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Julie Worley
Portland State University

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Oviposition site choice in a Neotropical treefrog, *Dendropsophus ebraccatus*

by
Julie Worley

Faculty Mentor:
Sarah Eppley

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Oviposition site choice in a Neotropical treefrog, *Dendropsophus ebraccatus*

JULIE L WORLEY

SARAH EPPLEY, FACULTY MENTOR

Abstract

An organism's fitness is determined by its ability to produce viable offspring. For animals that lack post-hatching parental care, assessing and discriminating between oviposition sites may increase offspring survival. The Neotropical treefrog, *Dendropsophus ebraccatus*, is the only vertebrate known to be able to lay eggs both aquatically and terrestrially, and can do so in a given night. Laying eggs in water can increase hypoxia and aquatic predation, whereas desiccation and terrestrial predation increase with oviposition on land. *Dendropsophus ebraccatus* therefore provides a unique opportunity to measure adult reproductive choices in response to opposing costs associated with aquatic versus terrestrial reproduction. To test this, I 1) quantified aquatic predation risk in a natural setting, 2) tested whether *D. ebraccatus* demonstrates oviposition site discrimination in response to an aquatic egg predator, and 3) measured the relative importance of egg desiccation and aquatic predation risk on *D. ebraccatus* oviposition decisions. Aquatic egg predation risk is high in nature and the presence of an aquatic egg predator altered reproductive mode choices by *D. ebraccatus*. Not only did *D. ebraccatus* discriminate against pools with aquatic egg predators, but aquatic egg predation risk outweighed terrestrial egg desiccation risk, causing frogs to lay eggs out of water even in unshaded habitats where desiccation risk is high.

Introduction

Most animals lack parental care. Parents choose a suitable environment for oviposition and lay eggs, at which point parental investment is completed and offspring are left to develop on their own. Oviposition site choice is therefore an extremely important decision for parents and selection should act strongly to shape oviposition decisions. Laying eggs in a suboptimal habitat has strong survival costs to offspring and parents should be able to correctly discriminate potential risks to their offspring (Resatarits 1996).

Multiple invertebrate and vertebrate animals, such as mosquitoes, phantom midges, aquatic beetles and amphibians, can discriminate between oviposition site based on both biotic and abiotic factors (Resatarits 1996, Kiesecker and Skelly 2000). For example, backswimmers (*Notonecta maculata*) and mosquitoes (*Culiseta longiareolata*) strongly avoid ovipositing in pools containing

predators (Arav and Blaustein 2006). Several amphibians, such as North American bullfrogs (*Rana catesbeiana*) and wood frogs (*R. sylvatica*), choose to oviposit eggs in warmer microclimates, which in turn increases the rate of development of eggs and tadpoles (Howard 1978, Seale 1982). Several other amphibian species preferentially avoid laying eggs in ponds containing competitors or pathogens (Resatarits and Wilbur 1989, Kiesecker and Skelly 2000). Such decisions as these can increase the likeliness of offspring survival.

The most important factor affecting recruitment in amphibian populations is the number of metamorphosing young (Berven 1990). Therefore, predation on eggs and tadpoles has a direct influence on anuran population sizes. When predators such as fish become established in temporary ponds, anuran reproductive success can drop to zero (Resatarits 1996). For certain species, such as gray treefrogs (*Hyla chrysoscelis*), complete reproductive failure often results from ovipositing in ponds containing fish (Resatarits and Wilbur 1989). Many amphibians, such as gray treefrogs, wood frogs, streamside salamanders (*Ambystoma barbouri*), American toads (*Bufo americanus*), pickerel frogs (*R. palustris*), pinewood treefrogs (*H. femoralis*), and treehole breeding frogs (*Phrynobatrachus guineensis*) avoid ovipositing in ponds with predators (Resatarits and Wilbur 1989, Resatarits 1996, Egan and Paton 2004, Hopey and Petranka 1994, Kats and Sih 1992, Holomuzki 1995, Reiger et al. 2004, Rudolf and Rodel 2005). However, some anurans, such as common frogs (*R. temporaria*), are not able to discern between oviposition sites with and without predators (Laurila and Aho, 1997). This may be true for many other anurans that don't have the capability to assess their environment or for anurans whose offspring are not strongly affected by predation.

The hourglass treefrog (*Dendropsophus ebraccatus*) has a unique advantage in choosing oviposition sites. This Neotropical treefrog is the first vertebrate known to exhibit reproductive mode plasticity; individual females can choose to oviposit their eggs either aquatically or terrestrially and can change reproductive mode during a single night (Touchon and Warkentin 2008a).

Dendropsophus ebraccatus lays eggs aquatically when in sunny environments where desiccation risk is high for terrestrial eggs (Touchon and Warkentin 2008a). However, aquatic eggs may suffer hypoxia, and thus, *D. ebraccatus* lays eggs terrestrially in shaded environments where desiccation risk is lower (Touchon and Warkentin 2008a). It is unknown whether factors other than shade influence their oviposition site choice. Since eggs can be laid above or below water, there may be more abiotic and biotic environmental factors that affect oviposition decisions. For example, temperature, vegetation, and predators may all vary in different ways in water or on land, and may influence oviposition site choices by females.

I measured aquatic egg predation and the ability of mating *D. ebraccatus* pairs to assess terrestrial and aquatic egg mortality risks. First, I quantified the risk of predation on aquatic egg masses in a natural setting. I then measured the ability of *D. ebraccatus* pairs to discriminate oviposition sites with and without aquatic egg predators. Lastly, I measured the oviposition site choices of *D. ebraccatus* pairs placed in unshaded mesocosms with and without aquatic egg predators. I hypothesized that 1) predation upon aquatic egg masses would be high and developmental costs to eggs would be low, 2) *D. ebraccatus* adults would choose to lay eggs in predator-free pools when given the option, and 3) pairs in unshaded pools would lay eggs aquatically when predators were absent, but would lay terrestrial eggs when predators were present.

Materials and Methods

Dendropsophus ebraccatus is a Neotropical treefrog known to lay eggs both terrestrially and aquatically around permanent and semi-permanent ponds (Touchon and Warkentin 2008a). At my field site, breeding occurs during the rainy season from May through November. Females lay 100–340 eggs in a given night, partitioned into 2–10 discrete egg masses (mean = 242 eggs/female; J. Touchon, unpublished data).

There are many potential aquatic egg predators in the ponds where *D. ebraccatus* breeds. Conspecific and heterospecific (e.g. *Leptodactylus pentadactylus*) tadpoles and fish are known to eat aquatic *D. ebraccatus* eggs (J. Touchon, pers. comm.). *Astyanax ruberrimus* (Characidae) is a diurnal fish that predaes on both *D. ebraccatus* eggs and larvae (Touchon and Warkentin 2008b). Chemical cues from *A. ruberrimus* predators induce morphological and color changes in *D. ebraccatus* tadpoles (Touchon and Warkentin 2008b).

I studied *D. ebraccatus* at two ponds near the Smithsonian Tropical Research Institute field station in Gamboa, Panama. The first pond, Quarry Pond, is where aquatic oviposition in *D. ebraccatus* was first discovered (Touchon & Warkentin 2008a). Quarry Pond is large, has little overhanging forest canopy, and has a layer of floating aquatic vegetation (*Salvinia*) which frogs use for laying aquatic egg masses. The second pond, Experimental Pond, is a man-made pond that lacks fish predators. It is surrounded by vegetation, with the rainforest edge approximately four meters away on one side and an open field on the other.

Natural Aquatic Predation Risks

To establish if predation is a threat to aquatic *D. ebraccatus* eggs, I exposed eggs to aquatic predators in a natural setting. I collected nine amplexant *D. ebraccatus* pairs at Quarry Pond on 9 and 12 August 2008. I placed pairs in plastic bags overnight in an open-air laboratory and allowed them to breed (Touchon and Warkentin 2008b). Pairs laid eggs by adhering them to the inside of the bag. All pairs were returned to Quarry Pond the following morning. The morning after oviposition I transferred eggs from the plastic bag to *Salvinia* in a manner consistent with *D. ebraccatus*' natural aquatic egg mass placement. I created 40 replicated egg masses of 50 eggs each. I placed aquatic egg masses on *Salvinia* in Quarry Pond ca. 10 hours after oviposition and monitored them for evidence of predation. To enable differentiation of predation mortality from hypoxia or our handling and

manipulation, I placed half of the egg masses inside predator exclosures. *Salvinia* plants (containing egg masses) were marked with flagging tape to ensure correct identification. After 36 hours, all egg masses were collected and returned to the lab. I recorded the number of viable and dead eggs remaining on the *Salvinia*, as well as the number of eggs missing due to predation.

Oviposition Site Discrimination Tests

To test the ability of *D. ebraccatus* adults to discriminate ponds with and without predators, I conducted oviposition site choice trials in mesocosms located at Experimental Pond. I constructed six 1.3 m³ mesh cages, each containing two 60L pools filled with aged tap water and emergent vegetation common to *D. ebraccatus* breeding ponds in Gamboa. I placed mesocosms under heavy forest canopy to promote terrestrial oviposition (Touchon and Warkentin 2008a). Two *A. ruberrimus* were randomly placed in one of the two tubs for all six mesocosms. The range of size for each *A. ruberrimus* in each pair was 48-69 mm. To create chemical cues which may indicate the presence of predators to adults, predators were fed 10 recently hatched *D. ebraccatus* tadpoles at least 24h before oviposition site choice trials began. Two predators died during the experiment and were replaced.

Between 15 and 21 June 2008 I tested oviposition site discrimination of 21 mating pairs of *D. ebraccatus* caught from Experimental Pond. I placed pairs in mesocosms between 2230 and 2300 h, randomly starting them on either the predator or predator-free pools. I left pairs to breed undisturbed overnight. The following morning, I released pairs from mesocosms and recorded the number of eggs laid in the predator or predator-free pools.

Aquatic/Terrestrial Oviposition Site Choice Tests

To measure the oviposition site choices of *D. ebraccatus* pairs facing conflicting risks of aquatic egg predation and terrestrial egg desiccation, I conducted oviposition site choice trials in

unshaded mesocosms with and without predators. I constructed six 1.3 m³ mesocosms, each with a single pond (1.3 m diameter x 0.25 m tall). All mesocosms were placed in an unshaded environment, where *D. ebraccatus* prefer to lay eggs aquatically (Touchon and Warkentin 2008a). I filled ponds with aged tap water, four common emergent plants, and a layer of floating *Salvinia* vegetation (Touchon and Warkentin 2008a). Four *A. ruberrimus* were randomly placed into three mesocosms, while the other three were left as controls (mean total length 29-33 mm). To create chemical cues in predator pools, predator ponds were seeded with 25 recently hatched *D. ebraccatus* tadpoles 24 h before beginning oviposition site choice tests. Predators were also fed ca. 55 eggs each every 2-3 days throughout the experiment.

Between 26 July and 16 August I tested oviposition site choices of 20 mating pairs of *D. ebraccatus*, caught from Experimental Pond (located ca. 30 m away from cages). I randomly placed pairs in mesocosms between 2230 and 2300 h. Pairs were left undisturbed overnight to breed, and released the following morning. I recorded the location of egg masses (aquatic, terrestrial, or on the water surface) and number of eggs within the masses for each clutch. Masses were recorded as aquatic if each egg was submerged or in contact with the water. Masses were considered to be on the water surface if some eggs were submerged or in contact with the water, but others were completely out of the water. The number of eggs below and above the water was recorded for each mass at the water's surface. A terrestrial mass was defined as any mass in which all of the eggs were completely out of the water.

Statistics Analyses

All statistical analyses were conducted in R version 2.7.1. (R Development Core Team 2007). Aquatic predation rates were modeled using a generalized linear model (GLM) with underlying quasibinomial distribution and logit link function. A quasibinomial distribution accounts for overdispersion of the data (Pinheiro & Bates 2000). I tested for both treatment and block effects

and an interaction effect. Neither the block effect nor the interaction were significant and both were left out of the final model. I used a χ^2 test to quantify if eggs were laid non-randomly in oviposition site discrimination tests. I modeled aquatic and terrestrial oviposition site choices using a multinomial logistic regression (MLM). I tested for block effects of date and pair of frogs, and interaction effects, and compared models with Akaike's Information Criterion (AIC). Date never had a significant effect and was left out of the final model.

Results

Natural Aquatic Predation Risks

After 36 hours in Quarry Pond, egg masses exposed to predators had 43.4% more eggs missing than egg masses in predator exclosures (Figure 1; GLM, $F_{1,37} = 22.39$, $P < 0.00001$).

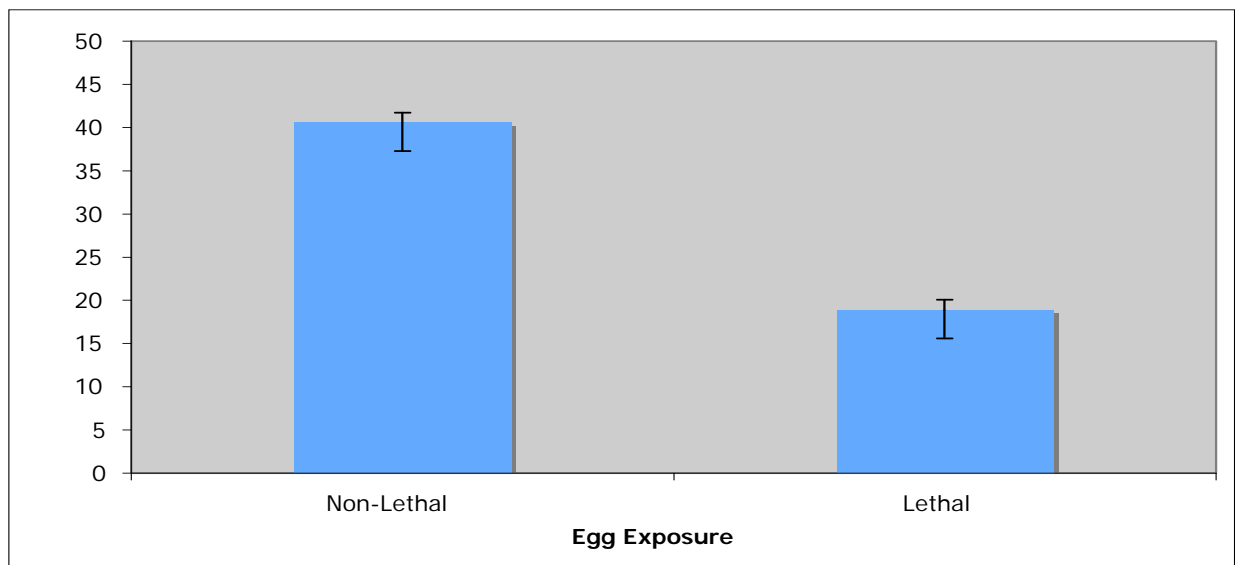


Figure 1. Number of *Dendropsophus ebraccatus* eggs remaining in egg masses placed in Quarry Pond and exposed to predators or in non-lethal predator exclosures. More eggs were missing from egg masses exposed to predators than in egg masses within predator exclosures. ($P < 0.00001$, $N = 20$).

The average number of dead eggs did not significantly differ between treatments (non-lethal = 6.2 ± 1.13 ; lethal = 4.6 ± 1.69).

Oviposition Site Discrimination Tests

When given a choice between ovipositing in predator versus predator-free pools, *D. ebraccatus* pairs laid eggs non-randomly; pairs oviposited an average of 197.33 eggs over the control pools and 111.86 eggs over the predator pools (Figure 2; $\chi^2 = 2379.47$, $P < 0.00001$).

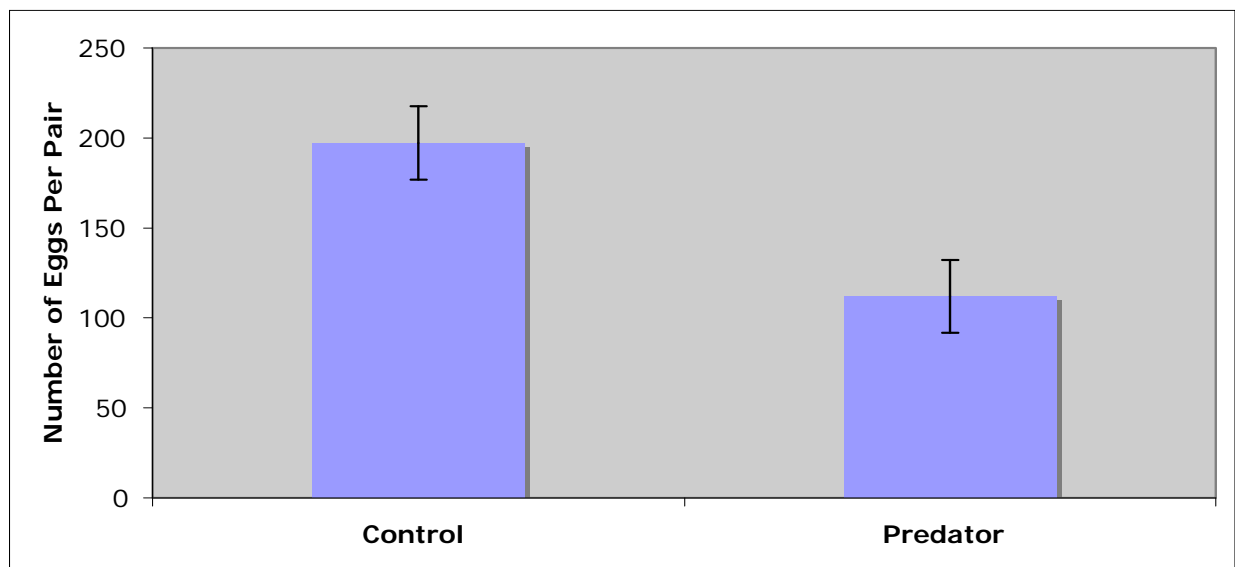


Figure 2. The average number of eggs laid by *Dendropsophus ebraccatus* pairs over control and predator pools. Pairs laid more eggs predator-free control pools than over pool with predators ($P < 0.00001$, $N = 21$)

Aquatic/Terrestrial Oviposition Site Choice Tests

There was a significant effect of predator presence on aquatic versus terrestrial oviposition; in pools containing predators, pairs laid more terrestrial eggs, whereas in predator-free pools more aquatic eggs were laid (Figure 3; MLM, $F_{2,89} = 25.69$, $P < 0.00001$).

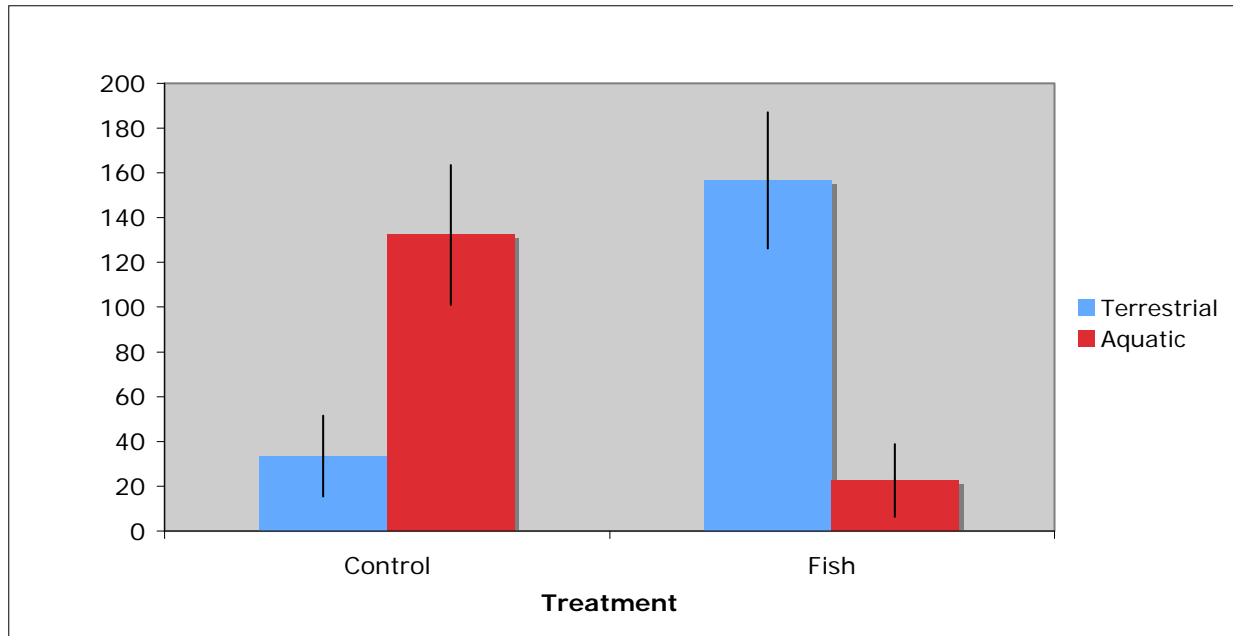


Figure 3. The average number of *Dendropsophus ebraccatus* eggs per pair laid terrestrially and aquatically in predator pools and control pools. Pairs chose to lay more aquatic eggs in pools without predators, whereas the presence of *Astyanax ruberrimus* in the water caused frogs to lay more eggs terrestrially ($P < 0.00001$, $N = 20$).

There was a significant effect of pair on the model as well ($F_{2,89} = 11.56$, $P = 0.00309$). An ANOVA test showed that there was no significant difference between the number of eggs laid per pair in predator cages or predator-free cages ($F_{18,24} = 2.095$, $P > 0.165$).

Discussion

I have demonstrated that egg predation is a realistic threat to aquatic *D. ebraccatus* egg masses and that adults can not only detect aquatic predators, but alter oviposition behavior in response to aquatic egg predation risk. Gamete investment and oviposition site selection are the only forms of parental care demonstrated in most organisms (Howard 1978). Oviposition site choice strongly affects the reproductive success of pond-breeding amphibians (Kiesecker and Skelly 2000). Therefore, the ability to alter oviposition behavior in response to multiple factors, such as sun exposure and predator presence, can greatly increase parental fitness

Aquatic *D. ebraccatus* egg masses exposed to predators had significantly more eggs missing than egg masses protected from predation. Although a small percentage of eggs were missing from control egg masses, over 40% more eggs were missing from masses exposed to aquatic predation. There was no difference between the number of dead eggs in exposed masses versus controls (6-14% of egg mortality), indicating that hypoxia was similar in both treatments. There was, however, a difference between dead eggs in the two blocks of experiments. This was not likely due to handling differences, since both blocks were set up in the same manner. Oxygen levels, however, may have fluctuated between blocks, perhaps because of rainfall or water temperature (Benson and Krause 1984). Terrestrial predation may have occurred to a small degree, as control cages did have some eggs missing out of their masses. We did not observe which terrestrial or aquatic predators were responsible for eating *D. ebraccatus* eggs, but likely terrestrial predators are fishing spiders (J. Worley, pers. obs.) and *A. ruberrimus* or conspecific tadpoles are good aquatic egg predators (J. Touchon, pers. comm.).

Many organisms have demonstrated the ability to discriminate between oviposition sites, increasing offspring survival rates (Arav and Blaustein 2006, Egan and Paton 2004, Holomuzki 1995, Hoey and Petranks 1994, Howard 1978, Kats and Sih 1992, Kiesecker and Skelly 2000, Rieger et al. 2004, Resatarits 1996, Resatarits and Wilbur 1989, Rudolf and Rodel 2005, Seale 1982, Touchon and Warkentin 2008a). Fish are strong predators, capable of decimating anuran larvae populations (Resatarits 1996). Natural selection should favor behavior that minimizes larval contact with predators (Kats and Sih 1992). Therefore, ovipositing in a predator-free area should be selected for. My oviposition site choice tests demonstrated that *D. ebraccatus* can discriminate between oviposition sites with and without predators. The predator pools used in this experiment contained two fish, although there were times when one fish would die, temporarily leaving a single fish in the pools. Rieger et al. (2004) found that a single fish has much less of an effect than two or

more fish on the strength of anuran oviposition site discrimination. This is probably due to less emission of predator chemical cues from a single fish. The strength of discrimination might be intensified if more predators were used. The results from this experiment show that even with minimal amounts of predators present, *D. ebraccatus* can still discriminate between pools.

Desiccation is a serious risk for terrestrial *D. ebraccatus* eggs and sunny environments cause frogs to lay eggs aquatically instead of terrestrially (Touchon and Warkentin 2008a). *Dendropsophus ebraccatus* oviposit around 80% of their eggs aquatically in sunny environments (Touchon and Warkentin 2008a). This is consistent with my results, where frogs in control mesocosms also laid 80% of their eggs aquatically. There was no difference in the total number of eggs laid by pairs in predator or control mesocosms. Thus, the variation in aquatic/terrestrial oviposition site choices between the two treatments was not due to predators eating the aquatic eggs before I collected them from the cages. Along with treatment, pair was a significant predictor of where the eggs were oviposited, indicating that there is variation between pairs' oviposition decisions. Some pairs in predator cages oviposited 100% of their eggs terrestrially while some oviposited masses in both aquatic and terrestrial environments. The variation could be due to many factors including weather, the presence of terrestrial predators, or variation in the ability of different frogs to assess their environment.

I have demonstrated that *D. ebraccatus* adults can assess abiotic and biotic egg-stage risks in both aquatic and terrestrial environments simultaneously during a single night and use this information to decide between aquatic and terrestrial reproductive modes. Like most animals, *D. ebraccatus* lacks parental care; oviposition site choices are therefore critical for increasing offspring survival. The cues that *D. ebraccatus* use to evaluate oviposition sites are currently unknown. Future studies should separate likely environmental cues, such as ambient starlight and air or water temperature, which may indicate the presence of shade, or olfactory or chemical cues that may

denote the presence of egg predators. Understanding how *D. ebraccatus* assess their environment will offer insight into how animals perceive the world around them and evaluate their surroundings.

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