

## Dryness affects burrowing depth in a semi-fossorial amphibian

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### ABSTRACT

The fossorial life-history emerged as a response to hostile environmental conditions, such as extreme temperatures and dryness. It evolved independently in different lineages of amphibians, which are particularly vulnerable to dehydration and require a continuous level of humidity because of their permeable skin. For the first time, we test the hypothesis that amphibians are able to adapt their fossorial behaviour to substrate humidity, using the Pacific horned frogs (*Ceratophrys stolzmanni*) from the Ecuadorian dry forest as a model. When exposed to dry substrate, the animals burrowed much deeper than the ones in wet soil. The capacity to excavate was particularly high in this species: frogs from the dry treatment could be found at more than one-meter depth after just a few hours. Our results reveal a conditional strategy that contributes to explain the persistence of amphibians in dry environments.

### Keywords:

Aridity, burrowing, conditional strategy, fossorial amphibian, xeric environments

## 1. Introduction

Fossorial life is considered to be a behavioural strategy that offers protection from unfavourable environmental conditions such as extreme temperatures and dehydration (Bentley, 1966; Kinlaw, 1999), and has evolved repeatedly in various taxa (Kinlaw, 1999; Nomura et al., 2009). Among the species inhabiting xeric environments, amphibians are particularly dependent on underground shelters because of their permeable skin (Navas et al., 2008). Although most amphibians can use burrows opportunistically, some species are obligate burrowers (semi-fossorial), spending most of their life underground, and only emerging to forage and reproduce (Wells, 2010). The majority of semi-fossorial anuran species dig backwards (Emerson, 1976), and resulting burrows do not have a permanent structure due to gravitational collapse (Johnson and Hembree, 2015).

Detailed description of morphological adaptations to digging and of physiological traits that allow semi-fossorial anurans to spend extended periods underground are available for some taxa (Emerson, 1976; Secor and Lignot, 2010), but hardly any information is available regarding behavioural aspects of burrowing, with few studies focusing on burrow depth (Reynolds et al., 2011; Ruibal et al., 1969), and data generally coming from accidental excavation of individuals (Šklíba et al., 2016). The few studies in controlled laboratory conditions exploring dryness effects on burrowing behaviour only investigate the occurrence of emergence (Hoffman and Katz, 1989; Jansen et al., 2001). Amphibians are able to rehydrate by absorbing water from surrounding soil (Booth, 2006; Ruibal et al., 1969), so it had been suggested that burrowing anurans exposed to dry conditions would dig deeper into the substrate to seek moisture and rehydrate (Cartledge et al., 2006b). However, this hypothesis remained unconfirmed.

Given this background, we tested the hypothesis that burrowing depth is a conditional response to soil dryness. Specifically, we expected a greater depth to be reached in a dryer soil. We chose as a model a semi-fossorial species living in xeric environments: the Pacific horned frog (*Ceratophrys stolzmanni*). This species is active only during the rainy season (January–May), when individuals spend the days buried in the ground, and emerge during humid nights to feed and reproduce (Székely et al., 2018a,b). For the rest of the year, the frogs remain buried in the ground in a dormant state, covered in a cocoon of dead skin layers that diminishes water loss.

## 2. Material and methods

We collected 24 adult *C. stolzmanni* (12 males, 12 females) from Arenillas Ecological Reserve, Ecuador (03°34'S, 80°08'E, 40 m a.s.l.). The animals were brought to the lab and allowed to acclimate for at least one month before the start of the experiments. During this time, they were kept at a density of 4 individuals/terrarium (50 x 37 cm, 30 cm height), lined with a 15-cm layer of humid soil, and fed crickets *ad-libitum*. The snout-vent length and tibiofibular length of the

frogs were measured with a dial calliper. Experiments took place during the active season (April–May), after the reproductive period but before aestivation. Individuals were randomly assigned to one of two treatments: wet or dry (6 males and 6 females in each). Experimental conditions followed natural ones: temperature (16–25° C), air humidity (65–85%), and photoperiod (12L/12D). Individuals were not fed for three days before and during the experiment. The lack of feeding for several days is normal in fossorial amphibians under natural conditions (e.g. Wells, 2010). To eliminate hydration differences between individuals, immediately before placement in the experimental enclosures, the frogs were kept in shallow water (3 cm) for 30 min. As a burrowing substrate, we used Sungro Sunshine® Advanced Mix.

The experiments were set in high and narrow glass enclosures (65 x 6.5 cm, 150 cm height), allowing a clear view of the animal position even while buried, but giving the animal enough room to move and providing a burrowing depth of up to 130 cm (Fig. 1). The soil-filled part of the terrarium was covered with an opaque cloth (imitating underground darkness), which was partially removed only when checking for the position of the animal. To determine soil moisture corresponding to treatment, we oven dried soil until reaching a constant weight, then we added water until saturation (35 g dry soil hold 150 ml water). This water volume was considered 100% humidity. All soil used in the experiments was oven dried (110° C) until constant weight, then a corresponding volume of water was added, as follows: dry treatment 10% humidity, wet treatment 50% humidity. This procedure was repeated before each trial.

The burrowing behaviour was monitored during two consecutive nights, for a total of 39 hours. The frogs were introduced into the enclosures immediately after sunset (18:30), one individual per enclosure. The location of animals was checked every 3 hours during the night, when the animals are active (21:30; 00:30; 03:30; 06:30), and once during the day (09:30). Each animal was tested once. At the end of experiments, individuals were released at the capture site.

Statistical analyses were done using SPSS 21.0 (IBM Corp., Armonk, NY), with  $\alpha = 0.05$ . Because burrowing data (maximum depth, time necessary to reach maximum depth) were not normally distributed, we compared the treatment effects using Mann-Whitney U non-parametrical tests.

### **3. Results**

The frogs burrowed backwards (Appendix A in Supplementary material). They formed an approximately vertical tunnel, loosely filled by the surrounding substrate and ending in a chamber where the animal was located (Appendix B in Supplementary material). Individuals from the dry treatment dug deeper (median: 130 cm, range: 98–130 cm) compared to the wet treatment (33 cm, 13–79 cm;  $U = 0$ ,  $n = 24$ ,  $Z = -4.2$ ,  $P < 0.001$ ; Fig. 1). The time required to reach maximum depth

did not significantly differ between the dry and wet treatments (dry: median: 9h, range 3–27h; wet: 12h, range 9–39h;  $U = 45.5$ ,  $Z = -1.56$ ,  $n = 24$ ,  $P = 0.118$ ; Fig. 1). Burrowing parameters (i.e. depth of burrows and time to reach maximum depth) were not influenced by sex ( $U$  tests,  $U = 69$ ,  $P = 0.859$ , and  $U = 66$ ,  $P = 0.723$ , respectively), snout-vent length (Spearman correlation,  $\rho = 0.02$ ,  $P = 0.907$  and  $\rho = -0.24$ ,  $P = 0.251$ , respectively) or tibiofibula length (Spearman correlation,  $\rho = 0.002$ ,  $P = 0.993$  and  $\rho = -0.34$ ,  $P = 0.109$ , respectively) of the frogs.

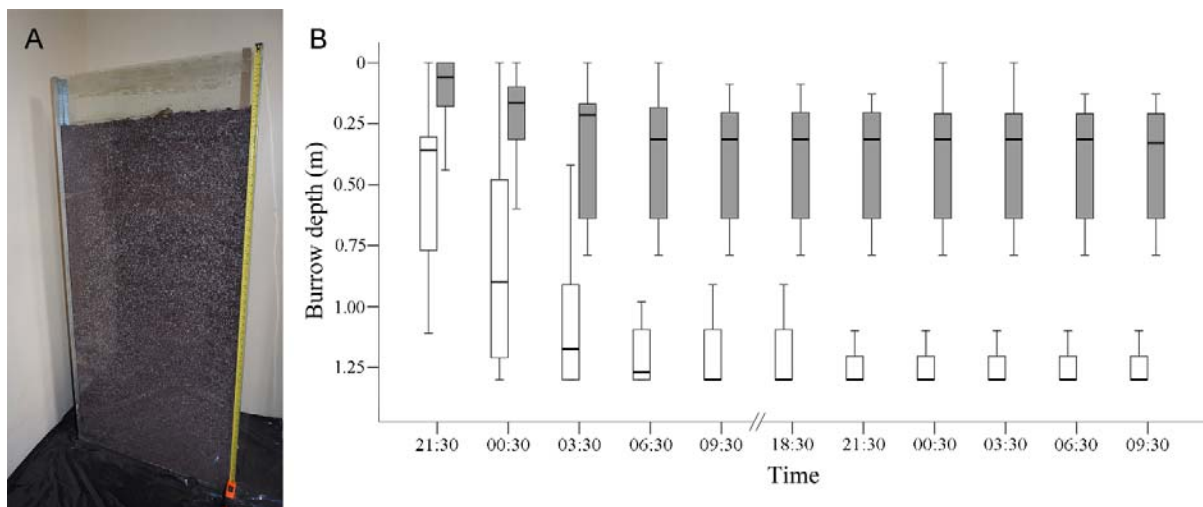


Fig. 1. A. experimental design and B. burrowing depth in *Ceratophrys stolzmanni* from wet (dark boxes) and dry (light boxes) treatment ( $n = 12$ /treatment) over the experiment duration (39 hours), that started at 18:30 when individuals were introduced in experimental enclosures. Interval between 9:30 and 18:30 (during daylight) was not checked. Box plots show the median (dark midline), percentiles 25–75 and minimum–maximum value not higher than 1.5 IQR.

#### 4. Discussion

The ethology of semi-fossorial amphibians is particularly difficult to document (Székely et al., 2017). The few studies investigating the behavioural adaptations that help semi-fossorial amphibians to cope with a decrease in humidity showed them to reduce their emergence frequency and activity time outside burrows (e.g. Ryan et al., 2015). Our experiment emphasizes a new key-aspect of this life-history strategy: amphibians exposed to drier conditions dig deeper into the soil.

The frogs were able to excavate to the maximum depth of soil provided (130 cm), and the majority reached their deepest location during the first 12 hours. The observed burrowing depth fits with maximum records of excavated anurans in the wild (90 cm in *Spea hammondi*, Ruibal

et al., 1969, and 240 cm in *Notaden nichollsi*, Thompson et al., 2005), but it is greater than other records for *Ceratophrys* species (15 cm in *C. ornata*, McClanahan et al., 1976). Our results relating to depth and speed are not directly applicable to all field situations: some soil characteristics (e.g. moisture tension and compaction) can be different in natural settings, compared to the ones we provided in the lab. Future studies on different kinds of soils are needed to determine how soil characteristics affect burrowing activity, and ultimately the success of amphibians across different environments.

We do not know if the frogs react to a change in osmotic pressure, searching for moister microhabitats to rehydrate through cutaneous water uptake, or if the dryness of the substrate acts as a trigger to retreat to deeper, aestivation burrows. In *S. hammondi* (Ruibal et al., 1969), summer burrows (daily retreating sites with shallow depth of 1–5 cm and high moisture) differed from winter burrows (depth of up to 91 cm, where toads remained the whole dry season). In other amphibians, burrowing for aestivation seems to be triggered not directly by a perceived decrease in humidity, but instead by some internal factor like a circannual rhythm or an energetic storage threshold (Tracy et al., 2007), such that individuals retreat for aestivation while the substrate is still moist (Cartledge et al., 2006a).

Whereas the semi-fossorial life is an obligate life-history trait for species such as the Pacific horned frog, we showed that the burrowing depth is clearly a response to soil dryness. Through their behavioural plasticity, the studied frogs are able to avoid the cost of digging deeper in wet soils, while keeping the potential to go deeper in case of dryness risk. This adaptation is of paramount importance to the persistence of semi-fossorial amphibians in arid landscapes.

**Ethics.** Experiments were conducted following the ASAB/ABS and ARRIVE guidelines on the use of animals in research, and permits were issued by Ministerio del Ambiente de Ecuador (MAE-DNB-CM-2015-0016) and UTPL Ethics Committee (UTPL-CBEA-2016-001).

**Authors' contributions.** DS and PS collected the data; all authors were involved in study design and manuscript writing, and approved the final version of the manuscript.

**Competing interests.** We declare we have no competing interests.

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## Appendix A. Supplementary data

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