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Effects of temperature and desiccation on the morphology, body condition, and metamorphosis of tadpoles and froglets of *Agalychnis moreletii*

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ABSTRACT

Hypothesis: Temperature and desiccation will increase growth rates in *Agalychnis moreletii* tadpoles, reducing size at metamorphosis and other morphological traits. Time to metamorphosis will also be reduced for froglets, and synchrony of metamorphosis will be decoupled.

Organism: Tadpoles and early froglets of the black-eyed tree frog (*Agalychnis moreletii*).

Site of experiments: Área de Protección de Flora y Fauna Nahá-Metzabok, Chiapas, Mexico.

Methods: We performed controlled experiments where we exposed tadpoles from natural populations to contrasting desiccation and temperature conditions. We measured snout–vent length (SVL) and tail length to obtain growth rates. Once metamorphosis was complete, we recorded day of metamorphosis and the number of conspecifics remaining in each replicate. We measured froglet mass, SVL, head width, forelimb length, hindlimb length, and three different body condition indices. Finally, we calculated the synchrony index and coefficient of variation of metamorphosis. We used a Kruskal-Wallis test or analysis of variance (ANOVA) to compare residuals of morphological traits among treatments, 'derived variable statistics' to analyse growth rate, a generalized linear model (GLM) to analyse the effect of number of individuals on morphological traits, and ANOVA and χ^2 to compare the synchrony index and the coefficient of variation.

Results: Growth rates differed significantly between treatments for body size and head width. Time to water emergence was shorter under high temperature conditions, but delayed under desiccation. In addition, we found a negative relationship between all morphological traits and body condition with number of individuals. Synchronization of metamorphosis was also different among treatments. Thus, temperature and desiccation affect the morphology, time of metamorphosis, and synchrony of tadpoles of *A. moreletii*, but there is also an effect of the number of individuals on morphological traits. In contrast to other studies, we found no increase in growth rate, or early metamorphosis, associated with desiccation risk. Also contrary to other studies, body condition did not differ among treatments, but there was an effect of number of individuals in each replicate. Finally, the synchrony of metamorphosis and coefficient of variation differed among treatments.

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INTRODUCTION

Phenotypic plasticity is the modification of phenotypic characters due to the interaction of genotype with environmental factors (Via and Lande, 1985). Plastic traits are controlled by both ancestry (genetics and parental investment) and environmental factors (resources, temperature, density, stress), which, together, lead to a variety of plastic responses (Crump, 1984; Loman, 2002; Castellano *et al.*, 2004; Dziminski and Alford, 2005; Tejedo *et al.*, 2010). Several morphological and life-history traits with plastic responses have been documented in numerous anuran species. Plasticity during the larval stages is particularly important because it allows larvae to develop successfully in heterogeneous and changing habitats (Newman, 1988; Székely *et al.*, 2010). Therefore, it is important to study the effects of intrinsic (e.g. parental effects) and extrinsic (e.g. environment effects) factors during anuran larval development.

Today, phenotypic plasticity likely plays a major role in buffering the effects of habitat change, as shifts in land use and global warming affect rainfall patterns and temperature (Cox *et al.*, 2000; Foley *et al.*, 2005; Radić *et al.*, 2013). Changes in rainfall patterns affect the amount of precipitation (mean rainfall per year), the continuity (if rainfall occurs on consecutive days) and seasonality of rains (delaying it or speeding it up). Furthermore, global warming is having an effect on the mean temperature of some habitats around the world, while also increasing variability in minimum and maximum temperatures (Rahmstorf and Ganopolski, 1999; Zhang and Delworth, 2005). Together, these aspects of environmental change can affect hydroperiods of ephemeral wetlands and patterns of reproductive activity in anurans, which are directly linked with larval growth, development, and successful metamorphosis (Prado *et al.*, 2005).

During and after metamorphosis, morphological traits are important for individual performance, and most have direct impacts on fitness. During tadpole development, tail length influences locomotor performance, while overall body size affects survivorship (Huey, 1980; Smith, 1987; Pfennig, 1992). Once metamorphosis is complete, other morphological traits increase in importance. For example, head width affects food acquisition, as a wider head permits the capture and consumption of larger and more nutritious prey (Lima and Moreira, 1993; Emerson, 1985); forelimb length is important for prey manipulation and locomotor performance; while hindlimb length determines jumping performance, which may be critical in avoiding predation and facilitating dispersal (Emerson, 1978; Tejedo *et al.*, 2000; Manzano *et al.*, 2008). Body condition after metamorphosis reflects the quantity of resources consumed, which provides the energy reserves necessary for survival and growth when metamorphosis is complete (Schulte-Hostedde *et al.*, 2005; Sztatecsny and Schabetsberger, 2005).

Traditionally, studies of tadpole development have focused mainly on the effects of environmental conditions, such as temperature, food availability, population density, or desiccation risk, on morphological and metamorphic traits (Wilbur, 1977a, 1977b; Merilä *et al.*, 2000; Álvarez and Nicienza, 2002; Relyea and Hoverman, 2003; Gonda *et al.*, 2010; Reading, 2010; Richter-Boix *et al.*, 2010; Székely *et al.*, 2010; Tejedo *et al.*, 2010). Few of these studies, however, have analysed the interacting effects of more than one environmental factor and have tended to focus on species from temperate regions. The thermal tolerance of amphibians is related to the temperatures experienced in the environment they inhabit. Given that mean temperature decreases and temperature range increases with increasing latitude, tropical anuran species may respond differently to changes in temperature compared with temperate anuran species, as a result of long-term adaptations and the greater stability of ecological conditions across seasons (Miller and Packard, 1974; Snyder and Weathers, 1975; Reading, 2007; Deutsch *et al.*, 2008).

Furthermore, there is a lack of information on how the conditions of larval development affect the synchrony of metamorphosis. In most study systems, synchrony is a trait that is tightly linked to individual fitness and anti-predator responses (Emlen and Demong, 1975; Augspurger, 1981). In anurans, individuals that emerge relatively late have lower fitness than those that emerge together earlier due to the risk of predation (Petranka and Thomas, 1995). In addition, several species of tadpoles form aggregations to reduce predation risk through a dilution effect (Watt *et al.*, 1997; DeVito, 2003; Spieler, 2003). Based on these studies, it is likely that the synchrony of metamorphosis is also relevant for anticipating the outcomes of environmental change for anuran performance.

Studying the interactions of environmental factors is thus important for understanding their influence on metamorphosis in tropical species. The aim of this study was to determine whether growth rate, morphological traits (in tadpoles and froglets), and metamorphosis (both timing and synchrony) are affected by variation in temperature, desiccation risk, and number of conspecifics.

METHODS

Study species and site

The tree frog *Agalychnis moreletii* (Duméril, 1853) occurs throughout Central America. During the rainy season (May to October), individuals congregate at ephemeral ponds to mate. Females lay their eggs on vegetation overhanging ponds, so larvae can complete their development in water. Larval development in *A. moreletii* is reported to be 120–121 days on average, depending on parental size (Briggs, 2013).

The present study was performed in Nahá, Chiapas, Mexico (16°56′–17°02′N, 91°33′–91°39′W; 800–1200 m above sea level), a protected area consisting mostly of evergreen tropical forest (Miranda and Hernández, 1963) where the mean annual temperature is 25°C and total annual precipitation is 2500 mm (Muench-Navarro, 1978).

Experimental design

We collected 20 clutches located on leaves in and around an ephemeral pond on 18 June 2017. Clutches were maintained under a 12/12 hour light/dark photoperiod at room temperature, and moistened once a day during the hours of darkness (to simulate light rain) with a water-spray to avoid egg desiccation. We photographed each clutch alongside a ruler and measured the diameter of yolks in each clutch using ImageJ (v.1.51) software to determine whether yolk size differed among clutches.

Once tadpoles hatched, individuals from different clutches were mixed (as we detected differences in yolk size; see Results section) to eliminate maternal effects on treatments and replicates. We assigned 31 ± 2 individuals at random to different treatments; each treatment had five replicates. To dissociate the effects of evaporation on natural conditions, we performed desiccation risk and high temperature treatments. Treatments were as follows:

1. Control (CT): constant water volume (5 litres) and low temperature (16–22°C).
2. Desiccation risk (DT): decreasing water volume during the experiment (300 mL per week) and low temperature.

3. Moderate temperature (MT): constant water volume (5 litres) and temperatures between 26°C and 28°C.
4. High temperature (HT): constant water volume (5 litres) and temperatures between 29°C and 31°C.

Temperatures of treatments were chosen based on the range of temperatures observed in the pond where clutches were collected (16.5–34.9°C). However, we did not choose temperatures above 31°C, as temperatures under natural conditions vary throughout the day, and constant high temperatures might have led to high mortality. We used commercial fish tank thermostats to regulate temperature in the moderate and high temperature treatments. Tadpoles were fed fish flakes (Wardley) daily at a rate of 0.2 g per replicate during the first month, then 0.4 g per replicate until metamorphosis. The water was changed when necessary to keep the water transparent enough to make individual measurements. We removed dead individuals from each replicate to avoid cannibalism and water eutrophication.

We photographed each replicate twice a week with a measurement reference (1 mm accuracy) using ImageJ (v.1.51) software. We measured the head width, body length, and tail length of each tadpole where possible – some individuals were not swimming horizontally when the photographs were taken. We calculated the mean of individual measurements per replicate for body growth rate estimation because we were unable to identify individual tadpoles.

Once froglets emerged from the water, identified as Gosner stage 43 or 44 (Gosner, 1960), we transferred each individual to a terrarium until their tail was completely reabsorbed. We recorded day of metamorphosis, counted the number of individuals present in each replicate at water emergence, and measured mass (g), snout–vent length (mm), head width (mm), forelimb length (mm), and hindlimb length (mm) using a scale (0.01 g) and callipers (0.01 mm). We calculated three body condition indices using data obtained on the day of emergence:

1. Fulton's index (K): $K = (W/\mathrm{SVL}^3) \times 10^5$ (Sztatecsny and Schabetsberger, 2005);
2. the relative mass condition index (W_r): $W_r = 100 \times (M/M_s)$, where M_s is body mass predicted in a linear regression of M and SVL (Hansen, 2005); and
3. the residual condition index (RCI) taken from the residuals of the linear regression in the relative mass condition (Denoël *et al.*, 2002).

We used these three body condition indices as they are based on different assumptions (isometric growth and comparison of the same life stage for the Fulton index; comparison of each individual's weight to a standard for a population for the residual and relative mass indices), and thus may reveal different patterns (Denoël *et al.*, 2002; Hansen, 2005; Sztatecsny and Schabetsberger, 2005; Băncilă *et al.*, 2010). Finally, we used the package 'flower' in R v.3.3.2 (Wang, 2015) to calculate the synchrony index of Augspurger (1983) and the coefficient of variation of each treatment. In this study, individuals that emerged from the water by day were considered analogous to and replaced data that would correspond to flowers opening by day. Synchronization indices range from 0 (synchronization totally absent) to 1 (mass synchronization).

Statistical analyses

For the analysis of morphological traits in tadpoles, we log-transformed the data and then performed linear regressions on each trait (head width, tail length, and total length) against body

length from the control (CT) data. We obtained the residuals of each trait from the linear models described above. Our aim was to analyse the effects of treatments with respect to the control, which represents the hypothetical basal conditions. For froglet data, we calculated the log of the value for each trait (head width, SVL, forelimb length, and hindlimb length), then corrected by log mass to avoid false results because of plastic responses occurring simultaneously on several traits. For example, reduced SVL in one treatment could result in longer limbs if corrected by that trait, so mass is a better corrector to assess differences because of its independence from the other traits, and assuming that all tadpoles had the same access to food. We performed Shapiro-Wilk normality tests on all datasets. As the distribution of data was not normal, we performed Kruskal-Wallis tests to assess differences in morphological traits in tadpoles and froglets among treatments and Dunn tests for paired differences.

We analysed growth rate using 'derived variable statistics' (Crowder and Hand, 1990; Diggle *et al.*, 1994; Davis, 2003), which consist of linear regressions of the mean data of each replicate, with time as the independent variable and body measurements (head width, body length, and tail length) as dependent variables. We considered the slope of each linear regression to be equivalent to growth rate and obtained a mean growth rate for each treatment, performed Shapiro-Wilk tests to ascertain normality of the data, and conducted analyses of variance (ANOVA) or Kruskal-Wallis tests to assess differences in growth rates among treatments. In addition, we performed Kruskal-Wallis tests to compare morphological traits among treatments and a full generalized linear model (GLM) that included the effect of number of individuals (as mortality was different among treatments), treatment, and their interaction on tadpole morphological traits to determine if there were isolated or additive effects of treatment and number of individuals. We ran the same analyses for body condition indices and morphological traits of froglets. Finally, we compared the synchrony index among treatments with an ANOVA and the coefficient of variation of synchrony with χ^2. All statistical analyses were performed in R v.3.5.0 (R Core Team, 2018).

RESULTS

Yolk diameter differed among clutches ($df = 15$, $F = 39$, $P < 0.001$, mean = 3.31 ± 0.37 mm). Tadpoles in the moderate temperature (MT) treatment did not metamorphose, while all froglets in the high temperature (HT) treatment were obtained from two replicates, as all the others died. The mean values of traits in tadpoles were higher in the control (CT) treatment, followed by the HT, desiccation risk (DT), and MT treatments. Furthermore, mass, head width, and SVL measures were higher in froglets in the HT treatment, followed by the CT and DT treatments, and both limbs were larger in the CT treatment than in the DT and HT treatments (Table 1).

Several morphological traits differed among treatments. Body length was different among treatments ($\chi^2 = 47.93$, $df = 3$, $P < 0.001$); individuals in the CT treatment were larger than those in the DT and MT treatments. In contrast, individuals in the MT treatment were smaller (Fig. 1A). Moreover, comparisons performed using data corrected by size did not follow the pattern exhibited by the mean values. Residuals showed differences in head width among treatments ($\chi^2 = 52.51$, $df = 3$, $P < 0.001$), with individuals in the DT treatment having narrower heads (Fig. 1B). The residuals of tail length were also different among treatments ($\chi^2 = 13.00$ $df = 3$, $P = 0.004$); individuals in the CT treatment had shorter tails, whereas individuals in the MT treatment had longer tails (Fig. 1C). In addition, total length was not different among treatments ($\chi^2 = 5.69$, $df = 3$, $P = 0.127$); individuals in the MT treatment were longer than those in all other treatments (Fig. 1D).

Table 1. Means and standard errors of morphological traits of tadpoles and froglets

	Tadpoles				Froglets				
Treatment	HW (mm)	BL (mm)	TL (mm)	Total L (mm)	Mass (g)	HW (mm)	SVL (mm)	FL (mm)	HL (mm)
Control	9.03 ± 0.13	15.70 ± 0.27	23.22 ± 0.53	39.34 ± 0.83	0.84 ± 0.01	8.39 ± 0.02	21.99 ± 0.35	18.61 ± 0.35	31.40 ± 0.19
Desiccation risk	8.36 ± 0.11	14.43 ± 0.23	21.74 ± 0.75	36.23 ± 0.75	0.77 ± 0.01	8.31 ± 0.02	21.39 ± 0.09	17.53 ± 0.02	30.25 ± 0.18
Moderate temperature	7.44 ± 0.21	12.12 ± 0.41	18.14 ± 0.90	29.87 ± 1.39	—	—	—	—	—
High temperature	8.65 ± 0.34	14.55 ± 0.67	21.42 ± 1.40	34.92 ± 2.14	0.90 ± 0.04	8.60 ± 0.08	22.40 ± 0.29	17.33 ± 0.29	30.59 ± 0.31

Note: Treatments are CT, control; DT, desiccation risk; MT, moderate temperature; and HT, high temperature. Traits are HW, head width; BL, body length; TL, tail length; Total L, total length; M, mass; SVL, snout–vent length; FL, forelimb length; and HL, hindlimb length.

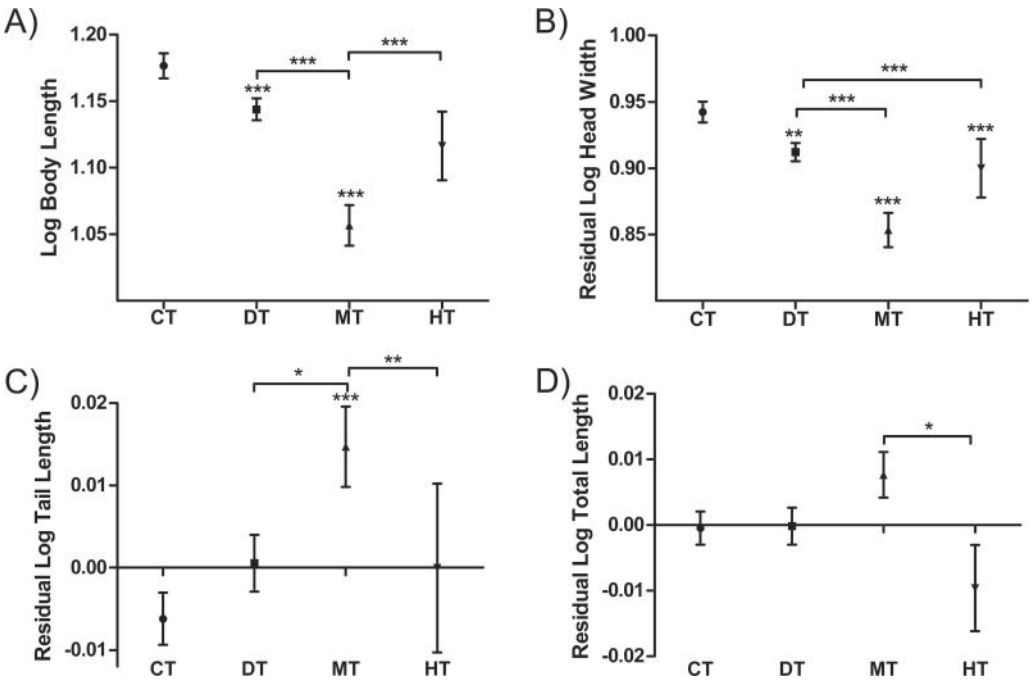

Fig. 1. Means and standard errors of morphological traits in tadpoles (corrected by log body length in panels B–D). Treatments are CT, control; DT, desiccation risk; MT, moderate temperature; and HT, high temperature. Data compared using the Kruskal-Wallis test. Asterisks represent significant differences with respect to the control treatment, while horizontal bars represent differences between pairs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

We found that treatment affected mass-corrected morphological traits of froglets. First, mass differed between treatments ($\chi^2 = 20.57$, $df = 2$, $P < 0.001$); individuals in the DT treatment had lower mass (Fig. 2A). However, there were no differences in head width among treatments ($\chi^2 = 2.33$, $df = 2$, $P = 0.311$) (Fig. 2B). There were differences in SVL among treatments ($\chi^2 = 7.92$, $df = 2$, $P = 0.018$); individuals in the CT treatment were larger than in the DT and HT treatments (Fig. 2C). Furthermore, forelimb length was different among treatments ($\chi^2 = 49.29$, $df = 2$, $P < 0.001$); individuals in the CT treatment had longer forelimbs than those in the DT and HT treatments, while individuals in the HT treatment had the shortest forelimbs (Fig. 2D). Finally, we found differences in hindlimb length among treatments ($\chi^2 = 22.05$, $df = 2$, $P < 0.001$); individuals in the CT treatment had longer hindlimbs than in the DT and HT treatments, while individuals in the HT treatment had the shortest hindlimbs (Fig. 2E).

Our results indicate that growth rates differed between treatments. Head width differed among treatments ($F = 72.79$, $df = 3$, $P < 0.001$); individuals in the DT treatment had narrower heads than those in the MT and HT treatments (Fig. 3A). Body length also differed among treatments ($F = 20.14$, $df = 3$, $P < 0.001$); it was shorter in the DT treatment than in the MT and HT treatments (Fig. 3B). Furthermore, tail length was different among treatments ($F = 52.38$, $df = 3$, $P < 0.001$), except in the DT vs. CT and MT vs. HT treatment comparisons (Fig. 3C). Finally, total length was different among treatments ($F = 29.90$, $df = 3$, $P < 0.001$), except in the DT vs. CT and MT vs. HT treatment comparisons (Fig. 3D).

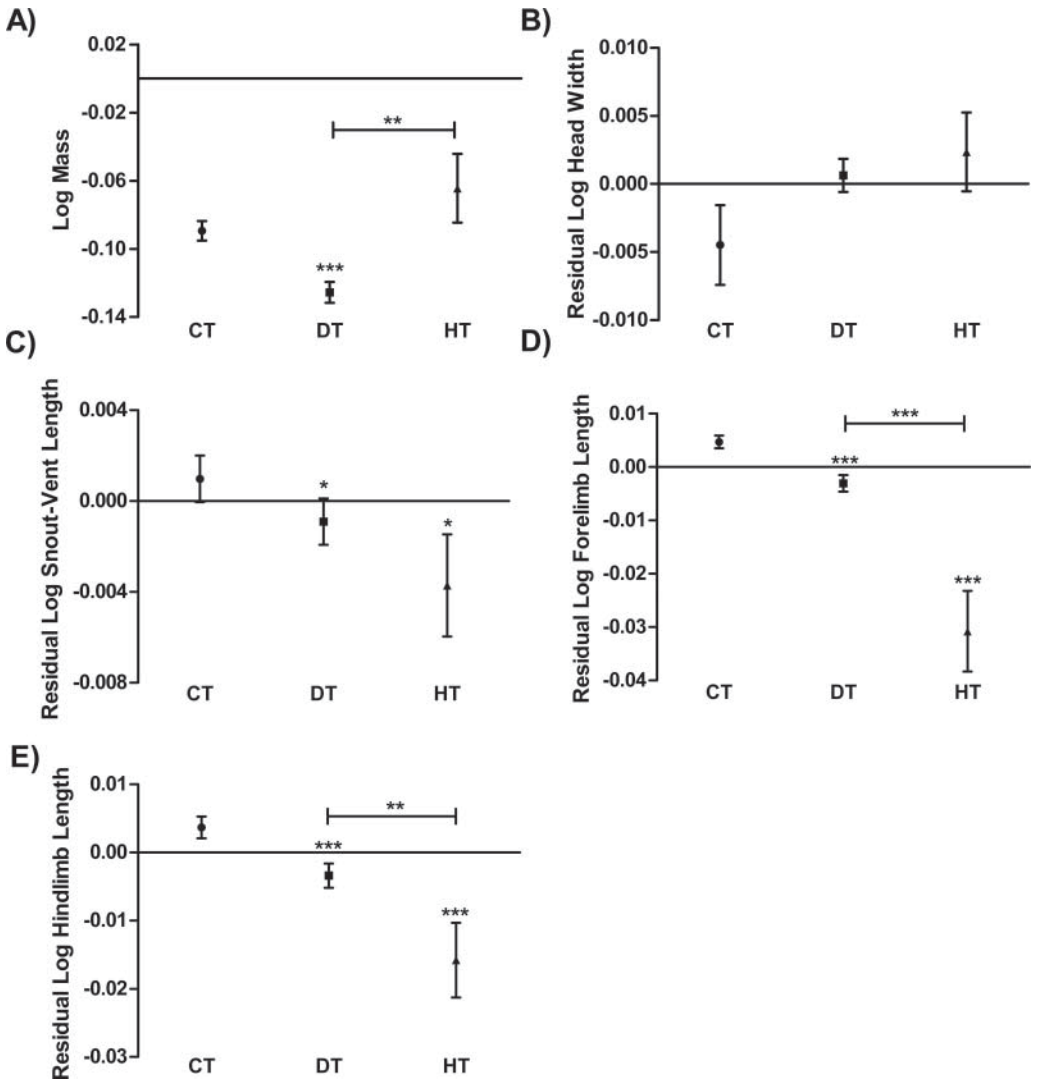

Fig. 2. Means and standard errors of morphological traits in froglets (corrected by log body length in panels B–E). Treatments are CT, control; DT, desiccation risk; MT, moderate temperature; and HT, high temperature. Data compared using the Kruskal-Wallis test and *post hoc* Dunn test. Asterisks represent significant differences with respect to the control treatment, while horizontal bars represent differences between pairs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The results of the full generalized linear model (GLM) that included the effect of number of individuals, treatment, and their interaction on morphological traits in tadpoles and froglets are shown in Table 2 and Table 3, respectively. In tadpoles, we found that the DT treatment increased tail length and total length; the MT treatment increased head width but reduced total length; and the HT treatment increased head width but reduced body length. The number of individuals increased head width and reduced body length and tail length. For the interaction between

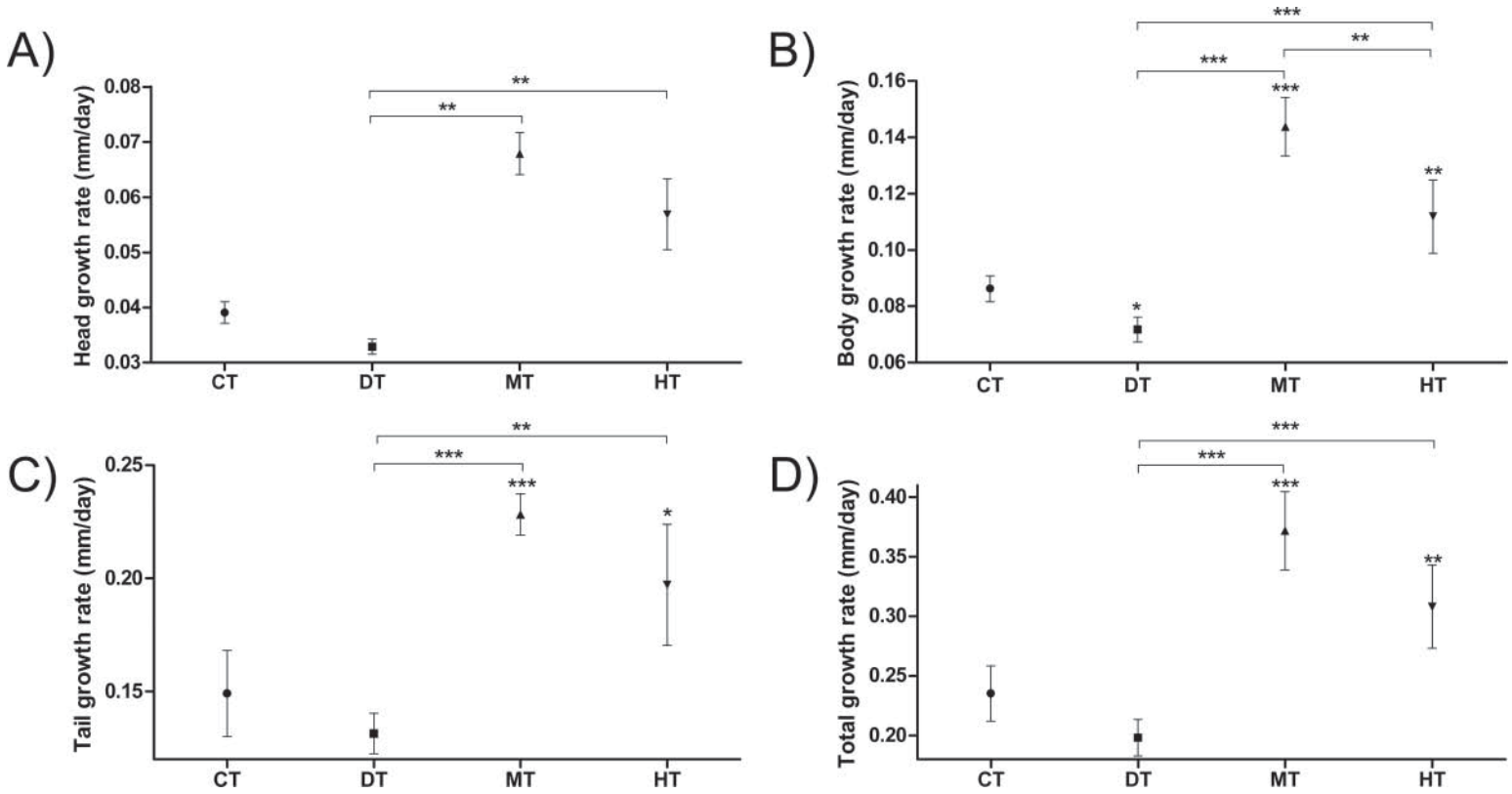

Fig. 3. Means and standard errors of growth rates by morphological trait in tadpoles. Treatments are CT, control; DT, desiccation risk; MT, moderate temperature; and HT, high temperature. Data compared using the Kruskal-Wallis test. Asterisks represent significant differences with respect to the control treatment, while horizontal bars represent differences between pairs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2. Results of GLM full model including treatment, number of individuals, and their interaction for morphological traits of tadpoles

Trait		Estimate	SE	*Z*	*P*
Head width	CT.DT	−0.0060	0.0052	−1.152	0.250
	CT.MT	**0.0343**	**0.0048**	**7.094**	**<0.001**
	CT.HT	**0.0275**	**0.0040**	**6.754**	**<0.001**
	Individuals	**0.0006**	**0.0001**	**4.980**	**<0.001**
	CT.DT × Individuals	<0.0001	0.0002	0.366	0.715
	CT.MT × Individuals	**−0.0016**	**0.0002**	**−5.983**	**<0.001**
	CT.HT × Individuals	**−0.0007**	**0.0001**	**−5.340**	**<0.001**
Body length	CT.DT	0.0481	0.0282	1.703	0.089
	CT.MT	−0.0444	0.0262	−1.696	0.090
	CT.HT	**−0.2119**	**0.0220**	**−9.599**	**<0.001**
	Individuals	**−0.0101**	**0.0006**	**−15.365**	**<0.001**
	CT.DT × Individuals	**−0.0027**	**0.0011**	**−2.510**	**0.012**
	CT.MT × Individuals	**−0.0109**	**0.0015**	**−7.117**	**<0.001**
	CT.HT × Individuals	**0.0057**	**0.0007**	**7.778**	**<0.001**
Tail length	**CT.DT**	**0.0331**	**0.0145**	**2.275**	**0.0233**
	CT.MT	−0.0279	0.0152	−1.835	0.0670
	CT.HT	0.0218	0.0121	1.799	0.0726
	Individuals	**−0.0009**	**0.0003**	**−2.888**	**0.0040**
	CT.DT × Individuals	−0.0010	0.0005	−1.827	0.0682
	CT.MT × Individuals	**0.0018**	**0.0008**	**2.052**	**0.0470**
	CT.HT × Individuals	0.0003	0.0003	0.876	0.3816
Total length	**CT.DT**	**0.0237**	**0.0112**	**2.102**	**0.0360**
	CT.MT	**−0.0245**	**0.0118**	**−2.075**	**0.0385**
	CT.HT	0.0161	0.0094	1.717	0.0865
	Individuals	0.0003	0.0002	−1.240	0.2156
	CT.DT × Individuals	**−0.0009**	**0.0004**	**−2.140**	**0.0328**
	CT.MT × Individuals	0.0009	0.0006	1.336	0.1823
	CT.HT × Individuals	<0.0001	0.0002	−0.319	0.7496

Note: Treatments are CT, control; DT, desiccation risk; MT, moderate temperature; and HT, high temperature. Significant differences are shown in **bold** font ($\alpha \leq 0.05$).

individuals and treatment, the results were as follows: individuals × DT reduced body length and total length; individuals × MT reduced head width and body length but increased tail length; and individuals × HT reduced head width but increased body length. In froglets, we only found a reduction in mass, body length, and hindlimb length as a result of number of individuals, although there was a general negative trend of individuals for all traits measured (Fig. 4).

The body condition indices revealed no significant differences among treatments. The relative mass condition index (W_r) was not influenced by either treatment or the number of individuals. Fulton's index (K) was impacted negatively by the number of individuals ($t = -2.023$,

Table 3. Results of GLM full model including treatment, number of individuals, and their interaction for morphological traits of froglets

Trait		Estimate	SE	*Z*	*P*
Mass	CT.DT	0.0253	0.0348	0.726	0.4685
	CT.HT	0.1083	0.1100	0.985	0.3260
	Individuals	**−0.0136**	**0.0008**	**−15.981**	**<0.001**
	CT.DT × Individuals	−0.0020	0.0017	−1.139	0.2562
	CT.HT × Individuals	−0.0233	0.0124	−1.876	0.0622
Head width	CT.DT	−0.0145	0.0134	−1.083	0.280
	CT.HT	0.0226	0.0365	0.619	0.537
	Individuals	−0.0004	0.0003	−1.304	0.194
	CT.DT × Individuals	0.0007	0.0006	1.099	0.273
	CT.HT × Individuals	−0.0026	0.0042	−0.628	0.531
Body length	CT.DT	−0.0049	0.0093	−0.531	0.5957
	CT.HT	0.0180	0.0256	0.903	0.4828
	Individuals	**−0.0005**	**0.0002**	**−2.598**	**0.0100**
	CT.DT × Individuals	0.0003	0.0004	0.641	0.5223
	CT.HT × Individuals	−0.0017	0.0030	−0.588	0.5569
Forelimb length	CT.DT	0.0120	0.0194	0.620	0.5361
	CT.HT	0.0787	0.0531	1.482	0.1397
	Individuals	−0.0034	0.0004	−7.335	<0.001
	CT.DT × Individuals	−0.0013	0.0009	−1.404	0.1617
	CT.HT × Individuals	−0.0177	0.0062	−2.843	0.0049
Hindlimb length	CT.DT	−0.0210	0.0151	−1.386	0.1674
	CT.HT	−0.0426	0.0413	−1.031	0.3039
	Individuals	**−0.0009**	**0.0003**	**−2.510**	**0.0129**
	CT.DT × Individuals	0.0008	0.0007	1.071	0.2852
	CT.HT × Individuals	0.0031	0.0048	0.656	0.5124

Note: Treatments are CT, control; DT, desiccation risk; MT, moderate temperature; and HT, high temperature. Significant differences are shown in **bold** font ($\alpha \leq 0.05$).

df = 206, *P* = 0.044). The residual condition index (RCI) was also impacted negatively by the number of individuals ($t = -5.806$, $df = 206$, $P < 0.001$), suggesting that density has a general negative effect on body condition indices (Table 4).

Time to metamorphosis was, at most, marginally different among treatments ($\chi^2 = 5.77$, $df = 2$, $P = 0.055$), being shorter when individuals were exposed to the HT treatment. The synchrony index differed among treatments ($F = 105.7$, $df = 2$, $P < 0.001$); in the HT treatment, synchrony of metamorphosis was non-existent. Individuals took from 43 to 151 days to metamorphose. High variability was reflected in the coefficient of variation of metamorphosis, which was highest in the HT treatment (Table 5).

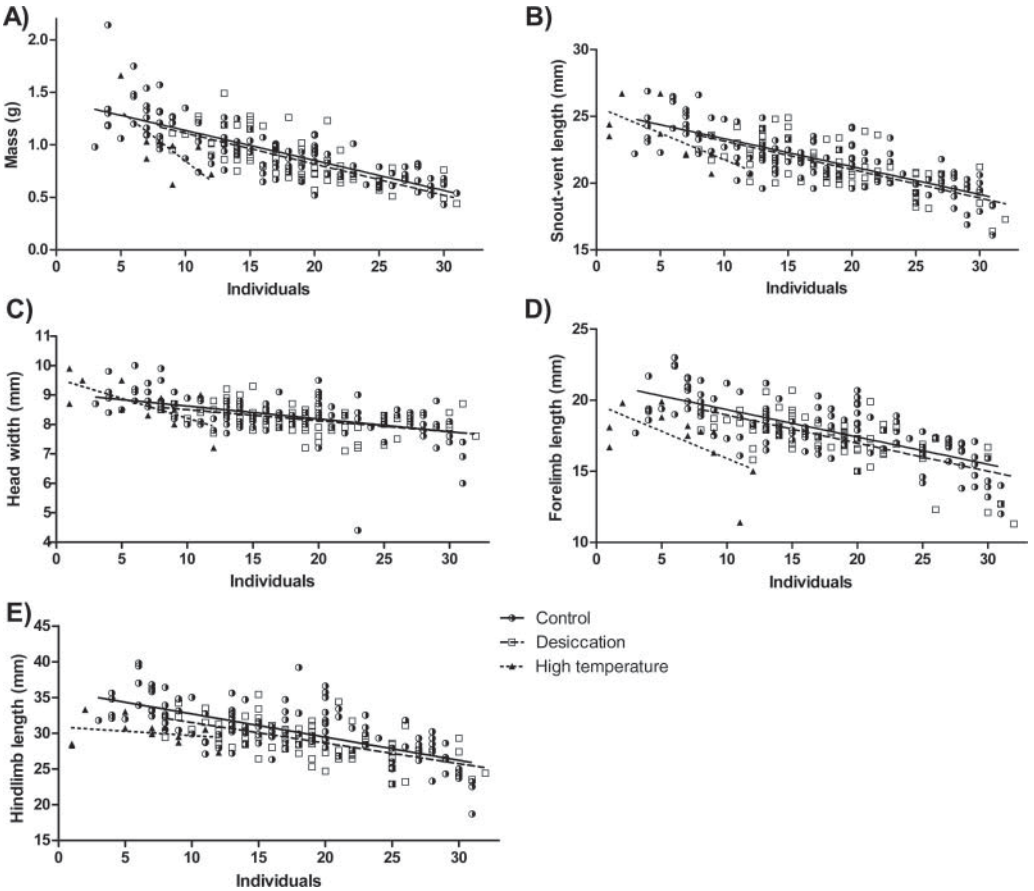

Fig. 4. Linear relationship between morphological traits of froglets and number of individuals when metamorphosis was complete: (A) mass, (B) snout–vent length, (C) head width, (D) forelimb length, and (E) hindlimb length. Treatments are ○, control; □, desiccation risk; and ▲, high temperature.

Table 4. Mean values (± standard errors) of body condition indices

Treatment	Fulton's index (K)	Relative mass condition index (W_r)	Residual condition index (RCI)
Control	7.82 ± 0.06	98.50 ± 1.46	0.0008 ± 0.005
Desiccation risk	7.82 ± 0.07	98.57 ± 1.79	0.0015 ± 0.007
High temperature	7.80 ± 0.22	97.68 ± 5.27	−0.0038 ± 0.018

Table 5. Mean values of time to metamorphosis (± SE), synchrony index (± SD), and coefficient of variation of all treatments

Treatment	Time to metamorphosis	Synchrony index	Coefficient of variation
Control	112.29 ± 2.47	0.45 ± 0.004	0.86
Desiccation risk	116.72 ± 2.92	0.29 ± 0.03	0.74
High temperature	106.41 ± 4.87	0.0 ± 0.0	1

DISCUSSION

We found differences in yolk size among clutches. Egg-yolk provisioning is a variable trait in anurans, particularly for species that reproduce in ephemeral ponds, where such variability may work as a bet-hedging strategy in response to environmental unpredictability (Crump, 1984; Dziminski and Alford, 2005). In ephemeral ponds, the environmental conditions (e.g. permanency, amount of resources, predators) are unpredictable and highly dynamic, thus differential yolk provisioning may be advantageous under changing conditions and, ultimately, may facilitate metamorphosis in *Agalychnis moreletii*.

Environmental factors can modify several tadpole traits that likely affect individual fitness, such as time to metamorphosis, body size, head width, tail size, limb length, and body condition (Huey, 1980; Emerson, 1985; Reading and Clarke, 1995; Tejedo *et al.*, 2000; Vega-Trejo *et al.*, 2014). Body size, mass, and body condition are tightly linked to fecundity and survival when individuals become adults. In general, females prefer calls that are of low frequency, high duration, and highly repetitive, and only males with large size and mass and good body condition are capable of producing such calls. Thus, these traits are likely under strong sexual selection (Forester and Czarnowsky, 1985; Reading and Clarke, 1995; Bastos and Haddad, 2002). Therefore, it is necessary to understand the role of each morphological trait on individual performance (locomotion, feeding, mate selection), and how such traits might change with environmental conditions during metamorphosis, to assess the potential long-term consequences for fitness and population performance.

Tadpole performance is related to temperature, as higher temperatures promote faster growth rates by accelerating metabolic processes such as food absorption (Marian and Pandian, 1985). However, high temperatures can also have negative effects on tadpole locomotor performance, or even increase morality (Miller and Packard, 1974). Most studies of the effects of temperature on tadpoles have focused on temperate ecosystem species, which have higher tolerance to temperature fluctuations than tropical species (Snyder and Weathers, 1975; Navas, 1996; Deutsch *et al.*, 2008). In the present study, mortality was higher in the high and moderate temperature treatments (higher in the HT than the MT treatment). This mortality leads to a reduction in the number of individuals, and higher food availability per capita for survivors. Low food availability has been associated with lower average larval growth and size at metamorphosis (Travis, 1984). We found that tadpoles in the HT treatment were of similar size to, or even larger than, those in the control (CT) treatment, and there was a similar pattern in froglets. These results suggest that there is a complex relationship between temperature and individual performance that is mediated by temperature effects on mortality and competition. This effect was clear, as individuals in the MT treatment had lower mortality, were smaller, and had higher densities than those in the HT treatment, which had negative effects on growth and size (Wilbur, 1977a; Dash and Hota, 1980).

We found that higher temperatures increased growth rate, a commonly observed pattern in anurans (Álvarez and Nicienza, 2002). However, we also observed that individuals in the higher temperature treatments were larger and heavier than those in the low temperature treatments, which is inconsistent with other studies in which individuals subjected to high temperatures tended to grow faster but were smaller at metamorphosis (Marian and Pandian, 1985; Reading, 2010). However, this result might stem from changes in the amount of food available to each individual, as explained previously. Other studies have shown that amphibians are capable of reducing their susceptibility to variation in pond desiccation as an environmental stressor by increasing their growth and developmental rate for survival (Székely *et al.*, 2010). Our results indicate that rate of growth in terms of head width and body length was significantly lower in the desiccation risk (DT) treatment than in the CT treatment, contrary to previous studies of species inhabiting sites with marked seasonal changes in rainfall patterns (Mogali *et al.*, 2017).

The relative inability of *A. moreletii* to accelerate its growth and development in response to pond drying could signal severe demographic consequences under future drought conditions.

In addition to temperature and desiccation, the number of individuals present in ponds had negative effects on growth, body size, limb length, and mass (Wilbur, 1977a; Morin, 1986; Loman, 2001; Relyea, 2004; Relyea and Auld, 2005). Our results are consistent with the literature, as we found an effect of number of individuals in all treatments, but we also found an interaction between treatment and number of individuals for several traits. In future studies, it would be prudent to consider the interactions between factors and consider even more variables (predation, density, amount of food) to understand how environmental change might ultimately shape morphological traits and performance. Additionally, we found that the interaction of number of individuals and treatment affects body condition, highlighting the importance of considering the number of individuals in the manner described above. In anurans, body condition is a trait that is critical for fitness, as individuals with better body condition have higher survival and fecundity (Reading and Clarke, 1995). In this study, we did not find differences in body condition among treatments, but there were differences in mass and size. Individuals in the DT treatment had reduced body size and mass, although these differences were not reflected in body condition because of the correlation between these traits.

There were no differences in time to metamorphosis among treatments, likely due to the high coefficient of variability in time to metamorphosis among individuals in treatments; some individuals metamorphosed on day 43, whereas others took over 151 days (more than a three-fold difference). This variability may relate to variability in parental genotypes and phenotypic traits (maternal effects) in *A. moreletii* (Briggs, 2013), but may also be the result of environmental conditions. Synchrony of metamorphosis differed among treatments. The DT and HT treatments showed reduced synchronization in time to metamorphosis. In habitats where many other species breed at the same time and resources are limited, low synchrony could benefit individual fitness by reducing competition for food (Wilbur, 1977b; Smith, 1983). In other ecological contexts with high numbers of predators, both in aquatic and terrestrial habitats, marked synchrony could benefit survival by reducing predation risk per capita (Watt *et al.*, 1997; DeVito, 2003; Spieler, 2003). Therefore, the effect of synchrony on fitness likely depends on the ecological context. Because we only examined the larval stage here, we encourage future researchers to examine synchrony in metamorphosis under natural conditions to understand the role of this trait from an evolutionary perspective.

In conclusion, temperature and desiccation have different effects on several morphological traits, growth rate, and synchrony of metamorphosis. We have also shown that the number of local individuals affects morphological traits and body condition indices. *Agalychnis moreletii* does not exhibit a response to desiccation risk via increased growth rate or reduced time to metamorphosis. Although studies of anuran larval development are abundant, there is still a gap in our understanding as to how different environmental factors interact to produce specific plastic responses. Filling these gaps is critical given that future global change scenarios predict changes in rainfall patterns in the tropics, together with increases in temperature (Rahmstorf and Ganopolski, 1999; Cox *et al.*, 2000; Zhang and Delworth, 2005). If the predictions are accurate, these changes could lead to catastrophic consequences for our study species because of its low thermal tolerance limit compared with other species and its poor response to desiccation risk. However, individuals that survived under higher temperatures were larger (in size and mass) and had a reduced time to metamorphosis, which might offset the mortality effects of increasing temperatures.

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