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Oviposition site choice under conflicting risks demonstrates that aquatic predators drive terrestrial egg-laying

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Laying eggs out of water was crucial to the transition to land and has evolved repeatedly in multiple animal phyla. However, testing hypotheses about this transition has been difficult because extant species only breed in one environment. The pantless treefrog, *Dendropsophus ebraccatus*, makes such tests possible because they lay both aquatic and arboreal eggs. Here, we test the oviposition site choices of *D. ebraccatus* under conflicting risks of arboreal egg desiccation and aquatic egg predation, thereby estimating the relative importance of each selective agent on reproduction. We also measured discrimination between habitats with and without predators and development of naturally laid aquatic and arboreal eggs. Aquatic embryos in nature developed faster than arboreal embryos, implying no cost to aquatic egg laying. In choice tests, *D. ebraccatus* avoided habitats with fish, showing that they can detect aquatic egg predators. Most importantly, *D. ebraccatus* laid most eggs in the water when faced with only desiccation risk, but switched to laying eggs arboreally when desiccation risk and aquatic predators were both present. This provides the first experimental evidence to our knowledge that aquatic predation risk influences non-aquatic oviposition and strongly supports the hypothesis that it was a driver of the evolution of terrestrial reproduction.

1. Introduction

Without question, the transition from aquatic to terrestrial environments is one of the most important evolutionary events in the history of life. For animals, the success of this transition not only stemmed from the ability of adults to survive on land, but also to breed and lay eggs away from water. The ability to lay eggs out of water has evolved separately and repeatedly in multiple phyla including gastropods, arthropods and vertebrates [1–3]. Indeed, laying eggs out of water may have even preceded the movement of vertebrates onto land [4].

The primary hypothesis for the evolution of non-aquatic reproduction is the avoidance of aquatic predators, although other hypotheses such as escaping aquatic parasites and low oxygen in tropical ponds have also been considered [5–8]. The terrestrial environment is not a panacea, however, and comes with its own suite of predators (e.g. [9–11]) as well as new risk factors, such as the potential for eggs to desiccate (e.g. [12–14]). The relative importance of these various selective agents on reproduction has proved difficult to study because extant reproductive behaviours and embryo development are generally fixed for one environment or the other, precluding a direct comparison across aquatic and terrestrial habitats. For example, terrestrial amphibian eggs are usually much larger than aquatic ones [15], which limits their ability to obtain oxygen in water. Similarly, aquatic eggs rapidly dry out if removed from water [16]. Therefore, the fact that extant embryos are adapted to their normal environment prohibits an experiment that might directly compare survival of each type of egg in each environment.

Amphibians were the first vertebrates to successfully transition to life on land and anurans possess the most diverse array of reproductive modes in the tetrapod vertebrates, with nearly 40 different forms of reproduction documented [17]. Although aquatic egg laying is considered ancestral in amphibians, various

forms of non-aquatic oviposition have evolved more than 50 times independently in frogs alone [18]. Furthermore, terrestrial forms of reproduction appear to have spurred several major radiations of frog species [19–21]. The diversity of reproductive forms therefore makes anurans particularly interesting and useful for studying the evolution of reproduction. Recent phylogenetic studies have shown an association between terrestrial forms of amphibian reproduction and areas of high precipitation [18] and forest cover [22]. However, these studies do not provide tests of different selective agents on reproduction, thereby only allowing us to infer the conditions that might allow terrestrial reproduction to evolve but not the selective agents responsible for driving such events.

One potential way to evaluate the relative importance of these different selective factors is through the oviposition site choices of reproducing adults. Animals commonly choose among potential sites for their eggs in an effort to increase offspring survival, among other hypotheses [23]. Such behaviour is widespread, occurring in mosquitoes [24,25], damselflies [26], moths and butterflies [27], beetles [28], amphibians [29–31] and reptiles [32], among others. These oviposition site choices are important for fitness, as choosing poor quality or incorrect habitats can lead to total offspring mortality or reduced performance later in life [33,34]. Furthermore, assessing behavioural decisions in extant animals provides a tractable way to assess the importance of selective agents on the evolution of reproduction, as behaviours and soft tissues (such as embryos) do not fossilize.

Animals making choices about where to place their offspring are faced with many different axes of habitat quality. Mothers may choose a particular oviposition site to avoid predators [26,29,35] or competitors [28,31,35] or because of advantageous physical characteristics of the site [30,36,37]. However, oviposition site choices may entail complex decisions that require incorporating multiple, potentially conflicting, types of threats and benefits. For example, Texas field crickets (*Gryllus texensis*) prefer to oviposit in warmer burrows, but will shift to using cooler ones when preferred sites incur predation risk [38]. Similarly, squirrel treefrogs (*Hyla squirella*) generally avoid ovipositing in ponds with fish predators, but will do so when breeding activity is high to decrease competition with conspecifics [39]. Oviposition site choices are thus not as simple as they may at first appear and provide an excellent way to evaluate how organisms perceive and rank various risks.

The pantless treefrog, *Dendropsophus ebraccatus* [40], provides a unique opportunity to use oviposition site choice to evaluate the importance of various selective agents on terrestrial reproduction because this species has plasticity in its reproductive mode, flexibly laying either aquatic or arboreal eggs in different environmental contexts [41,42]. We refer to the non-aquatic eggs of *D. ebraccatus* as ‘arboreal’ to avoid confusion with direct-developing anuran eggs, which are often referred to as ‘terrestrial’. For *D. ebraccatus*, laying eggs out of the water comes with a high risk of desiccation, which is ameliorated by shade and rainfall; in unshaded habitats, 98% of arboreal eggs die during the first two days after oviposition if not rained upon [14]. Eggs that receive more than approximately 10 mm of rain during the first day after oviposition have essentially no mortality and approximately 50% of days during the rainy season receive rainfall [42]. Ovipositing eggs directly into the pond removes the threat of drying out, but potentially exposes eggs to aquatic predation, which poses, on average, higher mortality risk than egg

desiccation out of water [42]. Pond water unavoidably contains less oxygen than air and therefore embryos may also face developmental costs when laid in water [42]. Lastly, rainfall in the future is not predictable and climate change is making it even less so [42]. *Dendropsophus ebraccatus* females therefore likely face conflicting risks from aquatic predation, oxygen deprivation in water and desiccation risk in air when choosing where to lay their eggs. Understanding the ways that *D. ebraccatus* evaluates these risks will inform our understanding of the relative influence of each on the evolution of aquatic versus non-aquatic reproduction in this species.

Here, we investigate the conflict between aquatic and arboreal oviposition in *D. ebraccatus*. We first quantified the development of aquatic and arboreal eggs in the field to estimate potential developmental costs associated with aquatic oviposition. We then measured the ability of *D. ebraccatus* to discriminate between small breeding habitats with and without fish, a known aquatic egg and tadpole predator. Lastly, we experimentally measured reproductive mode choices (laying aquatic or arboreal eggs) in desiccation-prone environments (unshaded mesocosms) with and without fish predators. We hypothesized that (i) naturally laid eggs in water would develop more slowly than those laid on leaves above water, (ii) when given a choice, *D. ebraccatus* would avoid breeding in the presence of fish, (iii) in unshaded mesocosms lacking fish, frogs would lay most eggs directly in the water and (iv) in unshaded mesocosms containing fish, frogs would lay most eggs arboreally, out of the water.

2. Material and methods

(a) Study system

Dendropsophus ebraccatus is a Neotropical treefrog, commonly found from southern Mexico to coastal Ecuador [43]. Reproduction takes place throughout the wet season, from approximately May to November at our study site in central Panama. The following experiments were conducted at the Gamboa field station of the Smithsonian Tropical Research Institute between June 2007 and August 2008. Males call at night from vegetation to attract females [44,45]. Females lay 243 ± 60 eggs in a single night, partitioned into one to eight separate egg masses (mean ± 1 SD, here and below; $N = 67$ pairs of frogs, J.T. 2006, unpublished data). Eggs are deposited in a single layer on the upper side of emergent plants or directly in the water attached to floating vegetation or other substrate, which is necessary to keep them near the surface of the water as they do not float [41].

(b) Field survey: developmental rate in air and water

In order to estimate the effects of different oviposition sites on *D. ebraccatus* embryo development, we preserved a time series of embryos from naturally laid egg masses above and at the water surface at two ponds near our study site. Egg masses were laid on the night of 21 June 2007 and located and flagged the following morning. At Bridge Pond ($9^{\circ}6'50.26''$ N, $79^{\circ}41'48.13''$ W), only arboreal egg masses were found ($n = 8$), whereas at Quarry Pond ($9^{\circ}7'22.71''$ N, $79^{\circ}41'36.37''$ W), we found both aquatic and arboreal egg masses ($n = 10$ each). Quarry Pond is a relatively deep permanent pond with relatively little canopy, whereas Bridge Pond is a shallow seasonal pond that fills at the start of the wet season and generally holds water until the dry season and that has moderate canopy overhead. Aquatic egg masses were laid just under the surface of the water on floating *Salvinia* plants. We used midnight as the estimated time of oviposition for embryo age calculations; most clutches are laid between 23.00 and 01.00 (J.T. 2006, personal observation). We removed one egg at a time from each egg mass at

approximately 16, 32 and 40 h after oviposition; embryos were preserved immediately upon removal from the egg mass in 10% neutral buffered formalin and Gosner stages [46] were later estimated using a dissecting microscope (8× magnification). After approximately 40 h, aquatic embryos are capable of hatching [11], thereby making observations of development difficult. Owing to the time needed to travel within and between ponds, embryos at Quarry Pond were preserved approximately 30 min earlier than those at Bridge Pond at each time interval.

All statistical analyses here and elsewhere were conducted in R v. 3.1.1 [47]. Embryo developmental stage was analysed using a linear mixed model (LMM; [48]) testing for effects of time and egg pond/environment (Quarry Pond aquatic, Quarry Pond arboreal, Bridge Pond arboreal) and their interaction, with time also as a random slope (to account for multiple observations per time period) and egg mass as a random intercept (to account for the fact that multiple embryos observed over time came from a single egg mass). We conducted *post hoc* analyses to compare between the three egg pond/environment combinations at each time point using a Tukey test [49]. As *post hoc* tests were conducted within each time point separately, it was not necessary to include random effects and as such the base model was simplified to a linear model. Here and below, significance of predictors in mixed models was assessed using likelihood ratio tests and model fit was verified visually by examining quantile–quantile plots.

(c) Experiment 1: oviposition site discrimination test

To test the ability of *D. ebraccatus* adults to discriminate between oviposition sites with and without predators, we conducted oviposition site choice trials in mesocosms located near Experimental Pond (9°7'14.88" N, 79°42'14.11" W) in Gamboa, Panama. We constructed six 1.3 m³ mesh cages, each containing two 60 l plastic tubs (40 cm deep × 44 cm diameter) filled with aged tap water and emergent vegetation common to *D. ebraccatus* breeding ponds at our study site; we did not provide frogs with an option for aquatic oviposition (although it would be feasible for frogs to lay aquatic eggs attached to the wall of the plastic tub). Furthermore, we placed mesocosms under thick forest canopy to promote arboreal oviposition [41]. We assigned two *Astyanax ruberrimus* fish to one of the two tubs within each mesocosm (the 'predator' tub) and the other tub was a predator-free control. *Astyanax ruberrimus* is often found in ponds where *D. ebraccatus* breeds at our study site (J.T. 2004, personal observation) but does not normally occur in Experimental Pond. We placed 10 recently hatched *D. ebraccatus* tadpoles in each predator tub 24 h before oviposition site choice trials to create chemical cues of predation.

We tested 21 mating pairs of *D. ebraccatus* caught from Experimental Pond between 15 and 21 June 2008. We collected pairs that were in amplexus but had not yet begun laying eggs and placed them in mesocosms between 22.30 and 23.00. We started trials by haphazardly placing pairs on the vegetation above either the predator or predator-free tub. Thus, pairs were ready to breed at the pond and had no experience with the predators in our experiment until being transplanted to a given cage. We left pairs to breed overnight and released them at the pond the following morning. Only one pair of frogs was ever placed in a cage at a time. We counted and recorded the location of all eggs laid in both predator and predator-free tubs. After each test, we removed eggs from each tub, allowed them to hatch in the laboratory and returned them to the pond as tadpoles.

Oviposition site discrimination was analysed using a LMM testing for an effect of tub type (predator or control) on the number of eggs laid above it, with random intercept effects controlling for the date of the trial (as multiple trials were conducted per night, and weather on different nights might influence oviposition choices) and the cage the trial was conducted in (as cages may have been slightly different from one another and were used multiple times

over the course of the experiment). In addition, because eggs laid in each tub were considered separately despite being laid by a single pair of frogs, 'pair' was included as a random slope effect. This model is equivalent to a paired *t*-test, but allows for control of random effects [50].

(d) Experiment 2: reproductive mode choice under conflicting egg desiccation and predation risk

To measure the choices of aquatic/arboreal egg laying by *D. ebraccatus* pairs facing conflicting risks of egg predation and low oxygen in water and egg desiccation above water, we conducted oviposition site choice trials in unshaded mesocosms (where eggs face high desiccation risk) with and without fish predators. We constructed six 1.3 m³ mesocosms, each with a single pool (25 cm deep × 130 cm diameter) filled with aged tap water and containing both emergent vegetation and a layer of floating aquatic vegetation (*Salvinia*, similar to [41]). Four *A. ruberrimus* were placed into each of three mesocosms ('predator' mesocosms), while the other three were left as predator-free controls. To initiate chemical cues in predator mesocosms, we placed 25 recently hatched *D. ebraccatus* tadpoles into predator mesocosms 24 h before beginning oviposition site choice tests. To maintain predation cues during the course of the experiment, we placed approximately 50 *D. ebraccatus* eggs in the water of predator mesocosms every 2–3 days. All eggs were consumed by predators at each feeding.

Between 26 July and 16 August, we tested oviposition site choices of 18 mating *D. ebraccatus* pairs caught from Experimental Pond. We collected pairs that were in amplexus but had not yet begun laying eggs and placed them in mesocosms between 22.30 and 23.00. We haphazardly assigned pairs to either predator or control mesocosms. As above, pairs had no experience with the predators nor the mesocosm environment until being transplanted to the experiment and only one pair of frogs was placed in a cage at a time. We left pairs undisturbed overnight to breed and released them at the pond the following morning. We recorded the number of eggs within each egg mass and categorized the location of all egg masses as aquatic, arboreal or at the water surface (with some eggs both in and out of water; [41]). Furthermore, we recorded the location of all individual eggs as either aquatic (in contact with the water surface or below) or arboreal (no contact with the water). After counting, all eggs were removed from the mesocosms and, with the exception of eggs fed to predators to create chemical cues, were allowed to hatch in the laboratory before being returned to the pond.

We analysed choices of reproductive mode in two separate but complementary ways. First, we used a generalized linear mixed model (GLMM) assuming an underlying binomial error distribution and logit link function. The model tested for effects of treatment on the proportion of eggs laid arboreally or aquatically and included the date of the trial (as multiple trials were conducted per night) as a random intercept effect. An observation-level random effect (pair of frogs) was also included to account for overdispersion in the model. Second, we used a multinomial logistic regression to test for effects of predator presence on the probability of frogs laying different types of egg masses (aquatic, water surface or arboreal), similar to [41]. The multinomial model did not include random effects but instead included both date and cage as fixed effects in the model.

3. Results

(a) Field survey: developmental rate in air and water

The Gosner developmental stage [46] of embryos laid in the three egg pond/environment combinations differed significantly,

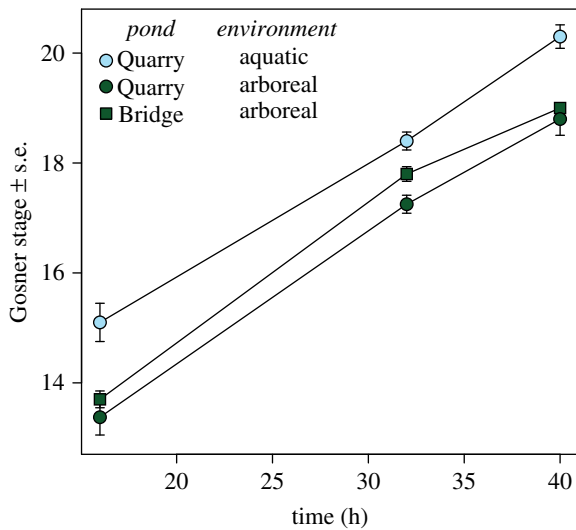


Figure 1. The developmental stage [46] of *D. ebraccatus* embryos preserved from naturally laid aquatic or arboreal egg masses at two ponds near Gamboa, Panama. Embryos developing in water at Quarry Pond were developmentally advanced compared with embryos in air, both at the same pond as well as at one other pond. Bars are mean \pm s.e. $N = 8$ arboreal masses at Bridge Pond, $N = 10$ arboreal masses at Quarry Pond, $N = 10$ aquatic masses at Quarry Pond. (Online version in colour.)

with eggs in the water at Quarry Pond developing more rapidly than those in air at either pond (figure 1: pond/environment, $\chi^2 = 37.5$, $p < 0.00001$). Developmental stage increased over time, as would be expected, and there was no interaction between aquatic or arboreal position and time, indicating that the effect of being in air or water was consistent across the three times we preserved embryos (time, $\chi^2 = 99.1$, $p < 0.00001$; time \times pond/environment, $\chi^2 = 1.0$, $p = 0.59$). A Tukey comparison of the three pond/environment combinations revealed that, at each time point, developmental stage was always significantly greater for eggs in the water at Quarry Pond than eggs in the air at either Bridge or Quarry Ponds (all $p < 0.02$). Development was not different between arboreal eggs at Bridge and Quarry ponds at 16 or 40 h (both $p > 0.71$), but was marginally different at 30 h ($p = 0.052$).

(b) Experiment 1: oviposition site discrimination test

Female *D. ebraccatus* used emergent vegetation for laying arboreal eggs and strongly discriminated against ovipositing in tubs containing fish ($\chi^2 = 8.4$, $p = 0.004$). Females laid approximately two-third of their eggs (197 ± 93 eggs) in tubs without fish and one-third in tubs with fish (112 ± 93 eggs). Six of the 21 pairs tested laid all eggs in the tub without predators, whereas only one female laid all of her eggs with predators. The number of eggs laid by a given female was not influenced by the proportion of eggs she laid with predators, indicating that fish did not consume eggs and alter our results ($\chi^2 = 0.24$, $p = 0.63$).

(c) Experiment 2: reproductive mode choice under conflicting egg desiccation and predation risk

The presence of fish in unshaded mesocosms had a dramatic effect on reproductive mode choices, making female *D. ebraccatus* much more likely to lay arboreal eggs (figure 2a: $\chi^2 = 18.1$, $p = 0.00002$). When there were no predators in the water,

females laid 88% of their eggs aquatically, whereas females only laid 13% of their eggs in water with predators. The number of eggs laid by females did not differ for predator or control mesocosms, indicating that fish did not alter our results by consuming eggs before we arrived to remove them nor did females withhold eggs in the presence of fish (232 ± 48 eggs, 254 ± 32 eggs, t -test: $t_{13.8} = -1.17$, $p = 0.26$). Looking at the types of egg masses laid, females were more likely to lay arboreal egg masses when fish were present but more likely to lay egg masses either fully aquatically or at the water surface in the absence of fish (figure 2b: multinomial logistic regression, $\chi^2 = 17.9$, $p = 0.0001$). The relative types of egg masses laid also varied across dates, most likely owing to the influence of weather on different nights ($\chi^2 = 26.5$, $p = 0.0009$).

4. Discussion

The ability to lay eggs away from water was likely crucial to the success of vertebrates transitioning onto land [1,3]. However, given that this event (or more likely, these events) occurred long ago, and because behaviours and soft-tissues like embryos do not fossilize, it has been difficult to quantify the relative strength of selection pressures that may have pushed ancestral organisms to leave the water and oviposit eggs on land. Here, we present evidence that the risk of frog eggs being predated in water strongly outweighs the risk of those eggs becoming desiccated out of water, at least from the perspective of the ovipositing parent. We assume that oviposition site choices reflect the same selection that has shaped the evolution of reproduction in general and thus, our results provide the first true experimental support for the hypothesis that aquatic predation risk was an important driving factor behind the evolution of non-aquatic egg laying. However, it is important to acknowledge that our experiment does not recreate the environmental conditions that led to the evolution of non-aquatic oviposition *per se*.

For oviparous vertebrates, the choice of where to lay one's eggs is the final and most important choice for enhancing offspring survival, and therefore fitness, as most fish, amphibians and reptiles lack parental care after oviposition [51]. Such choices are increasingly understood to be quite sophisticated; for example, mosquitoes can not only discriminate predator presence but quantify varying degrees of risk [25] and turtles appear to choose oviposition sites in order to balance offspring sex ratio [52]. However, the ways that females make decisions when facing conflicting indicators of offspring survival and development is less well understood (but see [38,39]).

We exposed females to predators under two different scenarios. First, females discriminated against laying arboreal eggs in habitats containing fish when given the option of choosing a fishless habitat. Despite the fact that such behaviour is known for many taxa (e.g. [25,26,31]), it still seems remarkable that *D. ebraccatus* successfully discerned predators in relatively small containers in close quarters and with no prior experience, including the lack of fish in the pond where frogs were collected for experiments. Second, we presented females with conflicting risks to their eggs: desiccation risk from a lack of shade above the habitat versus fish predators in the water. As expected based on previous work [41], the desiccation-prone environment caused females to lay most of their eggs directly in contact with or under the water surface when there were no predators present (figure 2). However, when fish were in

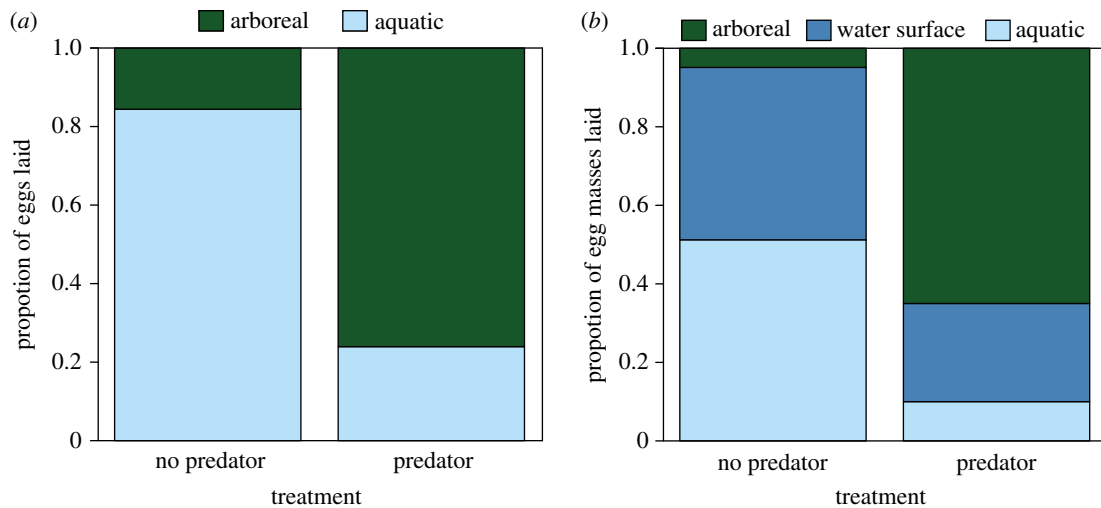


Figure 2. The proportions of (a) aquatic or arboreal eggs and (b) aquatic, water surface or arboreal egg masses laid by *D. ebraccatus* in unshaded mesocosms with and without fish predators. In the absence of predators, frogs laid most eggs and egg masses in the water, whereas the presence of predators caused frogs to lay more eggs and egg masses arboreally. $N = 18$ pairs of frogs. (Online version in colour.)

the pool, females ignored desiccation risk and switched to laying most eggs out of the water. Our field survey suggests little or no developmental costs of aquatic egg laying (figure 1), removing it as an important risk factor influencing oviposition site choice, at least in the present scenario. Thus, we can conclude that females viewed aquatic predators as a greater risk to their eggs than desiccation.

Our experiment took place in artificial mesocosms and did not attempt verisimilitude, only being a simplified version of a pond that might occur in nature. Similarly, our experiments did not attempt to recreate the conditions that might have driven the evolution of non-aquatic reproduction in the first place. Actually testing the evolution of reproductive mode seems impossible with vertebrate animals at the current time, but may be feasible with a cleverly chosen invertebrate with a rapid generation time. *Dendropsophus ebraccatus* likely evolved reproductive mode plasticity long ago and we will never know the exact conditions that drove such a shift in behaviour. Given that behaviours do not fossilize, measuring the decisions of extant species remains the best way to assess the role of various selective agents on the evolution of egg laying. Furthermore, conducting experiments like ours under more natural conditions (e.g. in actual ponds) is unfeasible given the need to find every embryo that a female lays during the course of an evening (females each laid 250–300 eggs in the experiments here).

Aquatic frog embryos are rapidly consumed by predators (e.g. [53]) and previous results show that aquatic predators are the single greatest source of mortality for *D. ebraccatus* eggs [42]. Thus, the presence of fish represents a reliable indicator of risk. On the other hand, arboreal oviposition represents an unknown possibility. Unshaded habitats come with a high risk of desiccation, and the rainfall that will follow in the days after breeding is unknowable [14,42]. Rain falls on approximately 50% of days during the breeding season of *D. ebraccatus* [42] and if it does rain, the risks associated with arboreal oviposition are largely negated [14]. However, rain is decreasing in regularity, at least in central Panama [42]. Thus, females appear to make decisions based on the concrete information they gain during the actual oviposition event. This is supported by the fact that frogs that are about to breed can be transplanted from heavily shaded

ponds to unshaded mesocosms at night and will still oviposit in the water [41]. The fact that predators are present appears to represent a sufficient risk to cause mothers to gamble on unknown future rainfall and the potential for eggs to not die from desiccation.

Touchon & Warkentin [41] first demonstrated that aquatic oviposition occurs in *D. ebraccatus*, but here we show that it is a viable and potentially advantageous strategy for *D. ebraccatus* in the absence of predators. Aquatic eggs just under the surface of the water and exposed to daytime sun developed more quickly than arboreal egg masses (figure 1). Aquatic predation can be very high [42], but if adults find themselves in predator-free or low predation risk habitats, laying eggs in the water may be developmentally advantageous. Even in the presence of fish, it may be possible to lay eggs at the water surface in a way that would protect them from predation from below, such as on top of a submerged or floating leaf. We have found fish in Quarry Pond, where we encountered naturally laid aquatic eggs. Similarly, some of the females in our experiment laid aquatic eggs even in the presence of fish (figure 2). Thus, laying eggs in the water is a high risk/high reward decision for *D. ebraccatus*. There is a high likelihood your eggs will get eaten if you lay them aquatically, but if they are not, they will develop quickly and without risk of desiccation. Furthermore, the fact that some females still laid aquatic eggs in the presence of fish implies that there is variation across individuals in how they assess risk or perceive cues of habitat quality. The small sample size of our field survey does not negate its importance as a proof of concept that aquatic oviposition in this species is not necessarily detrimental. Two other ponds were studied previously and also found almost no cost to aquatic development of *D. ebraccatus* embryos [42]. Our goal was not to provide an all-encompassing estimate of the effects of developing in water but merely to show that aquatic oviposition is feasible and does not necessarily delay development. If eggs get sunken in the pond, for example, there are likely developmental costs. Similarly, developmental costs may be seen in ponds with different abiotic conditions from the one studied here.

Müller *et al.* [22] recently found a strong association between terrestrial amphibian reproduction and forest habitat in East African amphibians. Similarly, Gomez-Mestre *et al.*

[18] found a relationship between terrestrial reproduction in frogs and climates with high precipitation and humidity. Based on our results here, we propose a two-step process through which non-aquatic reproduction might evolve, which should apply to amphibians as well as other taxa. First, species must persist in environments with high amounts of rainfall and high ambient humidity, which removes the constant and strong selection pressure of desiccation. Only in these habitats can the terrestrial niche be opened and made available for exploitation. Second, the presence of egg-consuming aquatic predators drives the evolution of choosing oviposition sites out of the water. Once females begin to oviposit out of the water and the terrestrial niche has been opened, the various extant terrestrial embryos and forms of terrestrial reproduction we see today may evolve.

5. Conclusion

Given that the shift from aquatic to non-aquatic egg laying has occurred many times in very different taxa, it is not likely that there is a single driving force behind all these evolutionary events. Our results do not rule out potentially important roles for waterborne parasites, low oxygen levels in tropical ponds or enhanced development out of water (although in our field survey development was greatest in water) [5–8]. All are potentially important in determining the success of the transition to terrestrial reproduction. However, our results do strongly support a role, and potentially the most important role, of aquatic predators in the shift to breeding out of water. Reproducing adults not only discriminated against ovipositing above habitats with fish, but when faced with conflicting egg desiccation versus predation risk, frogs chose to lay the

majority of their eggs out of the water. Aquatic parasites are not known to be a problem in this system, but future work should investigate the potential role of oxygen levels in reproductive mode plasticity. In the years since Touchon & Warkentin [41] first documented this plasticity in *D. ebraccatus*, several other frog species have been found to have flexible reproductive modes as well [54,55], and these species may provide additional independent insights into the factors that influence the shift to laying eggs on land. Similarly, it is possible that the radiations of non-aquatic reproducing frogs in Asia and Africa contain species with similar flexibility that would provide valuable independent measures of selection on reproduction.

Ethics statement. This research was conducted under Boston University IACUC protocol no. 05–022 and was also approved by STRI IACUC.

Data accessibility. Data for the field survey and experiments are accessible at doi:10.5061/dryad.n6c78.

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Authors' contributions. J.C.T. conceived of and designed the study, analysed the data and drafted the manuscript. J.L.W. participated in the design of the study, collected the data and edited the manuscript. Both authors gave final approval for publication.

Conflict of interests. We have no competing interests.

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