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Original Article

Habitat-specific constraints on induced hatching in a treefrog with reproductive mode plasticity

Justin C. Touchon,^{a,b} Jenny Urbina,^c and Karen M. Warkentin^{a,b}

^aDepartment of Biology, Boston University, Boston, MA 02215, USA, ^bSmithsonian Tropical Research Institute, Apartado Postal 0843-03092, Balboa, República de Panamá, and ^cGrupo Herpetológico de Antioquia, Instituto de Biología, Universidad de Antioquia, A.A. 1226, Medellín, Antioquia, Colombia

Many organisms show adaptive phenotypic plasticity in response to environmental variation. Some environmental factors may, however, impose constraints on the ability of organisms to respond to other factors. The neotropical treefrog *Dendropsophus ebraccatus* lays eggs both above water on leaves and directly in water, thereby exposing embryos to different abiotic conditions and predator communities. Rising pond levels can also flood arboreal clutches after rainstorms. We tested for predator-induced hatching in submerged and arboreal *D. ebraccatus* egg masses and assessed effects of prior hydration or desiccation on escape success in attacks by a terrestrial predator, *Azteca* ants, and an aquatic predator, large conspecific tadpoles. Embryos responded to both aquatic and terrestrial predator attacks by hatching prematurely, as much as 67% earlier than the peak of hatching in undisturbed clutches. Desiccation reduced the hatching response of terrestrial embryos, resulting in substantially lower escape rates. This desiccation effect disappeared rapidly with flooding; all embryos showed high escape hatching success underwater. The occurrence of predator-induced escape hatching in response to 2 different predators, in 2 different physical environments, suggests that this is a general response of *D. ebraccatus* to egg predator attack. Both ant attack and sublethal desiccation are common in nature, thus the inhibition of escape hatching in this context likely impacts tadpole recruitment. More generally, we demonstrate that an adaptive plastic response to risk is contingent on additional environmental variables and suggest that many instances of plasticity may similarly be modified by environmental constraints. **Key words:** abiotic–biotic environmental variation, adaptive phenotypic plasticity, anura, complex life cycle, *Hyla ebraccata*, interaction modification, predator-facilitated hatching. [*Behav Ecol* 22:169–175 (2011)]

Phenotypic plasticity is a ubiquitous and important response of organisms to environmental variation (Karban and Baldwin 1997; Pigliucci 2001; West-Eberhard 2003). Plastic responses include both adaptive environmentally cued changes in morphology, behavior, and life history (Boersma et al. 1998; Lardner 2000; Johansson et al. 2001; Benard 2004) and nonadaptive phenotypic changes resulting directly from environmental effects on development. As a result of variation in one environmental factor, an organism's adaptive plastic response to another factor can sometimes be altered, ablated, or rendered ineffective (Gilbert and Epel 2008). Changing levels of rainfall, oxygen availability, ultraviolet radiation, or temperature can interact with biotic environmental variation to alter the expression of adaptive plasticity (Newman 1998; McIntyre and McCollum 2000; Blaustein et al. 2001).

Hatching represents a major life-history switch point. Many animals are now known to adaptively alter their hatching timing in response to abiotic conditions, natural enemies, or resources affecting egg or larval survival (reviewed in Warkentin and Caldwell 2009). Embryos can hatch early in response to egg-stage threats (Warkentin 1995; Chivers et al. 2001; Li 2002; Touchon et al. 2006), delay hatching if they detect a predator of the subsequent life stage (Sih and Moore 1993; Laurila et al. 2002; Miner et al. 2010), or hatch when abiotic conditions

are most appropriate for larval survival (Martin 1999; Ehlinger and Tankersley 2003; Gunzburger 2003). Hatching plasticity, like other forms of adaptive plasticity, often reflects trade-offs. For instance, animals that hatch early to escape from egg predators are generally smaller and less developed than later hatchlings, which in turn can affect survival with larval predators (Warkentin 1995; Gomez-Mestre et al. 2006, 2008).

In the case of inducible hatching, the environment may potentially limit the expression of plasticity. Because embryos must escape from their egg capsules, physical alteration of the egg capsule or clutch or effects on the developing embryo itself may alter the ability or propensity of embryos to hatch. For example, the probability that terrestrial killifish (*Fundulus heteroclitus*) embryos hatch when inundated with seawater increases after prolonged bouts of desiccation (Tingaud-Sequeira et al. 2009). Terrestrial amphibian eggs are also highly vulnerable to desiccation (Duellman and Trueb 1986). Amphibian egg clutches are comprised of embryos surrounded by perivitelline fluid, a perivitelline membrane, and a variable number of layers of jelly (Duellman and Trueb 1986; Altig and McDiarmid 2007). The physical properties and structure of these clutches can change with hydration and desiccation, potentially altering the ability of embryos to hatch (Neckel-Oliveira 2004; Touchon and Warkentin 2009).

We studied the potentially interacting effects of the physical environment (aquatic or terrestrial), prior clutch desiccation or hydration, and predator attack on hatching age and escape success in the neotropical treefrog *Dendropsophus ebraccatus*. *Dendropsophus ebraccatus* is currently the only vertebrate known to exhibit aquatic/terrestrial reproductive mode plasticity;

Address correspondence to J.C. Touchon. E-mail: jtouchon@bu.edu.

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eggs are laid on leaves above water in shaded habitats but are laid directly in the water in unshaded ponds where terrestrial egg desiccation risk is high (Touchon and Warkentin 2008). Thus, *D. ebraccatus* embryos can develop in 2 very different abiotic environments—in air or underwater—with different predator communities. This species thus provides a unique opportunity to assess the potential effects of prior and current environmental conditions on embryo responses to predators without subjecting embryos to ecologically and physiologically unrealistic conditions. Eggs in both environments develop to hatching in 3–4 days (Touchon and Warkentin 2010). Rainfall, which hydrates clutches, is important for egg survival both because terrestrial predators prefer desiccated eggs and because extreme desiccation kills eggs directly (Touchon and Warkentin 2009). In addition, arboreal *D. ebraccatus* eggs frequently become flooded when pond levels rise (Touchon and Warkentin 2009). It is unknown if *D. ebraccatus* embryos can respond to egg predators by hatching early. Furthermore, it is unknown if developing or being attacked in different physical environments affects any possible embryo responses to egg predators.

We hypothesized that 1) *D. ebraccatus* embryos can hatch prematurely in response to predator attack in both air and water and 2) if embryos can hatch early, their ability to do so in air is reduced by clutch desiccation. To test this, we quantified the hatching age and survival of embryos in hydrated and desiccated clutches attacked by either an arboreal predator (*Azteca* sp. ants) or an aquatic predator (large cannibalistic *D. ebraccatus* tadpoles). We compared these results with the spontaneous hatching age of matched hydrated and desiccated egg clutches not exposed to predators.

MATERIALS AND METHODS

We conducted egg predation trials between 18 July and 2 August 2007. To obtain egg masses, we collected 31 mating *D. ebraccatus* pairs from 2 ponds in Gamboa, Panama (Bridge Pond, lat 9°6′50.26″ N, long 79°41′48.13″ W and Experimental Pond, lat 9°7′14.88″ N, long 79°42′14.11″ W). We placed pairs in individual plastic bags with a small amount of water and allowed them to breed overnight in an ambient temperature laboratory. All pairs laid 2 or more egg masses inside their bag. Frogs were returned to their pond the following day. We use midnight as the time of oviposition for embryo age calculations; most clutches are laid between 11 PM and 1 AM (Touchon J, personal observation). The morning after oviposition, individual egg masses were removed from bags by cutting the plastic around them without disturbing eggs. We counted the eggs and obtained the initial weight of each egg mass by weighing it with the attached plastic, then subtracting the weight of an equal-sized piece of plastic cut from the same bag (Touchon and Warkentin 2009).

Because *D. ebraccatus* egg predators, including *Azteca* ants, prefer desiccated eggs over hydrated ones (Touchon and Warkentin 2009), we conducted predation trials in a no-choice manner, exposing only one type of egg mass (hydrated or desiccated) to predators at a time. Pairs of similarly sized egg masses laid on the same night were randomly assigned to be either hydrated or desiccated and within each pair masses were randomly assigned to predator or control treatments. Healthy undisturbed arboreal *D. ebraccatus* eggs at our field site hatch 80–85 h postoviposition (Touchon and Warkentin 2010). Thus, to determine how early *D. ebraccatus* embryos might be able to hatch, predation trials were begun 31–69 h postoviposition. Eggs were allowed to desiccate naturally under shaded ambient temperature and humidity conditions without rainfall until the start of predation trials. We hydrated egg masses by placing them in a shallow bath of aged tap water until the start of trials. No developmental effect of our hydra-

tion treatment was evident at the start of predation trials. All embryos in both treatments were alive and developing normally when presented to predators.

Desiccated and hydrated egg masses did not vary significantly in their starting number of eggs (58 ± 1 and 59 ± 1 eggs, respectively, mean \pm standard error here and throughout; *t*-test, $t_{128} = -0.50$, $P = 0.62$) nor in their initial mass (desiccated = 0.64 ± 0.02 g, hydrated = 0.61 ± 0.02 g; *t*-test, $t_{128} = 0.60$, $P = 0.55$). Likewise, egg masses used as controls or exposed to predators did not differ in their starting number of eggs (58 ± 1 and 58 ± 1 eggs, respectively; *t*-test, $t_{128} = 0.13$, $P = 0.9$) nor initial mass (control = 0.61 ± 0.02 g, predator = 0.64 ± 0.02 g; *t*-test, $t_{128} = -0.78$, $P = 0.44$).

Ant predation trials

We conducted 21 predation trials with ants ($N_{\text{hydrated}} = 11$, $N_{\text{desiccated}} = 10$), all begun at 56 h postoviposition and lasting until all eggs had either hatched or been eaten or until night-fall (length of trials: 7.8 ± 0.2 h). Each trial consisted of an egg mass exposed to predators and a similar sized control. We exposed egg masses to predators by placing them in small plastic cups attached to trees near *Azteca* sp. nests and trails. Cups contained a small amount of water to catch *D. ebraccatus* hatchlings. Ants usually discovered egg masses on their own soon after trials began (time until ants seen on clutch: 2.0 ± 0.2 h). Occasionally, if ants took longer to discover egg masses, we used forceps to place a few ants near or on egg masses; these ants then recruited additional foragers. Predation trials took place at both Bridge and Experimental Ponds. To ensure that control egg masses experienced the same handling and environmental conditions as those in predation trials, we brought controls to ponds along with predator egg masses but kept them protected from predation throughout the trial. There were 3 possible outcomes for embryos: 1) missing from the clutch and not in the cup, thus eaten by ants; 2) missing from the clutch but found as a tadpole in the cup, thus hatched; and 3) unhatched and remaining in the egg. We recorded the number of eggs in each category in both predator and control egg masses hourly during the predation trials. After predation trials, a subset of control egg masses ($N = 9$ hydrated and 4 desiccated) were returned to the laboratory and monitored to determine spontaneous hatching age (total observation time: 33.0 ± 4.8 h). All egg masses were misted after returning to the laboratory to enable embryos to continue developing without direct mortality from desiccation. We also assessed the effect of hatching age on hatchling phenotype. See Supplementary Materials for details.

Tadpole predation trials

Dendropsophus ebraccatus eggs are laid directly in the water in some habitats, and arboreally laid eggs often become flooded after large rainstorms (Touchon and Warkentin 2008, 2009). A common predator of aquatic *D. ebraccatus* eggs is large con-specific tadpoles, which opportunistically cannibalize other tadpoles or embryos in the water (Touchon J, personal observation). We conducted 45 predation trials with tadpole predators ($N_{\text{hydrated}} = 22$, $N_{\text{desiccated}} = 23$). As above, each trial consisted of both a clutch exposed to predators and a similar sized control. We exposed previously hydrated or desiccated *D. ebraccatus* egg masses to groups of 3 large *D. ebraccatus* tadpoles for 10.7 ± 1.9 h. Tadpole predators were dipnetted from Bridge Pond. Predation trials were conducted in $18 \times 14 \times 7$ -cm plastic containers filled with aged tap water. To ensure that large tadpoles could not eat hatchlings, which would confound assessment of hatching rates, predation trial venues

were fitted with a mesh false bottom that allowed hatchlings to fall out of reach of the larger *D. ebraccatus* tadpoles. Control egg masses were placed in cups filled with aged tap water and observed throughout the predation trial. We monitored both predator and control egg masses hourly during predation trials, recording the number of eggs missing from the egg mass but not in the container (eaten by tadpole predators), missing from the egg mass but hatched into the water, and those remaining unhatched. As in the ant predation trials, to determine spontaneous hatching age, we monitored a subset of control egg masses ($N = 19$ desiccated, 19 hydrated) that were left in water until all eggs had hatched (total observation time: 56.7 ± 4.9 h). To explore the relationship between escape hatching ability and development (age since oviposition), we began predation trials 30.5–69.3 h postoviposition. See Supplementary Materials for information about the effects of hatching age and treatment on hatchling development and phenotype.

Statistical analyses

All statistical analyses were conducted in R version 2.10.1 (R Development Core Team 2007). Initial mass of eggs and initial number of eggs in each egg mass were analyzed with paired *t*-tests. In all models described below, we began by fitting the maximal model with all possible interactions and then used model simplification to achieve the minimal adequate model, including at least all main predictors (Crawley 2007). In addition, because some pairs of frogs contributed multiple egg masses to the experiments, we initially included family in all models. However, family never had a significant effect, and we thus removed it from final analyses.

We modeled the proportion of eggs hatched from all egg masses (both predator exposed and controls) and the proportion of eggs escaped in egg masses attacked by predators, using generalized linear models (GLM) with underlying quasibinomial error function and logit link functions. 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 and Bates 2000). Specifically, we tested for effects of hydration and predation treatments and their interaction on the proportion of eggs hatched during predation trials. We then tested for effects of hydration treatment alone on the proportional escape hatching success of embryos exposed to predators (the number of hatchlings found out of the number of eggs missing from the egg mass). Because we started tadpole predation trials at different times postoviposition, we included trial time in models of proportion hatched during predation trials as well as escape hatching success.

In the ant experiment, because all trials began at the same time postoviposition, we simply tested for effects of hydration and predator treatments on mean hatching age with a linear model (LM). In the tadpole experiment, where clutches were submerged at different times, we tested for effects of hydration treatment, predator treatment, and trial time on age at the onset of hatching (the point when 5 embryos had hatched from a given egg mass) with LM. To facilitate comparison with previous research, we also calculated mean and modal (peak) hatching ages of control egg masses that we observed until all eggs had hatched, testing for an effect of hydration treatment in the ant experiment and of hydration treatment and trial time in the tadpole experiment with LMs. We also compared both mean and modal hatching times of desiccated and hydrated control egg masses across the 2 experiments with LM.

For additional statistical analyses of hatchling size and developmental stage, see Supplementary Materials.

RESULTS

Ant predation trials

Prior to exposure to *Azteca* sp. ants, the mass of hydrated egg masses had increased $268 \pm 47\%$ due to water absorption, and desiccated egg masses had lost $34 \pm 7\%$ of their original mass to evaporation. Both hydration treatment and exposure to ants strongly influenced the proportion of embryos that were hatched at the end of predation trials, with more eggs hatching from predator egg masses than controls and fewer from desiccated than hydrated masses (GLM, predator, $F_{1,32} = 22.3$, $P < 0.0001$ and hydration, $F_{1,32} = 19.6$, $P = 0.0001$). There was also a marginally nonsignificant interaction between predators and clutch hydration (GLM, predator \times hydration, $F_{1,32} = 3.3$, $P = 0.07$). Only $1 \pm 0.7\%$ of hydrated and $1 \pm 0.1\%$ of desiccated control eggs hatched during the predation trials. However, $22 \pm 1\%$ of hydrated eggs hatched after exposure to ants, whereas only $1 \pm 1\%$ of ant-exposed desiccated eggs hatched.

In embryos that were attacked by ants, clutch hydration strongly affected escape hatching success (Figure 1A; GLM, $F_{1,13} = 30.9$, $P < 0.001$). In hydrated egg masses, 60 \pm 13% of missing embryos had successfully hatched and escaped ant predation, whereas only 4 \pm 3% of missing desiccated embryos had escaped (Figure 1A). Ants consumed significantly more embryos from the desiccated than the hydrated clutches that they attacked during predation trials ($52 \pm 11\%$ and $6 \pm 2\%$, respectively; GLM, $F_{1,19} = 22.6$, $P = 0.0001$). Six of the 9 desiccated egg masses attacked by ants were entirely consumed with no hatchlings escaping.

Predator and hydration treatments significantly affected the mean age at hatching, and there was an interaction between the 2, indicating that the effect of hydration differed for

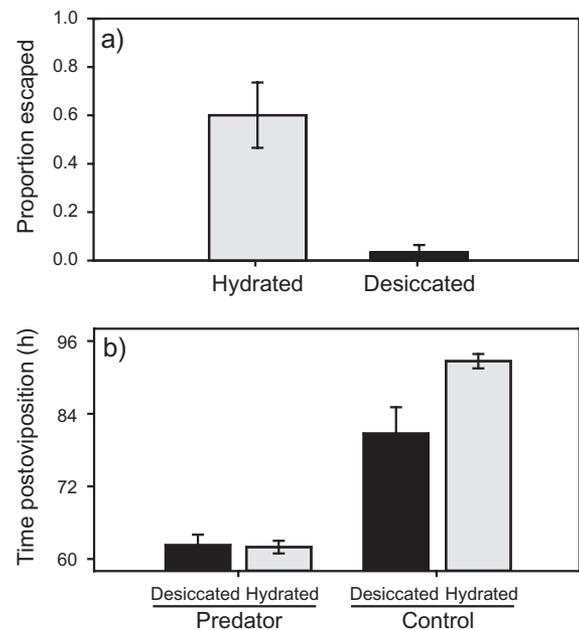


Figure 1

Ant predator-induced and spontaneous hatching of *Dendropsophus ebraccatus* embryos from hydrated and desiccated egg clutches. (a) Escape hatching success, measured as the proportion of eggs missing from egg masses attacked by *Azteca* sp. ants that escaped by hatching prematurely. Other missing eggs were presumed eaten. (b) The hatching age of hydrated or desiccated eggs that were exposed to *Azteca* sp. ant predators or were left as undisturbed controls. Bars are mean \pm standard error.

embryos hatching spontaneously or when attacked by ants (Figure 1B; LM, predator, $F_{1,19} = 137.5$, $P < 0.00001$, hydration, $F_{1,19} = 9.8$, $P = 0.005$, and predator \times hydration, $F_{1,19} = 6.7$, $P = 0.02$). Hydrated embryos and the few desiccated embryos that hatched successfully did so at similar ages if attacked by ants (61.9 ± 1.0 and 62.3 ± 1.7 h postoviposition, respectively), whereas desiccated embryos spontaneously hatched earlier than hydrated embryos (80.7 ± 4.4 and 92.6 ± 1.2 h postoviposition, respectively). Likewise, hydration treatment affected the modal hatching age of desiccated and hydrated control eggs (79.3 ± 4.2 and 94.9 ± 4.3 h postoviposition, respectively; $F_{1,12} = 5.6$, $P = 0.04$). Embryos induced to hatch early in ant attacks were smaller and less developed than those that hatched later from control clutches. See Supplementary Materials for details.

Tadpole predation trials

Prior to tadpole predation trials, the mass of hydrated egg masses had increased $197 \pm 13\%$ from water absorption, whereas desiccated egg masses had lost $26 \pm 5\%$ of their mass to evaporation. Large *D. ebraccatus* tadpoles readily attacked all egg masses offered to them, including hydrated and desiccated egg masses of all ages. The proportion of eggs that hatched during the ~ 10 -h long predation trials was strongly influenced by predator presence but not hydration treatment (Figure 2A; GLM, predator, $F_{1,84} = 57.0$, $P < 0.00001$ and hydration, $F_{1,84} = 0.5$, $P = 0.47$). Hatching in both control and predator treatments increased as trial time was later in development, and the effect of predation treatment on hatching changed over time (Figure 2A; GLM, trial time, $F_{1,84} =$

123.4 , $P < 0.00001$ and predator \times trial time, $F_{1,84} = 15.4$, $P = 0.0002$). There was also an interaction between the hydration and predation treatments; slightly more hydrated eggs hatched and escaped than desiccated eggs in predation trials, whereas the opposite pattern of hatching was true in controls (Figure 2A; GLM, predation \times hydration, $F_{1,84} = 8.9$, $P = 0.003$).

In egg masses that were attacked by tadpoles, embryo escape hatching success was not affected by hydration treatment but did increase as trial times were later in development (GLM, hydration treatment, $F_{1,41} = 1.0$, $P = 0.31$, trial time, $F_{1,41} = 55.6$, $P < 0.00001$, and hydration \times trial time, $F_{1,41} = 0.41$, $P = 0.55$).

The onset of hatching varied with predation treatment, hydration treatment, and trial time (Figure 2B; LM, predator, $F_{1,76} = 248.1$, $P < 0.00001$, hydration, $F_{1,76} = 11.5$, $P = 0.001$, and trial time, $F_{1,76} = 101.7$, $P < 0.00001$). There was also an interaction between trial time and predator treatment (Figure 2B; LM, predator \times trial time, $F_{1,76} = 145.6$, $P < 0.00001$) such that the onset of hatching was correlated with trial time in egg masses exposed to predators but not in controls. Egg masses exposed to predators began hatching 2.0 ± 0.2 h after the start of the trial, regardless of when trials began (Figure 2B; LM, hydrated predator $R^2 = 0.99$ and dehydrated predator $R^2 = 0.99$). In contrast, the onset of hatching in desiccated control eggs began at 66.7 ± 2.1 h after oviposition and in hydrated control eggs at 76.2 ± 1.9 h after oviposition and was not related to trial time (Figure 2B; hydrated control $R^2 = 0.12$ and dehydrated control $R^2 = 0.04$). There was also an interaction between hydration and predator treatments; hydration did not affect the onset of hatching in predator exposed egg masses, but, following submergence, previously hydrated control embryos began hatching significantly later than desiccated controls (Figure 2B; LM, predator \times hydration, $F_{1,76} = 14.3$, $P < 0.0003$).

The mean and modal ages of spontaneous hatching in submerged hydrated and desiccated control egg masses that were observed until all eggs hatched differed; desiccated eggs hatched on average 85.5 ± 3.1 h postoviposition, whereas hydrated eggs hatched 94.5 ± 1.8 h postoviposition (LM, $F_{1,36} = 6.3$, $P = 0.017$). Peak hatching occurred at 84.0 ± 4.6 h postoviposition in desiccated eggs and 97.5 ± 2.6 h postoviposition in hydrated eggs (LM, $F_{1,36} = 6.4$, $P = 0.016$). The earliest predator-induced hatching occurred at 31.5 h postoviposition from hydrated eggs and 32.5 h postoviposition from desiccated eggs, 67% and 61% earlier than the age of peak hatching in their respective submerged controls. When comparing undisturbed control egg masses from both the ant and the tadpole experiments, only initial hydration treatment but not experiment environment (air or water) affected mean or peak hatching age (LM, mean hatching age: hydration, $F_{1,49} = 19.5$, $P < 0.00001$, experiment, $F_{1,49} = 0.7$, $P = 0.41$ and peak hatching age: hydration, $F_{1,49} = 10.7$, $P = 0.002$, experiment, $F_{1,49} = 0.5$, $P = 0.47$). Hatchlings escaping from tadpole predators were smaller and less developed than animals that hatched spontaneously from control egg masses; see Supplementary Materials for details.

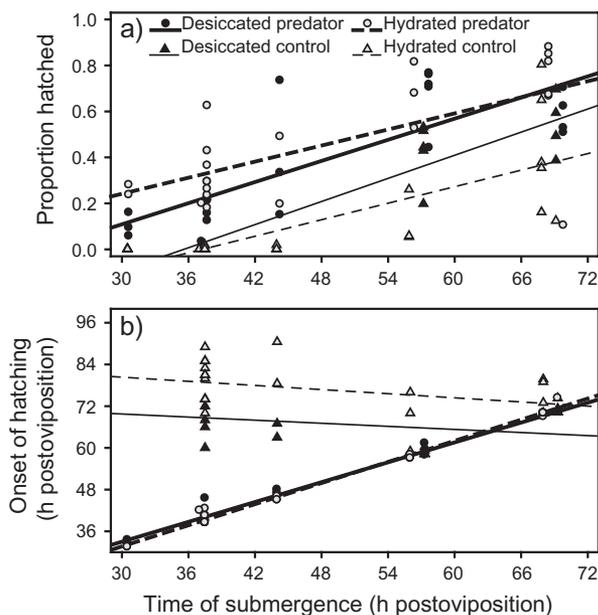


Figure 2
Tadpole predator-induced and spontaneous hatching of *Dendropsophus ebraccatus* embryos from hydrated and desiccated egg clutches after flooding. (a) The proportion of hydrated and desiccated *D. ebraccatus* eggs that hatched during trials for egg masses that were submerged and exposed to large *D. ebraccatus* tadpole predators (thick lines) or submerged without exposure to a predator (thin lines). (b) The onset of hatching in hydrated and desiccated egg masses that were attacked by tadpoles and in flooded control clutches. Data in (a) are the proportion hatched, out of the entire egg mass, in individual masses submerged at different times and in (b) are the times when the first 5 embryos had hatched from an egg mass.

DISCUSSION

We demonstrate that embryos of the neotropical treefrog *D. ebraccatus* hatch prematurely in response to attacks by 2 different predators in 2 different environmental contexts. Because the reproductive mode of *D. ebraccatus* is plastic, eggs naturally develop both in air and in water (Touchon and Warkentin 2008), exposing embryos to aquatic and terrestrial predators. Thus, antipredator responses have presumably been under selection in both contexts. Premature hatchlings are less

developed and smaller than undisturbed hatchlings (Supplementary Material, Supplementary Figures S1 and S2), a phenotype that likely carries a cost during the larval stage (Warkentin 1995). Furthermore, we demonstrate that the hatching response of *D. ebraccatus* embryos to predators is hydration dependent, at least when out of water; embryos developmentally capable of hatching early and escaping predation are inhibited by clutch desiccation.

Hydration-contingent hatching response to ant attack

Our predation experiments with *Azteca* ants revealed that hydrated *D. ebraccatus* embryos hatched up to 33% early, successfully escaping predation. Despite the fact that desiccated embryos were capable of hatching eventually, desiccation inhibited early hatching leading to high predation. *Dendropsophus ebraccatus* clutches lose water from both the jelly layers and the perivitelline space when desiccated; the jelly becomes thin and tacky and the perivitelline space shrinks, constricting the movement of the embryo (Touchon and Warkentin 2009). When eggs are well hydrated, however, the perivitelline space is large and the jelly is thick, protecting embryos from both drying out and predator attacks (Touchon and Warkentin 2009). Because desiccation does not slow development (Touchon and Warkentin 2010), our results suggest that hydrated egg masses are physically easier for embryos to hatch from than desiccated egg masses. Embryos in desiccated egg masses begin hatching later but hatch more synchronously and on average earlier than those in submerged or hydrated egg masses (Touchon and Warkentin 2010). Desiccation may create a stressful environment for developing embryos as well as impeding their ability to hatch early, so that once embryos are capable of hatching, they leave the clutch quickly. Alternatively, the hatching of some embryos may facilitate hatching for embryos that otherwise have difficulty leaving the clutch, contributing to hatching synchrony. Clearly the changes to the clutch structure caused by desiccation severely limit the ability of developmentally hatching-competent embryos to escape when attacked by predators.

Hatching response to tadpole attack

Although hydrated *D. ebraccatus* eggs hatched substantially prematurely (33 %) when attacked by ants, they can hatch even earlier. Flooded embryos hatched in all our tadpole predation trials, including those begun at just 30.5 h postoviposition, although their escape success improved with development. Predator attacks in the developmentally earliest trials caused embryos to begin hatching 51% and 59% earlier, respectively, than the start of hatching by previously desiccated or hydrated embryos in water and 61–67% earlier than their peak hatching time. To our knowledge, this is the largest predator-induced acceleration of hatching documented to date. In addition, induced hatching began rapidly after the predation trials with tadpoles commenced, ~2 h after egg submergence. This delay most likely reflects the time needed by predatory tadpoles to detect the eggs and chew through the jelly as well as the time required for embryo responses. In the very earliest tadpole predation trials, we did not observe embryos breaking out of their capsules themselves, but instead their movements in response to attacks appeared to increase their chance of escape as the predator broke open the egg capsule. Thus, physical disturbance from predators may enable *D. ebraccatus* embryos to exit the egg capsule earlier or more rapidly than they can on their own.

Embryos did not hatch in response to submergence itself. Flooding early in development kills some amphibian eggs that normally develop in air (Pyburn 1970; Kam et al. 1998) and can cause premature hatching of more developed eggs due to

oxygen stress (Petranka et al. 1982; Bradford and Seymour 1988; Warkentin 2002; Gomez-Mestre et al. 2008). *Dendropsophus ebraccatus* embryos, however, are often laid directly in water and can develop normally in both terrestrial and aquatic environments (Touchon and Warkentin 2008, 2010); thus, it is not surprising that flooding did not induce early hatching.

Hatching constraints and reproductive mode evolution

Developing in air or water exposes embryos to separate selective forces that favor different adaptations. Because adaptations to different environments have strong trade-offs, most amphibian eggs can only survive in the single environment where they are oviposited, for example, in water, on a leaf in the air, or in a foam nest (Duellman and Trueb 1986; Wells 2007). Our work with the reproductively plastic *D. ebraccatus* demonstrates a previously undescribed risk of terrestrial or arboreal egg development: Desiccation can impair the ability of embryos to hatch early and escape predation. Because hatching appears to be constrained by a change in the physical structure of the egg mass, similar effects may also occur in other terrestrial amphibian eggs. This seems particularly likely for species with egg clutches that are relatively well adapted to aquatic development, such as *D. ebraccatus* (Wells 2007). Terrestrial eggs have multiple independent evolutionary origins in amphibians, suggesting that the benefits of terrestrial oviposition in each case exceeded not only the risk of direct desiccation mortality but also any increased predation rates and decreased escape hatching following sublethal clutch desiccation (Touchon and Warkentin 2009). Although escape hatching is a more consistently effective defense for *D. ebraccatus* eggs in water than in air, this species only reproduces aquatically in habitats where terrestrial egg desiccation risk is very high (Touchon and Warkentin 2008). This suggests that the overall risks facing aquatic eggs are also substantial. Further work will evaluate mortality from multiple sources above and below water to quantify selection on aquatic and terrestrial eggs.

Developmental effects of desiccation and hydration

Desiccation and hydration affected the spontaneous hatching age of embryos, regardless if eggs were still in air or had been submerged. In both the ant and the tadpole experiments, desiccated control embryos hatched 9–12 h before hydrated eggs. Submergence clearly rehydrated embryos, and previously desiccated embryos that were submerged escaped from tadpole predator attacks as well as did previously hydrated eggs. We had greater resolution to detect developmental effects of hydration and desiccation in the tadpole experiment, where we found that initially desiccated and hydrated control eggs developed on slightly different trajectories. Embryos that were always hydrated grew more slowly underwater, hatching later, and at smaller sizes, although not less developed, than animals from initially desiccated clutches (Supplementary Material, Supplementary Figure S2). Eggs in air that are hydrated by simulated rainfall also hatch later than desiccated embryos (Touchon and Warkentin 2010). Oxygen demand increases as development progresses, and depending on how hydration changes clutch structure, oxygen availability to embryos might differ in hydrated and desiccated egg masses in air or after a period of submergence. We do not know the extent to which physical changes in clutch structure during desiccation carry over to affect clutch structure after rehydration.

Potential costs of premature hatching

Hatching in response to an egg predator attack is clearly adaptive; embryos escape predation and move into the next life stage. Although we did not test for it here, there is likely

a trade-off for premature hatchlings compared with full-term hatchlings. Early-induced hatchlings may be more vulnerable to aquatic predators either at hatching (Warkentin 1995; Gomez-Mestre et al. 2008) or later in development (Vonesh 2005) or they may be smaller at metamorphosis (Vonesh and Bolker 2005). Such trade-offs are the selective context in which plasticity is favored and a defining characteristic of adaptive phenotypic plasticity (Pigliucci 2001; West-Eberhard 2003). Well-developed full-term *D. ebraccatus* hatchlings from hydrated eggs survive better with a hatchling predator than do hatchlings that are small because of developing underwater or hatchlings from desiccated eggs in air (Touchon and Warkentin 2010). Based on this, the extremely premature induced hatchlings we document here are probably highly vulnerable, especially considering their lack of tail fin or eye development. In fact, our tadpole predation venues contained false bottoms to allow hatchlings to move away from the large predatory tadpoles, which would otherwise have eaten hatchlings (Touchon J and Urbina J, personal observation). Early-induced hatchlings are likely easily captured in nature if discovered; however, they may be able to avoid detection in a pond with complex microhabitats.

Conclusions

Using *D. ebraccatus*, a treefrog capable of breeding aquatically and arboreally, we document habitat-specific constraints on the expression of hatching plasticity. Embryos can hatch early in response to both terrestrial and aquatic predators, but escape hatching is inhibited by desiccation in the terrestrial environment where eggs most often occur. Desiccation, even for brief periods prior to rehydration, also affects hatching age and development in the absence of predators in comparison with eggs that are consistently hydrated. Although embryos are more consistently able to utilize escape hatching as an egg defense in aquatic habitats, frogs most often oviposit out of the water, suggesting that overall risk of mortality for aquatic eggs has often exceeded that for arboreal eggs. The expression of adaptive antipredator responses and the outcome of predator–prey interactions depend on both environmental history and current environmental context. We demonstrate that to understand how environmental variation shapes predator–prey interactions, it is important to consider nonadaptive plastic responses to environmental variation and how they interact with evolved adaptive responses.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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