

# Short- and long-term effects of the abiotic egg environment on viability, development and vulnerability to predators of a Neotropical anuran

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## Summary

1. Environmental variation during development is common and often has long-lasting effects on phenotypes and survival. In organisms with complex life cycles, such effects may carry over from one life stage into the subsequent stages, affecting reproductive success and survival in both direct and indirect manners. Much research has focused on the transition from the larval to adult stages, but fewer studies have addressed how egg-stage variation may affect the larval stage, development to metamorphosis, and adult fitness.

2. We assessed the short- and long-term consequences of abiotic environmental variation during egg development of the Neotropical treefrog *Dendropsophus ebraccatus*. Typically, *D. ebraccatus* eggs are oviposited terrestrially on leaves above water and hydrated by rainfall. However, at our field site in Panama ~25% of terrestrial eggs become desiccated during development and ~19% become submerged underwater. In addition, in unshaded ponds *D. ebraccatus* lays eggs directly in water. We assessed immediate and carryover effects of egg development under hydrated, desiccated and submerged conditions. We measured morphology and vulnerability to predators at hatching and at three time points during the larval period. We also measured morphology at metamorphosis.

3. Submerged eggs hatched less developed and later and had higher baseline mortality after hatching than either hydrated or desiccated terrestrial eggs. By 10 days after hatching, the morphology of tadpoles from the three egg environments was indistinguishable. Nonetheless, the egg environment affected predation, with tadpoles from submerged eggs being least vulnerable; later in development egg environment ceased to affect vulnerability. Tadpoles from all egg environments grew to a size refuge from dragonfly nymphs, whereas vulnerability to water bugs remained constant throughout development. At metamorphosis, froglets from submerged eggs were the largest and those from hydrated eggs were the smallest.

4. With anticipated climate change in the Neotropics, rainstorms are predicted to become more sporadic but larger when they do occur, potentially increasing the chances of both egg desiccation and flooding. The incidence of different egg environments may therefore change, potentially affecting amphibian phenotypes, interactions with predators, and survival across multiple life stages.

**Key-words:** anura, carryover effects, complex life history, *Hyla ebraccata*, predator-prey

## Introduction

Few organisms develop in uniform and stable environments. It is well-documented that variation in early life experiences can lead to delayed effects later in life, which in turn can influence individual fitness, population dynamics,

and even the course of evolution (Pechenik, Wendt & Jarrett 1998; Lindstrom 1999; Metcalfe & Monaghan 2001; Beckerman *et al.* 2002; West-Eberhard 2003). Variation in abiotic or biotic factors such as climate, larval density or food resources during development can alter the phenotype and survival of adult forms (Scott 1994; Olsson & Shine 2002; Solberg *et al.* 2004; Engels & Sauer 2007). These effects are common in a broad array of taxa, including

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insects, cephalopods, crustaceans, and vertebrates (e.g. Ward & Slaney 1988; Scott 1994; Gimenez, Anger & Torres 2004; Gorman & Nager 2004; Solberg *et al.* 2004).

In organisms with complex life histories, variation in one life stage can carry over to affect the phenotype of the next (Goater 1994; Pechenik, Wendt & Jarrett 1998; Engels & Sauer 2007; Benard & McCauley 2008). Much research to date has focused on the transition between larval and adult life stages. Many factors in the larval environment can influence the timing of, and size at, metamorphosis, which then can lead to variation in post-metamorphic success (Semlitsch, Scott & Pechmann 1988; Scott 1994; Taylor, Anderson & Peckarsky 1998; Altwegg & Reyer 2003; Engels & Sauer 2007; Jannot, Brunea & Wissinger 2007; Benard & McCauley 2008). Fewer studies have addressed how environmental variation during the egg stage carries over to affect larval development, metamorphosis or adult phenotype and fitness (but see Olvido, Busby & Mousseau 1998; Broomhall 2004; Roussel 2007).

Carryover effects can occur in two ways. Early environments can directly affect phenotypes that affect later performance; for instance greater body mass at metamorphosis often increases post-metamorphic survival and reproductive success (Smith 1987; Semlitsch, Scott & Pechmann 1988; Taylor, Anderson & Peckarsky 1998). There may also be indirect costs associated with compensatory growth, potentially from accrued cellular damage, occurring after periods of resource deprivation (Metcalf & Monaghan 2001; Munch & Conover 2003; Mangel & Munch 2005; Alonso-Alvarez *et al.* 2007). Compensatory growth may therefore lead to organisms that appear phenotypically similar, but have different long-term survival or reproductive success (Altwegg & Reyer 2003; De Block & Stoks 2005; Alvarez & Metcalfe 2007). If stressful environmental conditions during egg development create suboptimal hatchlings, we might expect long-term consequences resulting from these conditions early in life even if the larvae 'catch-up' phenotypically.

We measured the short- and long-term consequences of naturally occurring abiotic environmental variation during the egg stage of the Neotropical treefrog *Dendropsophus ebraccatus* (Fig. 1, = *Hyla ebraccata* Cope, Faivovich *et al.* 2005). This species is common from southern Mexico to northern Columbia, most often reproducing by laying eggs on leaves above water (Duellman & Trueb 1986; Wells 2007). Eggs develop in 3–4 days and hatch as aquatic tadpoles into the pond below. *Dendropsophus ebraccatus* breeds during the Neotropical rainy season and egg clutches are most often rained on and well-hydrated. However, rainfall is highly variable and clutch hydration depends on the amount of rain received (Touchon & Warkentin 2009). When large rainstorms occur, pond levels rise and ~19% of clutches become flooded at our field site (Touchon & Warkentin 2009). We also recently discovered that *D. ebraccatus* lays eggs directly in water when ponds are unshaded (Touchon & Warkentin 2008a). Eggs laid in water are usually attached to floating or submerged vegetation which keeps them near the top of the water column, whereas flooded clutches may be much deeper

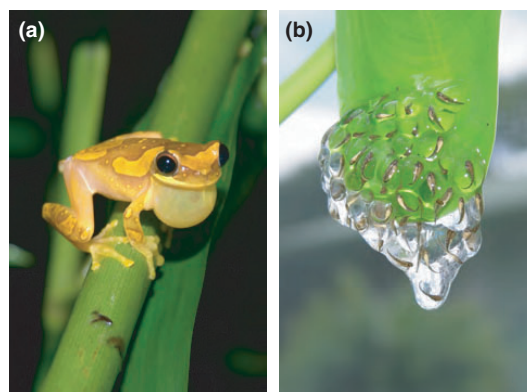


Fig. 1. (a) A calling male *Dendropsophus ebraccatus* and (b) a well-hydrated arboreal clutch of *D. ebraccatus* eggs.

depending on initial oviposition height and size of the storm (Touchon & Warkentin 2008a).

We simulated three extremes of *D. ebraccatus* egg environments: (1) above water, rained on and well-hydrated; (2) above water but not rained on and therefore desiccated; and (3) submerged underwater, such as occurs with flooding. We monitored embryo development to hatching, then raised tadpoles from each egg environment to metamorphosis, and measured phenotypes at four time points during the larval period. We also conducted predation trials with common larval predators at four points during the larval period to assess effects of egg environments on tadpole vulnerability. We hypothesized that desiccated and submerged egg environments would cause suboptimal embryo growth, resulting in both short- and long-term phenotypic alterations and reduced survival when tadpoles were exposed to predators.

## Materials and methods

### COLLECTION AND REARING OF EGGS

We collected 17 pairs of *D. ebraccatus* adults from Bridge Pond, in Gamboa, Panama, on 8 August 2005 and brought them to an ambient-temperature laboratory. We collected both amplexant pairs that had not yet begun laying eggs and unpaired females who were assigned to random calling males. To obtain clutches, we placed *D. ebraccatus* pairs in 1 gallon plastic bags with a small amount of water and allowed them to breed overnight in the laboratory. All pairs mated successfully, adhering their eggs to the inside of the bag. Frogs were returned to the pond the following day. Pairs laid  $237 \pm 12$  eggs (mean  $\pm$  SE, here and throughout). Each family of eggs was split into three equal-sized egg masses 8–10 h post-oviposition and these were randomly assigned to the three egg environment treatments: simulated heavy rain (hereafter, 'hydrated'), desiccated or submerged. We did not remove eggs from the plastic, but cut out pieces of plastic with the eggs attached and mounted them onto rigid plastic cards. The initial size of egg masses in the three treatments did not differ (hydrated =  $78.7 \pm 4.0$  eggs, desiccated =  $79.5 \pm 3.9$ , submerged =  $78.9 \pm 3.8$ ; linear model (LM),  $F_{2,48} = 0.0074$ ,  $P = 0.99$ ). Egg masses in the three treatments were suspended vertically and placed in 240 mL plastic cups. Cups with hydrated and desiccated egg masses contained ~50 mL of water for hatchlings to

fall into and submerged egg masses were flooded with aged tap water. Submerged egg masses were suspended in the water column so that eggs at the top of the mass were a few mm below the surface and eggs at the bottom were 3–6 cm below the surface.

We built an irrigation system to slowly drip aged tap water onto egg masses to mimic the hydrating effects of heavy rainfall. Egg masses from the hydrated treatment were 'rained on' twice daily for two hours each, from 08.00–10.00 h and again from 18.00–20.00 h. The hydrated treatment was designed to ensure that egg masses were maximally hydrated; hydrated egg masses received  $c. 300 \pm 15$  mL of aged tap water during each 'rain'. We removed the accumulated water after each 'rain' to ensure egg masses did not become flooded. Egg masses in the desiccated treatment were misted infrequently but as necessary to keep eggs alive while maintaining them substantially desiccated. Starting at 58 h post-oviposition, when we first observed an egg hatch, we monitored egg masses every 2–4 h until all eggs had hatched or died.

Once 15 eggs had hatched from any given egg mass five hatchlings were randomly selected and preserved in 10% buffered formalin for later morphological analyses. We randomly assigned the remaining 10 hatchlings into a predation trial with a common hatchling predator, a backswimmer, *Buenoa antigone antigone* Kirkaldy (Notonectidae), or a no-predator control treatment. Thus, hatchling predation trials did not begin simultaneously but in accordance with the hatching pattern of individual egg masses. Backswimmer sizes were the same for hatchlings from all three egg environments (total length =  $8.0 \pm 0.3$  mm; LM,  $F_{2,7} = 2.11$ ,  $P = 0.19$ ). We collected all backswimmers from ponds near Gamboa where *D. ebraccatus* is common. Predation trials took place in opaque round containers containing 5 L aged tap water, two large *Anacardium sp.* leaves and a stick. We placed backswimmers in containers at least 24 h before the first tadpole hatched to give them time to acclimate to the arena. Predation trials lasted for 24 h, at which point we removed the predator and recorded the number of surviving tadpoles. Two backswimmers died during the predation trial and those replicates were excluded from analyses. In addition, we attempted predation trials using two other potential hatchling predators (large *D. ebraccatus* tadpoles and Libellulid dragonfly nymphs), but they failed to prey on hatchlings. Those replicates are thus excluded from the analyses presented here (accounting for 25 total replicates). The final number of replicates was as follows: backswimmer –  $N_{hydrated} = 3$ ,  $N_{desiccated} = 3$ ,  $N_{submerged} = 4$ ; control –  $N_{hydrated} = 4$ ,  $N_{desiccated} = 5$ ,  $N_{submerged} = 5$ .

The remaining hatchlings from each mass (i.e. all hatchlings after the first 15) were pooled into three common containers, one for each egg treatment. We combined tadpoles from the different families to create a genetically mixed pool for each egg environment from which to draw replicates for the tadpole portion of the experiment. Thus, individual egg-stage replicates were not continued through to metamorphosis. Prior work had indicated that *D. ebraccatus* tadpoles are particularly susceptible to mortality if handled during the first 3 days after hatching, therefore they were not disturbed during this time.

#### REARING TADPOLES TO METAMORPHOSIS

Three days after the final eggs had hatched (hereafter, 'post-hatching'), we randomly sampled tadpoles from our pooled tubs to create replicates of 40 tadpoles each from each egg treatment. Despite not handling the hatchlings, mortality of hatchlings from submerged eggs was high (see results below), limiting the number of replicates possible for the tadpole portion of the experiment ( $N_{hydrated} = 20$ ,  $N_{desiccated} = 20$ ,  $N_{submerged} = 14$ ). The 54 containers were randomly

arranged on four shelves (blocks) in an open air laboratory and covered with a fine mesh. Tadpoles were reared in containers holding 5 L of aged tap water and two *Anacardium sp.* leaves. We checked tadpole containers and fed tadpoles every 1–2 days. Tadpoles were fed pellets of rabbit chow *ad libitum* and excess food and faeces were removed as needed.

To track the development of tadpoles and assess their vulnerability to predators, we measured tadpole morphology and conducted predation trials at three time points during the larval period (10, 20 and 30 days post-hatching). At each time period, we removed 10 tadpoles from each container. Five of these tadpoles were preserved in 10% buffered formalin for morphological analyses. The five remaining tadpoles were combined with five tadpoles from a separate, randomly chosen replicate of the same original egg environment and randomly assigned to a 24 h predation trial with either a libellulid dragonfly nymph (*Pantala flavescens* Fabricius: Libellulidae;  $16.8 \pm 0.5$  mm) or a giant water bug (*Belostoma sp.*: Belostomatidae;  $33.6 \pm 1.2$  mm). Predators were released after each predation trial and new predators were caught 2 days before subsequent trials. Predators were not fed before predation trials to ensure they were hungry. Predator sizes did not differ across the three time periods (dragonfly: LM,  $F_{2,28} = 2.34$ ,  $P = 0.12$ ; water bug:  $F_{2,33} = 0.03$ ,  $P = 0.98$ ) nor across treatments (dragonfly: LM,  $F_{2,28} = 1.83$ ,  $P = 0.18$ ; water bug:  $F_{2,33} = 1.14$ ,  $P = 0.33$ ). At each time point we conducted 10 predation trials with tadpoles hatched from both the hydrated and desiccated egg treatments (five randomly assigned to each predator), and seven predation trials with tadpoles from the submerged egg treatment (four with one predator, three with the other, all randomly assigned). Previous work indicated that baseline mortality of tadpoles was minimal during these periods and thus we did not include a no-predator control treatment. Predation trial arenas were set up as above. After 24 h we removed the predator and recorded the number of tadpoles surviving. Due to occasional predator mortality, the final number of replicates for each time point was as follows: 10 days – water bug:  $N_{hydrated} = 5$ ,  $N_{desiccated} = 5$ ,  $N_{submerged} = 3$ , dragonfly nymph:  $N_{hydrated} = 5$ ,  $N_{desiccated} = 3$ ,  $N_{submerged} = 2$ ; 20 days – water bug:  $N_{hydrated} = 5$ ,  $N_{desiccated} = 5$ ,  $N_{submerged} = 3$ , dragonfly nymph:  $N_{hydrated} = 5$ ,  $N_{desiccated} = 4$ ,  $N_{submerged} = 4$ ; 30 days – water bug:  $N_{hydrated} = 4$ ,  $N_{desiccated} = 4$ ,  $N_{submerged} = 4$ , dragonfly nymph:  $N_{hydrated} = 4$ ,  $N_{desiccated} = 3$ ,  $N_{submerged} = 3$ . We reared the final 10 tadpoles in each container until metamorphosis. Once tadpoles developed hindlimbs, containers were checked every day and metamorphs were removed as soon as their forearms emerged.

#### MORPHOLOGICAL ANALYSES

Preserved hatchlings and 10, 20 and 30 days old tadpoles were digitally photographed using a Nikon Coolpix E8700 camera attached to a Leica Wild M10 dissecting scope  $6.1 \pm 0.8$  days after predation trials. Preserved tadpoles were randomly assigned a new identification label prior to photography and image analyses were conducted blindly to ensure no measurer bias. Morphology and pigmentation were analyzed using ImageJ 1.34s (NIH). We measured tadpole total length (TTL), body length (BL), head width at the eyes (HW), tail length (TL), tail muscle width at the base of the tail (TMW), tail muscle depth at the base of the tail (TMD), maximum tail fin depth (TD), tail spot area (TSA), and hindlimb length (HL) if present. We also measured the pigmented area (PA) of each tadpole. *Dendropsophus ebraccatus* tadpoles develop large red tails in response to certain predators (Touchon & Warkentin 2008b). However, in this experiment we raised tadpoles without predators and were thus interested in

variation in pigmentation resulting from different egg environments, not colour differences induced by predators. Using the Threshold function in ImageJ, we measured the area of pixels on the tadpole with a brightness less than 100 (on a scale of 0–255, 0 being pure dark and 255 being pure light). This method was verified by three independent observers as correctly isolating pigmented areas from the lighter areas of the tadpole and from the background of the photo. For hatchlings, we assessed the developmental stage according to a standard staging table (Gosner 1960) as well as specific traits that allowed us to subdivide Gosner stage 23. To increase our resolution of developmental differences at hatching, we defined four substages of Gosner stage 23 [operculum covering the base of bilateral external gills, oral disk and denticles differentiating (Gosner 1960)]: (1) The operculum was distinguishable first, followed by; (2) mouthpart formation; (3) body and tail melanization, and; (4) yolk vascularization.

When tadpoles emerged at metamorphosis each was placed in a cup with a small amount of water and given 2 days to fully resorb its tail, at which point we measured snout-vent length (SVL), forelimb length (FL), hindlimb length (HL), head width (HW), and mass. Length measurements were made by hand using callipers. Forelimbs and hindlimbs were stretched and measured from the point where they met the body to the end of the longest toe and head width was measured at the eyes. No animals were harmed during the measurement process and all metamorphs were released at their natal pond. Five replicates were excluded from morphological analyses because fewer than three tadpoles survived to metamorphosis (final replicates;  $N_{\text{hydrated}} = 18$ ,  $N_{\text{desiccated}} = 18$ ,  $N_{\text{submerged}} = 13$ ).

#### STATISTICAL ANALYSES

All statistics were conducted in R v.2.6.0 (R Development Core Team, 2007). Mortality during egg development and in hatchling predation trials was analyzed with generalized linear models (GLM) with underlying quasibinomial distributions and logit link functions (also known as logistic regression). A quasibinomial error function is the same as a binomial error function, except that it accounts for overdispersion of the model and penalizes  $P$ -values accordingly (Pinheiro & Bates 2000). We tested for effects of egg environment, predator treatment (backswimmer or control), clutch and all possible interactions. Hatching time and stage, tadpole total length at hatching, length of the embryonic period and hatching synchrony (time elapsed from first egg hatched to last egg hatched in each clutch) were analyzed with linear models testing for effects of the egg environment, clutch and the interaction between them. Clutch never had a significant effect on hatching traits or hatchling survival with predators (all  $P > 0.29$ ) and was excluded from final analyses. We used Tukey's post-hoc tests to compare effects of the original egg environment.

We analyzed survival in larval predation trials as a single analysis for both predators and across the three time points. The data were not overdispersed and we used a binomial GLM testing for effects of predator, egg environment, and age of tadpoles, as well as all possible interactions. Since the tadpoles used in each predation trial were combined from two different rearing tubs, we could not test for block effects of the shelf tadpoles were reared on.

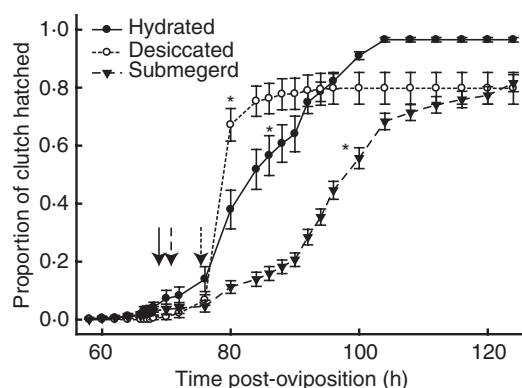
To look for changes in tadpole and metamorph phenotypes, we first assessed if animals from different egg environments differed in TTL or SVL, respectively, using LM. If animals differed in TTL or SVL, we log-transformed the morphological measurements and corrected for the effect of size using common principal components analysis (CPCA) with Burnaby's back projection method (BBPM) (McCoy *et al.* 2006). CPCA is a more appropriate and effective method for size-correction of morphological traits than the more

commonly used methods of shearing or residuals analysis, which are not designed for comparing among groups (McCoy *et al.* 2006). CPCA tests for shared allometry between groups by comparing covariance matrices; in this case, covariance matrices are of log-transformed morphological measures (Flury 1988). If a common size axis is present, BBPM tests for size-independent variation in shape (McCoy *et al.* 2006). If no common size axis is present, the covariance of morphology is so different between groups that size-correction is not possible (McCoy *et al.* 2006). It is not currently possible to test for blocking factors such as the shelf tadpoles were reared on in conjunction with CPCA. To assess condition at metamorphosis, we analyzed mass and SVL with LM, testing for effects of the original egg environment and density of tadpoles surviving to metamorphosis, as well as their interaction. We also tested for the effect of the shelf tadpoles were reared on. Separate from morphological analyses, we analyzed the effect of original egg environment and shelf on tadpole pigmentation using LM's, controlling for TTL as a covariate.

## Results

### EFFECTS OF EGG ENVIRONMENT ON EMBRYO DEVELOPMENT

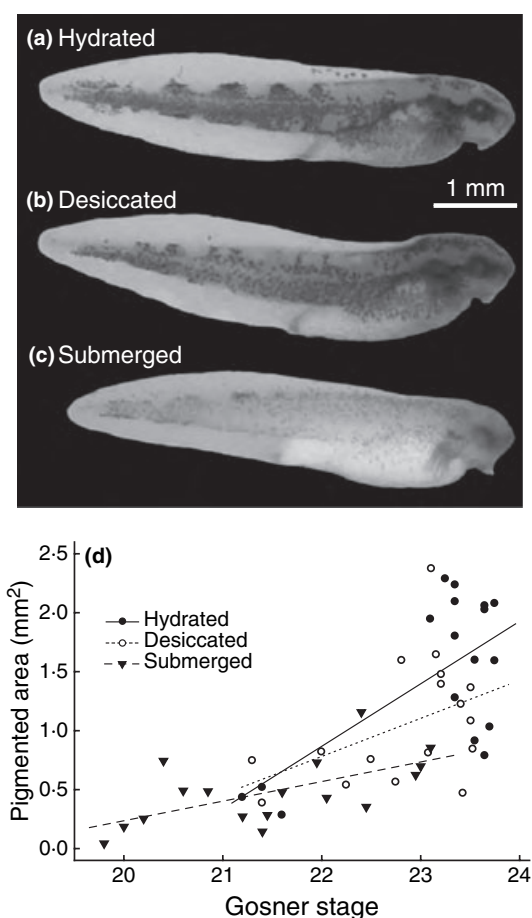
The environment that *D. ebraccatus* embryos were reared in affected both survival to hatching and hatching timing. Mortality was ~16% higher for eggs in the desiccated and submerged treatments than for eggs in the hydrated treatment (Fig. 2; GLM,  $F_{2,48} = 8.79$ ,  $P = 0.0006$ ). Mean hatching time and the synchrony of hatching varied for egg masses in the three environments (Fig. 2; hatching timing: LM,  $F_{2,48} = 72.82$ ,  $P < 0.00001$ ; hatching synchrony: LM,  $F_{2,48} = 119.45$ ,  $P < 0.00001$ ; all Tukey's post-hoc comparison,  $P < 0.001$ ). Desiccated eggs hatched earliest and most synchronously,  $80.3 \pm 0.5$  h post-oviposition and in  $10.2 \pm 1.4$  h from the onset of hatching



**Fig. 2.** The time-course of hatching of *Dendropsophus ebraccatus* eggs reared in three different abiotic environments: terrestrial and hydrated, terrestrial and desiccated or submerged underwater. Arrows indicate the mean start of hatching for each treatment. Asterisks indicate mean hatching time for each treatment. Hydrated eggs began hatching first but desiccated eggs had the earliest mean hatching time. Desiccated eggs also hatched the most synchronously whereas submerged eggs hatched the most gradually. Hydrated eggs had higher survival than either desiccated or submerged eggs.  $N = 17$  clutches per replicate. Data are mean proportion of eggs hatched  $\pm$  SE.

(Fig. 2). Hydrated eggs hatched later ( $85.9 \pm 1.4$  h post-oviposition) and over a greater period of time ( $32.4 \pm 2.0$  h). Submerged eggs did not hatch until  $97.0 \pm 0.9$  h post-oviposition and took  $52.6 \pm 2.3$  h to hatch (Fig. 2). Hydrated eggs began hatching  $69.5 \pm 1.9$  h after oviposition, significantly earlier than desiccated eggs ( $75.8 \pm 0.6$  h; Fig. 2; LM,  $F_{2,48} = 3.73$ ,  $P = 0.031$ ; Tukey's post-hoc comparison,  $P = 0.027$ ). Neither group differed from submerged eggs, which began hatching at  $71.6 \pm 2.0$  h (Fig. 2).

Despite hatching later, hatchlings from submerged eggs were less developed than hatchlings from either terrestrial environment [Fig. 3; Gosner stage: hydrated =  $23.1 \pm 0.2$ , desiccated =  $22.9 \pm 0.2$ , submerged =  $21.5 \pm 0.3$  (Gosner 1960); LM,  $F_{2,48} = 16.24$ ,  $P < 0.00001$ ; Tukey's comparisons, submerged – hydrated,  $P < 0.0001$ , submerged – desiccated,  $P = 0.0002$ , hydrated – desiccated,  $P = 0.65$ ]. Hatchlings from submerged eggs were also smaller than those from hydrated eggs, but neither was different in size from hatchlings from desiccated eggs (Fig. 3; TTL: hydrated =  $5.56 \pm 0.06$  mm, desiccated =  $5.41 \pm 0.06$  mm,



**Fig. 3.** Representative *Dendropsophus ebraccatus* hatchlings from (a) hydrated, (b) desiccated and (c) submerged eggs. (d) Hatchling pigmentation varied with both developmental stage and egg environment. Hatchlings from submerged eggs were less developed than hatchlings from either terrestrial egg treatment; they were smaller overall and had more elongate yolks and less developed gills, eyes and mouthparts. Hatchlings shown are at the mean developmental stage for each egg treatment.

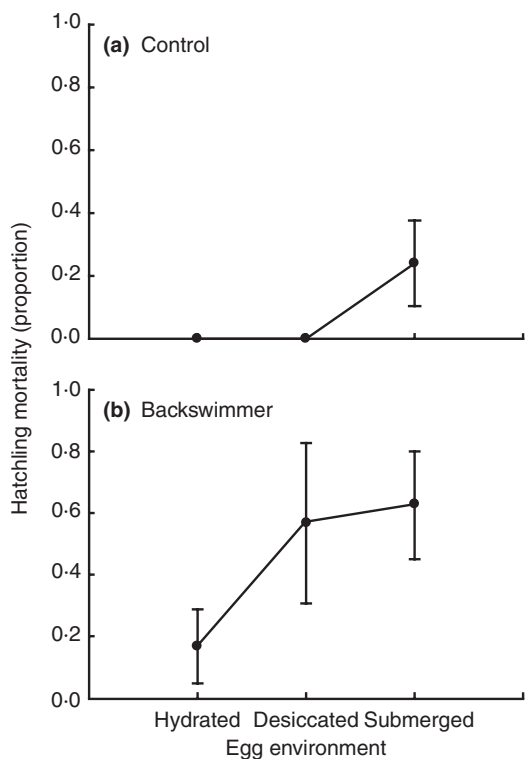
submerged =  $5.26 \pm 0.09$  mm; LM,  $F_{2,48} = 4.50$ ,  $P = 0.016$ ; Tukey's comparison, submerged – hydrated,  $P = 0.011$ ). Hatchling pigmentation varied by both egg treatment and stage at hatching, and there was no significant interaction between the two (Fig. 3d; LM, egg treatment:  $F_{2,45} = 3.79$ ,  $P = 0.029$ ; stage:  $F_{1,45} = 19.27$ ,  $P = 0.00001$ ; egg treatment  $\times$  stage,  $F_{2,45} = 2.58$ ,  $P = 0.087$ ). Pigmentation area increased with developmental stage, and was greatest in hatchlings from terrestrial egg treatments (Fig. 3d). However, because hatchlings were at different developmental stages from one another, they did not share a common size axis (i.e. the covariance of morphological measures was different between groups) and morphology could not be size corrected for comparisons of shape (Fig. 3; CPCA,  $P = 0.0002$ ).

#### EFFECTS OF EGG ENVIRONMENT IMMEDIATELY AFTER HATCHING

Egg environment affected survival immediately post-hatching. Hatchlings were placed in predation trials in accordance with their hatching pattern (after 15 eggs had hatched in a clutch), and as a result predation trials did not all begin simultaneously (start of predation trials, hydrated =  $76.6 \pm 1.8$  h, desiccated =  $78.0 \pm 0.8$  h, submerged =  $82.0 \pm 2.0$  h; LM,  $F_{2,48} = 3.36$ ,  $P = 0.04$ ). Predation trials with hatchlings from submerged eggs began significantly later post-oviposition than did predation trials with hatchlings from hydrated egg masses (Tukey's comparisons, submerged – hydrated,  $P = 0.04$ , all others,  $P > 0.16$ ). There was an effect of egg environment and backswimmer presence on hatchling survival in 24 h predation trials (Fig. 4; GLM, predator:  $F_{1,18} = 15.96$ ,  $P = 0.001$ , egg environment:  $F_{2,18} = 4.84$ ,  $P = 0.021$ ). Despite entering predation trials latest, hatchlings from submerged eggs had  $24 \pm 13\%$  baseline mortality whereas hatchlings from hydrated or desiccated eggs had no mortality (Fig. 4a). Backswimmers consumed more hatchlings from desiccated and submerged eggs than from hydrated eggs (Fig. 4b) although we did not detect a statistical difference in hatchling vulnerability to predators resulting from egg environment (Fig. 4; GLM, predator  $\times$  egg environment:  $F_{2,18} = 1.89$ ,  $P = 0.18$ ). This may be due to low power because of our small number of replicates. As in our no-predator controls, we observed no mortality in pooled tadpoles from hydrated and desiccated egg treatments during the first 3 days post-hatching. By contrast,  $\sim 30\%$  of hatchlings from submerged eggs died during this period; this mortality was within the range of standard error of the baseline mortality in no-predator controls (Fig. 4a).

#### EFFECTS OF EGG ENVIRONMENT ON TADPOLE DEVELOPMENT AND PREDATION RISK

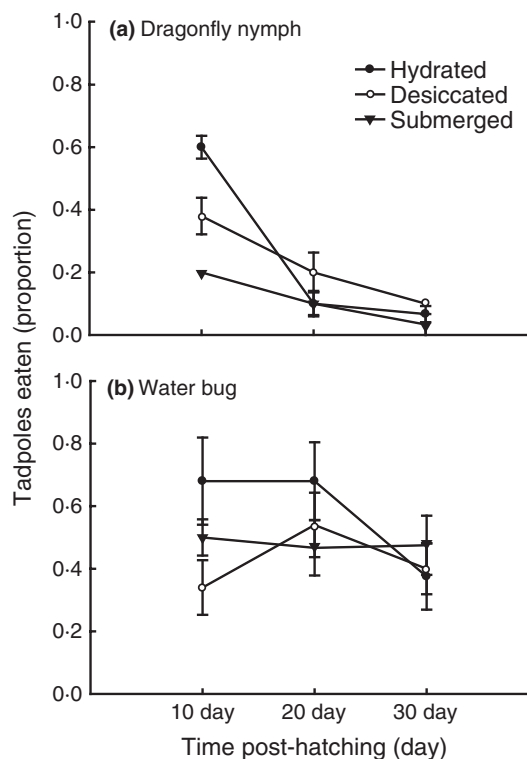
At 10 days post-hatching the morphology of tadpoles from all egg environments had converged and the effect of egg environment on the total length of tadpoles was marginally non-



**Fig. 4.** Mortality of *Dendropsophus ebraccatus* hatchlings from hydrated, desiccated and submerged eggs (a) in a no-predator control or (b) exposed to backswimmer predators. There were significant effects of predator presence and egg environment, but no significant interaction between them suggesting that hatchlings were equally vulnerable to backswimmers. Data are mean  $\pm$  SE.

significant (LM,  $F_{2,51} = 2.65$ ,  $P = 0.08$ ). Indeed, the size trend had reversed, such that tadpoles from submerged eggs were larger than those from desiccated or hydrated eggs (submerged =  $11.1 \pm 0.1$  mm, desiccated =  $10.8 \pm 0.1$  mm, hydrated =  $10.8 \pm 0.1$  mm). Tadpole morphology was indistinguishable at 20 and 30 days post-hatching (all  $P > 0.5$ ). Tadpole pigmentation at all three time intervals was positively correlated with TTL (larger tadpoles had larger pigmented areas), but was not affected by the egg rearing environment (LM, all egg treatment effects,  $P > 0.08$ , all TTL effects,  $P < 0.0004$ , all interaction effects,  $P > 0.30$ ).

Variation in the egg environment had lasting effects on tadpole survival with predators. There were significant predator, egg environment and age effects on tadpole vulnerability to dragonfly nymphs and water bugs (Fig. 5; GLM, predator,  $F_{1,53} = 67.36$ ,  $P < 0.00001$ , egg environment,  $F_{2,53} = 8.36$ ,  $P = 0.015$ , age,  $F_{2,53} = 22.50$ ,  $P < 0.00001$ ). In addition, the effect of tadpole age differed between predators; vulnerability to dragonfly nymphs decreased as tadpoles grew whereas tadpoles remained vulnerable to water bugs throughout development (Fig. 5; GLM, predator  $\times$  age,  $F_{2,53} = 24.53$ ,  $P < 0.00001$ ). The effect of tadpole age also differed across egg environments, or the effect of egg environment varied with age (Fig. 5; GLM, egg environment  $\times$  age,  $F_{4,53} = 12.57$ ,  $P = 0.014$ ). For example, tadpoles from hydrated eggs were most vulnerable to both predators early



**Fig. 5.** Predation by (a) dragonfly nymphs and (b) water bugs upon *Dendropsophus ebraccatus* tadpoles originating from hydrated, desiccated or submerged egg environments at 10 days, 20 days and 30 days post-hatching. The egg environment significantly affected tadpole vulnerability to predators, which also changed as tadpoles developed. Over time, tadpoles became less vulnerable to dragonfly nymphs but not to water bugs. Variation in predation upon tadpoles from different original egg environments changed over time as well. The egg environment had a larger effect on tadpole predation early in development than it did later in the larval period. Data are mean  $\pm$  SE. Two points lack error bars because all replicates had the same proportion of tadpoles eaten.

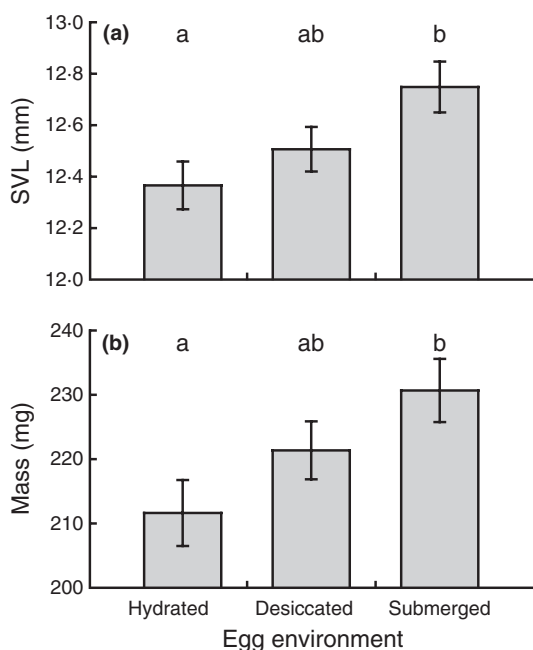
in development whereas the vulnerability of tadpoles from submerged eggs did not change with age (Fig. 5). Moreover, the egg environment had more effect on vulnerability earlier in development than it did later (Fig. 5). There was a marginally significant difference in the egg environment effect between the two different predators (Fig. 5; GLM, egg environment  $\times$  predator,  $F_{2,53} = 5.51$ ,  $P = 0.064$ ), and the three way interaction was not significant (GLM, predator  $\times$  egg environment  $\times$  age,  $F_{4,53} = 2.89$ ,  $P = 0.58$ ).

Egg environment had no effect on the number of tadpoles (out of 10 possible) that survived to metamorphosis (hydrated =  $6.6 \pm 0.6$  surviving tadpoles, desiccated =  $7.2 \pm 0.5$ , submerged =  $6.0 \pm 0.6$ ; LM,  $F_{2,51} = 0.98$ ,  $P = 0.38$ ). We do not know when during development tadpoles died. Egg environment did not affect the length of the larval period (hydrated =  $61.1 \pm 1.0$  days, desiccated =  $61.3 \pm 0.7$ , submerged =  $63.4 \pm 1.1$ ; LM,  $F_{2,46} = 1.70$ ,  $P = 0.19$ ). Despite having similar larval periods, egg environment did affect the SVL and mass of metamorphosis, whereas the density of tadpoles surviving to metamorphosis did not (LM, SVL: egg environment,  $F_{2,43} = 3.12$ ,  $P = 0.05$ , density,  $F_{1,43} =$

1.71,  $P = 0.20$ , egg environment  $\times$  density,  $F_{2,43} = 0.11$ ,  $P = 0.89$ ; mass: egg environment,  $F_{2,43} = 3.14$ ,  $P = 0.05$ , density,  $F_{1,43} = 0.10$ ,  $P = 0.75$ , egg environment  $\times$  density,  $F_{2,43} = 0.08$ ,  $P = 0.93$ ). Metamorphs that originated in submerged eggs were largest and those from hydrated eggs the smallest both in terms of SVL and mass (Fig. 6; Tukey's comparison of egg environment alone; SVL, hydrated – submerged,  $P = 0.021$ , all others  $P > 0.20$ ; mass, hydrated – submerged,  $P = 0.03$ , all others  $P > 0.31$ ). In addition, the distribution of metamorph sizes in each treatment were normal (Shapiro-Wilk normality test, SVL: hydrated,  $W = 0.91$ ,  $P = 0.10$ , desiccated,  $W = 0.96$ ,  $P = 0.61$ , submerged,  $W = 0.92$ ,  $P = 0.26$ ; mass: hydrated,  $W = 0.95$ ,  $P = 0.49$ , desiccated,  $W = 0.95$ ,  $P = 0.40$ , submerged,  $W = 0.95$ ,  $P = 0.57$ ) and the size variance did not differ among treatments (Bartlett's test of homogeneity of variances, SVL:  $K^2 = 0.30$ ,  $P = 0.86$ ; mass:  $K^2 = 0.63$ ,  $P = 0.73$ ). Metamorphs shared common allometry (CPCA,  $P = 0.41$ ) allowing for size-correction, which revealed no differences in metamorph shape (all  $P > 0.15$ ).

## Discussion

We demonstrate that common, naturally occurring variation in the environment has important short- and long-term effects on the early life stages of *D. ebraccatus*. Conditions experienced during the egg stage have immediate effects on embryo survival and development, and also carryover effects into the larval stage and through metamorphosis. Hatchling pheno-



**Fig. 6.** The (a) SVL and (b) mass of *Dendropsophus ebraccatus* metamorphs that originated from hydrated, desiccated or submerged eggs. Metamorphs that were originally in submerged eggs were the largest, both in terms of SVL and mass, whereas metamorphs from hydrated terrestrial eggs were the smallest.  $N_{\text{hydrated}} = 18$  replicates,  $N_{\text{desiccated}} = 18$  replicates,  $N_{\text{submerged}} = 13$  replicates. Letters indicate significantly different groups. Data are mean  $\pm$  SE.

type (Fig. 3), baseline hatchling survival (Fig. 4) and tadpole survival with predators (Fig. 5) vary with egg environment. Morphological carryover effects seem to disappear during most of the larval period but reappear at metamorphosis, affecting the size of metamorphosing frogs long after hatching (Fig. 6).

As global climate changes, the prevalence of environmental conditions suitable for egg development may change for many taxa (Doody *et al.* 2006; Huey & Janzen 2008). For example, in the Neotropics rainfall patterns are changing such that storms are becoming less frequent but larger when they do occur (Hulme & Viner 1998; Christensen *et al.* 2007; Allan & Soden 2008), potentially increasing both dry spells and pond level fluctuations. Thus, global climate change may increase the occurrence of both egg desiccation and submersion, altering the prevalence of carryover effects in *D. ebraccatus* populations.

Carryover effects of environmental variation experienced during the egg stage may be likely for many species. Most animals lack parental care and once eggs are laid they are subject to environmental changes potentially affecting survival and development. In organisms with complex life-histories, carryover effects may manifest in subsequent life stages. Most carryover effects are straightforward; for instance, greater mass at metamorphosis often translates to increased survival and reproduction of adults (Smith 1987; Altwegg & Reyer 2003; De Block & Stoks 2005; Benard & McCauley 2008). However, more subtle carryover effects can occur resulting from stress early in life. Compensatory growth following periods of starvation or resource depletion, for example, is generally associated with a cost, such as decreased burst swimming speed in sticklebacks (*Gasterosteus aculeatus*, Alvarez & Metcalfe 2007). Even when not associated with compensatory growth, rapid growth can have negative effects later in life, manifested as decreased viability, increased developmental errors or decreased fecundity (reviewed in Arendt 1997).

We observed a very clear short-term cost for *D. ebraccatus* eggs developing underwater; submerged eggs were developmentally retarded at hatching, probably due to oxygen limitation. Submerged eggs hatched both later and less developed than either terrestrial egg treatment (Figs 2 & 3). In addition, submerged eggs suffered  $\sim 20\%$  pre-hatching mortality, followed by an additional 20–30% post-hatching mortality. Most terrestrial amphibian eggs cannot survive underwater (Duellman & Trueb 1986; Wells 2007). For example, terrestrial *Agalychnis callidryas* eggs that are submerged drown if they are not old enough to hatch (Pyburn 1963; Warkentin 2000, 2002). However, *D. ebraccatus* embryos have a high surface area to volume ratio and are laid in a single layer with little jelly to impede oxygen diffusion, thereby improving oxygen supply to even eggs underwater (Strathmann & Strathmann 1995; Strathmann & Hess 1999). In fact, adults oviposit eggs directly in water in areas where terrestrial egg desiccation is likely to be high (Touchon & Warkentin 2008a). Desiccation is the most common source of mortality of *D. ebraccatus* eggs and terrestrial predation also increases when eggs are dehydrated (Touchon & Warkentin

2009). Moreover, aquatic egg predators such as fishes and conspecific tadpoles are common in *D. ebraccatus* breeding ponds (Touchon & Warkentin 2009). The immediate effect of developing in air or below water will therefore be habitat dependent; the risk of desiccation, the abundance of terrestrial and aquatic predators, and the amount of oxygen in the water will all affect the quality of different egg environments.

Tadpoles entering the water at premature developmental stages, such as those induced to hatch early by egg-stage predators or pathogens, can be more vulnerable to larval predators (Warkentin 1995; Gomez-Mestre, Touchon & Warkentin 2006), although this is not universal (Vonesh 2005; Vonesh & Bolker 2005). Hatchlings from both submerged and desiccated eggs appeared more vulnerable to backswimmers than hatchlings from hydrated eggs, although the interaction between effects of the hatchling predator and egg environment was not significant (Fig. 4). The lack of statistical significance likely results from low power; two more replicates of the hatchling predation trial with the same pattern as we observed would have rendered the interaction significant. It would not be surprising if hatchlings from submerged eggs were more vulnerable to predators, as they were substantially less developed than hatchlings from terrestrial eggs (Fig. 3) and may have not been able to swim as effectively to escape predation. It is less obvious, however, why hatchlings from desiccated eggs would be more vulnerable than those from hydrated eggs. Hatchlings from desiccated and hydrated eggs were developmentally similar and their predation trials started at approximately the same time (Figs 2 & 3). There could, however, be behavioural differences among hatchlings from the three egg environments that lead to differential levels of predation. Perhaps these differences are enough to increase vulnerability to backswimmers, although we did not detect such an effect here.

The advantage of developing in hydrated eggs, and the developmental cost of egg submergence, disappeared quickly. Ten days after all eggs had hatched, tadpoles from all three egg environments were similar in both size and shape. Such rapid compensatory growth is also seen between early induced and later, spontaneous hatchlings (Warkentin 1999; Vonesh & Bolker 2005). Despite their similar morphologies, the egg environment significantly altered vulnerability to predators (Fig. 5a). Tadpoles from submerged eggs were the least vulnerable to dragonfly nymphs throughout the larval period (Fig. 5a), indicating a potential benefit of developing underwater. Tadpole vulnerability may have been driven by behavioural differences caused by egg environment as, early in development, tadpoles from hydrated eggs suffered the highest predation from both dragonfly nymphs and water bug predators (Fig. 5). Both dragonfly nymphs and giant water bugs likely respond to tadpole movement and if tadpoles from hydrated eggs were more active than those from submerged or desiccated eggs, then they may have been subject to greater predation (Skelly 1994; Anholt & Werner 1995; Eklov & Werner 2000; Benard 2004). In addition, the tadpoles in our experiment were predator-naïve when placed in predation trials, and may have exhibited maladaptive activity

levels when first exposed to lethal predators. Beyond 10 days, vulnerability to dragonfly nymphs decreased for tadpoles from all egg environments as they grew towards a size refuge (Fig. 5a). On the other hand, tadpoles were equally vulnerable to the larger water bug predators throughout the larval period (Fig. 5b). Thus, although egg environment affects tadpole vulnerability, in the long term, differences in predator foraging are at least as important as differences among tadpoles. The relative importance of egg environment or predator effects will depend on specific predator communities and whether predation is focused on early or late larval stages.

At metamorphosis, we observed that metamorphs from submerged eggs were the largest (Fig. 6). We had predicted that if there were long-term effects of the egg environment, submerged eggs would experience a cost, not a seeming benefit. The differences in metamorph size were not due to variation in the larval period or the number of tadpoles surviving to metamorphosis. Also, at 30 days, tadpoles did not differ in size; we were therefore surprised to find differences in metamorph size. Mass at metamorphosis is commonly correlated with post-metamorphic survival and reproductive success in amphibians; increased mass translates to greater survival and higher fitness as an adult (Smith 1987; Semlitsch, Scott & Pechmann 1988; Altwegg & Reyer 2003; De Block & Stoks 2005; Benard & McCauley 2008). Assuming this is the case for *D. ebraccatus*, for tadpoles that survive beyond the first few days, the long-term consequence of developing underwater is beneficial. In a theoretical study, Mangel & Munch (2005) demonstrated that compensatory growth can lead to organisms that 'overshoot' the size of normally developing conspecifics, particularly when tissue damage from rapid growth is low. This has been demonstrated empirically in a *Lepomis* sunfish hybrid (Hayward, Noltie & Wang 1997). In the crab *Chasmagnathus granulata*, low larval mass leads to greater size and energy reserves later in life (Gimenez, Anger & Torres 2004). It was not feasible in our experiment to rear metamorphs to reproductive maturity, but this will be necessary to test if the size advantage at metamorphosis translates to a fitness benefit.

An alternative explanation for the effect of egg environment on metamorph size is that the early mortality of hatchlings from submerged eggs selected for animals that were larger at metamorphosis, via some correlated hatchling trait. A 30% truncation of the size distribution of metamorphs from hydrated eggs (similar to the level of mortality in submerged egg hatchlings) would shift the mean metamorph size to that of the submerged egg treatment. Although we cannot entirely rule this out, the increased mean size of metamorphs from submerged eggs does not appear to be a by-product of missing animals. The size distributions of metamorphs showed no evidence of such strong directional selection and the distribution and variance of metamorph sizes were similar for all egg environments.

We have demonstrated that environmental variation experienced during the egg stage has both short- and long-term carryover effects on the larval development of *D. ebraccatus*,

with effects lasting through metamorphosis. Our goal was to measure the effect of egg-stage environmental variation, without potential confounding factors, on hatching and tadpole development. We therefore raised tadpoles from the different egg environments in identical initial densities and without predators. However, different egg environments will lead to differential hatching input into a pond, and such density effects may alter the apparent long-term benefit of underwater egg development (Vonesh & Osenberg 2003; Vonesh & Bolker 2005). Egg-stage variation may also affect the time course or efficacy of predator-specific phenotype development. Tadpoles from submerged eggs may invest extra energy into compensatory growth and not be as able to develop predator-specific phenotypes, particularly the large red tail induced by dragonfly nymphs (Touchon & Warkentin 2008b). Alternatively, submerged-egg tadpoles exposed to predators may put energy into tail colour development and size instead of compensatory growth, therefore magnifying the extent of the short-term cost to underwater development. Nonetheless, our data indicate an immediate mortality risk for underwater egg development but, for the survivors, apparent long-term benefits in terms of decreased vulnerability to dragonfly nymphs and increased size at metamorphosis. Future studies of larval predator-prey interactions and developmental plasticity should consider the potential role of the egg environment in shaping the observed outcomes.

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## References

- Allan, R.P. & Soden, B.J. (2008) Atmospheric warming and the amplification of precipitation extremes. *Science*, **321**, 1481–1484.
- Alonso-Alvarez, C., Bertrand, S., Faivre, B. & Sorci, G. (2007) Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. *Functional Ecology*, **21**, 873–879.
- Altwegg, R. & Reyer, H.-U. (2003) Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*, **57**, 872–882.
- Alvarez, D. & Metcalfe, N.B. (2007) The tradeoff between catch-up growth and escape speed: variation between habitats in the cost of compensation. *Oikos*, **116**, 1144–1151.
- Anholt, B.R. & Werner, E.E. (1995) Interaction between food availability and predation mortality mediated by adaptive behavior. *Ecology*, **76**, 2230–2234.
- Arendt, J.D. (1997) Adaptive intrinsic growth rates: an integration across taxa. *Quarterly Review of Biology*, **72**, 149–177.
- Beckerman, A., Benton, T.G., Ranta, E., Kaitala, V. & Lundberg, P. (2002) Population dynamic consequences of delayed life-history effects. *Trends in Ecology & Evolution*, **17**, 263–269.
- Benard, M.F. (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology and Systematics*, **35**, 651–673.
- Benard, M.F. & McCauley, S.J. (2008) Integrating across life-history stages: consequences of natal habitat effects on dispersal. *American Naturalist*, **171**, 553–567.
- Broomhall, S.D. (2004) Egg temperature modifies predator avoidance and the effects of the insecticide endosulfan on tadpoles of an Australian frog. *Journal of Applied Ecology*, **41**, 105–113.
- Christensen, J.H., Hewitson, B., Busuioic, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.-T., Laprise, R., Rueda, V.M., Mearns, L., Menéndez, C.G., Räisänen, J., Rinke, A., Sarr, A. & Whetton, P. (2007) Regional Climate Projections. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor & H. L. Miller), pp. 847–940. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- De Block, M. & Stoks, R. (2005) Fitness effects from egg to reproduction: bridging the life history transition. *Ecology*, **86**, 185–197.
- Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M. (2006) Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evolutionary Ecology*, **20**, 307–330.
- Duellman, W.E. & Trueb, L. (1986) *Biology of Amphibians*. The Johns Hopkins University Press, Baltimore, MD.
- Eklov, P. & Werner, E.E. (2000) Multiple predator effects on size-dependent behavior and mortality of two species of anuran larvae. *Oikos*, **88**, 250–258.
- Engels, S. & Sauer, K.P. (2007) Energy beyond the pupal stage: larval nutrition and its long-time consequences for male mating performance in a scorpionfly. *Journal of Insect Physiology*, **53**, 633–638.
- Faivovich, J., Haddad, C.F.B., Garcia, P.C.A., Frost, D.R., Campbell, J.A. & Wheeler, W.C. (2005) Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History*, **294**, 1–240.
- Flury, B. (1988) *Common Principal Components and Related Multivariate Models*. Wiley and Sons, New York, NY, USA.
- Gimenez, L., Anger, K. & Torres, G. (2004) Linking life history traits in successive phases of a complex life cycle: effects of larval biomass on early juvenile development in an estuarine crab, *Chasmagnathus granulata*. *Oikos*, **104**, 570–580.
- Goater, C.P. (1994) Growth and survival of postmetamorphic toads: interactions among larval history, density, and parasitism. *Ecology*, **75**, 2264–2274.
- Gomez-Mestre, I., Touchon, J.C. & Warkentin, K.M. (2006) Amphibian embryo and parental defenses and a larval predator reduce egg mortality from water mold. *Ecology*, **87**, 2570–2581.
- Gorman, H.E. & Nager, R.G. (2004) Prenatal developmental conditions have long-term effects on offspring fecundity. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 1923–1928.
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183–190.
- Hayward, R.S., Noltie, D.B. & Wang, N. (1997) Use of compensatory growth to double hybrid sunfish growth rates. *Transactions of the American Fisheries Society*, **126**, 316–322.
- Huey, R.B. & Janzen, F.J. (2008) Climate warming and environmental sex determination in tuatara: the last of the Sphenodontians? *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2181–2183.
- Hulme, M. & Viner, D. (1998) A climate change scenario for the tropics. *Climatic Change*, **39**, 145–176.
- Jannot, J.E., Brunea, E. & Wissinger, S.A. (2007) Effects of larval energetic resources on life history and adult allocation patterns in a caddisfly (Trichoptera: Phryganeidae). *Ecological Entomology*, **32**, 376–383.
- Lindstrom, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, **14**, 343–348.
- Mangel, M. & Munch, S.B. (2005) A life-history perspective on short- and long-term consequences of compensatory growth. *American Naturalist*, **166**, E155–E176.
- McCoy, M.W., Bolker, B.M., Osenberg, C.W., Miner, B.G. & Vonesh, J.R. (2006) Size correction: comparing morphological traits among populations and environments. *Oecologia*, **148**, 547–554.
- Metcalfe, N.B. & Monaghan, P. (2001) Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, **16**, 254–260.
- Munch, S.B. & Conover, D.O. (2003) Rapid growth results in increased susceptibility to predation in *Menidia menidia*. *Evolution*, **57**, 2119–2127.
- Olsson, M. & Shine, R. (2002) Growth to death in lizards. *Evolution*, **56**, 1867–1870.
- Olvido, A.E., Busby, S. & Mousseau, T.A. (1998) Oviposition and incubation environmental effects on embryonic diapause in a ground cricket. *Animal Behaviour*, **55**, 331–336.

- Pechenik, J.A., Wendt, D.E. & Jarrett, J.N. (1998) Metamorphosis is not a new beginning. *BioScience*, **48**, 901–910.
- Pinheiro, J.C. & Bates, D.M. (2000) *Mixed Effects Models in S and S-Plus*. Springer, New York, NY.
- Pyburn, W.F. (1963) Observations on the life history of the treefrog, *Phyllomedusa callidryas* (Cope). *Texas Journal of Science*, **15**, 155–170.
- R Development Core Team (2007) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Roussel, J.M. (2007) Carry-over effects in brown trout (*Salmo trutta*): hypoxia on embryos impairs predator avoidance by alevins in experimental channels. *Canadian Journal of Fisheries and Aquatic Sciences*, **64**, 786–792.
- Scott, D.E. (1994) The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology*, **75**, 1383–1396.
- Semlitsch, R.D., Scott, D.E. & Pechmann, J.H.K. (1988) Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology*, **69**, 184–192.
- Skelly, D.K. (1994) Activity level and the susceptibility of anuran larvae to predation. *Animal Behaviour*, **47**, 465–468.
- Smith, D.C. (1987) Adult recruitment in chorus frogs: effects of size and date at metamorphosis. *Ecology*, **68**, 344–350.
- Solberg, E.J., Loison, A., Gaillard, J.M. & Heim, M. (2004) Lasting effects of conditions at birth on moose body mass. *Ecography*, **27**, 677–687.
- Strathmann, R.R. & Hess, H.C. (1999) Two designs of marine egg masses and their divergent consequences for oxygen supply and desiccation in air. *American Zoologist*, **39**, 253–260.
- Strathmann, R.R. & Strathmann, M.F. (1995) Oxygen supply and limits on aggregation of embryos. *Journal of the Marine Biological Association of the United Kingdom*, **75**, 413–428.
- Taylor, B.W., Anderson, C.R. & Peckarsky, B.L. (1998) Effects of size at metamorphosis on stonefly fecundity, longevity, and reproductive success. *Oecologia*, **114**, 494–502.
- Touchon, J.C. & Warkentin, K.M. (2008a) Reproductive mode plasticity: aquatic and terrestrial oviposition in a treefrog. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 7495–7499.
- Touchon, J.C. & Warkentin, K.M. (2008b) Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. *Oikos*, **117**, 634–640.
- Touchon, J.C. & Warkentin, K.M. (2009) Negative synergism of rainfall patterns and predators affects frog egg survival. *Journal of Animal Ecology*, **78**, 715–723.
- Vonesh, J.R. (2005) Egg predation and predator-induced hatching plasticity in the African reed frog, *Hyperolius spinigularis*. *Oikos*, **110**, 241–252.
- Vonesh, J.R. & Bolker, B.M. (2005) Compensatory larval responses shift trade-offs associated with predator-induced hatching plasticity. *Ecology*, **86**, 1580–1591.
- Vonesh, J.R. & Osenberg, C.W. (2003) Multi-predator effects across life-history stages: non-additivity of egg- and larval-stage predation in an African treefrog. *Ecology Letters*, **6**, 503–508.
- Ward, B.R. & Slaney, P.A. (1988) Life-history and smolt-to-adult survival of Keogh River steelhead trout (*Salmo gairdneri*) and the relationship to smolt size. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1110–1122.
- Warkentin, K.M. (1995) Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 3507–3510.
- Warkentin, K.M. (1999) Effects of hatching age on development and hatchling morphology in the red-eyed treefrog, *Agalychnis callidryas*. *Biological Journal of the Linnean Society*, **68**, 443–470.
- Warkentin, K.M. (2000) Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Animal Behaviour*, **60**, 503–510.
- Warkentin, K.M. (2002) Hatching timing, oxygen availability, and external gill regression in the tree frog, *Agalychnis callidryas*. *Physiological and Biochemical Zoology*, **75**, 155–164.
- Wells, K.D. (2007) *The Ecology and Behavior of Amphibians*. The University of Chicago Press, Chicago, IL.
- West-Eberhard, M.J. (2003) *Developmental Plasticity and Evolution*. Oxford University Press, New York, NY.

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