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Assessing behavioural and morphological responses of frog tadpoles to temporal variability in predation risk

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Abstract

Finely tuned adjustment of an individual's phenotype can offer substantial fitness benefits when it is closely matched with environmental change. For instance, prey may be safeguarded against unnecessary costs to growth or development when their responses to temporally variable predation risk include plastic anti-predator defences. Yet, the correspondence between perceived predation risk and related responses should differ between behavioural and morphological phenotypes when risk fluctuates because behaviour can be modified quickly, whereas morphological phenotypes require time to build. Theoretical models predict intermediate expression when risk fluctuates rapidly relative to the time required to mount a response, whereas traits that can be modified relatively quickly should more closely track current conditions. Using a tadpole-dragonfly larva system, we sought to compare the expression of behavioural and morphological defences following exposure to constant versus variable predation risk. By varying the pattern and total duration of predator cue exposure, but not cue concentration, we quantified phenotypic plasticity and trait reversibility. Our results show that strong behavioural responses were limited to early ontogeny but closely matched current level of risk. The morphology of prey experiencing a weekly changing predator environment was intermediate to that of prey in the no-predator and constantly exposed treatments. Yet, prey exposed to a predator environment for the same total duration as the weekly changing environment, but in a different exposure pattern, was morphologically unresponsive to the onset of predation risk. Finally, unexposed tadpoles gained deeper tails and smaller relative body size in late development, coincident with limb bud development. Such changes are consistent with anti-predator response and represent either an innate response when prey are more vulnerable or shape optimization when faced with increased drag. We conclude that phenotypic expression depends critically on patterns of temporal variability in the environment, although the actual extent of expression depends on the specific trait in question.

Introduction

Temporal variation in predation risk is an important factor governing the behavioural, morphological and life-history responses of prey. Because mounting an anti-predator defence can be costly (e.g. Pettersson & Brönmark, 1997; Rigby & Jokela, 2000; Van Buskirk, 2000), prey inhabiting areas characterized by variable levels of risk often possess defences that are expressed only when predators offer an imminent mortality threat (Harvell, 1990; Van Buskirk, 2002). Moreover, in systems where risk levels vary frequently during a prey's lifetime, inducible defences often are quickly reversible to accommodate predation risk variability (Gabriel, 1999; Relyea, 2003). Although many studies have assessed induced anti-predator responses across a variety of prey (e.g. see reviews by Laas & Spaak, 2003; Benard, 2004), such efforts typically evaluate prey responses in terms of a single prolonged exposure to predation risk (e.g. Trussell, 1996; Lardner, 2000; Vaughn, 2007). However, such a pattern of exposure is unrealistic for systems where predation risk is variable either across space or time during the course of their lifetime, as may be the case for most prey species. Indeed, the few empirical studies that have tracked prey responses to fluctuating predation risk indicate that variation in risk is a key determinant in phenotypic expression (e.g. Van Buskirk, 2002; Laurila *et al.*, 2004; Hoverman & Relyea, 2007). Notwithstanding these examples, considerable uncertainty remains in our understanding of how prey respond to variable predation risk and the broader mechanisms underlying the evolution and ecology of plastic anti-predator defences, specifically in the context of

predicting differences in correspondence of trait expression to current risk conditions among various plastic traits.

Research addressing prey behavioural responses to variable predation risk has largely centred on the risk allocation hypothesis (Lima & Bednekoff, 1999; Ferrari, Sih & Chivers, 2009). This model predicts that prey responses are (1) strongest during brief and infrequent pulses of risk and lessened when risk is sustained over longer periods; and (2) less plastic when risk exposure is chronic (Lima & Bednekoff, 1999). Ferrari *et al*. (2009) further emphasized that the predictions of the risk allocation hypothesis hinge on two critical assumptions: (1) prey are energy limited, with energy intake above a threshold securing no further gains in fitness; and (2) prey have perfect information about their current risk regime (i.e. prey know if they are in a location or time period conferring high or low risk) and can evaluate the proportion of time spent at high- versus low-risk regimes. In contrast, a more general model proposed by Gabriel *et al*. (2005) invokes phenotypic expression as being responsive to the time required to mount a response (i.e. the response lag) and the amount of information regarding current environmental conditions (Gabriel *et al*., 2005). Specifically, when phenotypic change can be induced quickly relative to the rate of environmental change, and prey have complete information about their environment, then reversible plastic phenotypes should be continually refined to reflect a specialist strategy (Gabriel *et al*., 2005). In contrast, phenotypes should be intermediately expressed (e.g. a phenotype between a predator-exposed and non-predator phenotype) when either the response lag is relatively long or if information about current environmental conditions is incomplete (Gabriel *et al*., 2005).

The framework presented by Gabriel *et al*. (2005) provides a synthetic means to predict the expression of plastic phenotypes (i.e. behavioural and morphological) in temporally variable environments. In general, behavioural defences should be expressed or modified more quickly than morphological defences that require time to build (Padilla & Adolph, 1996; Gabriel *et al*., 2005). We should therefore expect the extent and pattern of response in behavioural and morphological phenotypes to differ in temporally heterogeneous environments. Yet, despite the recognized importance of phenotypic plasticity of all kinds on prey fitness (e.g. Chivers *et al*., 2008; Johnson, Burt & DeWitt, 2008), morphological change under temporally variable predation risk is infrequently tracked empirically or contrasted directly with associated behavioural responses. These key studies have not only found that morphological responses are both sensitive to temporal variation in risk and graded according to the duration of risk exposure (Laurila *et al*., 2004), but also that morphological responses can sometimes be just as reversible as behavioural changes (Van Buskirk, 2002; Relyea, 2003). However, it has yet to be fully revealed how behavioural and morphological phenotypes are manifest depending on the trait sensitivity and reversibility relative to the duration and pattern of risk exposure.

Using a tadpole-dragonfly larva system, we sought to compare the extent and pattern of phenotypic expression in behavioural and morphological defences following exposure to constant versus variable perceived predation risk. Specifically, we experimentally varied the pattern and total duration of predator cue exposure, but not predator cue concentration, and tracked the phenotypic expression. We predicted that prey exposed to constant risk would show greater overall expression of anti-predator phenotypes because they experience increased average risk levels (i.e. greater total duration of exposure) compared to prey experiencing variable risk. Further, if the pattern of exposure has little effect on phenotypic expression, then prey exposed to predation risk for equal durations should respond similarly even if the temporal pattern of exposure differs. Finally, we predicted that behavioural responses should track variability in perceived predation risk closely, whereas morphology should be intermediately expressed owing to the greater response lag.

Methods

Lab methods and experimental design

Rana pipiens eggs were collected in late April 2007 from ponds near Peterborough, Ontario (44°22′ N 78°03′ W). Four egg broods (~1500 eggs) were reared in 110-L containers to Gosner stage 24 (Gosner, 1960) and newly hatched tadpoles were fed commercial rabbit food (Purina Rabbit Chow, Purina Mills, St. Louis, MO, USA) daily. We used a single brood for the experiment (remaining broods were used as predator food, see below), and 45 subjects [mean ± standard error (SE) tadpole mass: 0.0127 ± 0.0003 g] were placed in each of 60 aquaria filled with 12 L of filtered river water. We sought to standardize tadpole responses to predation risk by using a single brood (e.g. Storfer & White, 2004; Hossie *et al*., 2010), with the understanding that this approach restricted our ability to generalize across genotypes. Yet, this approach was critical because we assessed differential expression of plastic traits across environments and needed to control the extent of genetic differences among treatments. Further, this approach has the benefit of reducing within-treatment variability and should increase our ability to detect fine-scale responses to predation risk (Hossie & Murray, 2011). As such, variation in our data represents variation in plastic response among full-sib individuals, rather than among genetically independent individuals. Newly hatched tadpoles frequently are found in high densities, and weekly removal of tadpoles (see below) simulated the natural thinning process (Relyea, 2003). The laboratory was kept at 19–20°C with a 12:12 h light-dark schedule and tadpoles in each tank were fed blended boiled spinach *ad libitum* twice a week. Tank water was changed (and experimental treatments re-administered, see below) twice per week, and tadpoles were enumerated three times per week. To preclude any effects of external cues, water used in our experiment had always been aged for at least 72 h (Peacor, 2006; Fraker, 2009). Full water changes ensured temporal accuracy of predator cues in both predator and no-predator conditions.

The experiment was initiated 14 days after egg collection. Experimental treatments consisted of four treatment groups representing variable perceived predation risk administered via exposure versus non-exposure to predator cues. Treat-

ments were as follows: (1) *Control*: no-predator exposure; (2) *Weekly*: constant exposure to predation risk only during alternating weeks (i.e. kairomone exposure during weeks 2, 4, 6); (3) *Half*: no-predator exposure for 3 weeks followed by predator exposure for 3 weeks (i.e. constant kairomone exposure during weeks 4–6); and (4) *Predator*: constant-predator exposure. Each treatment was replicated using 15 tanks, and the predation treatment was administered by housing a single dragonfly larvae (*Anax junius*) in a clear plastic cage (7.5 cm × 13 cm × 7.5 cm) suspended in each tank. Predator cages had slots allowing chemical cues to be freely transferred to the tank without allowing direct contact between predators and tadpoles (Ferland-Raymond & Murray, 2008; Hossie *et al*., 2010). Dragonfly larvae were wild caught and maintained on 3–4 *R. pipiens* tadpoles three times per week through the duration of the experiment. Predators were fed outside the tank so as to eliminate transfer of any alarm substances released by depredated tadpoles. This design allowed us to examine shifts in anti-predator behavioural and morphological response specifically due to variation in perceived predation risk (i.e. predator kairomones, and possibly cues from digested conspecifics) over the course of 6 weeks.

Anti-predator behaviour

Behaviour of tadpoles was measured at 09:00 and 16:00 h 4 days per week by counting the proportion of tadpoles per tank that were (1) active (i.e. tail movement of any kind during 20-s scans), and (2) feeding (i.e. actively consuming provided food). Tadpoles that were feeding were also considered active. We have shown elsewhere (Ferland-Raymond & Murray, 2008; Hossie *et al*., 2010) that when used in our experimental design, the above method is sufficiently precise to detect baseline changes in anti-predator behaviour. Disturbance was minimized by approaching aquaria slowly, performing the behaviour test while standing still and moving carefully between aquaria.

Body size, stage and morphometrics

Morphological responses to treatments were evaluated primarily from analysis of tadpole body and tail shape. At the end of each week (6 weeks), five tadpoles were selected randomly, weighed, staged (Gosner, 1960) and digital pictures were taken of each using a Nikon D70 digital camera (Nikon Inc., Tokyo, Japan) equipped with a Macro 1:1 lens. Tadpoles were photographed alive and assuming a natural resting position inside a water-filled transparent display container. Body size was evaluated by size estimates from morphometric analysis (i.e. centroid size) as well as body mass measurements (i.e. wet weight). Tadpole stage was recorded only in the last 3 weeks of the experiment (i.e. weeks 4–6) as there was no apparent change in Gosner stage during weeks 1–3. Using tpsDig2 v.2.05 software (Rohlf, 2006), 14 landmarks characterizing tadpole shape and size were digitized directly on each picture (Ferland-Raymond & Murray, 2008). We consider that our selected landmarks reflect basic morphological features of tadpoles (see Johnson *et al*., 2008; Hossie *et al*., 2010) and should capture any predator-induced changes such as altered tail fin depth, tail muscle depth or relative body to tail size (e.g. Relyea, 2001; Teplitsky, Plenet & Joly, 2003). Poor-quality pictures (e.g. bent or cut tail or poor tadpole posture, ~12% of $n = 1800$) were discarded. Centroid size for each tadpole ($n = 1587$) was calculated as the square root of the sum of the squared distances from each landmark to the centroid (middle of the digitized landmarks) and averaged per tank. Centroid size is a useful metric of size because it is independent of shape (Zelditch *et al*., 2004). Landmarks were aligned using a procrustes generalized least-squares superimposition and used to create a consensus tadpole for each tank using tpsRegr v.1.36 (Rohlf, 2009). From the consensus tadpole landmarks, coordinates were aligned using a generalized least-squares superimposition and then used to calculate uniform component and partial warps scores (i.e. shape variables) using CVAgen6j software (Sheets, 2003).

Statistical analysis

Proportion of tadpoles that were active and the proportion of active tadpoles feeding (i.e. no. feeding/no. active) was arcsine square root transformed (Krebs, 1999) and analysed by repeated-measures analysis of variance (ANOVA) with WEEK (i.e. 1–6), DAY (i.e. 1–4) and TIME OF DAY (i.e. AM/PM) as repeated measures (i.e. nested within-tank factors) and predator treatment as the factor. Tadpole centroid size, wet weight and stage were averaged per tank and analysed by repeated-measures ANOVA, with WEEK as the repeated measure and predator treatment as the factor.

From the landmark coordinates and centroid size of the consensus tadpoles, using all tanks in all weeks of the study (i.e. $n = 358$), we conducted canonical variates analysis (CVA) using CVAgen6 software (Sheets, 2003), to determine the morphology that best discriminated predator treatment groups (Zelditch *et al*., 2004). A Bartlett's test determined which canonical variates (CVs) differentiated treatment groups, and the significant shape variable (i.e. CV1) was used as our metric for morphological response to perceived predation risk. Once the CV1 was established for each tank in each week, we conducted a repeated-measures ANOVA using predator treatment as the factor and WEEK as the repeated measure. This approach is effective at controlling the common variation in morphology across ontogeny among treatments, while elucidating only the resulting variation among predation risk treatments. It is advantageous to use geometric morphometrics over other shape analyses because they provide a clear representation of the shape variation among treatments (Rohlf & Marcus, 1993; Marcus *et al*., 1996; Zelditch *et al*., 2004). All statistical tests other than CVA (conducted in CVAgen6j; Sheets, 2003) were conducted using Statistica 7 (StatSoft, Inc., 2004).

Results

Anti-predator behaviour

When exposed to perceived predation risk fewer tadpoles were active ($F_{3,56} = 4.75$, $P = 0.005$, Fig. 1a) and fewer of the active

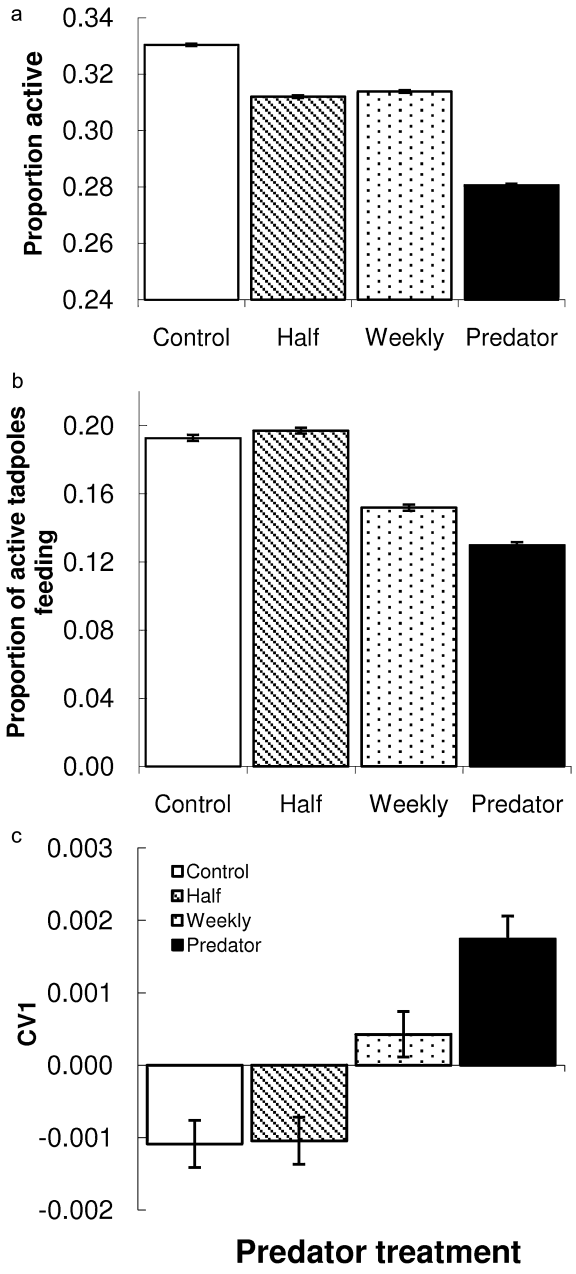

Figure 1 Proportion of *Rana pipiens* tadpoles active (top panel), proportion of active tadpoles foraging (centre panel), and induction of anti-predator morphology (bottom panel) following exposure to predator kairomones from *Anax junius*. CV1 is a shape variable where a large value indicates a relatively deep and long tail with a relatively small body. Error bars indicate standard error.

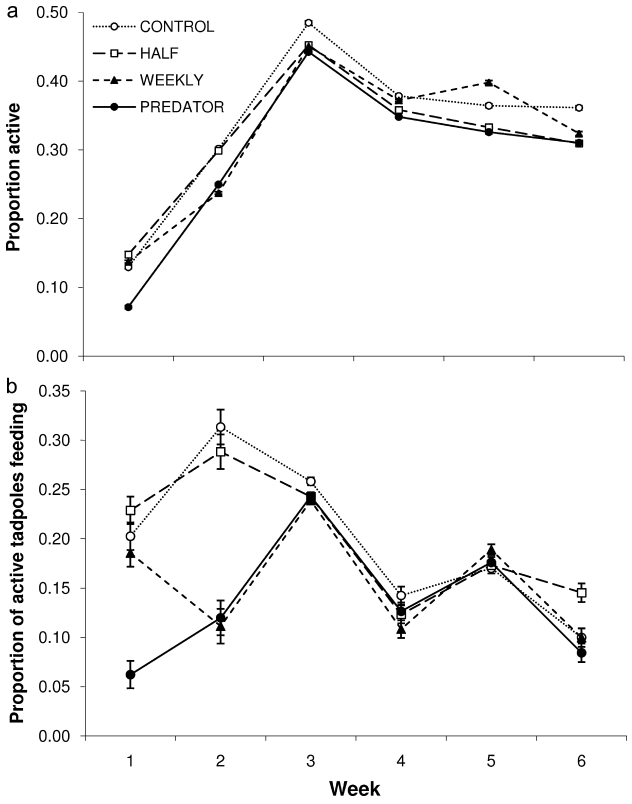

Figure 2 Proportion of *Rana pipiens* tadpoles active (top panel) and proportion of active tadpoles foraging (bottom panel) across ontogeny following exposure to predator kairomones from *Anax junius*. Error bars indicate standard error.

tadpoles were feeding ($F_{3,56} = 4.46$, $P = 0.007$, Fig. 1b). Interestingly, tadpoles in *Weekly* and *Half* treatments showed an intermediate and indistinguishable overall response in the proportion found to be active (mean ± SE, *Control*: 0.33 ± 0.0001, *Half*: 0.31 ± 0.0001, *Weekly*: 0.31 ± 0.0001, *Predator*: 0.28 ± 0.0001); only *Control* and *Predator* treatments were significantly different (Fisher's *post-hoc* $P = 0.003$, Fig. 1a). In comparison, fewer active tadpoles were feeding in the *Predator* treatment than both *Control* and *Half* treatments (Fisher's *post-hoc* $P = 0.026$ and $P = 0.016$, respectively; Fig. 1b). We detected significant interaction between the WEEK variable and treatment for both proportion of active tadpoles ($F_{15, 280} = 3.78$, $P < 0.001$) and proportion of active tadpoles feeding ($F_{15, 280} = 4.44$, $P < 0.001$). These interactions indicated that the response to perceived predation risk varied over the 6 weeks (Fig. 2a,b), with *post-hoc* tests showing that exposure to predator cues during weeks 1 and 2 resulted in fewer tadpoles that were active ($P < 0.05$), and actively feeding ($P < 0.005$). However, tadpole activity in the *Predator* treatment did not differ from the *Control* in week 2 ($P = 0.11$), and was instead intermediate to the *Control* and *Weekly* treatments (*Control*: 0.30 ± 0.002, *Half*: 0.30 ± 0.002, *Weekly*: 0.24 ± 0.002, *Predator*: 0.25 ± 0.002; Fig. 2a). No treatments differed from the *Control* for either behaviour during weeks 3–6 (all $P > 0.13$). Tadpole anti-predator behaviour was thus highly plastic at the outset of the experiment and plasticity was largely restricted to early ontogeny.

Morphological response

The CVA revealed one distinct CV that differentiated shape based on the predator treatments (Wilks $\lambda = 0.718$, $P =$

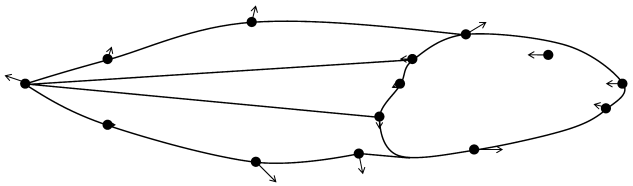

Figure 3 Vector plot showing morphological change following exposure to predation risk as generated from a canonical variates analysis on tadpole morphology. Dots and sketched tadpoles represent a zero CV1 score and vectors point in the direction and relative magnitude of an increasing CV1 (i.e. a morphological response to predation risk).

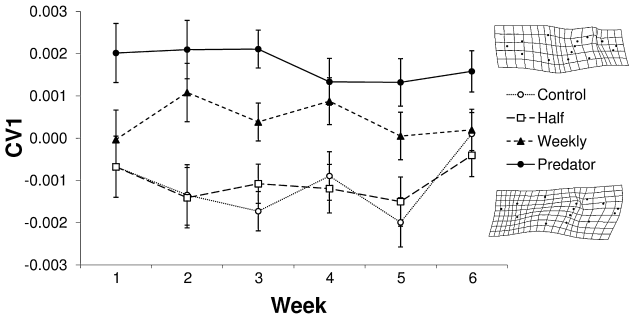

Figure 4 Change in tadpole anti-predator morphology across ontogeny following exposure to predator kairomones from *Anax junius*. An increasing CV1 score translates to a deeper and longer tail combined with a reduction in relative body size. Error bars indicate standard error.

0.00128, d.f. = 75, eigenvalue = 0.286). An increase in CV1 represents an increase in tail depth, tail length and a decrease in relative body size, and thus conformed to our *a priori* expectation of tadpole anti-predator morphology (Fig. 3). Individual treatments differed in their response to predation risk ($F_{3,54}$ = 17.96, $P < 0.001$; Fig. 1c); all main effect treatment pairs differed significantly (Fisher's *post-hoc*: $P < 0.005$) with the exception of the *Control* and *Half* treatment ($P = 0.92$, Fig. 1c). Furthermore, neither WEEK ($F_{5,270} = 1.24$, $P = 0.29$) nor the treatment × WEEK interaction ($F_{15,270} = 0.83$, $P = 0.63$; Fig. 4) affected anti-predator morphology. However, Fisher's *post-hoc* tests revealed that tadpole morphology generally followed the expected patterns. *Control* morphology differed significantly from the *Predator* treatment in weeks 1–5 (all $P < 0.05$), but not week 6 ($P = 0.19$). *Weekly* tadpole morphology was intermediate to *Control* and *Predator* treatments, only differing significantly from *Control* morphology in week 2 (week 1, $P = 0.56$; week 2: $P = 0.033$; week 3: $P = 0.062$; week 4: $P = 0.15$; week 5: $P = 0.071$; week 6: $P = 0.93$), but being indistinguishable from *Predator* morphology throughout weeks 1–6 (week 1, $P = 0.066$; week 2: $P = 0.36$; week 3: $P = 0.12$; week 4: $P = 0.67$; week 5: $P = 0.25$; week 6: $P = 0.21$). Finally, the morphology of tadpoles in the *Half* treatment never differed significantly from the *Control* (all $P > 0.57$), and differed significantly from the *Predator* treatment in all but week 6 (weeks 1–5 all $P < 0.027$; week 6: $P = 0.079$). *Post-hoc* results were confirmed by week-specific one-way ANOVAs. Thus, tadpoles in the *Weekly* treatment responded to variable predation risk by expressing an intermediate anti-predator phenotype, whereas the *Half* treatment failed to mount a morphological response to predation risk.

Table 1 Results from separate repeated-measures ANOVA tests conducted on centroid size, wet weight (g) and developmental stage (Gosner stage, Gosner, 1960). Treatment factor represents the four experimental manipulations of perceived predation risk and the repeated measure was the week of assessment

Term	*F*-value	*P*-value
Centroid size		
Treatment (T)	$F(3,54) = 0.49$	$P = 0.69$
WEEK (W)	$F(5,270) = 416.05$	$P < 0.001$
T × W	$F(15,270) = 0.56$	$P = 0.90$
Wet weight		
Treatment (T)	$F(3,50) = 0.06$	$P = 0.98$
WEEK (W)	$F(5,250) = 227.39$	$P < 0.001$
T × W	$F(15,250) = 0.35$	$P = 0.99$
Gosner stage		
Treatment (T)	$F(3,56) = 0.83$	$P = 0.48$
WEEK (W)	$F(2,112) = 16.95$	$P < 0.001$
T × W	$F(6,112) = 1.12$	$P = 0.35$

Interestingly, it seems that tadpoles in the *Weekly* treatment continued to track variation in predation risk via morphological features in weeks 4 and 5, although tadpoles in the *Half* treatment did not respond morphologically to predation risk (Fig. 4). This indicates that morphological response to predation risk was likely possible late in ontogeny, despite the lack of response by the *Half* treatment tadpoles. Finally, both *Control* and *Half* treatments increased CV1, whereas the *Weekly* treatment remained stationary between weeks 5–6 such that by week, all three were equivalent in shape. As week 6 morphological change was observed in both *Control* and *Half* treatments, apparently it does not represent an induced response to predation risk. Combined, these results indicate that (1) plasticity of the morphological defence may be limited as development progresses, and (2) an optimized morphological structure may exist for late-development tadpoles, which are largely independent of an individual's previous exposure to risk.

Growth, development and survival

Tadpole centroid size, wet weight and stage did not differ among treatment groups, but did change over time as tadpoles grew (week, mean ± SE tadpole mass (g): 1, 0.054 ± 0.001; 2, 0.107 ± 0.002; 3, 0.137 ± 0.003; 4, 0.244 ± 0.008; 5, 0.335 ± 0.013; 6, 0.590 ± 0.025; Table 1). The lack of a main effect of treatment or treatment × WEEK interaction (Table 1) indicates that despite variable exposure to predator cues, tadpoles showed common variation in size, wet weight and development across ontogeny. Tadpole survival over the 6 weeks also was high (mean mortality = ~6%, i.e. 2.75 ± 0.21 tadpoles per tank, range = 0–6 tadpoles) and was unrelated to treatment

($F_{3,56} = 0.34$, $P = 0.79$). Thus, exposure to predator kairomones failed to have a direct effect on tadpole growth, development or survival.

Discussion

By manipulating the duration and pattern of exposure to predator chemical cues, we examined behavioural and morphological responses of prey exposed to temporally variable perceived risk. Our results show that strong behavioural responses were constrained to early ontogeny, but as predicted, tadpole behavioural responses closely matched the appropriate level of risk in the environment. Interestingly, only overall feeding response, not activity *per se*, was affected by the pattern of exposure to predator cues. The morphology of prey experiencing a weekly changing predator environment was intermediate to the shape of prey in the no-predator and constantly exposed treatments. Yet, prey exposed to a predator environment for the same total duration as the weekly changing environment but with a different exposure pattern (i.e. exposed only during weeks 4–6), were morphologically unresponsive to the onset of perceived predation risk. Thus, in our experiment, temporal pattern of exposure to predation risk was at least as important as duration of exposure in governing expression of plastic phenotypes, and associated response lag of a given plastic phenotype predictably affected expression under fluctuating environmental conditions.

Our results suggest that feeding behaviour may be more sensitive to the pattern of predation risk exposure than activity. Despite different patterns of expression during the 6 weeks of study, overall activity response was equivalent between the *Half* and *Weekly* treatments (Fig. 1) and intermediate relative to the no-predator and constant-predator treatments, where the constant-predator treatment had twice as long total exposure. Thus, when combined, weak reductions in activity expressed late in ontogeny in the *Half* treatment roughly matched the strong early response observed in the *Weekly* treatment (Figs 1, 2). In contrast, active feeding in the *Half* treatment was not strongly reduced in late ontogeny, resulting in a greater overall reduction to active foraging in the *Weekly* treatment. This indicates that prey in the *Weekly* treatment did not exhibit compensatory foraging during periods of low risk to make up for energetic losses. Yet, tadpole body mass and survival were unaffected by predator treatments, suggesting that strong early reductions in foraging may be inconsequential when food is abundant throughout ontogeny. Accordingly, in the context of foraging behaviour our results do not match predictions of the risk allocation model, perhaps because our prey were not energy stressed and thus less responsive to levels of risk allocation (Ferrari *et al*., 2009). That behavioural responses were constrained to early ontogeny is consistent with previous work and likely reflects diminishing risk of predation as tadpoles grow larger (e.g. Puttlitz *et al*., 2001; Relyea, 2003). Although a moderated behavioural response might be expected in prey possessing predator-induced shape, we did not observe clear trade-offs between behavioural and morphological responses.

Our study revealed that the pattern of exposure can critically affect prey morphological response to perceived predation risk. A weekly changing environment invoked a morphological phenotype intermediate to the extreme induced and non-induced phenotypes (Fig. 4), whereas prey exposed to perceived risk only in the final 3 weeks (i.e. >5 weeks after egg laying and ~4 weeks after hatching) did not respond morphologically to perceived risk. Support for the Gabriel *et al*. (2005) model was most evident through the observed intermediate phenotypic expression of morphology: when frequency of environmental change is high relative to response lag of the phenotype, prey responses should be intermediate, as was revealed in by our data. The absence of any discernible morphological response among tadpoles from the *Half* treatment could indicate that retaining the 'no-predator' phenotype in late development secures superior fitness benefits through increased foraging efficiency or energy allocation compared to benefits afforded strictly by anti-predator morphology.

Plastic behavioural and morphological traits differed in their expression when confronted with an environment where predation risk varied weekly. Under fluctuating conditions, the behavioural response generally matched the constant-predator or no-predator phenotype that corresponded to the current conditions, whereas morphology was intermediately expressed throughout the experiment. Our study is thus consistent with Gabriel *et al*. (2005) in that expression of a plastic response to perceived risk is sensitive to time required to mount or reverse that response (i.e. the response lag). We note, however, that where reduction in perceived predation risk does not accurately reflect reduction in true predation risk, prey should be conservative in their allocation to anti-predator phenotypes.

Our results also identify a morphological change in unexposed (*Control*) prey consistent with anti-predator morphology (Fig. 4) that coincided with the development of limb buds (mean ± SE Gosner stage week 6 = 28.85 ± 0.23). As this shift in morphology also corresponds to a tadpole shape that has greater thrust (McCollum & Leimberger, 1997; Dayton *et al*., 2005; Johnson *et al*., 2008), we interpret this as either (1) increased tail thrust to maintain swimming efficiency as emergent limb buds increase drag (energetic efficiency hypothesis) or (2) morphological compensation for increased risk of capture late in development (innate predator defence hypothesis). Tadpole shape consistent with induced anti-predator morphology is known to provide greater thrust (e.g. Dayton *et al*., 2005; Johnson *et al*., 2008), and the fluid dynamics of tadpole swimming suggests that shape in metamorphic tadpoles is strongly influenced by swimming efficiency (Liu, Wasserberg & Kawachi, 1996). Conversely, increased vulnerability to predation during and following limb development (e.g. Crump, 1984; Brown & Taylor, 1995), and increased behavioural sensitivity to risk when close to metamorphosis (Laurila *et al*., 2004), support the innate anti-predator response hypothesis. Elucidating the purpose of these late-development changes to morphology requires experiments that determine the effects of specific late-development morphologies on performance in both survival and swimming speed experiments.

In conclusion, we consider that phenotypic expression depends critically on temporal variability in the environment, although the extent of expression varies according to the specific trait in question. One important limitation of our study is that all of the animals originated from a single egg brood meaning that the among-tank variation observed here represents intra-brood variability. Although this approach restricts generalization across genotypes, it was instrumental in allowing us to assess differential expression of plastic traits across treatments because it controlled genetic variation. The generality of our conclusions can be assessed by repeating the work and dividing tadpoles from multiple genetic lineages evenly among treatments. This would allow detection and control of inter-brood variation in reaction norms to temporal variation in predation risk not examined in this study. Future work should also test our conclusions more broadly by similarly examining how other taxa respond to temporally heterogeneous predator exposures. Ultimately, such efforts will provide a critical step towards developing a realistic and synthetic basis for our understanding of how prey respond to variable risk in natural systems.

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