entered into the analysis were independent of each other (Pearson's correlations, all $P > 0.22$). Tail fin depth was measured at the morphology sampling (33 days after hatching); behavioural data refer to the third observation period (30 days after hatching).

We analysed the outcome of predation trials (Experiment 3) with GZLM procedures with binomial error distribution and logit-link function. Survival (dead or alive) was entered as the dependent variable; the type of free-ranging predator in the predation trials and the tadpole treatment were entered as categorical factors. We also entered tub identity nested within the type of free-ranging predators as a categorical variable to control for the nonindependence of data on tadpoles used in the same trials.

We included all two-way interactions into initial models and applied a backward stepwise removal procedure to avoid problems because of the inclusion of nonsignificant terms. We re-entered removed variables one by one to the final model to obtain relevant statistics. We fitted linear mixed models using the restricted maximum likelihood approach. All tests were two tailed. Statistics were calculated using *SPSS* 15.0 for Windows (Somers, New York, USA).

Results

Experiment 1 – free-ranging predators

Survival of embryos and tadpoles was significantly affected by the type of predator present (Cox regression; Wald = 366.3, d.f. = 4, $P < 0.001$; Table 1; Fig. 2). Pairwise comparisons showed that survival was significantly lower in all treatments containing a predator compared to control treatments (all $P < 0.001$) and that newts and

Table 1 Average risk ratios during the four sampling intervals in the treatments containing a predator when compared to the control treatment [RR = $p(\text{risk in treatment})/p(\text{risk in control})$] in Experiment 1 (free-ranging predators).

Treatment	Average risk ratio (relative to control)			
	Day 0–18	Day 19–25	Day 26–33	Day 34–44
Leech	1.44	1.56	1.30	1.38
Water scorpion	2.05	2.41	0.64	0.64
Newt	5.21	17.74	3.99	1.99
Dragonfly larva	5.64	19.46	5.64	7.55

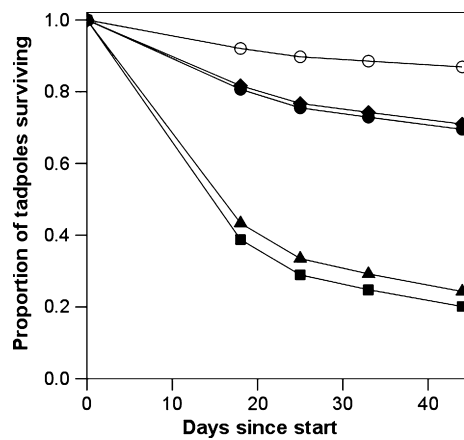


Fig. 2 Changes in the proportion of live tadpoles in Experiment 1 containing free-ranging predators. Treatments are symbolized by ○: control, ●: leech, ◆: water scorpion, ▲: newt, ■: dragonfly larva.

dragonfly larvae were more dangerous predators than leeches and water scorpions (all $P < 0.001$). However, we found no difference in dangerousness between newts and dragonfly larvae and between leeches and water scorpions (both $P > 0.16$). To determine how long the various predators affected tadpole survival, we gradually reduced the time span of the analyses on mortality rates to the last three, two and one monitoring interval. This revealed that in the presence of water scorpions mortality

after the first monitoring (18 days after start – d 18) was not different from mortality measured in control treatments ($P = 0.9$). The effect of leeches diminished after the second monitoring (d 25; $P = 0.084$), whereas the effect of newts (Wald = 3.96, d.f. = 1, $P = 0.047$) and dragonfly larvae (Wald = 28.58, d.f. = 1, $P < 0.001$) remained significant even during the last interval (ending on d 44).

Experiment 2 – caged predators

Both the ratio of tadpoles visible and the ratio of tadpoles swimming were significantly affected by treatment and changed over time, but the interaction between treatment and sampling date was also significant (Table 2, Electronic Appendix). Consequent analyses revealed that, except for the ratio of tadpoles swimming on day 30, both measures of tadpole behaviour significantly varied between treatments on all four sampling occasions (Table 2). According to multivariate GLMs performed post hoc on the four sampling dates separately, a higher ratio of tadpoles was visible in the dragonfly than in the control treatment on day 16 ($P = 0.035$), whereas this difference had started to diminish before day 23 ($P = 0.098$) and on day 30 and 37, fewer tadpoles were visible in the dragonfly than in the control treatment (both $P < 0.001$; Fig. 3a). Other treatments did not significantly differ from the control at any sampling occasion (all $P > 0.15$). On day 16, the ratio of tadpoles

Table 2 Impact of predators on the behaviour of tadpoles, as shown by a multivariate repeated-measures general linear model on data from Experiment 2 (caged predators). Behaviour was sampled 16, 23, 30 and 37 days after hatching. The two measures of behaviour were ratio of tadpoles swimming (RTswimming) and ratio of tadpoles visible (RTvisible). We also present results of multivariate general linear models on the effect of treatment at the four sampling dates separately, because the interaction between treatment and sampling date was significant. Bold indicates statistical significance at $P \leq 0.05$.

Effect	Multivariate tests				Dependent	d.f.	F	P
	d.f.	Wilk's λ	F	P				
Repeated-measures GLM						Tests of between-subjects effects		
Treatment	8, 40	0.170	7.114	< 0.001	RTvisible	4, 21	15.178	< 0.001
					RTswimming	4, 21	3.798	0.018
						Tests of within-subjects effects		
Sampling date	6, 16	0.022	116.191	< 0.001	RTvisible	2.44	284.517	< 0.001
					RTswimming	1.93	70.422	< 0.001
Treatment × Sampling date	24, 57.03	0.052	3.162	< 0.001	RTvisible	9.78	9.548	< 0.001
					RTswimming	7.74	7.319	< 0.001
GLM – effect of treatment						Tests of between-subjects effects		
On day 16	8, 40	0.216	5.748	< 0.001	RTvisible	4, 21	3.751	0.019
					RTswimming	4, 21	16.828	< 0.001
On day 23	8, 40	0.241	5.189	< 0.001	RTvisible	4, 21	6.137	0.002
					RTswimming	4, 21	8.848	< 0.001
On day 30	8, 40	0.452	2.437	0.030	RTvisible	4, 21	4.644	0.008
					RTswimming	4, 21	0.876	0.495
On day 37	8, 40	0.248	5.046	< 0.001	RTvisible	4, 21	6.733	0.001
					RTswimming	4, 21	7.101	0.001

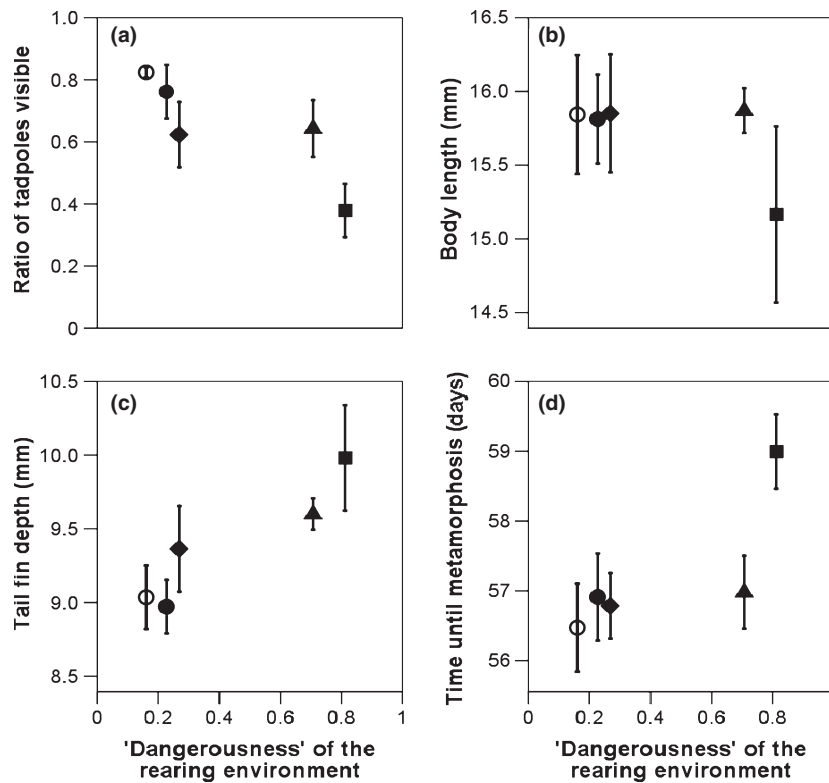


Fig. 3 Relationships between predator dangerousness and the magnitude of responses in behaviour, morphology and development. 'Dangerousness' was estimated by the average ratio of tadpoles that died in the treatments of Experiment 1 (free-ranging predators). Treatments are symbolized by ○: control, ●: leech, ◆: water scorpion, ▲: newt, ■: dragonfly larva. Ratio of tadpoles visible (a) refers to observations made 30 days after hatching. Body length (b) and tail fin depth (c) are measures taken 33 days after hatching in Experiment 2 (caged predators). Time until metamorphosis (d; from start of the experiment to reaching Gosner stage 42) was also estimated in Experiment 2. The figure is based on tub averages; means \pm SE are indicated.

swimming was higher in the water scorpion, newt and dragonfly treatment than in the control and leech treatment (all $P < 0.003$). On day 23, only the dragonfly treatment differed from the control and leech treatment (both $P < 0.003$, all other $P > 0.09$), and on day 30, there were no differences among treatments at all (all $P = 1$). On day 37, however, a lower proportion of tadpoles were swimming in the leech, water scorpion and dragonfly than in the control treatment (all $P < 0.01$; newt-control: $P = 0.077$).

Total tadpole length did not differ between treatments (LMM; $F_{4,30.04} = 0.7$, $P = 0.6$). Body shape depended on treatment (GLM; Wilks' $\lambda = 0.11$, $F_{16,55.63} = 3.63$, $P < 0.001$). Tests of between-subjects effects revealed that tail fin depth significantly varied between treatments ($F_{4,21} = 2.89$, $P = 0.048$; Fig. 3c), whereas body length ($F_{4,21} = 0.56$, $P = 0.69$), tail length ($F_{4,21} = 0.27$, $P = 0.89$) and tail muscle depth ($F_{4,21} = 0.38$, $P = 0.82$) did not seem to be largely affected by treatments. Time until metamorphosis differed among treatments (GLM; $F_{4,21} = 3.09$, $P = 0.038$), with tadpoles in the dragonfly treatment taking longer until the start of metamorphosis than those in the control treatment (dragonfly – control:

$P = 0.042$) and other treatments being intermediate (all other pairwise $P > 0.13$; Fig. 3d).

Several measures of phenotypic responses in tadpoles were in a close relationship with predator dangerousness (Table 3). Tests of between-subjects effects indicated negative relationships between dangerousness and the ratio of tadpoles visible (Fig. 3a), a positive relationship between dangerousness and tail fin depth (Fig. 3c) and time until metamorphosis (Fig. 3d), whereas there was no significant linear relationship between dangerousness of predators and the ratio of tadpoles swimming, body length, tail length or tail muscle depth (Table 3).

We found among-treatment differences in tadpole survival until metamorphosis (GZLM; Wald $\chi^2 = 12.92$, d.f. = 4, $P = 0.012$; Fig. 4a). Bonferroni-corrected pairwise comparisons of estimated marginal means revealed a significantly lower percentage of tadpoles surviving in the dragonfly treatment (58.18 ± 4.85 ; mean \pm SE) compared to the control (75.76 ± 3.45) and the leech treatments (77.27 ± 5.18 ; both $P < 0.029$), whereas all other comparisons (water scorpion: 70 ± 7.95 ; newt: 65.45 ± 9.6) were nonsignificant ($P > 0.34$). Metamorph

Table 3 The relationship between predator dangerousness and the magnitude of phenotypic responses and potential costs of induced defenses and the effect of life history changes in tadpoles on their survival and on metamorph mass and jumping performance. Analyses were performed on tub averages of measures taken at the third observation on behaviour (30 days after hatching) and at the morphology sampling occasion (33 days after hatching) in Experiment 2 (caged predators) using multivariate general linear models. Bold indicates statistical significance at $P \leq 0.05$.

Effect	Multivariate tests				Tests of between-subjects effects					
	d.f.	Wilk's λ	F	P	Dependent variable	d.f.	B	SE	F	P
'Dangerousness'	7, 18	0.254	7.572	< 0.001	Ratio of active tadpoles	1, 24	-0.477	0.139	11.718	0.002
					Ratio of swimming tadpoles	1, 24	-0.054	0.037	2.159	0.155
					Body length	1, 24	-0.664	0.640	1.075	0.310
					Tail length	1, 24	-0.221	1.272	0.030	0.864
					Tail muscle depth	1, 24	0.094	0.192	0.239	0.629
					Tail fin depth	1, 24	1.292	0.393	10.813	0.003
					Time until metamorphosis	1, 24	2.612	0.977	7.147	0.013
'Dangerousness'	3, 22	0.459	8.656	0.001	Survival until metamorphosis	1, 24	-24.186	10.112	5.721	0.025
					Metamorph mass	1, 24	0.125	0.037	11.631	0.002
					Jumping performance	1, 24	1.129	12.369	0.008	0.928
Ratio of active tadpoles	3, 20	0.771	1.981	0.149						
Ratio of swimming tadpoles	3, 20	0.874	0.959	0.431						
Tail fin depth	3, 21	0.382	11.321	< 0.001	Survival until metamorphosis	1, 23	-5.087	4.443	1.311	0.264
					Metamorph mass	1, 23	0.068	0.013	27.507	< 0.001
					Jumping performance	1, 23	12.334	4.644	7.053	0.014
Time until metamorphosis	3, 21	0.675	3.367	0.038	Survival until metamorphosis	1, 23	-3.963	1.889	4.402	0.047
					Metamorph mass	1, 23	0.007	0.006	1.818	0.191
					Jumping performance	1, 23	-2.477	1.974	1.575	0.222
All two-way interactions										> 0.120

mass also significantly varied among treatments (LMM; $F_{4,20.77} = 3.1$, $P = 0.038$; Fig. 4b), with tadpoles in the dragonfly treatment being larger than those in the control treatment ($P = 0.03$) and no other significant differences (all $P > 0.43$). Metamorphs exhibiting a larger body mass (LMM; $B = 140.63$, $SE = 24.08$, $F_{1,200.54} = 34.1$, $P < 0.001$) made longer hops; however, neither treatment ($F_{4,19.12} = 0.97$, $P = 0.45$) nor the interaction between treatment and metamorph mass ($F_{4,231.81} = 1.27$, $P = 0.28$) influenced jumping performance.

In the treatments containing more dangerous predators, tadpole survival was lower (Fig. 4a), metamorph mass was larger (Fig. 4b), whereas jumping performance was not affected (Table 3).

Metamorph mass and jumping performance of metamorphs were positively related, and survival was unrelated to tail fin depth (Table 3). Also, survival was negatively related, and metamorph mass and jumping performance were unrelated to time until metamorphosis (Table 3). Neither tadpole survival, metamorph mass nor jumping performance was related to behaviour of tadpoles (Table 3).

Experiment 3 – predation trials

At the first round of predation trials performed 12 days after hatching, when tadpoles taken from Experiment 2 were exposed to free-ranging water scorpions, newts and dragonfly larvae, survival was significantly affected by

both the type of free-ranging predators present (GZLM; Wald $\chi^2 = 19.92$, d.f. = 2, $P < 0.001$) and the tadpole treatment (Wald $\chi^2 = 42.02$, d.f. = 4, $P < 0.001$; Fig. 5). The interaction between type of free-ranging predators present and the tadpole treatment had no effect on survival of tadpoles ($P = 0.78$). Bonferroni-corrected pairwise comparisons of estimated marginal means indicated that more tadpoles survived in the presence of free-ranging water scorpions than with the other two predators (both $P < 0.001$), whereas there was no significant difference in survival between treatments containing free-ranging newts or dragonfly larvae ($P = 1$; water scorpions mean \pm SE: $87.5 \pm 3.55\%$; newts: $65.83 \pm 5.01\%$; dragonfly larvae: $65.83 \pm 5.81\%$). Also, tadpoles taken from the control treatments had the lowest survival (all pairwise $P < 0.001$), whereas we found no difference between the tadpoles taken from treatments containing predators (all pairwise $P > 0.19$; control: $41.67 \pm 6.06\%$; leech: $76.39 \pm 5.14\%$; water scorpions: $81.94 \pm 7.23\%$; newts: $80.56 \pm 5.18\%$; dragonfly larvae: $84.72 \pm 4.11\%$).

In the second round of predation trials performed 27 days after hatching, tadpoles tended to be more likely to survive trials in the presence of free-ranging newts than in the presence of dragonfly larvae (Wald $\chi^2 = 2.78$, d.f. = 1, $P = 0.095$; newts: $90 \pm 2.57\%$; dragonfly larvae: $82.5 \pm 3.42\%$). We observed no significant differences in survival between tadpole treatments ($P = 0.15$; Fig. 5; control: $75 \pm 6.88\%$; leech: $89.58 \pm 3.72\%$; water

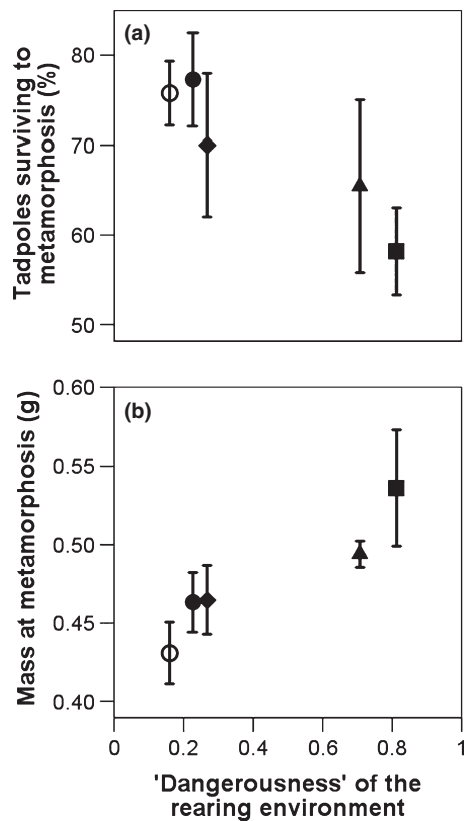


Fig. 4 Relationships between predator dangerousness and the magnitude of potential costs related to induced defences. Percentage of tadpoles surviving until metamorphosis (a) and body mass of individuals at the start of metamorphosis (b) were both measured in Experiment 2 (caged predators). 'Dangerousness' was estimated by the average ratio of tadpoles that died in the treatments of Experiment 1 (free-ranging predators). Treatments are symbolized by ○: control, ●: leech, ◆: water scorpion, ▲: newt, ■: dragonfly larva. The figure is based on tub averages; means \pm SE are indicated.

scorpions: $89.58 \pm 3.72\%$; newts: $85.42 \pm 4.83\%$; dragonfly larvae: $91.67 \pm 3.55\%$). As before, the interaction between the type of free-ranging predators present and the tadpole treatment had no effect on tadpole survival ($P = 0.33$).

Discussion

Free-ranging predators

Dangerousness of predators varied both between species and over time. Leeches and water scorpions were less voracious predators at any time than newts and dragonfly larvae, whereas neither leeches and water scorpions nor newts and dragonfly larvae largely differed from each other in dangerousness. Leeches and water scorpions were only dangerous to eggs and/or small tadpoles. Interestingly, leeches did not solely feed on eggs, but were capable of preying upon hatchlings as well. This

was also confirmed by observations of predation events. Newts and dragonfly larvae remained effective predators also of large tadpoles and during the last interval, the effect of dragonfly larvae seemed larger than that of newts. Consequently, gape-limited smooth newts may be less effective predators of very large tadpoles than dragonfly larvae. This is also supported by a nonsignificant tendency for large tadpoles being more likely to survive in the presence of newts than in the presence of dragonfly larvae in the second round of our predation trials and has been suggested by previous studies as well (Van Buskirk, 2001; Kishida & Nishimura, 2005).

Caged predators

Behaviour of tadpoles changed over time was strongly affected by treatments and reacted differently to the presence of predators early and late during the larval stage. Patterns in the ratio of tadpoles visible and the ratio of tadpoles swimming were similar. Large tadpoles raised in the presence of predators were less active than tadpoles in the control treatment, which aligns to a generally reported lowered activity as a response to predators. Small tadpoles, however, were more active in the predator treatments compared to controls. This result is surprising, because small tadpoles are generally more vulnerable to predation than large ones (Travis *et al.*, 1985; Semlitsch, 1990; Eklöv & Werner, 2000), and thus, elevated activity is likely to result in higher survival costs in their case (Stoks *et al.*, 2003; Laurila *et al.*, 2006; Takahara *et al.*, 2008). Nonetheless, benefits including elevated growth rates and a resulting early reaching of a size refuge from predation, more resources available for the expression and maintenance of morphological-induced defences, enhanced competitive ability, mating success or fecundity (Urban, 2007a,b; Biro *et al.*, 2005) may result in selection for the maintenance of high levels of activity during early life stages, even if these come at a cost of elevated immediate mortality risk.

Overall, tadpole activity was more strongly affected by the presence of more dangerous predators: smaller tadpoles appeared to increase, whereas large tadpoles to decrease their activity to a larger extent when they were exposed to more dangerous predators. This result also suggests that the benefits of a head start early during ontogeny, achieved by increased food intake resulting from elevated activity (*sensu* Werner & Anholt, 1993), may outweigh the costs. Further, in the presence of caged predators, the observed variation in tadpole activity did not translate into variation in tadpole survival, metamorph mass or jumping performance of froglets. This result supports the notion that changes in activity do not necessarily effect costs through lowered food intake (McPeck, 2004; Relyea & Auld, 2004; Steiner, 2007).

Tadpoles had a deeper tail fin in the presence of more dangerous predators, and body length was shortest in the presence of dragonfly larvae. A short body and high tail

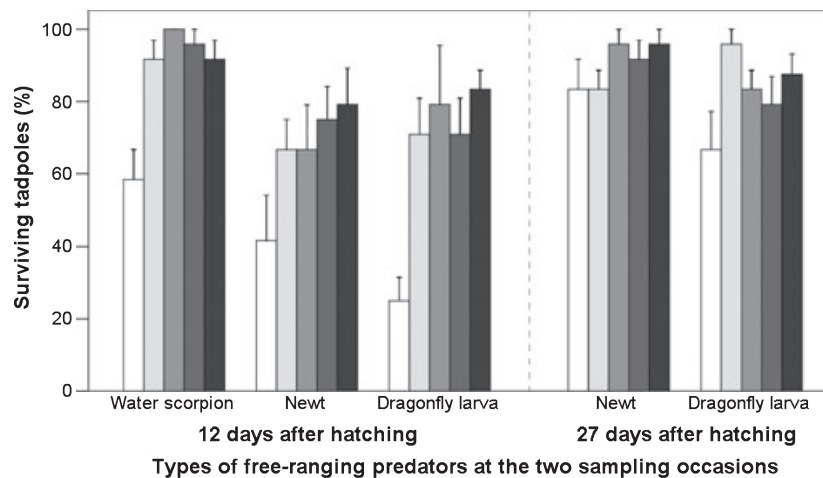


Fig. 5 Percentage of tadpoles surviving (mean \pm SE) in the predation trials (Experiment 3), when tadpoles raised in the presence of caged predators (Experiment 2) were exposed to free-ranging water scorpions, newts or dragonfly larvae of Experiment 1. White bars represent tadpoles originating from the control treatment, light grey bars represent tadpoles taken from tubs containing caged leeches, medium grey bars represent tadpoles taken from tubs containing caged water scorpions, dark grey bars represent tadpoles taken from tubs containing caged newts and black bars represent tadpoles taken from tubs containing caged dragonfly larvae.

fin may divert attacks away from the body or enhance swimming performance (Van Buskirk *et al.*, 1997; Doherty *et al.*, 1998; Johnson *et al.*, 2008). We did not find differences in total tadpole length among treatments. This result contradicts the predictions of theoretical models (e.g. Werner, 1986) and is all the more surprising as costs potentially arising from lowered activity and from alterations in morphology should have summed up rather than cancelled each other out. Nonetheless, the lack of an effect of caged predators on tadpole body size accords with previous results (Benard, 2004; Relyea, 2007).

Time until metamorphosis tended to be longer in the presence of predators and was longest in tadpoles raised together with the most dangerous predator. Again, contrary to our results, a theoretical model by Werner (1986) predicted a shortened larval phase in the presence of dangerous aquatic predators, and there is indeed strong selection on the timing of metamorphosis (Richter-Boix *et al.*, 2010). Nonetheless, our results again align to previous experimental studies using caged predators (Benard, 2004; Relyea, 2007). These discrepancies between theoretical predictions and empirical results may be explained by a dependence of responses on the environmental context or constraints imposed by costs that are paid for producing antipredator defences (Relyea, 2007).

We observed significant among-treatment variation in tadpole survival in the experiment containing caged predators, and mortality rates were related to predator dangerousness, mortality being highest in the presence of the most dangerous predator. We cannot be sure what caused elevated mortality rates, but as survival was lowest where morphological responses to predators were

strongest and where metamorphosis was delayed, we suggest that elevated tadpole mortality has arisen as a cost of induced defences. Severe costs of the expression of induced defences paid in other life history characters have been documented (Dixon & Agarwala, 1999; Van Buskirk, 2000; Hammill *et al.*, 2008), but organisms should rarely divert so much energy to antipredator defences that this itself puts a risk on survival (Werner & Anholt, 1993; Van Buskirk, 2000). This is one of only a few studies (e.g. McCollum & Van Buskirk, 1996; Van Buskirk & Relyea, 1998) which suggest that expressing antipredator defences may also incur a mortality cost, and further studies are needed to determine the circumstances under which this can arise.

Metamorph mass varied between treatments, and this variation was also related to the dangerousness of predators. However, this relationship is not indicative of a potential cost of antipredator responses, because metamorphs that emerged from tubs containing more dangerous predators were larger, and larger size at metamorphosis is beneficial for fitness (Smith, 1987; Semlitsch *et al.*, 1988; Altwegg & Reyer, 2003).

Even though the jumping performance of froglets depended on metamorph mass, we did not find systematic between-treatment variation in jumping performance. This suggests that escape ability of metamorphosed individuals is not strongly influenced by predators that were present in the aquatic rearing environment. Nonetheless, tadpoles showing strong phenotypic responses in terms of body shape (tail fin depth) developed into large metamorphs that had superior jumping abilities, most probably enjoying enhanced escape ability from predators (for similar results see Van Buskirk & Saxer, 2001). This outcome cannot be interpreted as a

manifestation of costs but rather as a benefit of expressing induced defences.

Predation trials

Small tadpoles exhibiting induced defences had a higher probability of survival in the predation trials than predator-naïve tadpoles. This is most probably a result of altered morphology and aligns to results of previous studies (e.g. McCollum & Van Buskirk, 1996; Kishida & Nishimura, 2005; Teplitsky *et al.*, 2005). However, tadpoles taken from tubs that contained more dangerous predators did not exhibit higher survival rates, possibly because positive effects of more pronounced morphological defences were countered by negative effects of elevated activity.

Even though two of the predators involved in the experiment use the sit-and-wait tactic (water scorpion and dragonfly nymph) and the other two (leech and newt) are active foragers, we did not observe qualitatively differing, predator-specific responses, as did Teplitsky *et al.* (2005) in the same tadpole species, probably because our study did not include fish as predators (also see Benard, 2006). The observed antipredator defences rather seem to have been universally effective against the tested predators (McCollum & Van Buskirk, 1996; Van Buskirk, 2001; Laurila *et al.*, 2006), and tadpoles did not have elevated survival when they were exposed to the type of free-ranging predator that they were raised with. Our results, thus, suggest that while the presence of induced defences did provide some protection, variation in the expression level of defences among predator treatments did not have large enough consequences on survival that we could have detected them in our experiment. Large tadpoles in general already had high survival, which was not further elevated measurably by the expression of induced defences. This may have been a result of a size refuge from predation: dragonfly larvae and especially newts may be less effective in capturing large tadpoles (Kishida & Nishimura, 2005; Urban, 2007a,b).

In summary, our results suggest that tadpoles generally reacted more intensely to more dangerous predators both in their behaviour and in their body proportions. We did not observe costs of induced defences manifested at or shortly after metamorphosis in the form of decreased mass at metamorphosis, or lowered jumping performance. Nonetheless, we obtained suggestive evidence for a survival cost suffered during the larval stage, where tadpoles experienced higher mortality in the nonlethal presence of more dangerous predators. When exposed to free-ranging predators, we observed a clear increase in the survival of tadpoles raised in the presence of any predator when compared to naïve tadpoles, but did not find benefits of stronger antipredator responses that developed in the presence of more dangerous predators. Our results, thus, suggest that while antipredator responses can be adjusted

to the dangerousness of predators, accordingly graded changes in both costs and benefits do not necessarily arise. Consequently, while our experimental set-up did not allow a formal test of this hypothesis, the relationship between costs and benefits appears to be nonlinear. Further studies are needed that simultaneously relate extent, costs and benefits of induced defences to predator dangerousness in other taxa to test the general applicability of our findings. Also, studies directly relating costs and benefits of induced defences to each other will be crucial for testing key assumptions of the theory of predator-induced plastic defences. Finally, it will be interesting to explore the conditions under which varying strengths of induced defences do or do not provide different levels of protection.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Tadpole behaviour (mean \pm SE) sampled at four occasions in Experiment 2 (caged predators).

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