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Morphological and behavioural responses of frog tadpoles to perceived predation risk: A possible role for corticosterone mediation?[1]

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Abstract: Predators can have an important influence on prey survival and fitness, and many prey species exhibit morphological or behavioural responses to perceived predation risk. Although basic characteristics of anti-predator responses have been well documented, physiological pathways underlying such responses are poorly understood. We sought evidence for a role of corticosterone, a major stress hormone in amphibians, in the behavioural and morphological anti-predator responses of leopard frog tadpoles (*Rana pipiens*) exposed to caged dragonfly nymphs (*Aeshna* spp.). By superimposing a metyrapone treatment (corticosteroid synthesis inhibitor) over chronic predator exposure in a 2 × 2 factorial design, we evaluated if tadpole anti-predator responses were mediated by corticosterone. Tadpoles were less active and more likely to exhibit a startle response when exposed to perceived predation risk, but direct and interactive effects of the metyrapone treatment on behaviour were negligible. Predator-exposed tadpoles grew larger and had deeper tail fins, whereas the metyrapone treatment resulted in smaller tadpoles with shallower tail fins. Tadpoles simultaneously exposed to metyrapone treatment and predation risk had reduced tail-fin depth and increased body:tail ratio compared to steroid-normal animals. Because both traits are implicated in tadpole vulnerability to predation, these results suggest that the corticosteroid pathway may mediate tadpole morphological response to perceived predation risk. We provide evidence supporting a possible role for corticosterone in anti-predator responses of amphibians specifically in terms of morphological responses. Our results suggest that corticosteroid adjustment may impact prey survival through phenotypic change upon exposure to predation risk and thereby suggest a possible functional role of this hormonal pathway in amphibian physiological ecology.
Keywords: amphibian, glucocorticoids, morphometrics, physiological ecology, predator–prey interactions, stress.

Résumé : Les prédateurs peuvent avoir une influence importante sur la survie et la valeur adaptative de leurs proies et plusieurs espèces de proies présentent des réponses morphologiques ou comportementales au risque perçu de prédation. Bien que les caractéristiques de base des réponses anti-prédateurs soient bien documentées, les processus physiologiques sous-jacents sont encore mal compris. Nous avons examiné si la corticostérone, une hormone de stress principale chez les amphibiens, joue un rôle dans les réponses anti-prédateurs comportementales et morphologiques chez les têtards de la grenouille léopard (*Rana pipiens*) exposés à des nymphes de libellules (*Aeshna* spp.) en cage. En superposant un traitement à la métyrapone (un inhibiteur de la synthèse corticostéroïde) à une exposition chronique à des prédateurs dans un plan factoriel 2 × 2, nous avons évalué si la corticostérone jouait un rôle dans les réponses anti-prédateurs des têtards. Les têtards étaient moins actifs et avaient plus tendance à sursauter lorsqu'ils étaient exposés à un risque perçu de prédation, mais les effets directs et interactifs du traitement à la métyrapone sur le comportement étaient négligeables. Les têtards exposés aux prédateurs ont atteint une plus grande taille et avaient des nageoires caudales plus longues, alors que les têtards ayant reçu le traitement à la métyrapone sont restés plus petits et avaient des nageoires caudales plus courtes. Les têtards exposés simultanément au traitement à la métyrapone et au risque de prédation avaient des nageoires caudales plus courtes et un ratio taille corporelle:queue plus élevé que les animaux ayant un taux de stéroïde normal. Puisque ces deux traits sont impliqués dans la vulnérabilité des têtards à la prédation, ces résultats suggèrent que la corticostéroïde puisse être impliquée dans la réponse morphologique des têtards au risque perçu de prédation. Nous fournissons ainsi des évidences d'un rôle possible de la corticostérone dans les réponses anti-prédateurs d'amphibiens spécifiquement en termes de réponses morphologiques. Nos résultats suggèrent qu'un ajustement de la corticostéroïde puisse avoir un impact sur la survie des proies via un changement phénotypique suite à une exposition au risque de prédation et suggèrent ainsi un rôle fonctionnel possible de ce processus hormonal dans l'écophysiologie des amphibiens.
Mots-clés : amphibien, écologie physiologique, glucocorticoïdes, interactions prédateur-proie, morphométrie, stress.

Nomenclature : Hillis, 1988.

Introduction

Predators are known to reduce prey fitness either directly by increasing mortality or indirectly by eliciting anti-predator responses leading to compromised survival or reproduction. Predation therefore is an important selective pressure, and morphological and behavioural attributes of many prey species can be shaped adaptively by predation risk (Tollrian & Harvell, 1999; Lind & Cresswell, 2005; Steiner, 2007). Yet, despite extensive literature documenting phenotypic responses of prey to perceived predation

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risk, most notably in larval amphibian systems (Anholt & Werner, 1998; Relyea, 2001; Dayton *et al*., 2005; Kishida & Nishimura, 2006), to date a mechanistic explanation for such responses has not been forthcoming. Indeed, it is well understood that predation risk can elicit complex physiological changes that influence prey morphology or behaviour (Orchinik, 1998; Sih, Bell & Kerby, 2004; Reeder & Kramer, 2005), but currently there is poor understanding of the specific physiological pathway(s) by which prey invoke responses that reduce their mortality risk. Doubtless, improved understanding of processes involved in the predation risk response will increase our ability to predict likely outcomes of complex predator–prey interactions.

The corticosteroid pathway is a logical candidate for anti-predator responses because various stressors can induce glucocorticoid release in many vertebrates, including amphibians (Glennemeier & Denver, 2002a; Moore & Jessop, 2003; Belden *et al*., 2005). It follows that associated neurons are known to respond to circulating corticosterone within minutes (Orchinik, 1998). Activation of the hypothalamic–pituitary–adrenal axis (hypothalamic–pituitary–interrenal axis in amphibians) resulting in corticosterone release may be critical for surviving or avoiding predator attacks (Wingfield, Breuner & Jacobs, 1997; Orchinik, 1998). In addition, dramatic and transient increases in glucocorticoid levels are a prominent feature of the vertebrate stress response (Wingfield, Breuner & Jacobs, 1997; Orchinik, 1998), and it is understood that glucocorticoids can alter dominant sensory modality, thereby shifting behaviours to those addressing potential threats (Rose, Moore & Orchinik, 1993; Orchinik, 1998). More generally, stress-induced responses are known to influence amphibian behaviour (*i.e*., suppression of courtship: Moore & Miller, 1984) and morphology (Glennemeier & Denver, 2002b) and thus may be integrally linked to anti-predator responses. Indeed, chronic predation risk is a candidate explanation for the unresolved among-pond variation in tadpole basal corticosterone levels (Belden *et al*., 2007).

In this paper we examine the role of corticosteroids in mediating behavioural and morphological responses of leopard frog (*Rana pipiens*) tadpoles to perceived predation risk. By superimposing a steroid block over chronic predator exposure in a 2 × 2 factorial design, we sought to determine if anti-predator responses of tadpoles were limited when corticosteroid synthesis was curtailed. We predicted that tadpole anti-predator responses would be impeded by application of the steroid block, such that the treatment would limit responses likely along both behavioural and morphological axes. It follows that successful reduction of the tadpole stress response when exposed to predation risk should result in a significant predator exposure × steroid-block interaction; such interaction should imply differential response to perceived predation risk depending on stress response functionality.

Methods

Laboratory methods and experimental design

Rana pipiens eggs were collected in April 2006 from ponds near Peterborough, Ontario (44° 22' N, 78° 03' W). Six egg-broods (~2000 eggs) were reared in 110-L containers to Gosner stage 25 (Gosner, 1960), and newly hatched tadpoles were fed commercial rabbit food (Purina Rabbit Chow, Purina Mills, St. Louis, Missouri, USA) daily. We used a single brood for the experiment (remaining broods were used as predator food, see below), and 25 subjects were placed in each of sixty 10-L aquaria filled with 8 L of filtered river water. The laboratory was kept at 19–20 °C with a 12:12 h light–dark schedule, and tadpoles in each tank were fed 2.46 mL blended boiled spinach. Water was changed (and experimental treatments re-administered, see below) 3 times per week.

Experimental treatments consisted of exposure to *i*) perceived predation risk and *ii*) corticosteroid synthesis inhibitor (metyrapone), with associated controls (*i.e*., no predation risk, no metyrapone), arranged in a 2 × 2 factorial design with 15 replicates. This design allowed us to seek evidence for direct and interactive effects of treatments in the following 4 treatment groups: *i*) predator-control and metyrapone-control; *ii*) predator-exposed and metyrapone-control; *iii*) predator-control and metyrapone-treated; and *iv*) predator-exposed and metyrapone-treated. The predation treatment was administered by housing a single dragonfly larvae (*Aeshna* spp.) in a clear plastic cage (7.5 × 13 × 7.5 cm) suspended in each tank; predator cages had slots allowing chemical cues to be transferred to the tank without allowing direct contact between predators and tadpoles (Ireland, Wirsing & Murray, 2007; Ferland-Raymond & Murray, 2008). Dragonfly nymphs were wild-caught and maintained on 3–4 *R. pipiens* tadpoles 3 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. We reduced corticosterone in tadpoles by administering a low concentration of metyrapone (MTP; Sigma-Aldrich, St. Louis, Missouri, USA) to each tank. MTP (2-Methyl-1,2-di-3-pyridyl-1-propanone) is an effective corticosterone synthesis inhibitor that has been used previously to demonstrate the role of corticosterone in the stress responses of tadpoles via significant corticosterone reduction (Glennemeier & Denver, 2002a,b; Yao, Hu & Denver, 2008). We dissolved MTP in ethanol and added it to metyrapone-treated tanks to achieve a final concentration of 110 µM MTP. This concentration is known to reduce total whole-body corticosterone content (> 50% reduction) in *R. pipiens* tadpoles not subject to either food limitation or density dependence (Glennemeier & Denver, 2002a,b), as well as decrease corticosterone to undetectable levels in juvenile *Xenopus laevis* (Yao, Hu & Denver, 2008). Accordingly, we expected that the treatment would elicit comparable responses in our experiment. MTP is not known to be toxic to tadpoles when applied in the above concentration (see Glennemeier & Denver, 2002a,b). Ethanol was added to treatments without MTP in an equivalent concentration (0.002% total water volume) to control for any effect of ethanol.

Anti-predator behavioural response

Behaviour of tadpoles was measured at 0900 and 1600, 5 d per week, by counting the tadpoles per tank that

were *i*) active (*i.e.*, tail movement of any kind during 20-s scans) and *ii*) burst-swimming (*i.e.*, any discrete rapid swim, reflecting fear-induced startle behaviour). Tadpoles that were burst-swimming were also considered active. We have shown elsewhere (Ferland-Raymond & Murray, 2008) that when used specifically in our experimental design, the above metrics are sufficiently precise to detect basic anti-predator behaviour in tadpoles.

BODY SIZE AND MORPHOMETRICS

Morphological responses to treatments were evaluated primarily from analysis of tadpole body and tail shape; body mass measurements also were collected but were correlated with tadpole size estimates from morphometric analysis (*i.e.*, centroid size); herein we present the latter data exclusively. At the end of the experiment (3 weeks) digital pictures were taken of each tadpole at a fixed distance using a Nikon D70 digital camera equipped with a Tamron 90-mm Macro 1:1 lens and using tpsDig2 v.2.05 software (Rohlf, 2006). Fourteen landmarks characterizing tadpole shape and size were digitized directly on each picture (Ferland-Raymond & Murray, 2008). Poor-quality pictures (*e.g.*, bent or cut tail or poor tadpole posture, $n = 285$, 19%) were discarded. Centroid size for each tadpole ($n = 1178$) 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.1.31 (Rohlf, 2005). We consider that our selected landmarks reflect basic morphological features of tadpoles (see Dayton *et al.*, 2005; Ferland-Raymond & Murray, 2008) and should capture any predator-induced changes, such as altered tail-fin depth, tail muscle depth, or relative body to tail size (*e.g.*, see Relyea, 2001; Teplitsky, Plenet & Joly, 2003).

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 tpsRegr v. 1.31 software (Rohlf, 2005). Uniform components are variations in shape that leave parallel lines (*i.e.*, having the same degree of change without morphological bending) (Zelditch *et al.*, 2004). Partial warps, on the other hand, are components that describe morphological deformation and are non-uniform (Zelditch *et al.*, 2004). Landmark-based geometric morphometrics have been used increasingly in morphometric studies (Birch, 1997; Dayton *et al.*, 2005; Olsson, Svanback & Eklöv, 2007).

STATISTICAL ANALYSIS

The proportion of tadpoles that were active was arcsine square-root transformed (Krebs, 1999) and analyzed by repeated measures ANOVA with time of day serving as a statistical block (*i.e.*, random variable, AM or PM). The number of tadpoles that were burst-swimming was analyzed using the Wald statistic in a generalized linear model adjusted for a Poisson distribution and with date and time of day blocked as random variables. Centroid size was analyzed by a two-way ANOVA with predation risk and MTP treatment as factors.

Using the landmark coordinates and centroid size of the consensus tadpoles we conducted a canonical variates analysis (CVA), using CVAgen6 software (Sheets, 2003), to determine the morphology that best discriminated treatment groups (Zelditch *et al.*, 2004). A Bartlett's test determined which canonical variates (CVs) discriminated between groups. We used thin-plate splines generated from TwoGroup6h (Sheets, 2000) to visualize deformations in shape between treatment pairs (Bookstein, 1991). To test how tadpole morphology differed between treatment groups, 5 pairwise comparisons were conducted using Goodall's *F*-test (Procrustes) in TwoGroup6h software (Sheets, 2000). Significance levels for these tests were corrected using Hochberg's Sequential Bonferroni (Quinn & Keough, 2002). It is advantageous to use geometric morphometrics such as CVA over other shape analyses because they provide a clear representation of the variation among treatments (Rohlf & Marcus, 1993; Marcus *et al.*, 1996, Zelditch *et al.*, 2004). We used multivariate analysis of covariance (MANCOVA) on uniform components and partial warps to evaluate the significance of treatment-induced differences in tadpole morphology, where predator and MTP treatments were independent variables and centroid size was the covariate. All statistical tests were conducted using Statistica 7 (StatSoft, 2004).

Results

ANTI-PREDATOR BEHAVIOURAL RESPONSE

The proportion of tadpoles active per tank declined by ~7% following exposure to predation risk ($F_{1, 115} = 132.64$, $P < 0.001$), whereas the MTP-treated tadpoles showed activity reduction by a nominal (but significant) ~1% ($F_{1, 115} = 4.37$, $P = 0.039$) (Figure 1a). A Tukey's HSD *post hoc* test showed that in the absence of predation risk, treatments with normal or reduced corticosterone levels did not elicit significantly different tadpole activity ($P = 0.07$); however, the trend suggests that in the absence of predation risk, application of MTP reduced activity levels. Most importantly, MTP treatment did not affect activity in the presence of predation risk (Tukey's HSD $P = 0.96$), and this finding was further supported by the absence of statistical interaction between treatments (predator × MTP treatment interaction: $F_{1, 115} = 1.91$, $P = 0.169$; Figure 1a). Thus, the MTP treatment failed to have an effect on tadpole activity that was related to predation risk. Time of day did not influence tadpole activity levels ($F_{1, 115} = 2.69$, $P = 0.103$).

Predator treatment elicited a significant increase (Control = 0.087 ± 0.015, Predator = 0.49 ± 0.015, Wald $\chi^2 = 150.48$, $P < 0.001$, Figure 1b) increase in the proportion of tadpoles exhibiting burst-swimming behaviour, whereas the MTP treatment failed to elicit comparable behavioural changes (Wald $\chi^2 = 1.62$, $P = 0.20$, Figure 1b). There was no significant interaction between treatments (Wald $\chi^2 = 0.64$, $P = 0.42$), indicating that burst-swimming behaviour was elevated in predator-exposed tadpoles irrespective of MTP treatment (Figure 1b). The blocked random effects time of day and date had a slight influence

on burst-swimming behaviour (Wald $\chi^2 = 4.83$, $P = 0.028$; Wald $\chi^2 = 119.17$, $P < 0.001$, respectively). Thus, we found that predation risk influenced both activity and burst-swimming in tadpoles; however, the MTP treatment had a negligible influence on activity level and was not related to perceived predation risk.

Body size and morphometrics

Tadpoles exhibited a variety of morphological changes following exposure to experimental treatments. Both predator exposure ($F_{1, 56} = 14.85$, $P < 0.001$) and MTP treatment ($F_{1, 56} = 35.17$, $P < 0.001$) influenced tadpole size, with the centroid size increasing by ~0.70 mm (*post hoc* test: $P < 0.05$) when predators were present and decreasing by ~1.1 mm (*post hoc*: $P < 0.05$) when MTP-treated (Figure 2). The non-significant predator × MTP treatment interaction term ($F_{1, 56} = 1.01$, $P = 0.32$) confirmed that predator presence increased centroid size irrespective of the MTP treatment (Figure 2). We noted a strong positive correlation between tadpole body mass and centroid size ($r = 0.961$, $P < 0.001$).

When we controlled for the effect of tadpole size on morphological features by scaling all centroid sizes to a common value, we noted that predator exposure influenced morphology by eliciting a deeper tail fin and proportionally smaller body size ($F_{24, 32} = 7.50$, $P < 0.001$, Figures 3a and 4a). In contrast, the MTP treatment caused tadpoles to have shallower tail depth and larger relative body size compared to MTP-control treatments ($F_{24, 32} = 16.02$, $P < 0.001$, Figures 3a and 4b). There was a significant predator × MTP treatment interaction ($F_{24, 32} = 2.22$, $P = 0.018$), which highlighted differential response to perceived predation risk depending on whether MTP was applied or not. This finding confirms that when tadpole shape was scaled for size, the MTP treatment influenced morphological response to perceived predation risk, resulting in a slight tail-fin depth reduction and slight increase in body:tail ratio relative to control tadpole morphology (Figure 4).

Canonical variates analysis yielded 3 significant CVs to distinguish treatment groups: CV1 Wilks' $\lambda = 0.004$, eigenvalue = 17.5992, $P << 0.001$; CV2 Wilks' $\lambda = 0.071$, eigenvalue = 4.2231, $P << 0.001$; CV3 Wilks' $\lambda = 0.370$, eigenvalue = 1.7063, $P = 0.003$; $n = 60$ (Figure 3). Our focus will be on CV1 because it contributed substantially more to the discrimination among treatment group morphologies (CV1 Partial Wilks' $\lambda = 0.054$; CV2 Partial Wilks' $\lambda = 0.191$; CV3 Partial Wilks' $\lambda = 0.370$). We interpret increase in CV1 as an increase in tail-fin depth with a reduction in relative body size (Figure 3a), increase in CV2 as a reduction in tail-fin symmetry with an increase in tail-fin area below the tail muscle (Figure 3a), and increase in CV3 as an increase in anterior body depth (Figure 3b).

Pairwise comparisons of treatment group morphology showed that all treatment groups differed from each other (Table I). Comparison between predator-control and

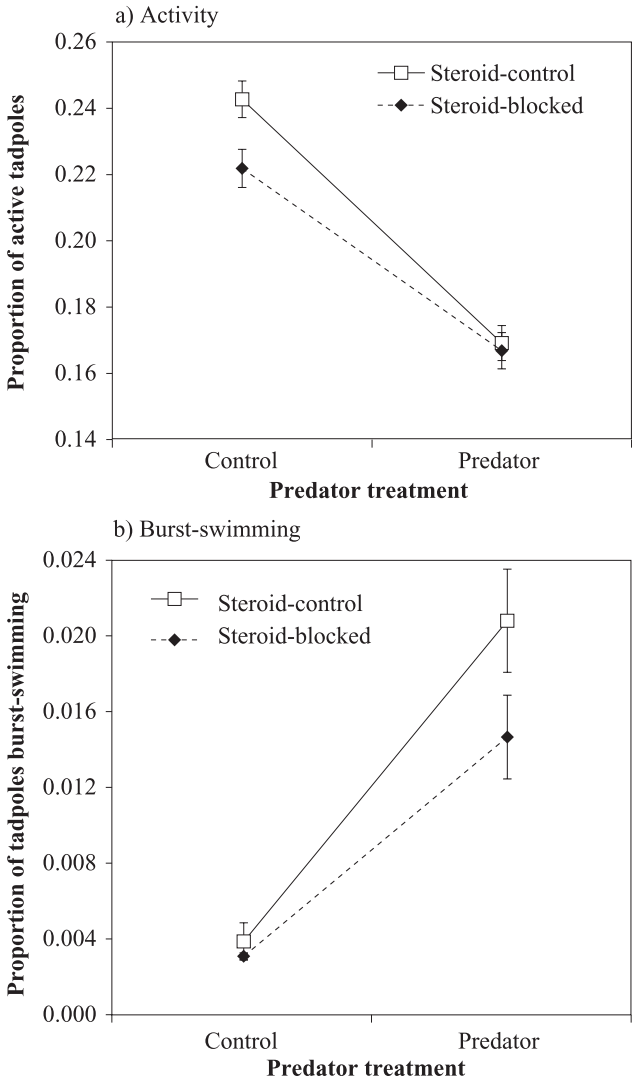

FIGURE 1. Effect of predator exposure and metyrapone treatment on mean (± SE, $n = 15$) a) proportion of active tadpoles and b) number of tadpoles burst-swimming. Observations were taken over 3 weeks.

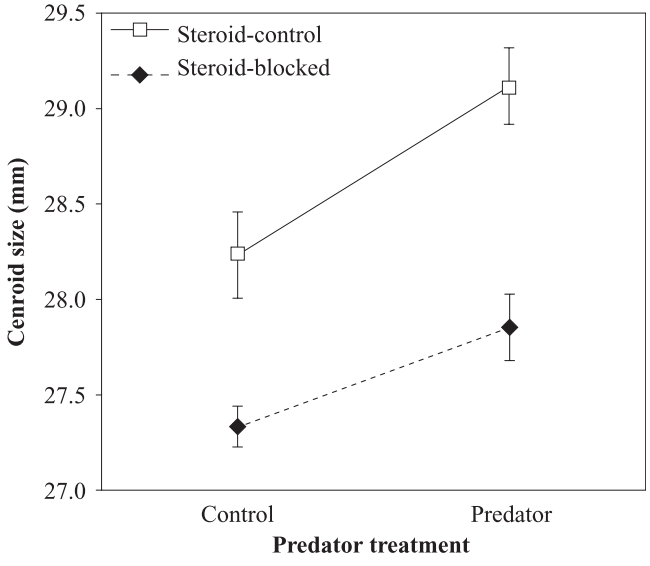

FIGURE 2. Effect of predator exposure and metyrapone treatment on mean (± SE, $n = 15$) centroid size of *R. pipiens* tadpoles. Centroids were averaged per tank and analyzed by factorial ANOVA.

predator-exposed treatments when tadpoles were not treated with MTP indicated that exposure to predation risk results in greater tail depth and smaller body size relative to tail size (Figure 3a). The same pattern was observed upon comparing morphology of predator-control and predator-exposed treatments when the MTP treatment was applied (Figure 3a), implying that tadpoles with impeded corticosterone production continued to exhibit some degree of morphological response to perceived predation risk. However, comparison of tadpole morphology between MTP-control and MTP-treated groups revealed that application of MTP results in shallower tail depth and larger body size relative to tail size, irrespective of predator treatment (Figure 3a). Thus, predator exposure and MTP treatment altered tadpole morphology in opposing directions, such that tadpoles from the predator-exposed/MTP-treated group showed the least deviation in morphology compared to predator-control/MTP-control tadpoles (Table I; Figure 3a). It should be

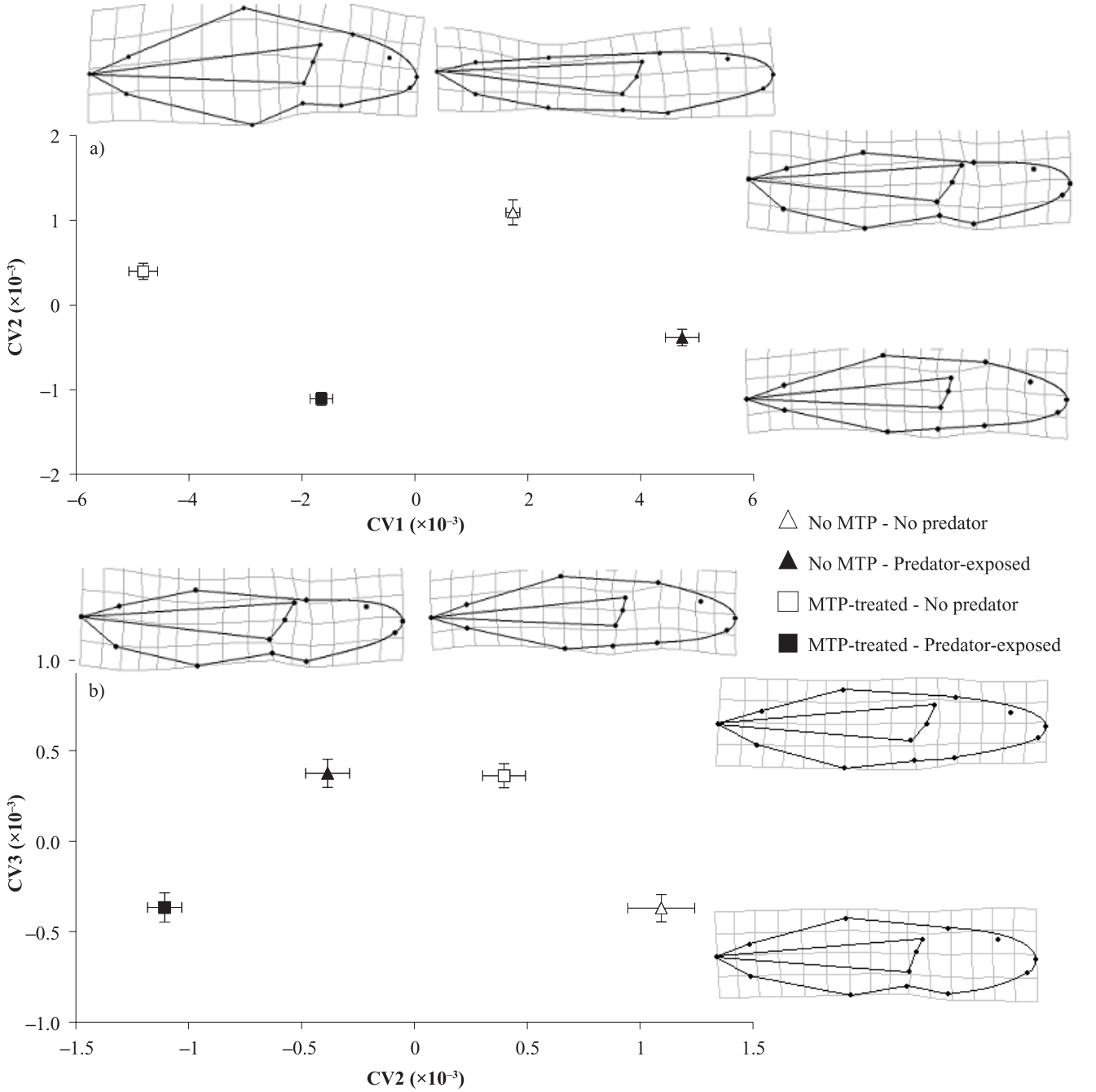

FIGURE 3. Mean (± SE, n = 15) for the first 3 morphological variables in a canonical variates (CV) analysis of *R. pipiens* tadpole morphology in relation to predator exposure and metyrapone (MTP) treatment. Each data point represents 1 treatment; filled symbols indicate predator-exposed, and open symbols represent predator-control treatments. Triangles represent MTP-control and squares represent MTP-treated treatments. Tadpole grid plots are visualizations from tpsRegr (Rohlf, 2005) depicting extreme morphologies for each CV. a) Grid plots are at the top of the Figure for CV1 and to the right for CV2; b) Grid plots are at the top of the figure for CV2 and to the right for CV3.

noted that tadpole stage did not change over the course of the experiment in any of the treatments.

To summarize, exposure to predation risk influenced all measured tadpole behaviours, but the MTP treatment did not have notable direct effects on tadpole behaviour nor any influence on perceived predation risk. In contrast, predator exposure and MTP treatment had opposite effects on tadpole centroid size, tail depth, and relative body size, and the interaction between treatments when morphology was scaled for body size clearly revealed the importance of the stress pathway in tadpole morphological responses to predation risk.

Discussion

We showed that application of MTP, a known corticosterone inhibitor, influenced morphological but not behavioural responses to chronic predation risk in tadpoles. The observed morphological changes are consistent with more general adaptive responses to predation risk in amphibian tadpoles (Relyea, 2001; 2003; Peacor & Werner, 2004) and therefore support the hypothesis that at least some anti-predator responses could be mediated by the corticosteroid pathway. Our study is among the first to evaluate experimentally the role of corticosterone in the production of anti-predator responses in larval amphibians (but see Fraker *et al*., 2009) and is to our knowledge the first to assess the potential role of corticosterone in tadpole morphological anti-predator defences. Our findings indicate that adaptive morphological responses to chronic perceived predation risk are likely to be integrally linked to endogenous corticosterone levels.

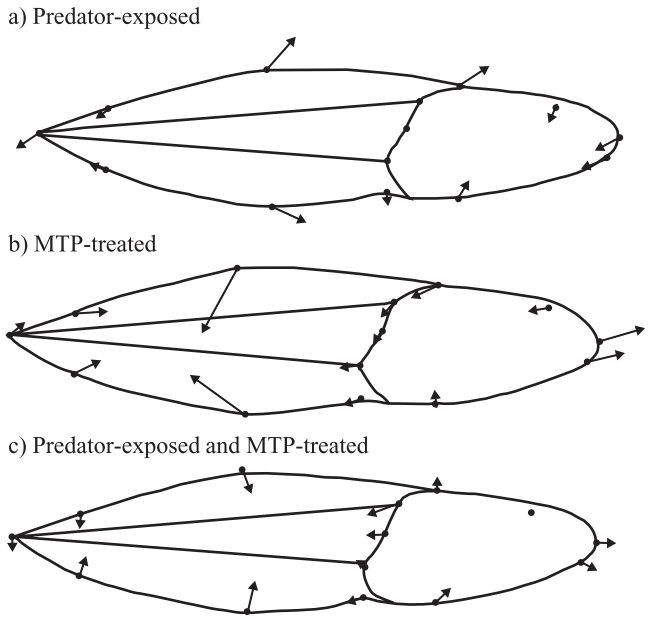

FIGURE 4. Vector plots showing morphological change resulting from the predator and metyrapone (MTP) treatments. Dots and sketched tadpoles represent predator-control and MTP-control tadpole morphology and vectors point in the direction and magnitude (with 15× exaggeration) of morphological change for respective treatments: a) Predator-exposed; b) MTP-treated; c) Predator-exposed and MTP-treated.

Because our experimental methods (*e.g*., frog species, tadpole stage, MTP dosage) were consistent with those adopted by Glennemeier and Denver (2002a), we infer that our MTP treatment was comparably effective at reducing endogenous corticosterone levels. This was further supported by our similar results showing morphological changes induced by MTP treatment (see Glennemeier & Denver, 2002b). The morphological response to predation risk seen even among MTP-treated tadpoles indicated that we likely failed to produce a complete blockage in endogenous corticosterone (see Glennemeier & Denver, 2002a), and the absence of any evidence of tadpole mortality due to MTP treatment supports the contention that MTP application was non-toxic. Furthermore, several studies on tadpoles have utilized MTP to block corticosterone and failed to observe pathological behaviour or other indication of toxic effects (Hayes & Wu, 1995; Glennemeier & Denver, 2002b; Crespi & Denver, 2004; Crespi, Vaudry & Denver, 2004; Yao, Hu & Denver, 2008). In fact, the MTP treatment had a larger absolute impact on morphology than our predation risk treatment, and thus we reasonably conclude that our MTP treatment was sufficiently effective to disentangle the complex role of corticosterone in anti-predator responses. Additionally, behavioural (Relyea, 2001; Watkins & McPeek, 2006; Ferland-Raymond & Murray, 2008) and morphological (Relyea, 2001; 2003; Peacor & Werner, 2004) responses similar to those we observed have been shown in the context of anti-predator responses of other frog species, so we conclude that our MTP treatment provided a biologically realistic manipulation of tadpole stress levels.

Although transient regulation of corticosterone has been suggested as underlying behavioural responses and selective sensory modulation in the amphibian system (Orchinik, 1998; Gasser & Orchinik, 2007; Fraker *et al*., 2009), we failed to observe a strong effect of MTP treatment

TABLE I. Distance between mean morphological scores for pairwise treatment comparisons of consensus *Rana pipiens* tadpoles exposed to predator and metyrapone (MTP) treatments. Tests of significance preformed with Goodall's *F*-test (df = 24, 672) and corrected using Hochberg's Sequential Bonferroni correction ($n = 30$).

Treatments compared	Distance between means	Goodall's *F*-test	Corrected α	*P*-value
Predator control-MTP control:Predator exposed-MTP treated	0.0061	1.8	0.05	0.01
Predator control-MTP control:Predator exposed-MTP control	0.008	2.85	0.025	< 0.001
Predator exposed-MTP control:Predator exposed-MTP treated	0.0105	5.32	0.0125	< 0.001
Predator control-MTP treated:Predator exposed-MTP treated	0.0113	5.98	0.00625	< 0.001
Predator control-MTP control:Predator control-MTP treated	0.0126	6.78	0.003125	< 0.001

on tadpole anti-predator behaviour. Interestingly, MTP-treated tadpoles in our experiment tended to show increased quiescence (*e.g.*, reduced activity and burst-swimming; Figure 1), suggesting that a reduction of corticosteroids may have resulted in decreased activity. This is consistent with a recent paper by Fraker and colleagues (2009) that showed that acute exposure to predation risk caused dose-dependent suppression of the tadpole neuroendocrine stress axis resulting in dose-dependent behavioural quiescence. Despite the occurrence of transient corticosterone suppression in the presence of an acute stressor (as suggested by Fraker *et al.*, 2009), chronic exposure to predation risk may cause increased basal corticosterone levels; ongoing research in our lab will test this hypothesis. Nevertheless, it remains evident that the mechanism for anti-predator behaviour and the specific role of corticosterone in such behaviour remain to be fully understood.

Our study demonstrates changes in tadpole morphology related to predation risk and MTP treatment that are consistent with our hypothesis that corticosterone mediates anti-predator morphological responses. Movement along the CV1 axis provides compelling evidence that changes in tail-fin depth are related to the quantity of endogenous corticosterone (Figure 3a). We observed opposite but analogous changes in morphology when we compared treatment-related tadpole morphologies; predator exposure increased tail-fin depth and tail:body ratio, while comparing MTP-control and MTP-treated tadpole morphologies revealed that a reduction in tail-fin depth and tail:body ratio resulted from application of the corticosteroid synthesis inhibitor. This is consistent with general findings showing that corticosterone administration increased tadpole dorsal–ventral tail morphology (Glennemeier & Denver, 2002b). Hence, morphological change associated with movement along the CV1 axis could be explained by the fact that chronic predator stress resulted in up-regulation of basal corticosterone and thereby produced increased tail-fin depth. We propose that treatment-related differences in morphology occurred as a result of the manipulation of endogenous corticosterone via the opposing mechanisms of chronic predator-stress *versus* the MTP treatment. It seems logical that chronic predator-stress resulted in up-regulation of basal corticosterone levels, which induced deeper tail-fin morphology, while shallower tail-fins resulted from the MTP treatment directly reducing the amount of circulating corticosterone. Thus, we provide support for the potential role of corticosterone as a physiological mediator involved in translating predator cues into an adaptive morphological response. However, additional research should focus more extensively on the specific linkage between corticosterone mediation and the range and magnitude of prey responses to predation risk.

We observed morphometric differences across the MTP treatments when predation risk was held constant; thus, it seems that the specific level of endogenous corticosterone may regulate the magnitude of expression for the aforementioned morphological phenotypes (tail depth, relative body-tail size). Assuming predator cues are a reliable estimate of predation risk, up-regulation of corticosterone in the presence of cues would allow tadpoles to adjust the degree of phenotypic expression based on the persistence of cues (*i.e.*, predation risk) over time and space. Since producing anti-predator phenotypes is energetically costly (Steiner, 2007), this mechanism would permit efficient regulation of the response magnitude and allow optimal expression of anti-predator morphology to maximize fitness in dynamic predator environments.

Tadpoles exposed to perceived predation risk were larger than those not exposed to predators. Such increases in body size may represent an adaptive strategy for tadpoles to attain a size-refuge, since it is well documented that larger tadpoles are less vulnerable to predation (Travis, Keen & Juilianna, 1985; McCoy & Bolker, 2008). In our experiment, tadpole size was reduced by the MTP treatment. It is conceivable that MTP-induced quiescence may have limited the rate of food acquisition in MTP-treated tadpoles relative to the MTP-control. Alternatively, the corticosteroid pathway may be linked with attaining a size refuge if predator-induced up-regulation of basal corticosterone leads to greater investment of energy into growth. Although up-regulation of corticosterone has been associated with growth reductions in tadpoles (Hayes, Chan & Licht, 1993; Glennemeier & Denver, 2002a,b) our findings remain consistent with previous tadpole research and suggest that elevated corticosterone concentrations could in some cases result in a slightly increased growth rate (*e.g.*, 8M treatment, Figure 2: Glennemeier & Denver, 2002a). Moreover, corticosterone-mediated growth reduction has been observed only during application of pharmacological concentrations of corticosterone or when testing tadpoles at extreme cases of high density or low resources (Glennemeier & Denver, 2002a,b; Belden *et al*., 2007). Thus, to our knowledge our results are the first to show significant change in tadpole growth rate related to corticosterone (*i.e*., via application of a corticosteroid inhibitor) in the absence of resource limitation, high density of conspecifics, or application of a pharmacological dose of corticosterone. Accordingly, our results may be indicative of an adaptive growth response by tadpoles upon chronic predation risk; however, further empirical testing is required. Tadpoles from only a single brood were used in our experiment, which potentially limits the generality of our results. However, the stress response in vertebrates is largely conserved (Yao, Hu & Denver, 2008), and observed behavioural and morphological change were consistent with studies on *R. pipiens* from locations as distant as Michigan (Relyea, 2000) and North Carolina (Glennemeier & Denver, 2002b). This suggests that although phenotypic variation among clutches may exist, it is likely to be inconsequential to the generality of the mechanism.

In conclusion, we show that experimental application of predation risk and MTP, a known corticosterone inhibitor, result in consistent changes in tadpole morphology and do so in opposing directions. Our results suggest a role for corticosterone as a mediator of morphological responses to predation risk. Future work should directly test the impact of acute and chronic predator stress on tadpole hypothalamic–pituitary–interrenal axis activity by directly measuring steroid levels and their binding globulin in tadpoles subjected to MTP and predation-risk treatments. Logistical constraints prevented us from performing an endocrine rescue treatment (*i.e*., simultaneous administration of corticosterone and a corticosteroid block); however, such a treatment is necessary to validate that our suppression of morphological

response to predation risk was mediated by corticosterone suppression. Future work should examine the physiological regulation of predator-specific responses so that the specific mechanisms underlying responses to predation risk are more fully revealed. An improved understanding of how corticosterone adjustment impacts prey survival rates through phenotypic change (*e.g.*, behavioural and morphological responses) will help clarify the functional role of this hormone in the physiological ecology of tadpoles. Finally, it would be helpful for comparative studies to explore the role of corticosterone among species or populations to provide insight into the evolutionary mechanisms that have shaped the diversity of animal phenotypes.

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