Egg clutch dehydration induces early hatching in red-eyed treefrogs, *Agalychnis callidryas* (#12904)

First submission

Please read the **Important notes** below, and the **Review guidance** on the next page.
When ready **submit online**. The manuscript starts on page 3.

Important notes

Editor
Donald Kramer

Files	4 Table file(s) 4 Other file(s) Please visit the overview page to **download and review** the files not included in this review pdf.
Declarations	**Involves vertebrate animals.** **Involves a field study on animals or plants.**

Review guidelines

Please in full read before you begin

How to review

When ready **submit your review online**. The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this **pdf** and upload it as part of your review

To finish, enter your editorial recommendation (accept, revise or reject) and submit.

BASIC REPORTING

- ✓ Clear, unambiguous, professional English language used throughout.
- ✓ Intro & background to show context. Literature well referenced & relevant.
- ✓ Structure conforms to **PeerJ standard**, discipline norm, or improved for clarity.
- ✓ Figures are relevant, high quality, well labelled & described.
- ✓ Raw data supplied (See **PeerJ policy**).

EXPERIMENTAL DESIGN

- ✓ Original primary research within **Scope of the journal**.
- ✓ Research question well defined, relevant & meaningful. It is stated how research fills an identified knowledge gap.
- ✓ Rigorous investigation performed to a high technical & ethical standard.
- ✓ Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- ℹ Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- ✓ Data is robust, statistically sound, & controlled.
- ✓ Conclusion well stated, linked to original research question & limited to supporting results.
- ✓ Speculation is welcome, but should be identified as such.

The above is the editorial criteria summary. To view in full visit **https://peerj.com/about/editorial-criteria/**

Egg clutch dehydration induces early hatching in red-eyed treefrogs, *Agalychnis callidryas*

María José Salica Corresp., , **James Vonesh** , **Karen Warkentin**

Corresponding Author: María José Salica
Email address: mjsalica@gmail.com

Terrestrial eggs have evolved repeatedly in tropical anurans exposing embryos to the new threat of dehydration. Red-eyed treefrogs, *Agalychnis callidryas,* lay eggs on plants over water. Maternally provided water allows shaded eggs in humid sites to develop to hatching without rainfall, but unshaded eggs and those in less humid sites can die from dehydration. Hatching responses of amphibian eggs to dry conditions are known from two lineages with independent origins of terrestrial eggs. Here, we experimentally tested for dehydration-induced early hatching in another lineage (*Agalychnis callidryas,* Phyllomedusidae), representing a third independent origin of terrestrial eggs. We also investigated how dehydration affected egg and clutch structure, and egg mortality. We collected clutches from a pond in Gamboa, Panama, and randomly allocated them to wet or dry treatments at age 1 day. Embryos hatched earlier from dry clutches than from wet clutches, accelerating hatching by ~11%. Clutch thickness and egg diameter were affected by dehydration, diverging between treatments over time. Meanwhile, mortality in dry clutches was six-fold higher than in control clutches. With this study, early hatching responses to escape mortality from egg dehydration are now known from three anuran lineages with independent origins of terrestrial eggs, suggesting they may be widespread. Further studies are needed to understand how terrestrial amphibian eggs can respond to, or will be affected by, rapid changes in climate over the next decades.

Egg clutch dehydration induces early hatching in red-eyed treefrogs, *Agalychnis callidryas*

María José Salica[1], James R. Vonesh[2] and Karen M. Warkentin[3, 4]

[1] Instituto de Ecorregiones Andinas (INECOA), Universidad Nacional de Jujuy - CONICET, San Salvador de Jujuy, Jujuy, Argentina
[2] Department of Biology, Virginia Commonwealth University, Richmond, VA, USA
[3] Department of Biology, Boston University, Boston, MA, 02215, USA
[4] Smithsonian Tropical Research Institute, Panamá, República de Panamá

Corresponding Author:
María José Salica[1]
Gorriti 237, San Salvador de Jujuy, Jujuy, 4600, Argentina

Email address: mjsalica@gmail.com

Abstract

Terrestrial eggs have evolved repeatedly in tropical anurans exposing embryos to the new threat of dehydration. Red-eyed treefrogs, *Agalychnis callidryas,* lay eggs on plants over water. Maternally provided water allows shaded eggs in humid sites to develop to hatching without rainfall, but unshaded eggs and those in less humid sites can die from dehydration. Hatching responses of amphibian eggs to dry conditions are known from two lineages with independent origins of terrestrial eggs. Here, we experimentally tested for dehydration-induced early hatching in another lineage (*Agalychnis callidryas,* Phyllomedusidae), representing a third independent origin of terrestrial eggs. We also investigated how dehydration affected egg and clutch structure, and egg mortality. We collected clutches from a pond in Gamboa, Panama, and randomly allocated them to wet or dry treatments at age 1 day. Embryos hatched earlier from dry clutches than from wet clutches, accelerating hatching by ~11%. Clutch thickness and egg diameter were affected by dehydration, diverging between treatments over time. Meanwhile, mortality in dry clutches was six-fold higher than in control clutches. With this study, early hatching responses to escape mortality from egg dehydration are now known from three anuran lineages with independent origins of terrestrial eggs, suggesting they may be widespread. Further studies are needed to understand how terrestrial amphibian eggs can respond to, or will be affected by, rapid changes in climate over the next decades.

Key words: climate change; hatching plasticity; Panama; Phyllomedusidae; tropical wet forest.

INTRODUCTION

Terrestrial eggs have evolved repeatedly in many species of teleost fishes and amphibians (Martin & Carter, 2013). In tropical anurans, Gomez-Mestre, Pyron & Wiens (2012) found 48 independent origins of terrestrial reproduction. The evolution of terrestrial breeding may be driven by the risk of aquatic predation in early life stages (Duellman & Trueb, 1986; Touchon, 2012). However, nonaquatic reproduction also entails risks. Terrestrial eggs are exposed to different threats than those affecting aquatic eggs, including terrestrial predators (Warkentin, 1995, 2000), pathogens (Warkentin, Currie & Rehner, 2001), and the novel threat of dehydration (Mitchell, 2002, Touchon & Warkentin, 2009). The risk of egg dehydration most strongly affects species without parental care, and this threat could be exacerbated by climate change (Donnelly & Crump, 1998). As well as temperature, rainfall patterns are changing in the tropics. Specifically, even if overall rainfall remains similar, in Neotropics rainfall events are becoming less frequent, resulting in an increase in dry spells during the rainy season (Hulme & Viner, 1998, Christensen *et al.,* 2007, Allan & Soden, 2008). Therefore, it is important to understand the potential responses of vulnerable life stages to such climate variations.

Environmentally cued variation in hatching time is widespread in many taxa (Warkentin, 2011a) and serves as an important defense mechanism against egg-stage risks. Environmentally cued hatching (ECH) is well documented in anurans (Warkentin, 2011b); much of this research addresses biotic threats to eggs and larvae, and a substantial subset addresses responses of embryos to hypoxia. The terrestrial eggs of red-eyed treefrogs, *Agalychnis callidryas,* one of the most studied species, hatch early in response to multiple environmental threats, including predator attack (snakes, Warkentin, 1995; wasps, Warkentin, 2000), fungal infection (Warkentin, Currie & Rehner, 2001) and flooding, which can kill embryos too young to hatch (Warkentin, 2002). Embryos presumably use some of the same mechanisms to respond to these different risks. For instance, all responses require a means to exit from the egg and the ability to regulate expression of this process (Cohen, Seid & Warkentin, 2016). Nonetheless, different types of threat provide very different types of cues. Their detection requires different sensors, and assessing different risks may require different cognitive mechanisms. Thus embryos that respond to one threat, using one type of cue, may be insensitive to other cues and unresponsive to other threats.

Only a few studies of ECH have examined how amphibian eggs respond to dry conditions (Warkentin, 2011b); thus, it is unclear how widespread hatching responses to egg dehydration might be. To date, such responses are known from two lineages with independent origins of terrestrial eggs. In the treefrog *Dendropsophus ebraccatus* (Hylidae: Dendropsophinae), eggs exposed to dehydration hatch earlier and more synchronously than well-hydrated clutches (Touchon & Warkentin, 2010, Touchon, Urbina & Warkentin, 2011). In the glassfrog *Hyalinobatrachium fleischmanni* (Centrolenidae: Hyalinobatrachinae) fathers hydrate their developing embryos during dry weather. When the caring parent is removed, increasing risk of egg dehydration, the embryos also respond by hatching Earlier And More Synchronously (Delia, Ramírez-Bautista & Summers, 2014). Here, we tested for dehydration-induced early hatching in another lineage (*Agalychnis callidryas,* Phyllomedusidae), representing a third independent origin of terrestrial eggs. We also investigated how dehydration affected egg and clutch structure, and egg mortality.

MATERIALS & METHODS

Study system

The recently redescribed family Phyllomedusidae (Amphibia: Anura: Arboranae, Duellman Marion & Hedges, 2016) are uniformly terrestrial egg layers. They place eggs on vegetation over water, into which tadpoles fall upon hatching. These treefrogs have evolved several strategies to avoid egg dehydration. Females absorb water from their environment before oviposition and deposit eggs surrounded by well hydrated jelly (Pyburn, 1970, 1980). In addition, some species wrap eggs in a funnel-shaped nest of leaves, and surround their eggs with eggless jelly capsules as water reservoirs (Faivovich *et al.,* 2010). Nonetheless, after the eggs are deposited, embryos must face dehydration and other risks with no further parental assistance. *Agalychnis callidryas* inhabits lowland wet forest from the Yucatan through Panama (Frost, 2016), breeding in seasonal ponds and swamps. This species lays their gelatinous egg masses exposed on vegetation, without wrapping them in leaves. Maternally provided water allows shaded eggs in humid sites to develop to hatching without rainfall. However, unshaded eggs and those in less humid sites can die from dehydration. We studied them at the Smithsonian Tropical Research Institute in Gamboa, Panama. At this locality egg mortality from dehydration has historically been low but detectable (e.g., 3% in 1998, vs. zero at a pond in Corcovado Park, Costa Rica, in 1993 and 1994; Warkentin, 2000, Gomez-Mestre & Warkentin, 2007). However, in the extremely dry El Niño of 2015 many entire egg cohorts laid in Gamboa perished from dehydration (K. Warkentin, pers. obs.).

Experimental Design

We collected 30 healthy egg clutches laid on the night of 24 July 2011 from the Experimental Pond in Gamboa, Panama (9°07'15" N, 79°42'14" W). All clutches were collected with the leaves on which they were laid, mounted on plastic cards for support and attached to the sides of plastic cups in a vertical orientation. Each cup contained aged tap water to catch hatched tadpoles. Eggs in each clutch were counted, and any dead or undeveloped eggs (possibly unfertilized) were noted. Clutches were paired based on overall similarity of clutch structure and size, and egg color and size, then one of each pair was randomly allocated to a wet treatment and the other to a dry treatment. Treatments were imposed starting at age 1 day. Wet clutches were heavily sprayed with aged tap water multiple times daily, taking care not to overspray onto dry clutches. Dry clutches were unsprayed or minimally sprayed in some cases where eggs were dying from dehydration. Clutches were located on the same table in a laboratory with slightly higher temperatures and less humidity than natural ponds, with a 12:12 light: dark photoperiod based on local sunrise/sunset times. All clutches were checked for hatching at least 5 times daily. Clutches were photographed daily with a ruler for egg size measurements, from age 1 to 4 days. At each age, for each clutch, we measured two orthogonal diameters for each of 10 eggs from the photographs, using ImageJ (NIH). We also measured the thickness of each clutch when it entered the experiment at 1 day old and after two days in the treatments, at 3 days old, by inserting a fine probe orthogonally through the thickest part of the clutch, between eggs, to the leaf surface. This measurement included both eggs and associated jelly thickness.

Statistical Analysis

All statistical analyses were conducted in R version 2.13.1 (R Development Core Team 2011). We used generalized linear models (GLM) with an underlying binomial error distribution to examine the influence of dehydration on time to hatching. To determine how dehydration affected clutch thickness and egg diameter we used a two way ANOVA. To highlight the effect of dehydration on egg mortality, an ANOVA was performed on a generalized linear model (family: quasibinomial).

RESULTS

Embryos in the dry treatment hatched earlier than embryos in the wet treatment, accelerating hatching by approximately 11% (Fig. 1, Binomial GLM, treatment $F_{1,\,445} = 91.9$, $P < 0.001$). In both treatments, hatching was gradual and asynchronous, but dry clutches started hatching sooner and the entire hatching curve was shifted earlier (Fig. 1).

Clutch thickness was altered by the treatment (ANOVA, day x treatment $F_{1,\,55} = 6.33$, $P = 0.015$). At the beginning of the experiment, at age 1 day, there was no difference in thickness between clutches assigned to different treatments; the mean for dry treatment was 7.03±0.44 mm (SE) while the mean for wet treatment was 6.73±0.40 mm (SE). However, two days later wet clutches were much thicker than dry clutches, the mean for dry treatment was 5.67±0.42 mm (SE), while the mean for wet treatment was 7.50±0.48 mm (SE).

The diameter of individual eggs was also affected by treatment, diverging over time (Fig. 2, ANOVA, treatment x day, $F_{1,\,115} = 15.9$, $P = 0.0001$). Initially, in both dry and wet clutches egg diameters increased due to absorption of water from the egg jelly into the perivitelline space; however, wet eggs swelled more rapidly. Eggs in wet clutches continued to swell, then stabilized in diameter at age 3 days. By contrast, from 2 days eggs in dry clutches shrank, with the difference between treatments increasing over time.

Egg mortality was also affect by treatment (Quasibinomial GLM, treatment $F_{1,\,28} = 6.91$, $P = 0.013$). Mortality in dry clutches was higher than in control clutches, 24 ± 0.09 % (SE) of the embryos in the dry treatment died due to dehydration, while egg mortality in the wet treatment was only 4 ± 0.01 % (SE). However, mortality level varied substantially among clutches; two clutches, with relatively little jelly, experienced 100% mortality in the dry treatment, whereas 3 clutches had no mortality at all. Mortality data did not include unfertilized or undeveloped eggs, only eggs showing normal development. Therefore, all the mortality was due to dehydration.

DISCUSSION

Our results show that red-eyed treefrogs can accelerate hatching when exposed to the gradual threat of dehydration over embryonic development. In this study, the acceleration in hatching timing (11%) was less than that reported for other frogs (*Dendrosophus ebraccatus*: 17%, Touchon & Warkentin 2010; *Hyalinobatrachium fleishmanni:* 59%, Delia, Ramírez-Bautista & Summers, 2014). It may be that, compared with those species, *A. callidryas* has a relatively limited capacity to accelerate hatching under the threat of drying. Indeed, based on field monitoring of eggs, both *D. ebraccatus* and *H. fleishmanni* both appear at higher risk of mortality from dehydration than does *A. callidryas.* Dehydration led to 98% mortality in terrestrial eggs of *D. ebraccatus* exposed to lack of rainfall during the first 48 h post-oviposition (Touchon & Warkentin, 2009). Similarly, in male removal experiments generating “orphan” clutches of *H. fleishmanni,* 78% of total mortality was due to dehydration (Delia, Ramírez-Bautista & Summer, 2013). Alternatively, because the mortality imposed by our drying treatment was moderate (24%), compared with the possible risk of mortality under more extreme weather conditions, it may not have tested the limits of *A. callidryas* capacity to accelerate hatching. The hatching pattern of drying clutches - accelerated but gradual hatching, over a period of days - was very similar to the hatching pattern of clutches infected by a pathogenic fungus which caused about 40% mortality and 17% acceleration of hatching (Warkentin, Currie & Rehner, 2001, Warkentin, 2011b). Both fungus and dehydration are chronic threats, that affect egg clutches gradually and potentially provide cues over extended periods of development. However, what those cues are, or how embryos detect them, is in both cases unknown. Red-eyed treefrog embryos use physical disturbance or vibrations to assess danger in predator attacks (Warkentin, 2005) and respond by hatching very rapidly, within seconds (Cohen, Seid & Warkentin, 2016; Warkentin *et al.,* 2007). They also use hypoxia as a cue to hatch from eggs that are flooded, responding to submergence in minutes (Warkentin, 2002). Like fungus infection, dehydration does not move eggs, and neither threat has a sudden, acute onset. Either vibrational cues or another sudden change in clutch conditions may be necessary to induce rapid or synchronous hatching.

Both clutch thickness and egg diameter were affected by dehydration, diverging between treatments over time. Dehydration began to affect these variables from age 3 days, when both clutch thickness and egg diameter decreased in dry treatment eggs (Fig. 2). Our results suggest that during early developmental stages water moves from the jelly layers into the perivitelline space, enlarging the eggs (Salthe, 1965), as egg diameter increased even in the dry treatment. Later in embryonic development (from 3 days), after available water from jelly layers has been absorbed, the eggs can absorb additional water from external sources, such as rainfall. Without external sources of water, egg diameter then begins to decrease, constricting the perivitelline space. Egg diameter of terrestrial breeding frogs usually decreases when they are exposed to dry conditions (e.g. *Kurixalus eiffinger*, Kam, Yen & Hsu, 1998a; *Bryobatrachus nimbus*, Mitchell, 2002), due to the semipermeable nature of their vitelline membrane (Salthe, 1965).

With this study, early hatching responses to escape mortality from egg dehydration are now known from three anuran lineages with independent origins of terrestrial eggs (Hylidae:

Dendropsophinae; Centrolenidae; Phyllomedusidae). Other responses to, and effects of, dehydration on terrestrial frog eggs have been explored in other lineages. For example, Kam, Yen & Hsu (1998a) found the opposite response in *Kurixalus eiffinger* (Rhacophoridae: Rhacophorinae); well-hydrated eggs hatched earlier than drier eggs. In this species, accelerating the time of hatching under wetter conditions has a clear adaptive significance. Tadpoles of *K. eiffinger* are oophagous. Females lay their first batch of trophic eggs before all the fertilized eggs have hatched, then return eight days later to feed the tadpoles again (Kam *et al.* 1998b). Tadpoles that hatch earlier obtain more trophic eggs, grow faster and reach metamorphosis earlier. Other studies have been conducted on terrestrial anuran embryos with a similar approach. Most of this research has focused on effects of different moisture conditions on phenotypic traits (Taigen, Pough & Stewart, 1984; Bradford & Seymour, 1988; Seymour, Geiser & Bradford, 1991a, 1991b; Kam*,* Yen & Hsu, 1998a, Mitchell, 2002). Anuran embryos exposed to dry conditions grow more slowly (*Pseudophryne bibroni*, Bradford & Seymour, 1988), have lower hatching success (e.g. *Kurixalus eiffingeri*, Kam, Yen & Hsu, 1998a; *Bryobatrachus nimbus,* Mitchell, 2002), produce smaller hatchlings (e.g. *Eleutherodactylus coqui*, Taigen, Pough & Stewart, 1984; Kam*,* Yen & Hsu, 1998a; Mitchell, 2002) and generate stunted and asymmetric morphologies at hatching (Mitchell, 2002). In *A. callidryas,* we observed no effects of drying on embryo development rate or morphology. Early-induced hatchlings are generally smaller and less developed than full term hatchlings (Warkentin 1995, 1999; Gomez-Mestre *et al.* 2008). Such differences, however, appear simply to be caused by differences in the period of embryonic development, not by differences in embryonic developmental trajectories. There is no evidence that hatching plasticity in this species occurs by altering the rate of embryo development (Warkentin, 2011a).

The anuran lineages now demonstrated to hatch early in response to drying vary in their degree of egg and clutch adaptation to terrestrial development. *Dendropsophus ebraccatus* egg size and clutch morphology are much like those of aquatic breeding congeners; they appear not to be strongly adapted to terrestrial development, and indeed can also develop aquatically (Touchon & Warkentin, 2008). In contrast, phyllomedusines have a long (34 to 50 million years) evolutionary history of terrestrial eggs (Gomez-Mestre, Pyron & Wiens, 2012) and *A. callidryas* eggs do not survive prolonged submergence (Pyburn, 1970). Considering that these highly adapted terrestrial eggs, which typically do not suffer high dehydration mortality, can show adaptive plastic responses to reduce mortality from this occasional threat, drying-induced early hatching may be a more general, broadly distributed phenomenon.

The risk of dehydration as a source of mortality for terrestrial-breeding frogs is particularly important in the context of global climate change. Local changes in weather and climate can affect the hydration of terrestrial embryos. In *D. ebraccatus* living in sympatry with *A. callidryas*, the survival of terrestrial eggs is affected both directly and indirectly by the amount of rainfall (Touchon & Warkentin, 2009). Directly, rain hydrates eggs and prevents mortality from drying. Indirectly, because the jelly surrounding eggs swells with hydration, rain decreases the risk of predation; dehydrated eggs are more susceptible to predation by ants and wasps. In *Phyllomedusa hypochondrialis*, which normally wraps its eggs in leaves, the mortality of embryos exposed directly to the air decreased during rainy periods (Pyburn, 1980). The tropics, where the highest biodiversity of amphibians is concentrated, are expected to become warmer and drier, and many tropical anuran lineages have evolved terrestrial eggs. Therefore, to understand how these terrestrial eggs can respond to, or will be affected by, rapid changes in climate over the next decades is relevant for conservation planning.

ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Monique Halloy, advisor to MJS, with gratitude for her support both personally and in herpetological research. We thank J. Charbonnier, R. Jimenez, S. Abinette, S. Bouchard, K. Cohen and M. Leavy for assistance. This research was conducted under permit SC/A-13-11 from the Panama Autoridad Nacional del Ambiente and IACUC approval 100625-1008-15 from the Smithsonian Tropical Research Institute (STRI).

REFERENCES

Allan RP, Soden BJ. 2008. Atmospheric warming and the amplification of precipitation extremes. *Science* 321: 148-1484

Bradford DF, Seymour RS. 1988. Influence of water potential on growth and survival of the embryo, and gas conductance of the egg, in a terrestrial breeding frog, *Pseudophryne bibroni*. *Physiological Zoology.* 61: 470-474

Christensen, JH, Hewitson B, Busuioc A, Chen A, Gao X, Held I, Jones R, Kolli RK, Kwon WT, Laprise R, Rueda VM, Mearns L, Menéndez CG, Räisänen J, Rinke A, Sarr A, Whetton P. 2007. Regional climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge: Cambridge University Press, 847-940

Cohen KL, Seid MA, Warkentin KM. 2016. How embryos escape from danger: the mechanism of rapid, plastic hatching in red-eyed treefrogs. *The Journal of Experimental Biology* 219: 1875-1883

Delia JR, Ramírez-Bautista A, Summers K. 2013. Parents adjust care in response to weather conditions and egg dehydration in a Neotropical glassfrog. *Behavioral Ecology and Sociobiology* 67: 557-569

Delia JR, Ramírez-Bautista A, Summers K. 2014. Glassfrog embryos hatch early after parental desertion. *Proceedings of the Royal Society. Biological sciences* 281: 20133237

Donnelly MA, Crump ML. 1998. Potential effects of climate change on two neotropical amphibian assemblages. In: Markham A, ed. *Potential Impacts of Climate Change on Tropical Forest Ecosystems*. Netherlands: Springer, 401-421

Duellman WE, Trueb L. 1986. *Biology of Amphibians*. Baltimore: JHU Press

Duellman WE, Marion AB, Hedges SB. 2016. Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). *Zootaxa* 4104: 1-109

Faivovich J, Haddad CFB, Baêta D, Jungfer KH, Álvares GFR, Brandão RA, Sheil CA, Barrientos LS, Barrio-Amorós CL, Cruz CAG, Wheeler WC. 2010. The phylogenetic relationships of the charismatic poster frogs, Phyllomedusinae (Anura, Hylidae). *Cladistics* 25: 1-35

Frost DR. 2016. Amphibian Species of the World: an online reference. Version 6.0. Available at http://research.amnh.org/herpetology/amphibia/index.html (accessed 27 May 2016)

Gomez-Mestre I, Wiens JJ, Warkentin KM. 2008. Evolution of adaptive plasticity: risk-sensitive hatching in neotropical leaf-breeding treefrogs. *Ecological Monographs* 78: 205-224

Gomez-Mestre I, Pyron RA, Wiens JJ. 2012. Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes in frogs. *Evolution* 66: 3687-3700

Gomez-Mestre I, Warkentin KM. 2007. To hatch and hatch not: similar selective trade-offs but different responses to egg predators in two closely related, syntopic treefrogs. *Oecologia* 153: 197-206

Hulme M, Viner D. 1998. A climate change scenario for the tropics. *Climatic Change* 39: 145-176

Kam YC, Yen CF, Hsu CL. 1998a. Water balance, growth, development, and survival of arboreal frog eggs (*Chirixalus eiffingeri*, Rhacophoridae) importance of egg distribution in bamboo stumps. *Physiological Zoology* 71: 534-540

Kam YC, Lin CF, Lin YS, Tsal YF. 1998b. Density effects of oophagous tadpoles of *Chirixalus eiffingeri* (Anura: Rhacophoridae): importance of maternal brood care. *Herpetologica* 54: 425-433

Martin KL, Carter AL. 2013. Brave new propagules: terrestrial embryos in anamniotic eggs. *Integrative and Comparative Biology.* 53: 233-247

Mitchell NJ. 2002. Low tolerance of embryonic desiccation in the terrestrial nesting frog *Bryobatrachus nimbus* (Anura: Myobatrachinae). *Copeia* 2002: 364-373

Pyburn WF. 1970. Breeding behavior of the leaf-frogs *Phyllomedusa callidryas* and *Phyllomedusa dacnicolor* in Mexico. *Copeia* 1970: 209-218

Pyburn WF. 1980. The function of eggless capsules and leaf in nests of the frog *Phyllomedusa hypochondrialis* (Anura: Hylidae). *Proceedings of the Biological Society of Washington* 93:153-167

Salthe, SN. 1965. Increase in volume of the perivitelline chamber during development of *Rana pipiens* Schreber. *Physiological Zoology* 38: 80-98

Seymour RS, Geiser F, Bradford DF. 1991a. Gas conductance of the jelly capsule of terrestrial frog eggs correlates with embryonic stage, not metabolic demand or ambient PO_2. *Physiological Zoology* 64: 673-687

Seymour RS, Geiser F, Bradford DF. 1991b. Metabolic cost of development in terrestrial frog eggs (*Pseudophryne bibronii*). *Physiological Zoology* 64: 688-696

Taigen TL, Pough FH, Stewart MM. 1984. Water balance of terrestrial anuran (*Eleutherodactylus coqui*) eggs: importance of parental care. *Ecology* 65: 248-255

Touchon JC. 2012. A treefrog with reproductive mode plasticity reveals a changing balance of selection for nonaquatic egg laying. *The American Naturalist* 180: 733-743

Touchon JC, Warkentin KM. 2008. 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 JC, Warkentin KM. 2009. Negative synergism of rainfall patterns and predators affects frog egg survival. *The Journal of Animal Ecology* 78: 715-723

Touchon JC, Warkentin KM. 2010. Short- and long- term effects of the abiotic egg environment on viability, development and vulnerability to predators of a Neotropical anuran. *Functional Ecology* 24: 566-575

Touchon, J. C., J. Urbina, and K. M. Warkentin. 2011. Habitat-specific constraints on induced hatching in a treefrog with reproductive mode plasticity. *Behavioral Ecology* 22: 169-175

Warkentin KM. 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 KM. 1999. The development of behavioral defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. *Behavioral Ecology* 10: 251-262.

Warkentin KM. 2000. Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Animal Behaviour* 60: 503-510.

Warkentin KM. 2002. Hatching timing, oxygen availability, and external gill regression in the treefrog, *Agalychnis callidryas*. *Physiological and Biochemical Zoology* 75: 155-164.

Warkentin KM. 2005. How do embryos assess risk? Vibrational cues in predator induced hatching of red-eyed treefrogs. *Animal Behaviour* 70: 59-71.

Warkentin KM. 2011a. Environmentally cued hatching across taxa: embryos respond to risk and opportunity. *Integrative and Comparative Biology* 51: 14-25.

Warkentin KM. 2011b. Plasticity of hatching in amphibians: evolution, trade-offs, cues and mechanisms. *Integrative and Comparative Biology* 51:111-127.

Warkentin KM, Currie CC, Rehner SA. 2001. Egg-killing fungus induces early hatching of red-eyed treefrog eggs. *Ecology* 82: 2860-2869.

Warkentin KM, Gomez-Mestre I, McDaniel JG. 2005. Development, surface exposure, and embryo behavior affect oxygen levels in eggs of the red-eyed treefrog, *Agalychnis callidryas. Physiological and Biochemical Zoology* 78:956-66.

Warkentin KM, Caldwell MS, Siok TD, D'Amato AT, McDaniel JG. 2007. Flexible information sampling in vibrational assessment of predation risk by red-eyed treefrog embryos. *The Journal of experimental biology.* 210: 614-619.

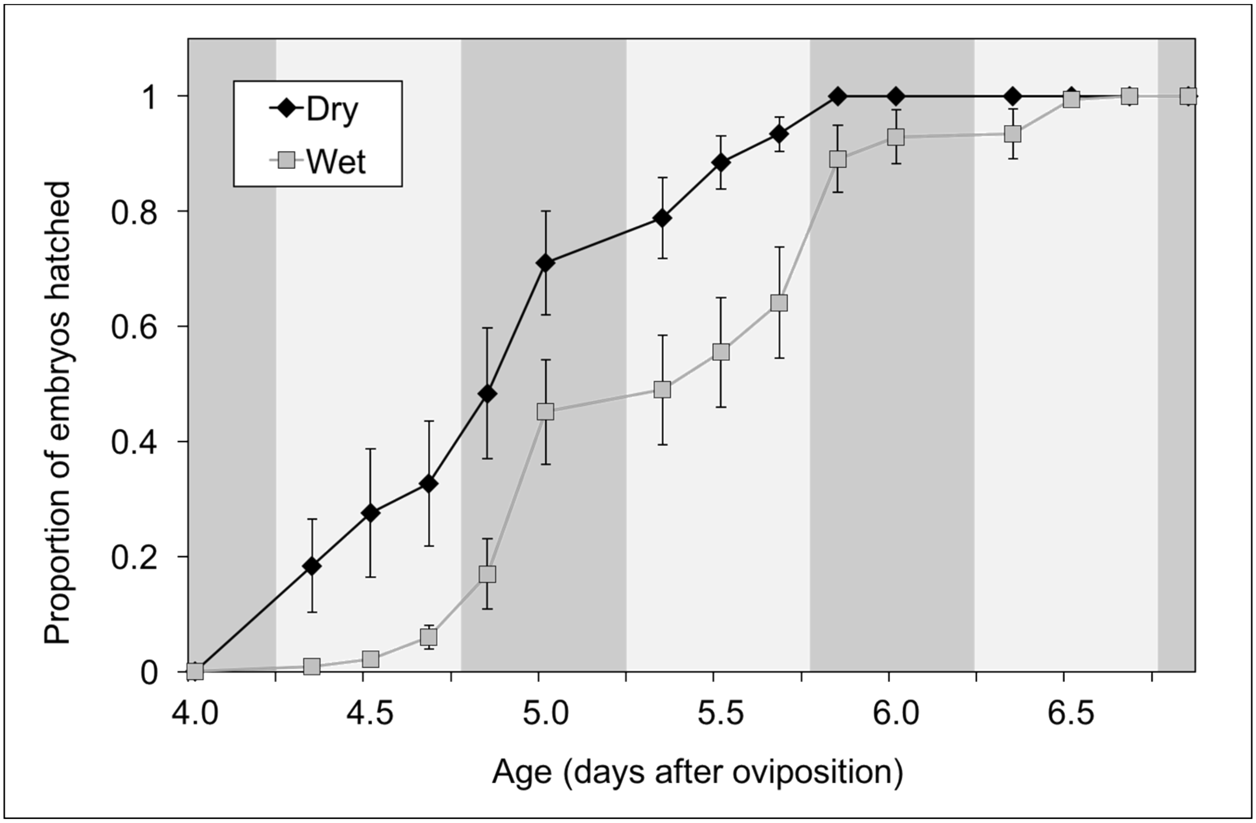

FIGURE 1. Embryos hatched ≈11% earlier from drying vs. wet clutches (Binomial GLM, treatment $F_{1,\,445} = 91.9$, $P < 0.001$). Data are mean proportion hatched at each age (± SE across clutches), of all that eventually hatched. Dark and light shading along the x-axis indicates photoperiod.

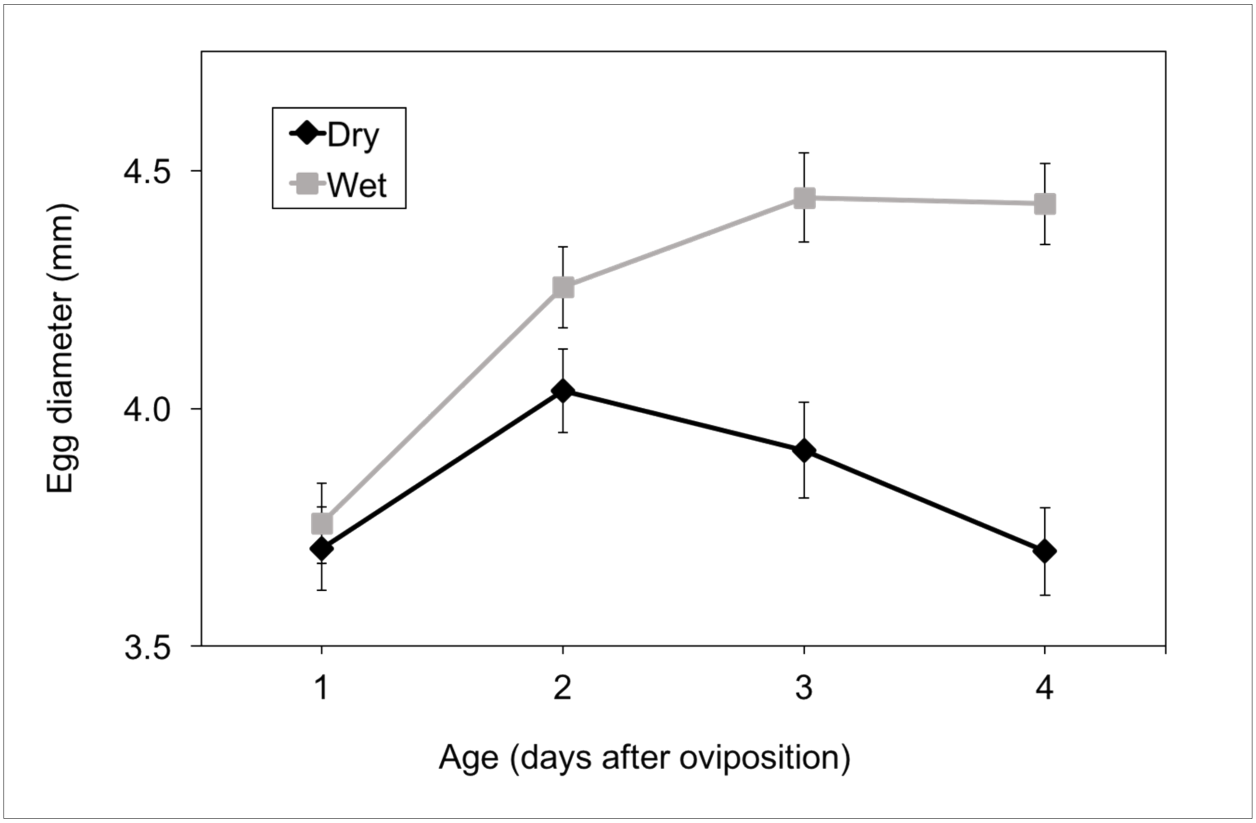

FIGURE 2. Effect of development and hydration treatment on *A. callidryas* egg diameter. Data are mean ± SE across clutches. Egg diameters diverged over time (ANOVA, treatment x day, $F_{1, 115}$ = 15.9, P = 0.0001).