

Predator-induced phenotypic plasticity in an arid-adapted tropical tadpole

CHRISTOPHER M. SCHALK

Ecology and Evolutionary Biology Program, Department of Wildlife and Fisheries Sciences, and Biodiversity Research and Teaching Collections, 210 Nagle Hall, Texas A&M University, College Station, Texas 77843, USA

Abstract Adaptive phenotypic plasticity is widespread and involves diverse phenotypes. Key environmental stressors, such as predation risk, can simultaneously induce changes in multiple traits, but the magnitude of response is dependent upon the environmental conditions. Species that utilize temporary ponds are expected to exhibit stronger predator-induced responses in the form of morphology than behaviour (i.e. reduced activity) to meet the demands of rapid development by maintaining high foraging activity while reducing predation risk via morphologically plastic traits. In a laboratory experiment, I examined the effects of predator chemical cues and conspecific alarm cues on activity, development and morphology on *Leptodactylus bufonius* tadpoles. This species has terrestrial oviposition and completes the early part of its development outside of ephemeral and temporary ponds in the Gran Chaco ecoregion of South America. Tadpoles in the predator treatments exhibited both behavioural and morphological predator-induced plastic responses. Tadpoles tended to possess shorter, deeper tails when exposed to predators. The greatest reduction in activity was observed in tadpoles exposed to both predator and conspecific alarm cues, which subsequently resulted in the slowest development. Temporary and ephemeral pond adapted species with terrestrial oviposition may capitalize on a head start in development by being able to afford reduced growth rates via a reduction in activity. This may occur when the constraints imposed by pond hydroperiod (e.g. risk of pond drying) are relaxed when compared with species with aquatic oviposition, which must undergo all stages of development during the pond's hydroperiod. Thus, in addition to the predator and hydroperiod gradients, examining phenotypically plastic responses along a 'terrestriality gradient' in a comparative framework would provide insights as to the costs and benefits of increasing terrestriality in anuran reproductive modes to environmental stressors.

Key words: amphibian, Neotropics, reproductive mode, terrestriality, trade-offs.

INTRODUCTION

Phenotypic plasticity is ubiquitous amongst organisms occurring across heterogeneous environments whereby they adjust to environmental variation by producing environmental-specific phenotypes (DeWitt *et al.* 1998; Pigliucci 2001). A wide array of environmental factors, including abiotic factors such as temperature and nutrients, and biotic factors such as competition and predation can induce plastic responses ranging from changes in behaviour, life-history and morphology in diverse taxa (Benard 2004). Predator-induced phenotypic plasticity, in particular, is pervasive across multiple taxa (Tollrian & Harvell 1999), often in the form of morphological or behavioural plasticity (Benard 2004), ranging from development of defences such as spines (Black & Dodson 1990; McCauley *et al.* 2008), reduction in activity (Van Buskirk & Yurewicz 1998) or use of different microhabitats or foraging sites (Petranka

1989; Heithaus & Dill 2002). Larval amphibians, in particular, have served as model systems to examine the trade-offs between predation risk, growth and costs of phenotypically plastic responses (Newman 1992; Van Buskirk *et al.* 1997; Relyea & Werner 2000; Relyea 2001, 2002a, 2002b, 2004).

In many instances, organisms exhibit multiple-trait plastic responses across disparate trait types. Given that plasticity occurs when a species experiences environmental variation, certain environments should favour different magnitudes of plasticity for different traits on the basis of their net fitness consequences (Pigliucci 2001). Species inhabiting lentic environments are arrayed along gradients of pond drying and predation (Wellborn *et al.* 1996). Species utilizing temporary ponds are vulnerable to desiccation because of reduced pond permanence, whereas permanent ponds contain a higher abundance and diversity of predators, thus exposing organisms to an increased risk of predation (Wellborn *et al.* 1996). Temporary pond species must maintain the demands of rapid growth and development before the pond dries. To meet the demands of rapid growth and development,

*Corresponding author.

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these species maintain a high activity level, which allows them to garner resources. Because these species must maintain high foraging activity, theory predicts that the predator-induced behavioural traits (i.e. reduction in activity) would come at a greater cost to the organism than predator-induced morphological traits because organisms are still able to maintain high foraging activity while reducing predation risk via morphological traits (e.g. deeper tail fins increase ability to escape) (Richter-Boix *et al.* 2007). As such, temporary pond-adapted species are therefore expected to exhibit predominately morphological predator-induced traits, allowing them to reduce predation risk (Anholt *et al.* 2000; Relyea & Werner 2000; Richter-Boix *et al.* 2007), although in extremely ephemeral ponds some species exhibit no plastic responses in the presence of predators (Dayton & Fitzgerald 2011). Permanent pond-adapted species are expected to exhibit both morphological and behavioural predator-induced plastic responses (Richter-Boix *et al.* 2007). These species are not constrained by shortened hydroperiods and behavioural responses, in the form of reduced activity, resulting in species developing more slowly, but this reduction in activity also reduces the species' ability to be detected by predators (Chovanec 1992; Anholt *et al.* 2000; Richter-Boix *et al.* 2007).

When examining the distribution of species along a hydroperiod gradient, we must differentiate between ponds that are temporary and ponds that are variable in pond drying (hereby defined as ephemeral ponds; Perotti *et al.* 2011). Many of the studies on the knowledge of plastic responses of larval amphibians are from temperate species where temporary ponds dry in a predictable fashion annually. Tropical anurans are more diverse in their modes of reproduction than temperate species (Duellman & Trueb 1994; Gomez-Mestre *et al.* 2012), with many species possessing complex oviposition behaviours such as terrestrial oviposition, or depositing their eggs in foam nests (Magnusson & Hero 1991; Duellman & Trueb 1994; Haddad & Prado 2005). One hypothesis is that these oviposition strategies are believed to have evolved as a means to reduce exposure of eggs and larvae to predators by reducing the amount of time spent in aquatic habitats (Magnusson & Hero 1991) (although predation on terrestrial clutches may be just as prevalent (Gomez-Mestre & Warkentin 2007)) as well as to protect eggs from desiccation associated with unpredictable pond drying (Crump 2015). While there is an overall evolutionary trend of increasing terrestriality in the life cycle of anuran amphibians (Gomez-Mestre *et al.* 2012), many species with terrestrial oviposition still rely on aquatic habitats to complete their life cycle (Heyer 1969; Duellman & Trueb 1994; Haddad & Prado 2005). However, less is known concerning behavioural or morphological plastic responses of these tropical

species with complex oviposition strategies, as compared with temperate species.

A study that examines how a species responds to different environments with multiple traits can provide insights as to the differences in plasticity amongst traits, the function of these traits and potential trade-offs amongst traits (Relyea 2001). Through a laboratory experiment, I tested whether predator chemical cues and alarm cues of injured conspecifics had any effect on development (time to metamorphosis), behaviour (activity) or morphology (overall size, body length, tail length and tail depth) on tadpoles of *Leptodactylus bufonius*, an arid-adapted tropical anuran with terrestrial oviposition. This is a common species in the semi-arid Gran Chaco ecoregion of South America with a complex oviposition strategy in terrestrial nest chambers, but the exotrophic larvae complete development in ponds. While this species utilizes ephemeral and temporary ponds, its reproductive mode allows it to complete part of its development outside the constraints of a pond's hydroperiod. Even though *L. bufonius* reproduces in ephemeral and temporary ponds, because of its terrestrial reproductive mode, I hypothesized that tadpoles would present reduced activity, slower development and deeper tails when exposed to predators.

METHODS

Study area and study organism

The study took place at a park guard camp (Yande Yari) in Kaa-Iya of the Gran Chaco National Park, Cordillera Province, Santa Cruz Department, Bolivia (S18°41' 30.516", W62°18' 6.9474"). The study site is located in the dry Chaco, a habitat characterized by semi-arid thorn forest with a distinct rainy season from December to April (see Schalk *et al.* 2013 for further details on the study site). Rainfall averages 512 mm annually (Navarro & Maldonado 2002), but it occurs sporadically during the rainy season, sometimes not occurring for weeks at a time, causing the breeding ponds utilized by the anurans in the region to be highly variable in their persistence on the landscape (Schalk & Saenz 2015). Breeding ponds utilized by *L. bufonius* can range in their hydroperiod from several weeks to months (Ceï 1980; C.M. Schalk, unpublished data, 2010; Crump 2015). *Leptodactylus bufonius* is a common, terrestrial anuran of the Gran Chaco that has adapted to the irregular nature of rainfall; calling activity of *L. bufonius* is highest during intermittent periods between rainfall events (Schalk & Saenz 2015). *Leptodactylus bufonius* oviposits terrestrially in a cone-shaped nest chamber that is constructed with mud by the male around the periphery of a breeding pond. The male calls from inside or close to the nests (Crump 1995; Schalk & Sezano 2014). The eggs are deposited in a foam nest, after which the entrance is capped off with mud by the female

(Philibosian *et al.* 1974; Crump 1995). The eggs hatch in the nest chamber after approximately 4 days (Gosner stage 20; Philibosian *et al.* 1974), but the tadpoles are unable to grow or develop beyond Gosner stage 25 (Gosner 1960) as the biochemical properties of the foam nest inhibits growth (Pisanó 1962). Tadpoles can persist in the nest chamber for over 40 days without water (Philibosian *et al.* 1974). When the next rainstorm occurs, the nest is flooded, and freed tadpoles enter the pond, where they complete the rest of their development. Tadpoles can metamorphose after approximately 20–30 days in the pond and are members of the generalized, benthic ecomorphological guild (Schalk & Leavitt *in press*).

Phenotypic plasticity experiment

I collected four sealed *L. bufonius* nests that were found around the periphery of ephemeral ponds. The date of oviposition of each nest was unknown, but the developmental stages and sizes were similar across the nests used for the experiment (Gosner stage (Gosner 1960) average = 25, SD = 0; total length average = 10.6 mm, SD = 0.62 mm). During the course of the experiment, tadpoles were fed a fixed diet of 15 mg per tadpole per day of a 3:1 ratio of crushed rabbit food pellets: TetraMin tropical fish food flakes. The experiment was conducted in a field house, so the ambient temperature fluctuated during the course of the experiment (min. = 22 °C, max. = 36 °C), but all replicates experienced the same temperature fluctuations, and these temperatures are representative of what the tadpoles experience in their natural habitats.

The experiment was a completely randomized design that had three treatments with eight tadpoles per tub. Each treatment was replicated seven times. The three treatments consisted of a predator-free treatment and two different predator treatments. Juvenile belostomatid water bugs (*Belostoma* sp., Hemiptera: Belostomatidae) were used as the predators in the experiment. Belostomatids are sit-and-wait predators occurring throughout the study area (C.M. Schalk, unpublished data, 2010) and have been documented as predators of amphibians (Toledo 2005; Schalk 2010). In aquatic habitats utilized by larval amphibians, chemoreception may be the most effective means for prey to detect and respond to predators and injured conspecifics (Kats & Dill 1998; Johnson *et al.* 2003; Saenz *et al.* 2003). The predators were placed in plastic mesh cages (8 × 8 × 8 cm, mesh size = 2 mm) and had no direct access to the tadpoles in either treatment but allowed the exchange of water between the cage and tub. The two predator treatments varied in the types of cues to which the tadpoles were exposed; the 'nonlethal' treatment contained a caged predator placed at one end of the tub, exposing tadpoles to chemical cues from the predator, whereas the 'lethal' treatment consisted of exposing tadpoles to multiple cues and contained a caged predator that was fed one conspecific tadpole daily, thereby exposing the tadpoles to chemical cues from the predator, plus alarm cues from the consumed tadpole. All the belostomatids used were not fed 24 h prior to their use in the lethal treatment. To control for the effect of the cage, the predator-free treatment contained an empty mesh cage, which was equal in size to the cages used in the predator treatments, at one side of the tub. There were some instances where tubs had one or more tadpoles die during the experiment (predator free = 1, nonlethal = 2, lethal = 1). Because this affects the amount of

food for each tadpole, which could affect growth and development, these tubs were excluded from analyses. Each plastic tub (34 × 22 × 14 cm) was filled with 3.5 L of well water, which was changed every 3 days.

To track the morphological changes during ontogeny, I preserved one tadpole from each replicate on four occasions during the course of the experiment (day of experiment: 3, 7, 13, 17). I also documented the Gosner stage (Gosner 1960) of each tadpole collected during the experiment. The experiment ended after 21 days when the first tadpoles reached Gosner stage 42 (emergence of forelimbs). At the end of the experiment, I measured the remaining four tadpoles in each replicate of each treatment. Those tadpoles that had reached Gosner stage 42 ($n = 2$) were not included in the final morphological measurements as they undergo rapid morphological changes, including absorption of their tail, at this stage. Using callipers (precision = 0.1 mm), I measured body length, tail length and tail depth on each tadpole, as these traits often exhibit a plastic response in defence from predators (Relyea & Werner 2000). Prior to examining differences in relative morphology, I adjusted for differences in overall tadpole size by conducting a principal components analysis on the three morphological traits and used the score from the first PC axis (PC-1) of each individual as a measure of overall size as the three traits loaded heavily and positively on PC-1 (Relyea & Werner 2000). Each of the three morphological traits were regressed against the PC-1 scores, and the residuals were saved (Bookstein 1991). Using the residuals from the morphology data and the log-transformed Gosner stages, I conducted ANOVA and Tukey's honestly significant difference to examine differences in morphology and development amongst treatments during each sampling interval. To examine behavioural differences, I measured the activity of the tadpoles amongst each treatment daily by standing approximately 0.5 m away from each tub with my eyes closed and counted the number of tadpoles moving at the instant I opened my eyes (Skelly 1995). I calculated the proportion of tadpoles moving in each tub to use as the response variable. Because the data were non-normal, I conducted a Kruskal–Wallis to test for differences in activity amongst the three treatments. All data were log transformed prior to analysis. All analyses were conducted in PAST (Hammer *et al.* 2001).

RESULTS

Morphology

Tadpoles increased in size over time, but tadpoles from both of the lethal and nonlethal predator treatments were significantly smaller than tadpoles from the control treatment on day 7 ($F_{2, 16} = 12.3$, $P < 0.001$) and were nearly smaller on day 13 ($F_{2, 15} = 3.31$, $P = 0.065$), but on day 17, only tadpoles in the lethal treatment were significantly smaller than the control treatment ($F_{2, 29} = 4.30$, $P = 0.023$), and at the end of the experiment (day 21), tadpoles in the lethal treatment were significantly smaller in size than tadpoles from the control and nonlethal treatments ($F_{2, 41} = 11.76$, $P < 0.001$) (as indicated by PC-1; Fig. 1a). The morphological responses of the other traits varied during ontogeny across each treatment. Body length fluctuated

across treatments during ontogeny; on day 7, body length was significantly greater in the absence of predators than in their presence in the nonlethal treatment ($F_{2, 16} = 3.74$, $P = 0.046$), whereas on day 13, body length was nearly significantly less in the lethal treatment when compared with the control treatment ($F_{2, 15} = 3.55$, $P = 0.055$) (Fig. 1b). This differed on days 17 and 21. I observed tadpoles from the nonlethal treatment had greater body lengths when compared with tadpoles from the lethal and control treatments ($F_{2, 29} = 4.12$, $P = 0.027$, and $F_{2, 41} = 32.08$, $P < 0.001$, respectively) (Fig. 1b). No differences in tail length were observed amongst the three treatments until day 17, when tadpoles in the lethal treatment had a greater tail length than tadpoles in the nonlethal treatment ($F_{2, 29} = 3.85$, $P = 0.033$). At the conclusion of the experiment on day 21, tadpoles in the control treatment had greater tail length than tadpoles in the nonlethal treatment ($F_{2, 41} = 3.25$, $P = 0.049$) (Fig. 1c). Tail depth did not differ amongst treatments until the last day of the experiment (day 21); tadpoles in the control and lethal treatments had greater tail depth than tadpoles in the nonlethal treatment ($F_{2, 41} = 17.96$, $P < 0.001$) (Fig. 1d).

Development

Leptodactylus bufonius tadpoles from the control (i.e. predator-free) treatment developed significantly faster than tadpoles from either predator treatment, which was observed on day 7 ($F_{2, 16} = 16.4$, $P < 0.001$), day 17 ($F_{2, 29} = 3.81$, $P = 0.034$) and day 21 ($F_{2, 41} = 11.53$, $P < 0.001$) of the experiment (Fig. 1e). Note that at the start of the experiment and on day 13, all tadpoles were of the same Gosner stage, and thus, an ANOVA could not be conducted.

Activity

The tadpoles exhibited a strong behavioural response in the form of reduced activity between the predator and control treatments. Tadpoles were the most active in the control treatment but were significantly less active when exposed to cues of the predator and were the least active when exposed to cues of a predator plus alarm cues of a consumed conspecific (Fig. 2, Kruskal–Wallis, $H = 50.65$, $P < 0.001$).

DISCUSSION

Leptodactylus bufonius exhibited plasticity in behaviour, morphology and development in response to chemical cues from a caged predator and alarm cues from consumed conspecifics. I observed reduced foraging activity under scenarios of increasing predation threat. Coupled with the reduction in foraging activity was a slower growth and developmental rate. Reduced activity levels

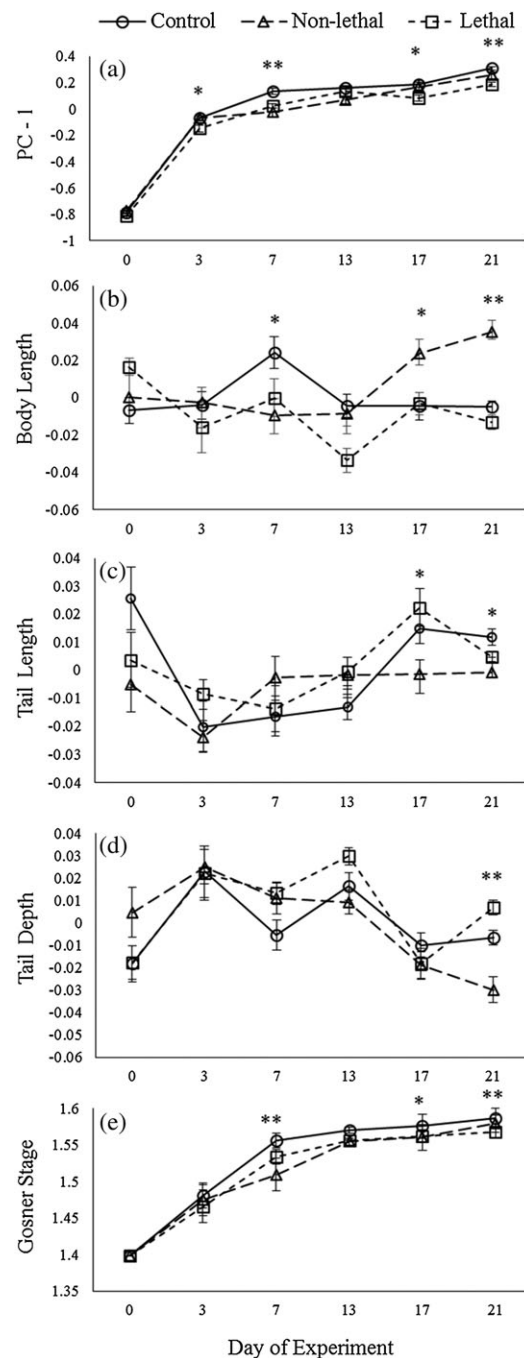


Fig. 1. Change in (a) overall size (PC-1), relative morphology (mean residuals + SE) of (b) body length, (c) tail length and (d) tail depth, and (e) development (log mean Gosner stage + SE) of *Leptodactylus bufonius* tadpoles across three treatments over the course of a 21-day experiment: the absence of chemical cues (i.e. control treatment, open circles), in the presence of chemical cues of belostomatid juvenile predators (i.e. nonlethal treatment, depicted by open triangles) and in the presence of chemical cues of belostomatid juvenile predators plus alarm cues of consumed conspecifics (i.e. lethal treatment, depicted by open squares). Significant differences amongst treatments on a given day are designated by a single asterisk (*) when $P < 0.05$ or double asterisk (**) when $P < 0.001$.

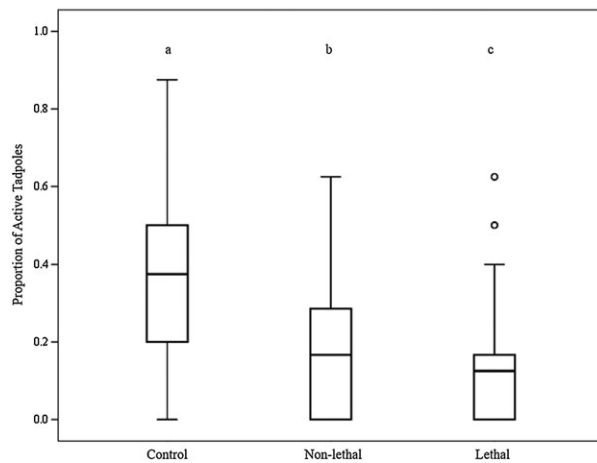


Fig. 2. Boxplots of proportion of active *Leptodactylus bufonius* tadpoles across the three experimental treatments. Significant differences in activity level between treatments are indicated by a different letter. Outliers are depicted by an open circle.

in the presence of predator and conspecific alarm chemical cues is expected to decrease an individual's risk of predation by reducing the ability to be detected by predators (Azevedo-Ramos *et al.* 1992; Skelly 1994). A trade-off occurs between maximizing food intake and predation risk; an increase in time spent foraging increases the amount of resources acquired, which in turn are available for increased growth, but an increased time spent foraging also increases predation risk (Werner & Anholt 1993). A behavioural trade-off is a mechanism that is believed to influence the distribution of anuran larvae along a gradient of pond permanency (Skelly 1995).

Ontogeny of morphological plasticity

Changes in morphology from predator and injured conspecific cues may be adaptive responses to reduce the risk of predation. Tadpoles generally increase their tail depth while decreasing their tail length in the presence of predators (Van Buskirk & Relyea 1998; McIntyre *et al.* 2004), which has been demonstrated to confer benefits of increased survivorship when exposed to free-ranging predators (McIntyre *et al.* 2004). Studies suggest that increased survivorship is the result of directing attacks towards the tail fin rather than the body (Caldwell 1982; Van Buskirk *et al.* 2003), rather than improved swimming performance as the effect of these predator-induced tails is small (Van Buskirk & McCollum 2000). Tadpoles in both predator treatments had significantly shorter tails when compared with the predator-free treatment, but tadpoles in the lethal treatment had deeper tail fins than the tadpoles in the nonlethal and predator-free treatment. These changes in larval morphology can have important ramifications for

interspecific and intraspecific interactions through changes in functional performance on an individual. Generally, predator-induced changes occur early in ontogeny and disappear later (Van Buskirk & Yurewicz 1998; Relyea & Werner 2000). Interestingly, in the present study, differences in the morphological traits associated with higher survivorship in anuran larvae under threats of predation (i.e. tail length and tail depth; Van Buskirk & Relyea 1998) did not emerge until the end of the experiment. As a result of colonization and extinction dynamics associated with pond drying, predators can be patchily distributed across the landscape (Relyea & Werner 2000; Werner *et al.* 2007), and this is especially prevalent in the Gran Chaco ecoregion where hydroperiod of breeding ponds is highly variable, with ponds drying and refilling multiple times during the rainy season (Schalk & Saenz 2015). Given that *L. bufonius* breeds across the entire rainy season (Schalk & Saenz 2015) and the variability in pond persistence on the landscape can cause the predator communities to be patchily distributed across space and time, tadpoles in these variable hydroperiod ponds may employ a strategy to delay inducing plastic responses to ensure that the predation threat is constant while the pond drying threat is minimal.

Tadpoles exhibit context-dependent phenotypic responses under different environmental conditions of predation risk and competition (Peacor & Werner 2004; Relyea 2004; Michel 2012) as well as adapt to the local conditions in their natal ponds, which has been attributed to localized selection of the predator and competitor regimes (Relyea 2002b). In Neotropical tadpoles, shifts in colour and morphology have been observed according to predator type (Touchon & Warkentin 2008). Wild-caught *L. bufonius* tadpoles have been observed with tails containing dark melanophores (Schalk & Leavitt *in press*), suggesting that tail colour may be another predator-induced phenotypic response in this species, although it was not measured in this study. The belostomatid predator used in this study employs a sit-and-wait foraging strategy (Kopp *et al.* 2006), but *L. bufonius* tadpoles may exhibit a different response when exposed to different predators that use alternative foraging tactics (e.g. active foragers). Other common potential predators in this region include carnivorous tadpoles of *Lepidobatrachus* spp. and *Ceratophrys cranwelli* (Schalk *et al.* 2013; Schalk *et al.* 2014a) and other predatory invertebrates such as dragonfly larvae (Odonata; C.M. Schalk, unpublished data, 2010), as well as annual killifishes (Montaña *et al.* 2012; Schalk *et al.* 2014b).

Complex oviposition strategies and phenotypic plasticity of anuran larvae

Many tadpoles that occur in ephemeral or temporary ponds exhibit a strong morphological predator-induced

plastic response while still maintaining high activity levels to garner resources (Anholt *et al.* 2000; Richter-Boix *et al.* 2007). However, I observed morphological and behavioural predator-induced plastic responses in tadpoles of *L. bufonius*, a species distributed in ephemeral and temporary ponds along the hydroperiod gradient. Species with terrestrial oviposition that utilize ephemeral and temporary ponds, like *L. bufonius*, are able to enter a newly formed pond with a head start in development (up to Gosner stage 25; Philibosian *et al.* 1974; Reading & Jofré 2003) and may be able to afford reduced growth rates via a reduction in activity because the risk associated with a shortened hydroperiod (i.e. risk of pond drying) may be reduced. Philibosian *et al.* (1974) noted that tadpoles of *L. bufonius* hatch in the nest after approximately 4 days (Gosner stage 20). *Leptodactylus bufonius* tadpoles can spend only 20–30 days in the pond developing, and they are able to get a considerable head start in their development given how short their larval period is in their breeding ponds. Compare this to an aquatic ovipositing species (e.g. most temperate species) that has a similar developmental period that occurs in a pond of the same hydroperiod; risks of mortality because of pond drying for an aquatic ovipositing species may be greater because all stages of development need to occur while in the breeding pond. Therefore, an aquatic ovipositing species may not be able to exhibit strong behavioural responses to predation (i.e. reduced activity) as compared with a terrestrial ovipositing species because it may be at higher risk for mortality as a consequence of pond drying compared with a species that has a considerable head start on development.

The paradigm of community structure in aquatic lentic habitats predicts that the costs and benefits of phenotypic plastic responses of amphibian larvae occur along a hydroperiod and predator gradient (Wellborn *et al.* 1996; Richter-Boix *et al.* 2007). However, this theory has emerged from studies that have utilized temperate species with primarily aquatic oviposition where eggs are deposited directly in the water (Wellborn *et al.* 1996). Predation and pond drying are still important factors organizing tropical pond communities (Heyer *et al.* 1975; Hero *et al.* 1998; Azevedo-Ramos *et al.* 1999), and the distribution of Amazonian tadpoles across ponds is reflected in their antipredator traits (e.g. palatability to predators) (Hero *et al.* 2001). Reproductive modes may also influence assemblage structure (Crump 2015). Thus, in addition to the predator and hydroperiod gradients, examining the responses of predator-induced plastic responses along a 'terrestrial gradient' would provide insights as to the costs and benefits of increasing terrestriality in anuran reproductive modes on phenotypic plastic responses. For those species that are able to complete part of their development outside of a breeding pond

(i.e. species with terrestrial reproductive modes), the pond's hydroperiod may not impose as strong a constraint in a species response to predators as compared with an aquatic ovipositing species with similar life-history attributes (e.g. use of same breeding sites and larval periods of similar duration). Species in the genus *Leptodactylus*, in particular, seem like model organisms to explore the costs and benefits of increasing terrestriality; species range in their degrees of terrestriality ranging from relying on an existing waterbody to oviposit a floating foam nest to reproducing in the absence of water in terrestrial nest chambers (e.g. *L. bufonius*) (Heyer 1969). For example, in a scenario where different species of *Leptodactylus* with different reproductive modes overlap in their use of breeding sites, those species that oviposit in nests that float on top of the water (e.g. members of the *ocellatus* and *melanonotus* species groups; Heyer 1969) may not exhibit as strong a behavioural response to predators as compared with species that oviposit in a terrestrial nest chamber (e.g. members of the *fuscus* species group; Heyer 1969) as the pond's hydroperiod may impose stronger constraints on the species of the *ocellatus* and *melanonotus* species groups as compared with the members of the *fuscus* species group. Given the high diversity and pervasiveness of complex oviposition strategies in the tropics (Haddad & Prado 2005), couching these hypotheses in a comparative framework would provide insights regarding the relative influence of oviposition strategies on interspecific variation in phenotypic plastic responses to environmental stressors.

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REFERENCES

- Anholt B. R., Werner E. E. & Skelly D. K. (2000) Effect of food and predators on the activity of four larval ranid frogs. *Ecology* **81**, 3509–21.
- Azevedo-Ramos C., Van Sluys M., Hero J. M. & Magnusson W. E. (1992) Influence of tadpole movement on predation by odonate naiads. *J. Herpetol.* **26**, 335–8.
- Azevedo-Ramos C., Magnusson W. E. & Bayliss P. (1999) Predation as the key factor structuring tadpole assemblages in a savanna area in central Amazonia. *Copeia* **1999**, 22–33.
- Benard M. F. (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Annu. Rev. Ecol. Evol. Syst.* **35**, 651–73.
- Black A. R. & Dodson S. I. (1990) Demographic costs of *Chaoborus*-induced phenotypic plasticity in *Daphnia pulex*. *Oecologia* **83**, 117–22.
- Bookstein F. L. (1991) *Morphometric Tools for Landmark Data*. Cambridge University Press, Cambridge.
- Caldwell J. P. (1982) Disruptive selection: a tail color polymorphism in *Acris* tadpoles in response to differential predation. *Can. J. Zool.* **60**, 2818–27.
- Cei J. M. (1980) Amphibians of Argentina. *Mon. Zool. Italiano Monogr.* **2**, 1–609.
- Chovanec A. (1992) The influence of tadpole swimming behaviour on predation by dragonfly nymphs. *Amphibia-Reptilia* **13**, 341–9.
- Crump M. L. (1995) *Leptodactylus bufonius* (NCN). Reproduction. *Herpetol. Rev.* **26**, 97–8.
- Crump M. L. (2015) Anuran reproductive modes: evolving perspectives. *J. Herpetol.* **49**, 1–16.
- Dayton G. H. & Fitzgerald L. A. (2011) The advantage of no defense: predation enhances cohort survival in a desert amphibian. *Aquat. Ecol.* **45**, 325–33.
- Duellman W. E. & Trueb L. (1994) *Biology of Amphibians*. John Hopkins University Press, Baltimore.
- DeWitt T. J., Sih A. & Wilson D. S. (1998) Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77–81.
- Gomez-Mestre I. & Warkentin K. M. (2007) To hatch and hatch not: similar selective trade-offs but different responses to egg predators in two closely related, syntopic treefrogs. *Oecologia* **153**, 197–206.
- Gomez-Mestre I., Pyron R. A. & Wiens J. J. (2012) Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes in frogs. *Evolution* **66**, 3687–700.
- Gosner K. L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–90.
- Haddad C. F. B. & Prado C. P. A. (2005) Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience* **55**, 207–17.
- Hammer Ø., Ryan P. & Harper D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **4**, 9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.
- Heithaus M. R. & Dill L. M. (2002) Food availability and tiger shark predation risk influence bottlenose dolphin habitat use. *Ecology* **83**, 480–91.
- Hero J., Gascon C. & Magnusson W. E. (1998) Direct and indirect effects of predation on tadpole community structure in the Amazon rainforest. *Aust. J. Ecol.* **23**, 474–82.
- Hero J. M., Magnusson W. E., Rocha C. F. & Catterall C. P. (2001) Antipredator defenses influence the distribution of amphibian prey species in the central Amazon rain forest. *Biotropica* **33**, 131–41.
- Heyer W. R. (1969) The adaptive ecology of the species groups of the genus *Leptodactylus* (Amphibia, Leptodactylidae). *Evolution* **23**, 421–8.
- Heyer W. R., McDiarmid R. W. & Weigmann D. L. (1975) Tadpoles, predation and pond habitats in the tropics. *Biotropica* **7**, 100–11.
- Johnson J. B., Saenz D., Adams C. K. & Conner R. N. (2003) The influence of predator threat on the timing of a life-history switch point: predator-induced hatching in the southern leopard frog (*Rana sphenoccephala*). *Can. J. Zool.* **81**, 1608–13.
- Kats L. B. & Dill L. M. (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**, 361–94.
- Kopp K., Wachlevski M. & Eterovick P. C. (2006) Environmental complexity reduces tadpole predation by water bugs. *Can. J. Zool.* **84**, 136–40.
- Magnusson W. E. & Hero J. M. (1991) Predation and the evolution of complex oviposition behaviour in Amazon rainforest frogs. *Oecologia* **86**, 310–8.
- McCauley S., Davis C. J. & Werner E. E. (2008) Predator induction of spine length in larval *Leucorhinia intacta* (Odonata). *Evol. Ecol. Res.* **10**, 435–47.
- McIntyre P. B., Baldwin S. & Flecker A. S. (2004) Effects of behavioral and morphological plasticity on risk of predation in a Neotropical tadpole. *Oecologia* **141**, 130–8.
- Michel M. J. (2012) Phenotypic plasticity in complex environments: effects of structural complexity on predator- and competitor-induced phenotypes of tadpoles of the wood frog. *Rana sylvatica*. *Biol. J. Linn. Soc.* **105**, 853–63.
- Montaña C. G., Schalk C. M. & Taphorn D. C. (2012) First record of Van den Berg's Pearlfish, *Austrolebias vanderbergi* Huber, 1995 (Cyprinodontiformes: Rivulidae) in Bolivia with comments on its diet and reproductive biology. *Check List* **8**, 589–91.
- Navarro G. & Maldonado M. (2002) *Geografía Ecológica de Bolivia: Vegetación y Ambientes Acuáticos*. Centro de Ecología Difusión Simon I Patiño, Santa Cruz de la Sierra, Bolivia.
- Newman R. A. (1992) Adaptive plasticity in amphibian metamorphosis. *Bioscience* **42**, 671–8.
- Peacor S. D. & Werner E. E. (2004) Context dependence of non-lethal effects of a predator on prey growth. *Israel J. Zool.* **50**, 139–67.
- Perotti M. G., Jara F. G. & Úbeda C. A. (2011) Adaptive plasticity of life-history traits to pond drying in three species of Patagonian anurans. *Evol. Ecol. Res.* **13**, 415–29.
- Petranka J. W. (1989) Response of toad tadpoles to conflicting chemical stimuli: predator avoidance versus “optimal” foraging. *Herpetologica* **45**, 283–92.
- Philibosian R., Ruibal R., Shoemaker V. H. & McClanahan L. L. (1974) Nesting behavior and early larval life of the frog *Leptodactylus bufonius*. *Herpetologica* **30**, 381–6.
- Pigliucci M. (2001) *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, Baltimore.
- Pisanó A. (1962) La espuma de los nidos de *Leptodactylus bufonius* y su acción inhibidora sobre el desarrollo de las larvas. *Arch. Bioquím. Quím. Farm.* **10**, 65–77.
- Reading C. J. & Jofré G. M. (2003) Reproduction in the nest building vizcacheras frog *Leptodactylus bufonius* in central Argentina. *Amphibia-Reptilia* **24**, 415–28.
- Relyea R. A. (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* **82**, 523–40.

- Relyea R. A. (2002a) Costs of phenotypic plasticity. *Amer. Nat.* **159**, 272–82.
- Relyea R. A. (2002b) Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. *Ecol. Monogr.* **72**, 77–93.
- Relyea R. A. (2004) Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* **85**, 172–9.
- Relyea R. A. & Werner E. E. (2000) Morphological plasticity in four larval anurans distributed along an environmental gradient. *Copeia* **2000**, 178–90.
- Richter-Boix A., Llorente G. A. & Montori A. (2007) A comparative study of predator-induced phenotype in tadpoles across a pond permanency gradient. *Hydrobiologia* **583**, 43–56.
- Saenz D., Johnson J. B., Adams C. K. & Dayton G. H. (2003) Accelerated hatching of southern leopard frog (*Rana sphenocphala*) eggs in response to the presence of a crayfish (*Procambarus nigrocinctus*) predator. *Copeia* **2003**, 646–9.
- Schalk C. M. (2010) *Physalaemus biligonigerus* (NCN) Predation. *Herpetol. Rev.* **41**, 202.
- Schalk C. M. & Saenz D. (2015) Environmental drivers of anuran calling phenology in a seasonal Neotropical ecosystem. *Aust. Ecol.* doi:10.1111/aec.12281
- Schalk C. M. & Leavitt D. J. (In press) *Leptodactylus bufonius*. *Cat. Amer. Amphib. Rept.*
- Schalk C. M., Senzano M. & Cuellar R. L. (2013) Inventory of the amphibians and reptiles from a locality in the Kaa-Iya of the Gran Chaco National Park, Bolivia. *Kempffiana* **9**, 26–33.
- Schalk C. M. & Sezano M. (2014) Observations on the use of tarantula burrows by the anurans *Leptodactylus bufonius* (Leptodactylidae) and *Rhinella major* (Bufonidae) in the Dry Chaco of Bolivia. *Acta Herpetol.* **9**, 99–102.
- Schalk C. M., Montaña C. G., Klemish J. L. & Wild E. R. (2014a) On the diet of the frogs of the Ceratophryidae: synopsis and new contributions. *S. Amer. J. Herpetol.* **9**, 90–105.
- Schalk C. M., Montaña C. G. & Libson M. (2014b) Reproductive strategies of two Neotropical killifish, *Austrolebias vanderbergi* and *Neofundulus ornatipinnis* (Cyprinodontiformes: Rivulidae) in the Bolivian Gran Chaco. *Rev. Biol. Trop.* **62**, 109–17.
- Skelly D. K. (1994) Activity level and the susceptibility of anuran larvae to predation. *Anim. Behav.* **47**, 465–8.
- Skelly D. K. (1995) A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. *Ecology* **76**, 150–64.
- Toledo L. F. (2005) Predation of juvenile and adult anurans by invertebrates: current knowledge and perspectives. *Herpetol. Rev.* **36**, 395–9.
- Tollrian R. & Harvell C. D., eds (1999) *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, Princeton.
- Touchon J. C. & Warkentin K. M. (2008) Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. *Oikos* **117**, 634–40.
- Van Buskirk J. & Relyea R. A. (1998) Selection for phenotypic plasticity in *Rana sylvatica* tadpoles. *Biol. J. Linn. Soc.* **65**, 301–28.
- Van Buskirk J., McCollum S. A. & Werner E. E. (1997) Natural selection for environmentally induced phenotypes in tadpoles. *Evolution* **51**, 1983–92.
- Van Buskirk J. & Yurewicz K. L. (1998) Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. *Oikos* **82**, 20–8.
- Van Buskirk J. & McCollum S. A. (2000) Influence of tail shape on tadpole swimming performance. *J. Exp. Biol.* **203**, 2149–58.
- Van Buskirk J., Anderwald P., Lupold S., Reinhardt L. & Schuler H. (2003) The lure effect, tadpole tail shape, and the target of dragonfly strikes. *J. Herpetol.* **37**, 420–4.
- Wellborn G. A., Skelly D. K. & Werner E. E. (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annu. Rev. Ecol. Evol. Syst.* **27**, 337–63.
- Werner E. E. & Anholt B. R. (1993) Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *Amer. Nat.* **142**, 242–72.
- Werner E. E., Yurewicz K. L., Skelly D. K. & Relyea R. A. (2007) Turnover in an amphibian metacommunity: the role of local and regional factors. *Oikos* **116**, 1713–25.