

# Negative synergism of rainfall patterns and predators affects frog egg survival

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## Summary

1. The importance of rainfall is recognized in arid habitats, but has rarely been explored in ecosystems not viewed as rainfall limited. In addition, most attempts to study how rainfall affects organismal survival have focused on long-term rainfall metrics (e.g. monthly or seasonal patterns) instead of short-term measures. For organisms that are short lived or are sensitive to desiccation, short-term patterns of rainfall may provide insight to understanding what determines survival in particular habitats.

2. We monitored daily rainfall and survival of arboreal eggs of the treefrog *Dendropsophus ebraccatus* at two ponds during the rainy season in central Panama. Desiccation and predation were the primary sources of egg mortality and their effects were not independent. Rainfall directly reduced desiccation mortality by hydrating and thickening the jelly surrounding eggs. In addition, rainfall reduced predation on egg clutches.

3. To elucidate the mechanism by which rainfall alters predation, we exposed experimentally hydrated and dehydrated egg clutches to the two *D. ebraccatus* egg predators most common at our site, ants and social wasps. Ants and wasps preferentially preyed on dehydrated clutches and ants consumed dehydrated eggs three times faster than hydrated eggs.

4. Rainfall patterns are expected to change and the responses of organisms that use rainfall as a reliable cue to reproduce may prove maladaptive. If rainfall becomes more sporadic, as is predicted to happen during this century, it may have negative consequences for desiccation-sensitive organisms.

**Key-words:** anura, complex life cycle, *Hyla ebraccata*, interaction modification, predator–prey interaction.

## Introduction

The importance of rainfall variability has long been acknowledged for arid ecosystems (Noy-Meir 1973; Meserve *et al.* 2003; Shachak *et al.* 2005), but has received little attention in ecosystems not typically considered rainfall limited, such as tropical rainforests. This is understandable since tropical rainforests experience extended periods of abundant regular rainfall. For example, on Barro Colorado Island (BCI), Panama, the probability of rain on any given day during the rainy season is > 70% (Windsor 1990). Despite this regularity, brief dry spells during the rainy season on BCI, even as short as 4 days, increase mortality of some developing trees by 20–40% (Engelbrecht *et al.* 2006). Dry spells during the rainy season also increase predation on *Anolis* lizard eggs by leaf-litter

ants (Chalcraft & Andrews 1999). For organisms such as these that are desiccation sensitive, brief dry periods may be important for determining individual survival.

One group of organisms that may be affected by dry spells are terrestrially and arboreally breeding amphibians. Not only are adult amphibians highly sensitive to desiccation (Duellman & Trueb 1986; Wells 2007), but many species in the tropics lay eggs out of water thereby exposing them to variable weather patterns. Annual and seasonal levels of rainfall in the tropics are predicted to change throughout this century (Hulme & Viner 1998; Christensen *et al.* 2007). Climate models also predict increased numbers of dry days during the rainy season which may alter rainfall regularity (Hulme & Viner 1998; Christensen *et al.* 2007). As a result, mortality of terrestrial amphibian eggs is predicted to increase in the tropics (Donnelly & Crump 1998). Global climate change is already affecting species interactions, altering plant and animal phenologies and shifting species ranges to higher elevations and latitudes

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(Miles 1994; Walther *et al.* 2002; Parmesan & Yohe 2003; Parmesan 2006). The potential importance of altered rainfall patterns have, however, not yet been explored.

While short-term rainfall variation may be important for many organisms, it may not be detectable in larger-scale metrics typically used to detect effects of rainfall (e.g., total annual rainfall). Many analyses of rainfall effects on individual growth and survival, or on interspecific interactions, have focused on only seasonal or annual weather records (e.g., Lima, Stenseth & Jaksic 2002; Morrison & Bolger 2002; Brown & Shine 2007). While such large-scale rainfall trends are useful for elucidating population and community-level dynamics (Stenseth *et al.* 2002; Hallett *et al.* 2004; Engelbrecht *et al.* 2007), they may not reveal the specific mechanisms that determine individual mortality and contribute to larger-scale processes. However, to elucidate how interspecific interactions and organismal growth and development are affected by short-term fluctuations in weather patterns, it may be necessary to look at weather patterns on a shorter time-scale (e.g., Donnelly & Guyer 1994; Engelbrecht *et al.* 2006).

We test the hypothesis that rainfall patterns act synergistically with predators to affect mortality of arboreal amphibian embryos. We monitored the survival of arboreal egg clutches of the Neotropical treefrog *Dendropsophus ebraccatus* during 3 years and correlated egg fate with daily rainfall. We also measured the foraging abilities and preferences of the most common predators of *D. ebraccatus* eggs on clutches reared under different simulated rainfall environments.

## Materials and methods

### STUDY SYSTEM

The Neotropical treefrog *D. ebraccatus* (= *Hyla ebraccata* Cope, Faivovich *et al.* 2005) ranges from southern Mexico to northern Columbia, and breeds in ponds found in rainforest and disturbed areas (Duellman 2001). *D. ebraccatus* has semi-terrestrial reproduction; eggs are laid on leaves above water, develop for 3–4 days, and then aquatic tadpoles hatch and fall into the pond below (Duellman & Trueb 1986; Wells 2007). *D. ebraccatus* was recently discovered to sometimes lay eggs in water (Touchon & Warkentin 2008a), but typically lays eggs arboreally at our main study ponds. As in many frogs, *D. ebraccatus* breeding activity is stimulated by rain (Donnelly & Guyer 1994), and reproduction is more common after heavy rains (J. Touchon, personal observation). At our field site in Gamboa, Panama, *D. ebraccatus* breeds throughout the rainy season, from May to November.

We studied *D. ebraccatus* at two ponds: Bridge Pond (9°6'50.26"N, 79°41'48.13"W) and Ocelot Pond (9°6'8.62"N, 79°40'56.96"W). Bridge Pond is small and shallow and frogs lay eggs mostly on low-lying emergent vegetation, which occurs throughout the pond. Clutches near the pond edges are shaded (~50% of pond area) while those in the centre are unshaded. Ocelot Pond is substantially larger and deeper than Bridge Pond, and clutches are laid primarily on fringing vegetation around the edge of the pond, under canopy shade. Because Ocelot Pond is deep, the centre of the pond lacks emergent vegetation. Several egg predators are found at both ponds: the most common are social wasps [*Polybia rejecta* (Fabricius) and *Agelaius centralis* (Cameron)] and ants (*Azteca* sp.). We frequently see

these predators feeding on *D. ebraccatus* eggs at Bridge and Ocelot Ponds, as well as other ponds near Gamboa. Eggs at both ponds can be flooded when the water rises after large rainstorms, exposing them to aquatic predators. Both ponds contain tadpoles that feed on flooded eggs and Bridge Pond contains fishes (e.g., *Astyanax ruberrimus* Eigenmann and *Brachyrhaphus* sp.).

### FIELD MONITORING

To identify the sources and quantify natural rates of *D. ebraccatus* egg mortality, we monitored 350 clutches of eggs at Ocelot and Bridge Ponds from 2003–2005. Because of random variation in rainfall patterns, monitoring did not take place at the same time each year. We monitored 150 clutches between 18 June and 5 July 2003, 100 clutches between 28 August and 30 September 2004 and 100 clutches between 23 September and 1 October 2005. Mean monthly rainfall and humidity do not vary at our field site during this period, and air temperature decreases slightly as the rainy season progresses. *D. ebraccatus* reproductive activity is high throughout this period (Fouquette Jr. 1960). We located clutches the morning after oviposition and recorded the initial number of eggs, then checked clutches twice daily – after dawn and before dusk – until all eggs hatched or died. On the afternoon of the second day, we hung a cup with a small amount of water underneath the clutch to catch hatchlings (Hayes 1983).

During each visit, we recorded the numbers of hatchlings, live eggs, eggs killed by desiccation, eggs failing to develop, predated eggs, and eggs submerged by rising pond water. Because hatchlings fell into the cup below the clutch, missing animals were assumed to be removed by predators. We also made direct observations of egg predation opportunistically. For submerged clutches, where hatchlings could not be contained by a cup, we assessed predation only for the first 48 h post-oviposition, before eggs were hatching competent. We also recorded drowned eggs, characterized by developmental retardation before death.

In 2003, we monitored rainfall twice daily with a rain gauge located in Gamboa (~2 km from Ocelot Pond and 0.5 km from Bridge Pond). In 2004 and 2005, we recorded rainfall with rain gauges at each pond (checked twice daily). We obtained daily humidity and air temperature measurements from the Meteorological and Hydrological Branch of the Panama Canal Authority (ACP) weather station in Gamboa. In 2004 and 2005, we recorded the initial height of each clutch above the pond (to the nearest centimetre), and measured clutch thickness (to the nearest 0.5 mm) at each check by inserting a fine probe through the clutch jelly to the leaf substrate. Clutch thickness serves as a measure of hydration since the jelly surrounding eggs swells with rainfall and shrinks as clutches desiccate (J. Touchon, personal observation).

### WASP PREDATION TRIALS

To measure the effect of clutch hydration on wasp foraging ability and preferences, we presented paired dehydrated and hydrated *D. ebraccatus* egg clutches to two species of social wasps regularly observed preying upon *D. ebraccatus* eggs, *P. rejecta* and *A. centralis*, between 21 June and 7 July 2004. To obtain clutches, we collected 10 mating pairs of *D. ebraccatus*, placed them in plastic bags with a small amount of water, and allowed them to breed overnight in an ambient-temperature laboratory. All pairs laid two or more masses of eggs inside the plastic bag. Frogs were returned to their pond the following day. We selected two approximately equal-sized clutches from each bag and assigned one to be hydrated and the other dehydrated. Clutches were removed from bags by cutting the plastic

around them, without disturbing eggs. We obtained the initial mass of each clutch by weighing it with the attached plastic, then subtracting the mass of an equal-sized piece of plastic cut from the same bag. Dehydrated and hydrated clutches did not vary significantly in their starting number of eggs ( $77 \pm 7$  and  $85 \pm 8$  eggs respectively, mean  $\pm$  SE here and throughout; paired  $t$ -test,  $t_9 = 0.66$ ,  $P = 0.53$ ) nor in their starting clutch mass (dehydrated =  $1.0 \pm 0.12$  g, hydrated =  $0.90 \pm 0.15$  g; paired  $t$ -test,  $t_9 = -1.16$ ,  $P = 0.28$ ).

Clutches were dehydrated in an environmental chamber at 30–31 °C and ~60% relative humidity, typical of a relatively hot, dry day during the rainy season in Gamboa (J. Touchon, unpublished data). To avoid direct mortality from desiccation, dehydration was stopped and clutches used in experiments when they had lost ~60% of their original mass as a result of evaporation. We allowed other clutches to absorb a maximal amount of water, as occurs in heavy rainstorms, by placing them in a shallow bath of aged tap water. Hydrated clutches were kept in water until predation trials began. Submergence can reduce the oxygen supply to developing embryos, slowing development. However, no developmental effect of our treatments was evident to the naked eye or from video recordings of eggs. All embryos in both treatments were alive and developing normally when presented to wasps.

Individually marked, free-ranging wasps were trained to come to a feeding station and forage on frog egg clutches attached to bricks set over water (for wasp training and marking methods, see Warkentin, Buckley & Metcalf 2006). We mounted age-matched hydrated and dehydrated clutches vertically beside each other, ~3 cm apart on the same brick, and allowed wasps to forage freely on the clutches until one had been 50% consumed. We alternated the side the hydrated and dehydrated clutches were on with each test. All wasp interactions with the clutches were videotaped. We recorded data from videotapes (length of predation trials =  $58.1 \pm 9.8$  min). We conducted trials with both 1- and 2-day-old clutch pairs ( $N = 4$  and 6, respectively). Twelve wasps (10 *P. rejecta* and 2 *A. centralis*) participated in the experiment, with one to seven individuals visiting each clutch pair ( $2.9 \pm 0.6$  wasps per trial). Most wasps participated in only one trial ( $2.2 \pm 0.4$  trials per trial), and predation rate did not change in those that participated in multiple trials [linear model (LM), trial number,  $F_{1,18} = 0.61$ ,  $P = 0.44$ ]. From the videotapes, we quantified the amount of time wasps spent foraging on each clutch, the number of foraging visits by wasps to each clutch, and the number of embryos killed by wasps (including embryos eaten, carried away, and left dead on the clutch).

We also wanted to compare foraging rates of wasps on hydrated and dehydrated clutches in a no-choice context. No-choice tests measure the ability of predators to feed on hydrated or dehydrated clutches, instead of preference for one type of clutch instead of another. We thus attempted three predation trials wherein wasps were presented with single desiccated or hydrated clutches. However, wasps presented with only a hydrated clutch did not forage and simply left the feeding station.

#### ANT PREDATION TRIALS

To measure the effects of clutch hydration on ant predation, we presented age- and size-matched pairs of hydrated and dehydrated *D. ebraccatus* egg masses to the most common ant predator at our ponds, *Azteca* sp. ants, on 8 and 9 November 2004. Egg clutches were obtained from 11 pairs of frogs as above. Two clutches were selected from each bag and standardized to 50 eggs each by carefully removing peripheral eggs. We obtained the initial mass of each egg clutch, and hydrated and dehydrated them as above. We used both

1- and 2-day-old clutch pairs ( $N = 6$  and 5 trials, respectively). Predation trials were conducted at both Ocelot Pond ( $N = 4$ ) and Bridge Pond ( $N = 7$ ) using a total of four different *Azteca* sp. colonies.

Predation trials were conducted by pinning pairs of hydrated and dehydrated clutches beside each other along the upper surface of a branch ~1 m from an *Azteca* sp. nest. We alternated placement of hydrated clutches towards and away from the nest among trials. Ants walked on all surfaces of the branch and moved readily over and around clutches. We checked clutches every hour until all eggs had been eaten from one clutch, or it began to rain and ants retreated to their nest (exposure time  $4.9 \pm 0.4$  h). At each observation, we recorded the number of eggs eaten and the number of ants feeding on each clutch.

We conducted no-choice predation trials to measure ant feeding rates on hydrated and dehydrated clutches on 16 and 23 June 2005, respectively. We obtained 10 clutches from seven pairs of frogs and hydrated and dehydrated them as above ( $N = 5$  trials each). Clutches were standardized to 50 eggs each by removing extra eggs. All no-choice tests were performed at Bridge Pond using two different *Azteca* nests. All trials used 2-day-old clutches and nests were offered both types of egg clutches during the experiment, but only one type of clutch on any given day. Clutches were pinned along tree branches at Bridge Pond ~1 m from *Azteca* nests and checked every hour until all eggs were eaten or until nightfall (mean exposure times: hydrated clutches = all trials 7 h, dehydrated clutches =  $4.4 \pm 0.2$  h). At each observation, we recorded the number of eggs missing and the number of ants on the clutch.

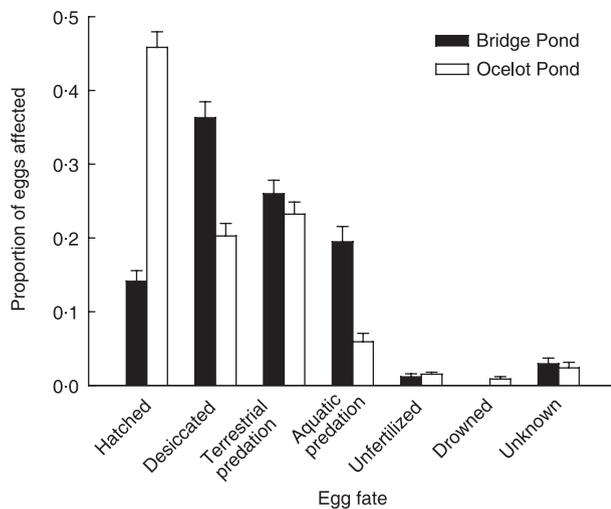
#### STATISTICAL ANALYSES

All statistical analyses were conducted in R version 2.6.0 (R Development Team 2007). Descriptive aspects of predation trials (e.g., number of wasp visits, total time wasps spent on a clutch, etc.) were analysed with paired  $t$ -tests or linear models (LM) as appropriate. For field-monitored egg clutches, we modelled variation in the average thickness of clutch jelly against total rainfall on each clutch, pond and year using LM. We modelled egg mortality from desiccation and terrestrial predation separately, against total rainfall on each clutch, pond and year using generalized linear models (GLM) with underlying quasibinomial error function and logit link functions (also called logistic regression). A quasibinomial error function is the same as a binomial error function, except that it accounts for overdispersion of the model and penalizes  $P$  values accordingly (Pinheiro & Bates 2000). We also modelled survival of field-monitored egg clutches that were rained on or not during the first 24 h, and 48 h, and egg survival in wasp and ant predation trials using quasibinomial GLMs.

## Results

#### NATURAL MORTALITY AND HATCHING PATTERNS

We observed a total of 22 533 eggs in 350 clutches from 2003–2005 (clutch size  $64 \pm 1.4$  eggs). At Bridge Pond, only  $14 \pm 2\%$  of eggs survived to hatch, whereas  $46 \pm 3\%$  of eggs at Ocelot Pond hatched (Fig. 1). Eggs that failed to hatch died from desiccation, were eaten by terrestrial predators, failed to develop (presumably unfertilized), became submerged and were eaten by aquatic predators before the start of the third day, or became submerged and drowned (Fig. 1). In addition,



**Fig. 1.** Fates of *Dendropsophus ebraccatus* eggs monitored at two ponds in Panama from 2003–2005. Embryos either hatched, or died before hatching from egg desiccation, terrestrial predation, drowning, or aquatic predation after submergence. Some eggs did not develop, and were presumably unfertilized. Some disappeared underwater after hatching competence; these could have hatched or been eaten.  $N = 350$  egg clutches. Data are mean proportion of affected eggs per clutch + SE.

some eggs disappeared underwater after the start of the third day; these may have hatched or been eaten, but their fate is unknown (Fig. 1).

We frequently observed social wasps (*P. rejecta* and *A. centralis*) and ants (*Azteca* sp.) preying on eggs, and damage patterns corroborate that these were the most important predators, accounting for all but one instance of observed arboreal egg predation. Wasp predation often leaves yolk spilled on the leaf, especially when wasps prey on young embryos (Warkentin 2000). Wasps also remove embryos from the jelly, leaving empty egg jelly behind (Warkentin 2000). Ants generally remove eggs and jelly completely; we rarely observed this without also seeing ants on the clutch. Wasp predation was more widespread as wasps could reach clutches throughout the ponds, whereas *Azteca* only preyed upon eggs in the same plant as their nest. Leaf-cutter ants killed one clutch while harvesting leaves from a plant containing *D. ebraccatus* eggs. We saw no evidence of snake predation on *D. ebraccatus* egg clutches, although it is common on *Agalychnis callidryas* egg clutches at Ocelot Pond (Warkentin 2000) and has been reported for *D. ebraccatus* elsewhere (Donnelly & Guyer 1994). From predation experiments (J. Touchon unpublished), we know that fishes (*A. ruberrimus* and *Brachyraphus* sp.) and tadpoles (including conspecifics and *Leptodactylus pentadactylus* Laurenti) consume flooded eggs.

The amount of rainfall that clutches received during development varied greatly during our observation periods (range 0–179.8 mm, mean  $33.3 \pm 1.9$  mm). The amount of rain that fell on clutches we observed was greater in 2005 than either 2003 or 2004, which did not differ from one another (2003 =  $13.1 \pm 0.98$  mm, 2004 =  $17.4 \pm 1.6$  mm, 2005 =  $77.1 \pm$

$3.7$  mm; LM,  $F_{2,347} = 267.27$ ,  $P < 0.0001$ ; Tukey's comparisons, 2003–2004,  $P = 0.30$ , 2003–2005 and 2004–2005,  $P < 0.0001$ ). In addition, dry days were more common during our 2003 and 2004 observation periods (proportion dry days: 2003 = 0.5, 2004 = 0.62, 2005 = 0.33). Mean daily temperature and relative humidity during our observations did not vary significantly between years, although 2005 was slightly cooler than 2003 or 2004 (2003 =  $25.9 \pm 0.2$  °C, 2004 =  $26.1 \pm 0.1$  °C, 2005 =  $25.5 \pm 0.2$  °C; LM, temperature,  $F_{2,57} = 2.74$ ,  $P = 0.073$ ; humidity,  $F_{2,57} = 1.54$ ,  $P = 0.22$ ; data from ACP).

Clutches were laid farther above the water surface at Ocelot Pond than at Bridge Pond, and oviposition height did not vary across years (Ocelot =  $62.1 \pm 3.6$  cm high, Bridge =  $20.9 \pm 1.9$  cm high; LM, pond,  $F_{1,196} = 105.2$ ,  $P < 0.0001$ ; year,  $F_{1,196} = 0.09$ ,  $P = 0.77$ ). The mean thickness of egg clutches throughout development increased with increasing rainfall and differed between ponds, but did not differ across years (Fig. 2a; LM, overall model,  $F_{7,192} = 17.41$ ,  $P < 0.0001$ ; rainfall,  $F_{1,192} = 90.39$ ,  $P < 0.0001$ ; pond,  $F_{1,192} = 12.35$ ,  $P = 0.0006$ ; year,  $F_{1,192} = 0.07$ ,  $P = 0.80$ ). The effect of rainfall on clutch thickness, however, differed between ponds and across years, as indicated by significant rainfall-by-pond and rainfall-by-year interactions (Fig. 2a; rainfall  $\times$  pond,  $F_{1,192} = 6.40$ ,  $P = 0.012$ ; rainfall  $\times$  year,  $F_{1,192} = 11.78$ ,  $P = 0.0007$ ).

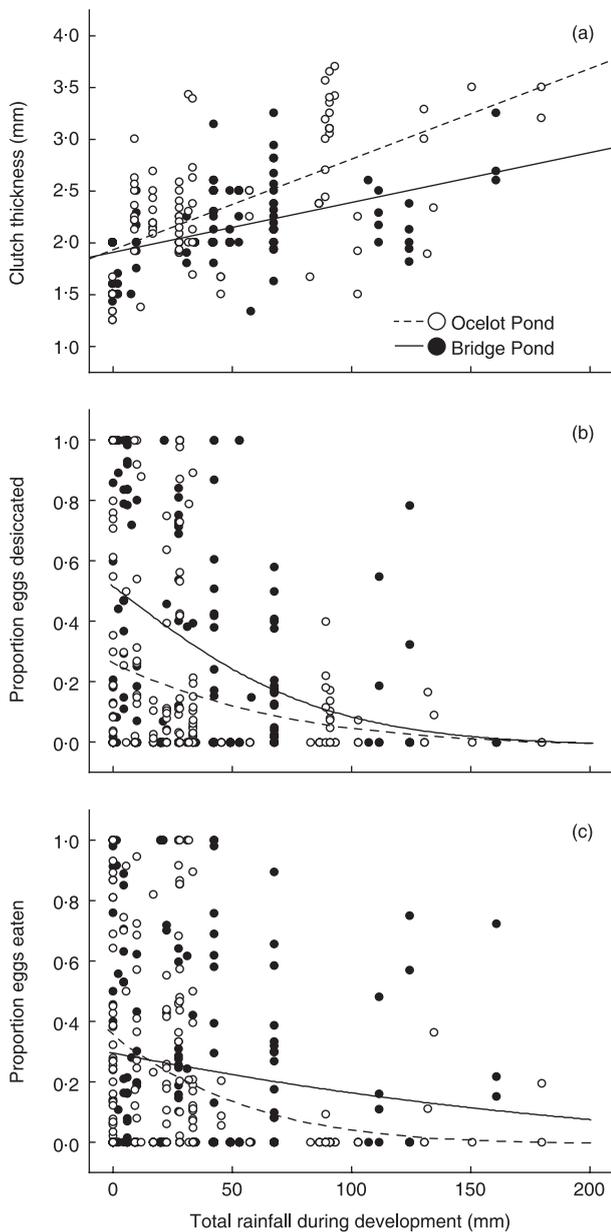
As rainfall increased, mortality from desiccation decreased (Fig. 2b, Table 1) and mortality was less at Ocelot Pond than at Bridge Pond (Fig. 2b, Table 1). In addition, there was a significant effect of year; egg desiccation mortality was lower in 2005 than either 2003 or 2004 (proportion of eggs desiccated, 2003 =  $0.38 \pm 0.03$ , 2004 =  $0.43 \pm 0.04$ , 2005 =  $0.23 \pm 0.02$ ). There were no interactions among rainfall, pond or year on egg mortality from desiccation (Table 1).

As rainfall on an egg clutch increased, predation from ants and wasps decreased (Fig. 2c; Table 1). There was no effect of pond on predation by ants and wasps, but predation did vary across years (Fig. 2c, Table 1; proportion eggs eaten by ants and wasps, 2003 =  $0.35 \pm 0.03$ , 2004 =  $0.23 \pm 0.03$ , 2005 =  $0.11 \pm 0.02$ ). In addition, there were interactions between pond and rainfall, and year and rainfall, such that the effect of rain on decreasing predation was not the same at both ponds or across years (Fig. 2c, Table 1).

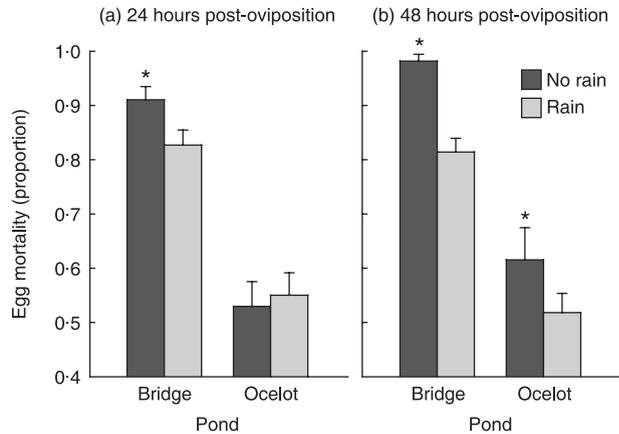
A lack of rainfall during the first 24 h after oviposition increased mortality of eggs at Bridge Pond approximately 10%, whereas it had no effect on mortality at Ocelot Pond (Fig. 3a; GLM, rain,  $F_{1,346} = 0.8$ ,  $P = 0.38$ ; pond,  $F_{1,346} = 66.4$ ,  $P < 0.0001$ ; rain  $\times$  pond,  $F_{1,346} = 5.2$ ,  $P = 0.024$ ). No rainfall during the first 48 h, however, increased egg mortality at both ponds (Fig. 3b; GLM,  $F_{1,346} = 11.7$ ,  $P = 0.0007$ ; pond,  $F_{1,346} = 68.3$ ,  $P < 0.0001$ ; rain  $\times$  pond,  $F_{1,346} = 12.2$ ,  $P = 0.0005$ ). Eggs that were not rained on for the first 48 h at Bridge Pond suffered  $98 \pm 1.3\%$  mortality (Fig. 3b).

#### PREDATION TRIALS

Before wasp predation trials, hydrated clutch mass had increased  $330 \pm 74\%$  due to water absorption, and dehydrated clutches had lost  $62 \pm 3\%$  of their original mass to evaporation.



**Fig. 2.** Effects of rainfall on *Dendropsophus ebraccatus* eggs at two ponds in Panama. (a) Increasing rainfall increases clutch thickness, as the jelly surrounding embryos absorbs water (linear model,  $F_{1,192} = 90.39$ ,  $P < 0.0001$ ). This relationship is steeper at Ocelot Pond (open circles and dashed lines), where clutches are more shaded, than at Bridge Pond (closed circles and solid lines) (linear model,  $F_{1,192} = 12.35$ ,  $P = 0.0006$ ). Increasing rainfall decreases mortality from (b) desiccation and (c) terrestrial predators (ants and wasps). Mortality from desiccation decreases with increasing rainfall (generalized linear model,  $F_{1,348} = 34.94$ ,  $P < 0.0001$ ) and varies between ponds ( $F_{1,347} = 26.62$ ,  $P < 0.0001$ ) and across years ( $F_{2,345} = 4.74$ ,  $P = 0.009$ ). Predation decreases with increasing rainfall ( $F_{1,348} = 20.46$ ,  $P < 0.001$ ) and varied across years ( $F_{2,345} = 13.45$ ,  $P < 0.0001$ ). Lines are predictions from (a) linear and (b and c) generalized linear models. Data points represent individual clutches monitored in the field. Clutch thickness is the average for each clutch across development, from twice-daily measurements.  $N = 200$  clutches observed from 2004–2005 in (a) and 350 clutches observed from 2003–2005 in (b and c).



**Fig. 3.** Timing of rainfall after oviposition affects *Dendropsophus ebraccatus* egg mortality at two ponds in Panama. (a) Total egg mortality increased in clutches not rained on during the first 24 h after oviposition (dark bars) at Bridge Pond but not at the more heavily shaded Ocelot Pond, where mortality was similar if eggs were rained on (light bars) or not during this period (generalized linear model,  $F_{1,346} = 0.8$ ,  $P = 0.38$ , pond,  $F_{1,346} = 66.4$ ,  $P < 0.0001$ , rain  $\times$  pond,  $F_{1,346} = 5.2$ ,  $P = 0.024$ ). (b) Eggs not rained during the first 48 h had higher mortality at both ponds, due mainly to increased desiccation and ant and wasp predation, compared to clutches that received rain during that period ( $F_{1,346} = 11.7$ ,  $P = 0.0007$ , pond,  $F_{1,346} = 68.3$ ,  $P < 0.0001$ , rain  $\times$  pond,  $F_{1,346} = 12.2$ ,  $P = 0.0005$ ). Asterisks indicate significantly different egg mortality.  $N_{24 \text{ hours}}$ : Bridge Pond – No rain = 70 clutches, Rain = 114 clutches; Ocelot Pond – No rain = 70 clutches, Rain = 96 clutches.  $N_{48 \text{ hours}}$ : Bridge Pond – No rain = 49 clutches, Rain = 135 clutches, Ocelot Pond – No rain = 40 clutches, Rain = 126 clutches. Data are mean  $\pm$  SE.

These amounts of hydration and dehydration correspond to the extremes of measured clutch thicknesses (Fig. 2a). Clutch hydration clearly affected wasp–egg interactions. Although hydrated and dehydrated clutches were positioned side by side, wasps visited dehydrated clutches more often than hydrated clutches ( $24.7 \pm 5.7$  vs.  $12.0 \pm 3.7$  total visits per clutch, respectively; paired  $t$ -test,  $t_9 = -3.21$ ,  $P = 0.01$ ). Wasps spent significantly more time feeding and exploring on dehydrated clutches than on hydrated ones ( $26.2 \pm 4.3$  vs.  $3.4 \pm 1.4$  min, respectively; paired  $t$ -test,  $t_9 = -5.41$ ,  $P = 0.0004$ ). Most importantly, wasps killed over seven times more embryos from desiccated clutches than from hydrated clutches (Fig. 4a; GLM, treatment,  $F_{1,18} = 87.629$ ,  $P < 0.00001$ ). Some embryos were killed and left in the clutch, some were eaten, and others were carried away, presumably to the wasp's nest. Neither age of the eggs nor clutch position on the brick (left or right) affected predation, and so these factors were excluded from the final model (GLM; age,  $F_{1,16} = 0.36$ ,  $P = 0.55$ ; side,  $F_{1,16} = 0.22$ ,  $P = 0.65$ ).

Clutches exposed to *Azteca* sp. ants varied in similar ways. Hydrated clutches had gained  $407 \pm 33\%$  of their original mass in water, while dehydrated clutches had lost  $58 \pm 2\%$  of their mass. Ants consumed 7.4 times more eggs from desiccated clutches than from hydrated clutches (Fig. 4b; GLM,  $F_{1,20} = 33.684$ ,  $P < 0.0001$ ). During predation choice trials, we

Model	d.f.	Deviance	Deviance explained	F	P value
Desiccation					
		17 505.7			
Rainfall	1,348	1318.6	0.075	34.94	<b>&lt; 0.0001</b>
Pond	1,347	1004.6	0.057	26.62	<b>&lt; 0.0001</b>
Year	2,345	358.1	0.020	4.74	<b>0.009</b>
Rainfall × Pond	1,344	18.5	0.001	0.49	0.48
Rainfall × Year	2,342	175.3	0.010	2.32	0.10
Pond × Year	2,340	107.8	0.006	1.42	0.24
Rainfall × Pond × Year	3,338	34.7	0.002	0.45	0.63
Total deviance explained			0.172		
Predation					
		14 023.3			
Rainfall	1,348	673.8	0.048	20.46	<b>&lt; 0.0001</b>
Pond	1,347	16.6	0.001	0.50	0.47
Year	2,345	885.7	0.063	13.45	<b>&lt; 0.0001</b>
Rainfall × Pond	1,344	230.3	0.016	6.99	<b>0.009</b>
Rainfall × Year	2,342	231.7	0.016	3.52	<b>0.031</b>
Pond × Year	2,340	181.3	0.013	2.75	0.065
Rainfall × Pond × Year	3,338	130.8	0.009	1.98	0.139
Total deviance explained			0.168		

**Table 1.** Summaries of quasibinomial GLM's of *Dendropsophus ebraccatus* egg mortality from desiccation and predation at two ponds in Panama from 2003–2005. The total deviance of each model is shown, along with the proportion of deviance accounted for by each predictor variable. The proportion of the deviance explained in a quasibinomial GLM is analogous to an  $R^2$  in a linear regression. Significant variables are highlighted in bold

observed more ants foraging on dehydrated clutches ( $15 \pm 3$  ants per clutch) than on hydrated clutches, where we never observed ants trying to eat eggs (paired  $t$ -test,  $t_{10} = 4.98$ ,  $P = 0.0005$ ). Although we did not see predation of hydrated eggs during our hourly observations, a small number were consumed during trials; we attribute this to ants since we saw no sign of other predators on these clutches. Ant predation was similar at both ponds where trials were conducted, and ants killed more 2-day-old eggs than 1-day-old eggs (GLM, pond,  $F_{1,19} = 0.55$ ,  $P = 0.47$ ; age,  $F_{1,18} = 6.9$ ,  $P = 0.02$ ).

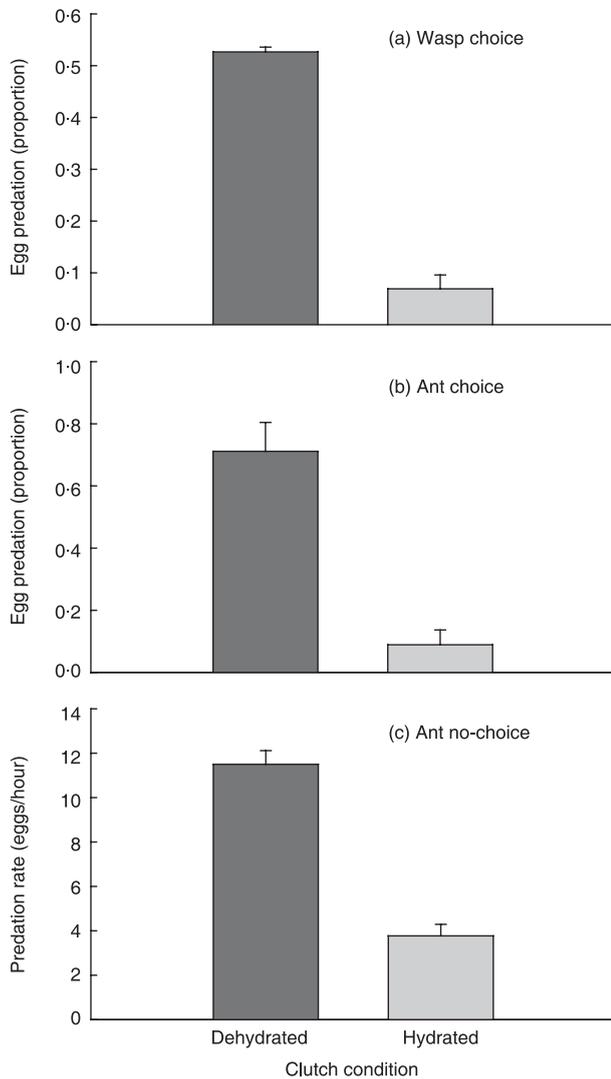
In no-choice tests ants also preyed on dehydrated clutches more than hydrated ones. All dehydrated eggs were eaten while only  $52 \pm 8\%$  of hydrated eggs were consumed, despite being exposed to ants for longer (GLM,  $F_{1,8} = 75.99$ ,  $P < 0.0001$ ). All dehydrated egg trials ended when all eggs were eaten, whereas all hydrated egg trials ended at nightfall. In addition, ants consumed dehydrated eggs three times faster than hydrated ones (Fig. 4c; paired  $t$ -test,  $t_4 = -15.22$ ,  $P = 0.0001$ ).

## Discussion

We demonstrate that short-term variation in rainfall patterns directly and indirectly influence the survival of a tropical anuran's arboreal eggs. It has long been recognized that overall precipitation limits biotic potential in arid ecosystems (Noy-Meir 1973; Leith 1975; Hawkins *et al.* 2003). Little work, however, has addressed how short-term fluctuations in rainfall affect survival and predator–prey interactions, particularly where rainfall is abundant, such as in rainforests during the rainy season. Nonetheless, even in ecosystems with abundant rainfall, organisms can be highly sensitive to changes in weather (Engelbrecht *et al.* 2006) and rainfall fluctuation can alter predator–prey interactions (Chalcraft & Andrews 1999). Rainfall patterns are predicted to change throughout this century (Hulme & Viner 1998; Christensen *et al.* 2007), and therefore warrant further attention.

*D. ebraccatus* lays its eggs on vegetation above ponds and, consequently, the eggs are vulnerable to terrestrial predators and desiccation – their primary sources of mortality (Fig. 1). Egg survival is highly dependent upon rainfall and the effect of predators and desiccation are not independent. Multiple environmental stressors are often more lethal than one alone (e.g., Relyea 2003; Rohr *et al.* 2004), and this appears to be the case with rainfall variation and predation. As more rain falls on a clutch the jelly around the eggs absorbs water, protecting eggs from desiccation (Fig. 2b) and thickening the physical barrier that blocks predator attacks (Figs 2a and 4). A lack of rainfall therefore diminishes clutch jelly thickness, facilitating easier predation by ants and wasps. The weather on the days following large rainstorms is critical. Large rainstorms stimulate more frogs to reproduce, and at one of our study ponds their eggs are 10–17% more likely to die if the ensuing days are dry (Fig. 3). Over 98% of eggs monitored at Bridge Pond died if not rained on during the first two days after oviposition.

Large-scale climate fluctuations, such as those that occur with the El Niño Southern Oscillation (ENSO), affect many types of organisms, including plants, herbivores and carnivores (Stenseth *et al.* 2002). These large-scale climate patterns have proved better predictors of population dynamics in some organisms, such as ungulates, than fine-scale weather measurements (Hallett *et al.* 2004; Owen-Smith, Mason & Ogutu 2005). However, for organisms that are short lived or are sensitive to changes in weather, fine-scale measurements may provide more accurate information about the conditions that influence individual survival and therefore provide insight into the mechanisms underlying population trends (Hallett *et al.* 2004). By monitoring weather on a day-to-day scale during our field observations, we detected the weather patterns important to the survival of *D. ebraccatus* eggs, revealing that even short dry spells lead to increased desiccation and predation. These findings would not have been evident looking at rainfall on longer time-scales, such as months, which vary little during the rainy season.



**Fig. 4.** Differential predation by ants and wasps on dehydrated (dark bars) and hydrated (light bars) clutches of *Dendropsophus ebraccatus*. (a) The proportion of eggs killed by social wasps (*Polybia rejeta* and *Agelaia centralis*) foraging on dehydrated and hydrated clutches in paired choice tests (generalized linear model,  $F_{1,18} = 87.629$ ,  $P < 0.0001$ ). Trials were stopped when mortality reached 50% in one clutch. (b) The proportion of eggs eaten by ants (*Azteca* sp.) from dehydrated and hydrated clutches in paired choice tests ( $F_{1,20} = 33.684$ ,  $P < 0.0001$ ). (c) The predation rate (number of eggs eaten per hour) on dehydrated and hydrated clutches exposed to ants during no-choice tests (paired  $t$ -test,  $t_4 = -15.22$ ,  $P = 0.0001$ ).  $N_{\text{wasp choice}} = 10$ ,  $N_{\text{ant choice}} = 11$ ,  $N_{\text{ant no-choice}} = 5$  clutches per condition. Data are mean + SE.

The effects of increased egg mortality on *D. ebraccatus* populations will depend on the nature and strength of density-dependent processes in later life stages, and might be negative, positive or neutral (Vonesh & De la Cruz 2002). Egg mortality can reduce population size (Vonesh & De la Cruz 2002). Reduced larval recruitment into a pond can also increase development rate and survival to metamorphosis via both density- and trait-mediated processes (Vonesh & Osenberg 2003; Vonesh 2005). Moreover, *D. ebraccatus* tadpoles invest in predator-specific defensive tail phenotypes (Touchon & Warkentin 2008b), and the magnitude of such morphological

changes may be affected by tadpole density (McCoy 2007). Further research is needed to elucidate potential community or population effects resulting from the weather variation documented here.

Rain increases *D. ebraccatus* egg defences by thickening the jelly around embryos (Fig. 2a). This hydrated jelly serves as both a water reserve for the eggs and a physical barrier between predator and prey, discouraging predators and reducing their foraging efficiency (Fig. 4c, Warkentin *et al.* 2006). When well hydrated, the jelly is thick and it is difficult for predator mandibles to reach through. However, when the jelly becomes desiccated and is thin, ants and wasps have much less difficulty reaching embryos (see Appendix S1 and S2, Supporting information for videos of these behaviours). Rainfall also has a direct benefit to *D. ebraccatus* eggs that we did not quantify here; rain alters predator behaviour. When it is raining, wasps and ants stop or decrease foraging (Spradbury 1973; Hölldobler & Wilson 1990). Most of our ant predation trials were ended by rainstorms, which caused the ants to return to their nests. Rainfall does not affect the behaviour of the prey in our system, since eggs are stationary. There might, however, be scent cues produced by desiccated clutches, or washed away when clutches are rained on, that increase detection of desiccated eggs or decrease detection of hydrated clutches.

The survival of arboreal *D. ebraccatus* eggs varied not just with rainfall levels, but also between ponds and across years (Figs 1, 2b and 3a, Table 1). The difference between ponds suggests that, in addition to rainfall, habitat structure has an important effect on survival. Oviposition sites at Ocelot Pond are generally more shaded than those at Bridge Pond, thereby staying cooler and more humid during the day (Williams-Linera 1990; Murcia 1995). During the peak of daily solar radiation, the air temperature at Bridge Pond routinely reaches upwards of 34 °C, whereas Ocelot Pond rarely gets above 28 °C (J. Touchon, unpublished data). Clutches are laid throughout Bridge Pond, even in areas that receive several hours of direct sunlight per day. *D. ebraccatus* is commonly found in disturbed habitats (Duellman 2001), which generally have less tree canopy than undisturbed sites (Peres, Barlow & Laurance 2006). Habitat disturbance and fragmentation will likely increase desiccation, resulting in higher mortality in comparison to more heavily shaded habitats.

The variation in egg desiccation and predation across years appear to be the result of much higher rainfall during our 2005 observations. The direct effect of year on desiccation and predation mortality is thus straightforward. However, we also found a significant interaction between year and rainfall on predation mortality (Table 1). Since egg jelly can absorb only a finite amount of water and predators can feed on hydrated eggs, although at a diminished rate (Fig. 4c), the relationship between increments of rainfall and predation varies with overall levels of rain. During drier periods, as in 2003 and 2004, increasing rainfall will have a strong effect on decreasing predation, whereas during wetter periods, as in 2005, increasing rain will have little effect because the egg clutches are already saturated.

Large rainstorms are predicted to become increasingly sporadic in the Neotropics throughout this century (Hulme & Viner 1998; Christensen *et al.* 2007). Larger rainstorms during the rainy season stimulate more frogs to reproduce in an evening; this response suggests that such storms have historically served as reliable cues of a suitable environment for egg development. However, if the ensuing days are dry, egg mortality increases dramatically. Other organisms developing during the rainy season may be similarly affected (e.g., Chalcraft & Andrews 1999; Engelbrecht *et al.* 2006). Less predictable rainfall and more disturbed habitats may compound the effects of desiccation and predation documented here.

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### Supporting Information

The following supporting information is available for this article online:

**Appendix S1. wasp\_feed\_dehyd.mpg:** A wasp (*Polybia rejecta*) feeding on a dehydrated *Dendropsophus ebraccatus* egg clutch during a predation trial.

**Appendix S2. wasp\_feed\_hyd.mpg:** A wasp (*Polybia rejecta*) feeding on a hydrated *Dendropsophus ebraccatus* egg clutch during a predation trial.

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