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The Correlates of in situ Larval Survivorship of the Threatened South American Toad *Melanophryniscus montevidensis* (Anura, Bufonidae)

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Abstract. Population declines are noticeable when adults disappear locally or massive die-offs are reported. However, populations can slowly decline if recruitment is unsuccessful. We aimed to determine the recruitment rates and the biotic factors involved in the metamorphosing success of a threatened species, *Melanophryniscus montevidensis.* Using in situ enclosures in ponds of a protected area (Laguna de Rocha, Rocha, Uruguay), we assessed larval survivorship, body size, and duration of development, from eggs to the end of metamorphosis. Also, we evaluated which factors of the larval dynamics (density, body size, predation, location, and depth) better explain the overall survivorship using generalized linear models (GLMs). We recorded metamorphs in only 8 of 25 clutches, accounting for a median success per clutch of 1.2%. Median development time until Gosner stage 42 was 26 days, with a median body length of 6.1 mm. The best simplified GLM for survival success included number of larvae (recorded 10 days before sampling) and depth as significant negative predictors and body length as a positive predictor. Depth and body length had the greatest effect. The body size at metamorphosis was negatively correlated to development time, suggesting a delayed growth possibly involved in the lack of response to pond duration (causing mass mortalities) in some enclosures. In spite of the potential plasticity of the species to respond to pond desiccation, we predicted that ponds that dry in less than 20 days might not yield any offspring. The in situ data provided by this study may help understanding the processes behind the decline of *M. montevidensis,* thus helping to establish proper conservation actions for the populations and the ponds where they breed.

Keywords. Amphibian conservation; Body length; Density; Larval development; Metamorphosis; Predation.

Resumen. Las declinaciones poblacionales son evidentes cuando los adultos desaparecen localmente o son reportadas muertes masivas. Sin embargo, las poblaciones pueden disminuir lentamente si el reclutamiento es bajo. Nuestro objetivo fue determinar las tasas de reclutamiento y los factores bióticos involucrados en el éxito de la metamorfosis de una especie amenazada: *Melanophryniscus montevidensis.* Para ello medimos la supervivencia larvaria, el tamaño corporal y la duración del desarrollo, desde la desova hasta el final de la metamorfosis, utilizando encierros in situ en charcas de un área protegida (Laguna de Rocha, Rocha, Uruguay). Además, se evaluó cuáles factores de la dinámica larvaria (densidad, tamaño corporal, depredación, sitio, profundidad) explicaron mejor la supervivencia global usando *generalized linear models* (GLMs). Registramos metamorfos sólo en 8 desovas de 25, lo que representó un éxito por desova del 1,2%. La mediana del tiempo de desarrollo hasta la etapa 42 de Gosner fue de 26 días, con una mediana del tamaño corporal de 6.1 mm. El mejor GLM simplificado para el éxito de supervivencia incluyó el número de larvas (registrado 10 días antes) y la profundidad como predictores negativos significativos, y el tamaño corporal como predictor positivo. La profundidad y el tamaño corporal fueron los factores de mayor peso. El tamaño corporal de los metamorfos se correlacionó negativamente con el tiempo de desarrollo, sugiriendo un retraso en el crecimiento posiblemente involucrado con la falta de respuesta a la duración de la charca (ocasionando muertes masivas) en algunos encierros. A pesar de la posible plasticidad de la especie para responder a la desecación de las charcas, aquellas con una duración menor a 20 días no permitirían el reclutamiento en estas poblaciones. Los datos in situ proporcionados por este estudio pueden ayudar a entender los procesos que están detrás de los declives de *M. montevidensis,* así como establecer acciones de conservación apropiadas para las poblaciones y las charcas donde se reproducen.

INTRODUCTION

In the context of the current amphibian declines, death and decreased recruitment (reproductive failure) are the immediate results of ultimate declining causes, such as atmospheric change, environmental pollutants, habitat loss, invasive species, and pathogens (Stuart et al., 2004; Hayes et al., 2010; Egea-Serrano et al., 2012; Carvalho et al., 2017). Although population declines are more noticeable when adults disappear or massive die-offs are reported, populations can decline slowly if healthy adults are not breeding or if offspring do not develop properly (Hayes et al., 2010). As most anurans have complex life cycles, to assess if a non-conspicuous decline of a given population is occurring (e.g., by using stage-structured models of the population dynamics), vital rates of both

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larval and adult stages in nature should be assessed first (e.g., Biek et al., 2002; McCaffery and Maxell, 2010).

Regarding larval stages, survivorship is the most direct measure of recruitment (Cecil and Just, 1979; Berven, 1990). However, body size at the end of metamorphosis plays an important role in the post-metamorphic fitness of many anuran species, with larger sizes at the end of metamorphosis being advantageous for later terrestrial stages (Werner, 1986; Berven, 1990; Altwegg and Reyer, 2003; Cabrera-Guzmán et al., 2013). Both survivorship and larval growth are usually density-dependent, with competition for food being one of the most relevant factors leading to the decrease of both parameters at high densities (Wilbur and Collins, 1973; Semlitsch and Caldwell, 1982). Alternatively, density can have positive effects on survivorship by reducing predation risk in aggregations (DeVito, 2003). Also, in order to avoid predation anuran, larvae are selected to grow fast, with a minimum threshold body size to be reached for successful metamorphosis (Wilbur and Collins, 1973; Werner, 1986).

Few studies have addressed the relative importance of larval and predator densities and body size on the metamorphosis success that leads to recruitment in natural populations of amphibians (e.g., Newman, 1998; Touchon et al., 2013). Moreover, most studies addressing the effects of parameters on larval survivorship have been carried out in laboratorial conditions, where survival rates may be biased (Melvin and Houlahan, 2012, 2014).

Possibly because of predator-avoidance selective pressures (Woodward, 1983; Kats et al., 1988), species of Bufonidae breed at highly ephemeral water bodies where they maximize developmental rate at the expense of growth, leading to very small body sizes at metamorphosis (Richter-Boix et al., 2011). Although these small body sizes would not be disadvantageous to their post-metamorphic fitness (Werner, 1986), bufonids grow at a maximum rate during larval stages, having little margin to respond to the stressful conditions of a drying pond (Richter-Boix et al., 2011) and reach their threshold body size for successful metamorphosis.

Melanophryniscus montevidensis (Philippi, 1902) is a bufonid from the coastal region of Uruguay and southern coast of Rio Grande do Sul (Brazil) that undergoes fast larval development in ephemeral ponds (Garrido-Yrigaray, 1989; Prigioni and Garrido, 1989). These features make it an excellent model for in situ studies on how larval and predator densities, body size, and development time interact and affect the larval recruitment of a bufonid species, helping understand the role these factors play in development during the drying process of ponds. Further, *M. montevidensis* is threatened both globally (Vulnerable for the IUCN; Langone, 2004) and regionally in both countries where it occurs (Critically Endangered in the Uruguayan Red List, Carreira and Maneyro, 2015; Near Threatened and Endangered in Brazilian national and state-level lists, respectively; MMA, 2014; GERGS, 2014). Assessing the metamorphosis success (recruitment) of this species in pristine habitats would improve understanding of its population declines, either by comparing with future reports or by including them in stage-structured models (e.g., Biek et al., 2002; McCaffery and Maxell, 2010).

The aim of this study was twofold. First, using in situ enclosures, we assessed the larval survivorship, body size, and duration of development (from egg to the completion of metamorphosis) of *Melanophryniscus montevidensis* in fairly natural conditions in ponds of a protected area. Second, we evaluated how different variables of the larval dynamics (density, predation, body size, and development time) interact to explain the in situ metamorphosing success and which biotic variables had the greatest effect on the overall survivorship.

MATERIALS AND METHODS

General background on the species

Melanophryniscus montevidensis is an explosive breeder (sensu Wells, 2007) that exploits shallow ponds (less than 30 cm deep) formed over psamophilic vegetation after heavy rains, possibly during warm months (Alonzo et al., 2002; Pereira and Maneyro, 2016; Bardier et al., 2019). Breeding activity is mainly diurnal; males floating on the water surface or grasping aquatic vegetation start calling immediately after rains. Amplectant pairs lay eggs in several small masses attached to pond vegetation (Alonzo et al., 2002), and metamorphosis finishes approximately 30 d after hatching (Garrido-Yrigaray, 1989; Prigioni and Garrido, 1989). No gregarious behavior has been described for the larvae. Although the larval diet has not yet been described, they seem to have benthic habits (Garrido-Yrigaray, 1989; Prigioni and Garrido, 1989; Alonzo et al., 2002; Baldo et al., 2014; Pereira and Maneyro, 2016).

Sampling design

We studied the larval development of *Melanophryniscus montevidensis* at three ephemeral ponds at Laguna de Rocha (for description see Table S1), a protected area of the National System of Protected Areas (34°37′S, 54°17′W, 3–10 m a.s.l.), Rocha Department, Uruguay. We observed three breeding events in the ephemeral ponds after heavy rains occurred in February 2014. We obtained climate data before and during sampling days from (Davis Instruments, 2020; Table S2). Each breeding event lasted 2–3 d (Table S2). During those days, we searched, collected, and stored amplectant pairs in plastic containers of ca. 1 L (one amplectant pair per container, with water and vegetation from the pond), a procedure previously

employed to obtain clutches from this species in field conditions (Alonzo et al., 2002; Pereira and Maneyro, 2018).

We collected 29 amplectant pairs; 28 were female-male and one was a male-male pair. Among the 28 female-male pairs, 25 spawned inside the containers within 24 h after capture: 6 from pond 1, 10 from pond 2, and 9 from pond 3. Following oviposition (regarded as day 1 of development), we released the adults in the same places where they were originally captured. We conducted an experimental design for which we recorded the number of eggs per clutch and then placed each clutch (from each amplectant pair) in an in situ semi-permeable enclosure at the exact point where the amplectant pair was first observed (Fig. 1). Enclosures were 32-L cages (43.0 × 27.4 × 27.1 cm, length × width × height) constructed from polypropylene, a safe, relatively inert polymer that does not contain Bisphenol-A and is used in food and water containers (Maier and Calafut, 1998). We made perforations to allow water flow in two sides of the cages and covered the sides with a thin plastic mesh (1 × 1 mm). We glued the mesh to the inner part of the cage using Sikaflex 1a (Sika AG, Zugerstrasse, Switzerland), which is used for potable water containers. To emulate natural conditions, we fixed the enclosures to the bottom of the pond and added some emergent vegetation to provide shelter while tadpoles exploit the whole water column. As the diet of the larvae was unknown, we paid special attention to the trophic behavior of the larvae in order to discard carnivore or macrophage diets. None of these behaviors were observed during the study. We observed larvae feeding only on the periphyton that grew on the walls and floor of the enclosures or on decomposing leaves of emergent plants, so we did not add extra food. A wide plastic mesh (14 × 19 mm) covered each enclosure to avoid avian or mammalian predation but not invertebrate predators. Common invertebrate predators for amphibians like coleopteran and odonate larvae were present in the three ponds outside the enclosures; we did not observe ponds without predators in the area during the sampling period. Although these predatory larvae enter the enclosures through the thin mesh, eggs smaller than 1 mm² could have entered (e.g., Joop et al., 2007) or been layed by adults through the wide mesh.

Figure 1. Enclosures in pond 1 at Laguna de Rocha, Rocha Department, Uruguay (photographed by C. Bardier).

We monitored the clutches once every 2 d until the end of metamorphosis or complete desiccation of the pond (no water in the enclosures) for a maximum of 35 d. We took pictures inside the enclosures during each sampling day to record the number of larvae and their body length using a Coolpix L810 16 megapixel camera (Nikon Corporation, Tokyo, Japan). We included a 1.0-mm ruler as a scale in the picture frames, and the distance and lighting remained roughly constant to standardize images and facilitate digital measurements. Metamorphs were photographed using the same criteria. Each time we took pictures (at 10:00–18:00), we measured water temperature and level inside the enclosures using a digital thermometer (Oakton® Instruments) and a wooden yardstick. To reduce the depth variation, each time the depth decreased below the level of the previous sampling day we moved the enclosures to the nearest deeper zone of the pond (if it existed; see the example plotted for three of the six enclosures from pond 1 in Fig. S1). Because of the shallow initial water level of the ponds, the water level of the enclosures was usually below 13 cm (half of the enclosure height; Table S1, Fig. S1).

We measured and counted larvae and eggs using the Micrometrics® SE Premium (ACCU-SCOPE, 2010) and ImageJ 1.48v (Rasband, 2012) software, respectively. We considered body length (BL) from the tip of the snout to the body terminus, excluding the tail (sensu McDiarmid and Altig, 1999). We observed surviving individuals in larval stages (stages 26–30; Gosner, 1960) 7 d after oviposition, so we considered that hatching success was achieved on this day. Predators (coleopteran and odonate larvae) became observable starting 6–7 d after oviposition and were observed predating *Melanophryniscus montevidensis* on subsequent days. On those days, we drew a random square of 12 × 12 cm on the floor of the enclosures to estimate predator density (predator/cm²). For the purpose of this study, we considered an individual to have successfully completed metamorphosis when it reached Gosner stage 42 because at this stage we observed the animals avoiding water by climbing the plastic mesh of the en-

closures above the water level. In captivity using proper humidity levels, specimens of *M. montevidensis* at this stage were already terrestrial (Bardier et al., 2017). Some specimens reached this stage 20 d after oviposition, so we considered this day to be the start of the metamorphosing stage of development in further analysis.

Analyses

We generated descriptive statistics for the following variables of each lutch of each amplectant pair: total number of eggs, hatching success (over total eggs, the percentage of surviving individuals 7 d after oviposition), metamorphosis success (over total eggs, percentage of surviving individuals that reached Gosner stage 42), median time of development, and median BL of larvae and metamorphs. We searched for differences in larval performance (number of eggs laid and percentages of larvae and metamorphs) between the three ponds. Whenever comparisons were possible and the assumptions of homoscedasticity (Bartlett and Fligner-Killeen tests for homogeneity of variances) were met, we used ANOVA tests. For paired comparisons between ponds, when the normality assumption of data was met (tested through Shapiro-Wilk tests), we performed Student's *t*-test; otherwise, we performed a Wilcoxon rank sum test. We plotted survivorship curves for each enclosure in each pond; the death of more than 50 larvae (representing more than 20% of the initial clutch size) between two sampling days was considered a mass mortality event. As we detected predators inside the enclosures of ponds 1 and 3, we performed Student's *t*-test to determine if there were differences in predator density between ponds.

To determine the correlation between variables and their relative importance on metamorphosis success, we fitted logit quasi-binomial General Linear Models (GLMs) to the data. The response variable of these models was metamorphosis success: the daily proportion of metamorphs from the number of eggs per clutch (enclosure). For this, we only considered data since day 20, when the first individual reached Gosner stage 42 (n = 61). The biotic predictors of the proportion of metamorphs were the daily measurements per enclosure of predator density (predator/cm^2) on day 7, number of larvae on days 10 and 20 (to assess the importance of larval density at different times of the development), and body length on day 20 (using the median BL of up to 15 larvae per enclosure). To consider inter-pond differences in metamorphosis success and water depth variation, we also included site (pond) and pond depth (measured inside each enclosure before relocation each day) as predictors in the models. As all larvae died before day 20 in three enclosures of pond 1 and all egg masses died before day 5 in enclosures of pond 2, data from those three enclosures of pond 1 and all data from pond 2 were excluded from the dataset for this analysis.

To assess correlations among the variables to be included in the GLMs and avoid multicollinearity, we identified highly correlated predictors based on pairwise Spearman's rank correlations matrix with $|r| > 0.7$. For each correlated pair, we retained the variable with the highest explanatory power on the response variable of larvae according to a univariate generalized linear model using the "select07" procedure in R (R Development Core Team, 2015; "select07" method in Dormann et al., 2013). Site is a factor with two levels (ponds 1 and 3), so it was not included in the "select07" procedure. The saturated model included site and each uncorrelated continuous predictor fitted as a covariate of site (Crawley, 2007). A backward stepwise procedure was applied to the saturated model to obtain a simplification in which a reduced set of predictors still contribute to predict the probability of success in larval survival and metamorphosis without significantly reducing the explanatory power of the model (Hosmer et al., 2013). Because of the overdispersion of errors in the fit, a quasi-binomial distribution of errors was assumed in the survival GLMs. There is no automatic simplification routine for this quasi-binomial adjustment as the Akaike Information Criterion cannot be calculated, so we used ANOVA to compare the residual deviance between saturated and simplified models (Crawley, 2007). To assess the relative importance of predictors within the final model, we used Student's *t* statistics provided by the summary of each model: the higher the *t* for each predictor, the higher the explanatory power of the predictor on the response variable.

We performed a Spearman's rank correlation between the BL of the metamorphs and time of development of these individuals, using the sample of metamorphs (n = 99). We considered an alpha of 0.05 in all analyses, using the statistical software R (R Development Core Team, 2015).

RESULTS

Descriptive statistics of larval performances are provided in Table 1. We found no differences in clutch size between the three ponds considering the results of the ANOVA (Bartlett's K-squared = 5.2, df = 2, P = 0.1; Fligner-Killeen's χ^2 = 2.7, df = 2, P = 0.3; F = 0.0, df = 2, P = 1.0); the average clutch size per amplectant pair was 135 (SD = 73) eggs. However in pond 2, all egg masses died; no egg hatched in the first 5 d after oviposition and the clutch showed signs of decomposition by the third day. Excluding this pond, we found significant differences in hatching success per clutch between ponds 1 and 3 (Shapiro-Wilk normality test of % of surviving hatchlings: W = 0.9, P = 0.2 pond 1; W = 0.9, P = 0.2 pond 3; t = -5.7, df = 12.1, $P < 0.001$) as all enclosures suffered mass mortalities during hatching stages in pond 1 (Table 1, Fig. 2). On day 5, the larvae reached Gosner stage 25; stages 26–

Table 1. Descriptive statistics of larval performance in the sampled ponds. "*n*" is the sample size (number of enclosures or metamorphs per pond) for each variable. "N eggs" is the number of eggs laid per clutch (*n* = enclosures). "% hatched" and "% metamorphosed" are the survival proportions from eggs until Gosner stages 26–30, counted on day 7, and until Gosner stage 42, respectively (*n* = enclosures). "Days" are statistics for the median time to reach Gosner stage 42 (*n* = metamorphs). "BL larvae" and "BL metamorphs" are, respectively, the statistics for the median body lengths at Gosner stages 31–39 (days 11–12, *n* = enclosures, based on the median size of a sample of up to 15 tadpoles within each enclosure) and median body lengths at Gosner stage 42 (*n* = metamorphs). In pond 3, we counted 95 metamorphs (Table S1) but only the BL of 94 could be assessed.

	Pond 1			Pond 2			Pond 3		
	n	**Median**	**min.–max.**	***n***	**Median**	**min.– max.**	***n***	**Median**	**min.– max.**
N eggs	6	124	79–221	10	124	51–216	9	95	26–317
% hatched	6	14.3	0–27.6	10	0	-	9	67.4	24.9–87.1
% metamorphosed	6	0.0	0–4.9	10	0	-	9	7.4	0–51.1
Days	5	25	23–27	0	-	-	95	26	20–34
BL larvae (mm)	3	4.3	4.1–4.6	0	-	-	9	4.5	3.5–5.2
BL metamorphs (mm)	5	6.1	5.1–6.5	0	-	-	94	5.8	5.0–7.2

30 were reached on day 7. Stages 31–39 were observed on days 11–12 of development, and median BL at these later stages was 4.3–4.5 mm (Table 1, Fig. 3).

We also found significant differences between ponds 1 and 3 in metamorphosis success per clutch (Shapiro-Wilk normality test of % of larvae that reached Gosner stage 42: W = 0.5, $P < 0.001$ pond 1; W = 0.8, $P < 0.05$ pond 3; Wilcoxon W = 8.0, $P < 0.05$). Only tadpoles from seven clutch in pond 3 and from one clutch in pond 1 completed metamorphosis (Table 1; Table S1). The surviving individuals in pond 1 completed metamorphosis before the pond dried. In pond 3, although metamorphosis success was higher than in pond 1, mass mortalities occurred in three enclosures because the pond dried before the larvae completed metamorphosis (Fig. 2). Considering only these ponds (with 8 successful enclosures out of 15), the median percentage of eggs that reached metamorphosis per clutch in this study was 1.2%, and the median development time was 26 d (25 and 26 d in ponds 1 and 3, respectively; Table 1). Newly metamorphosed individuals were a cryptic greyish color with dark patches on fore- and hind limbs, lacking the brilliant coloration patterns of adults, as described in Bardier et al. (2017); the median BL of these metamorphs was 5.8 mm (pond 3; Table 1, Fig. 3).

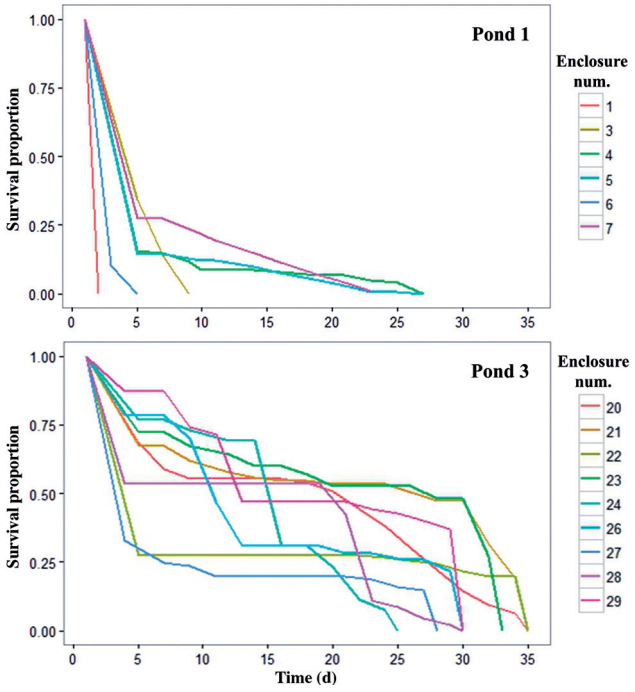

Figure 2. Survivorship curves in enclosures from ponds 1 and 3. Survival corresponds to the number of larvae divided by the number of eggs within each enclosure.

We observed larval invertebrate predators (Odonata: Anisoptera and Zygoptera, and Coleoptera: *Dytiscidae*) feeding on *Melanophryniscus montevidensis* larvae inside the enclosures from ponds 1 and 3. We found significant differences in the number of predators between ponds 1 and 3 (Shapiro-Wilk normality test of number of predators: W = 0.9, $P = 0.2$ pond 1; W = 0.9, $P = 0.7$ pond 3; Student's $t = 3.4$, $P < 0.005$). In pond 2, measurements ended by the fifth day, and we found no invertebrates inside the enclosures.

The number of larvae on days 10 and 20 were correlated ($|r| > 0.7$, $P < 0.05$), with the former variable having a higher explanatory power on the response variable (metamorphosis success) than the former. Thus, only the number of larvae day 10 was included in the saturated model (Table 2). Due to their low effect on the GLMs, predator densities were dismissed during the backward stepwise simplification procedure, as were interactions between site and other predictors, and site as a predictor itself (Table 2). The simplified model included number of larvae day 10 and depth as significant negative predictors and BL as a positive predictor of metamorphosis success, with depth and BL having the greatest effect (Table 3). Spearman's rank correlation between the BL of individual metamorphs and time of development was negative ($r_s = -0.5$, $P < 0.001$).

DISCUSSION

Information on the complete larval development of amphibians in natural conditions is generally scarce (Mel-

Table 2. Saturated and best simplified generalized linear models (logit) fitted to the metamorphosing success, and their contrast using ANOVA. "Site" is a nominal variable (levels: ponds 1 and 3); "N(larvae-10)" is the number of larvae counted on day 10; "Dens(pred)" is the predator density inside the enclosures (calculated on days 6–7); "BL" is the body length of larvae (using the median BL of up to 15 larvae per enclosure); "Depth" is the water depth inside each enclosure each day.

		Residual deviance	df	*P* value
Saturated	Site + N(larvae-10)*Site + Dens(pred)*Site + BL*Site + Depth*Site	68.2	51	
Simplified	N(larvae-10) + BL + Depth	87.5	57	
ANOVA	F = 2	7.6	5	0.1

Table 3. Coefficient estimation of the best simplified generalized linear model. For a general model $y = a + b_1x_1 + \ldots + b_nx_n$; "*b*" is the estimate of the coefficient of each variable *x;* "SE" is the standard error of the estimate *b;* "*t*" is the *t*-statistic value of each coefficient *b;* "*P*" is the significance value of the *t*-test of each coefficient *b.* "(intercept)" is the coefficient *a* of the model (intercept of the model with the y axis); "N(larvae-10)" is the number of larvae counted on day 10; "BL" is the body length of the larvae (using the median BL of up to 15 larvae per enclosure); "Depth" is the water depth inside each enclosure each day.

	b	SE	*t*	*P*
(intercept)	-12.4	3.4	-3.6	< 0.001
N(larvae-10)	-0.0	0.0	-2.5	< 0.05
BL	-1.6	0.5	3.1	< 0.05
Depth	-0.4	0.1	-3.5	< 0.001

vin and Houlahan, 2012) and had not been previously recorded for any *Melanophryniscus* species. In this study, we provide new data on survival proportions, body sizes, and development times, as well as other descriptive information, in semi-natural conditions.

The range of eggs per couple ($\bar{x}$ = 135, SD = 73) did not differ between ponds and was within the numbers reported in previous studies of this species (Garrido-Yrigaray, 1989; Alonzo et al., 2002; Pereira and Maneyro, 2018). Later in development, hatching and metamorphosis success differed locally (i.e., between ponds). Considering only ponds with metamorphs, we found local differences in predator densities, with lower percentages of metamorphs in ponds with higher predator densities. The presence of predators is expected to reduce larval survivorship in *Melanophryniscus* species, as some reports indicate that the ephemeral ponds in which these species breed usually do not harbor predators and larvae do not exhibit predator-avoidance behavior (Pereyra et al., 2011; Laufer et al., 2015). However, predator density was dismissed as a predictor of metamorphosis success during the simplification process of the saturated model. Instead, larval density on day 10, BL, and water depth were the main explanatory variables. Density during larval stages (mainly between days 10 and 20) had a more important (and detrimental) effect on metamorphosis success than larval density by the end of metamorphosis. During larval stages, density was higher than during metamorphosis stages (after day 20), thus its effects were possibly stronger during larval stages, reducing larval survivorship and leading to reduced metamorphosis success (e.g., Tejedo and Reques, 1994). High densities can reduce larval survivorship by reducing food availability, causing malnutrition diseases and starvation (Densmore and Green, 2007), enhancing the spread of different contagious diseases, even chytridiomycosis (Densmore and Green, 2007), or through the synergic effect of malnutrition and disease (e.g., Venesky et al., 2012). Although we did not test for the presence of chytridiomycosis in larvae, skin samples of adult *M. montevidensis* were analyzed by qPCR, and some individuals tested positive for *Batrachochytrium dendrobatidis* (Pontes, 2019), confirming its presence in this population.

Body lengths at Gosner stages 31–39 (4.3–4.5 mm) were smaller than lengths reported by Garrido-Yrigaray (1989) at stage 34 (5.6 mm). This could be due to in situ growth rates being lower than in laboratory conditions, which is likely due to the greater food supply in laboratory conditions (Melvin and Houlahan, 2014). Median body lengths inside enclosures during metamorphosis had the greatest positive effect on the metamorphosing success in our model. This positive effect has been reported in anuran and salamander species, both in laboratory and in situ venues (Berven, 1990; Scott, 1994; Morey and Reznick, 2004). In these studies, body size also had an inverse correlation with larval density, with larger body sizes and higher metamorphosing success reported at lower densities. Our results, however, do not support a correlation between body length and larval density, as both predictors were selected for the saturated model. The relationship between these two predictors, if significant, would be indirect or on previous stages not considered in the analysis (e.g., if larval density affects body size during larval stages but not during metamorphosis stages).

Size at metamorphosis (6.1 mm in pond 3) and its range of variation (5.0–7.2 mm) were similar to values reported for species in the *Melanophryniscus stelzneri* species group (Bustos Singer and Gutiérrez, 1997; Cairo et al., 2008; Kurth et al., 2014). Such low variation is consistent with the pattern previously observed by Werner (1986) and Richter-Boix et al. (2011) for bufonids. As a taxonomic group that breeds in ephemeral ponds, bufonids are under selective pressures exerted by such a high risk environment, which selects for maximum growth rates that poorly adjust to pond drying situations (Richter-Boix et al., 2011). The narrow range of body sizes at metamorphosis in *M. montevidensis* supports this hypothesis. The small body size at metamorphosis observed in this family, regardless of adult size, is probably due to the relatively low mortality of terrestrial life stages, which possess strong skin toxins that protect adults from predation (Werner, 1986). Indeed, this could be suggested for

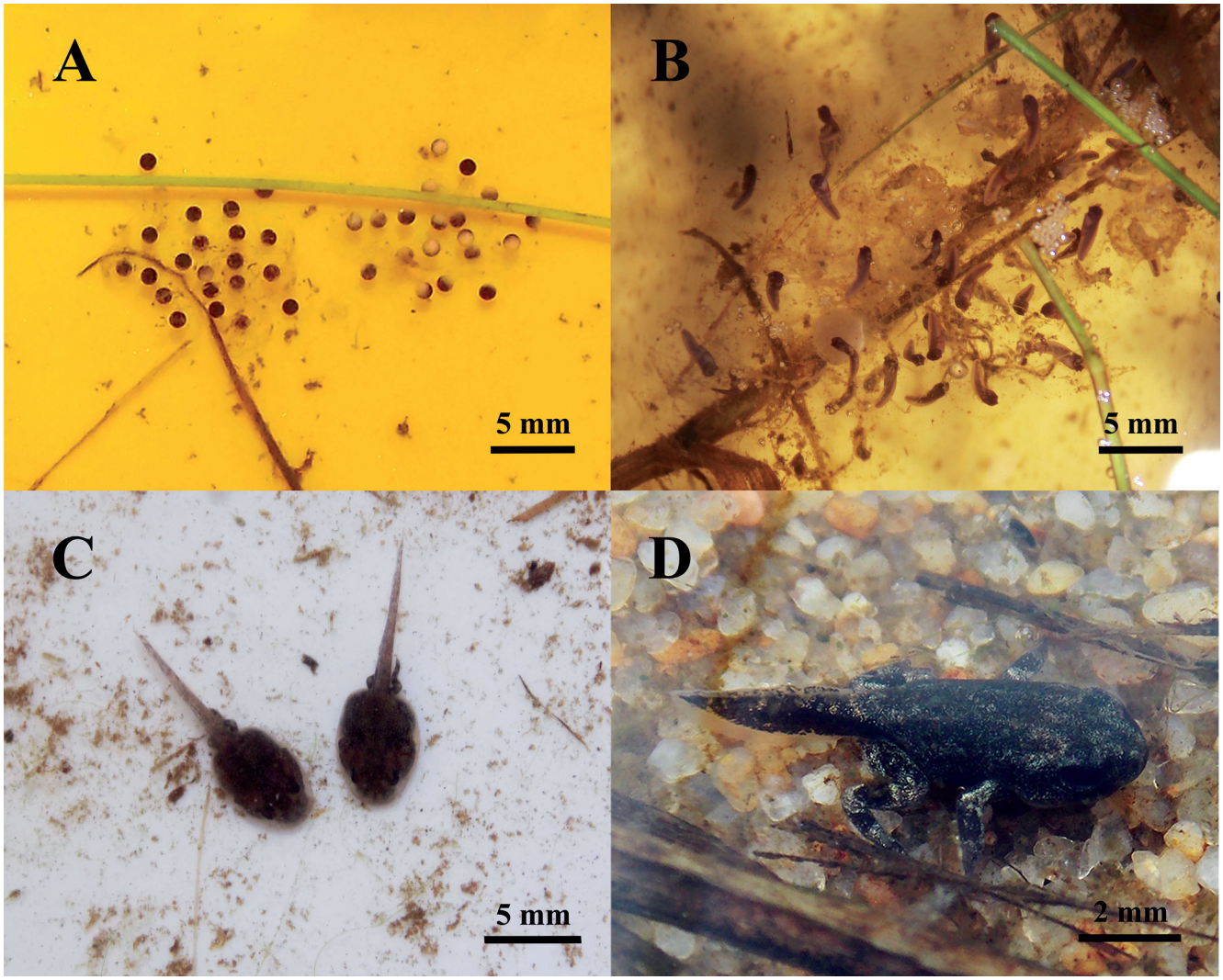

Figure 3. Developmental stages of *Melanophryniscus montevidensis*. **(A)** Two egg masses 5 h after oviposition (not a complete clutch) from pond 3; **(B)** embryos and hatchlings 2 d after oviposition in Gosner stages 19–21 from pond 3; **(C)** larvae 11 d after oviposition in Gosner stages 36–39 from pond 3; **(D)** metamorph 23 d after oviposition in Gosner stage 42 from pond 1 (photographed by C. Bardier).

the genus *Melanophryniscus*, whose adults are known to have high concentrations of pumiliotoxin and hydroquinone in skin and internal organs (Mebs et al., 2007; Grant et al., 2012).

The experimental design was meant to assess developmental parameters in field conditions, and we moved the enclosures in order to maximize the water availability for the larvae. However, an important negative effect of water depth inside the enclosures on metamorphosis success arose in the model. This effect can be interpreted as a signal of plasticity in development time in response to pond drying. Decreasing development time and higher metamorphosis rate has been reported for other anurans when the water level lowered (Laurila and Kujasalo, 1999; Altwegg, 2002; Morey and Reznick, 2004; Richter-Boix et al., 2006, 2011). Most of these studies also reported energetic costs associated with fast development, leading to smaller body sizes at shorter development times (Altwegg, 2002; Morey and Reznick, 2004). The narrow range of body sizes at metamorphosis and its negative correlation with development time might indicate that those costs were not evident in our study. In spite of the variability in development time (20–34 d), the median of 26 d (pond 3) was 10 d shorter than the maximum durations recorded by Garrido-Yrigaray (1989), which also supports developmental plasticity in this species.

Plasticity of development time has been suggested for other *Melanophryniscus* species (Bustos Singer and Gutiérrez, 1997; Cairo et al., 2008; Kurth et al., 2014). Bustos Singer and Gutiérrez (1997) reported accelerated development in *M. stelzneri* (Weyenbergh, 1875) when the water level was artificially lowered. However, Cairo et al. (2008) observed mass mortality of *Melanophryniscus* sp. when ponds dried out, similar to our observations in some enclosures. Tejedo and Reques (1994) suggested that, at low larval densities, tadpoles of *Epidalea calamita*

(Laurenti, 1768) were able of growing and developing faster in short-duration ponds but slower in long-duration ponds. It was observed that, at high larval densities, competition prevented accelerated growth in response to pond desiccation, resulting in similar development times for short- and long-duration ponds and mass mortalities in some cases (Tejedo and Reques, 1994). The present study recorded short development times in some enclosures and mass mortalities due to pond drying in others. Although we found no direct relationship between larval density and body length in metamorphosis stages, an indirect effect of larval density on the growth rate is still plausible, as reported for other ephemeral pond breeders (Travis and Trexler, 1986). Alternatively, other factors such as abiotic water conditions (e.g., dissolved oxygen, pH, or temperature) could be involved in the delayed growth of some individuals (Denver et al., 2002). Delayed growth would explain the negative correlation between body size at metamorphosis and development time and, consequently, the lack of response to pond duration (causing mass mortalities).

Regarding pond 2, where the hatching failure was total, we did not observe symptoms of fungal infections in the eggs, such as those caused by *Saprolegnia* spp. (i.e., we did not observe the presence of cotton-like masses around the eggs, following Fernández-Benéitez et al., 2008). Possibly, physicochemical conditions of the pond, critical for embryo success (e.g., dissolved oxygen or pH; see Boyer and Grue, 1995) were not within the ideal range for the eggs to hatch, as no free larvae were present during sampling period. This hypothesis might also be valid for pond 1 where mass mortalities during hatching stages (before predators could be detected) occurred in some enclosures, although we observed hatchlings and free larvae in this pond. Alternatively, low water depth in the enclosures of pond 2 (Table S1), which can also affect other variables such as water temperature and dissolved oxygen (Chapman, 1996), and its synergy with the composition of the vegetation at the margins of this pond (which was more dense and shaded than in the other ponds, Table S1) could be involved in the hatching failure (Skelly et al., 2012). Additional in situ studies should assess whether the low larval success in early stages is part of the normal variation for these populations, responds to natural conditions of the ponds, or has anthropic causes (Boone and James, 2005).

From this study, several causal relationships can be established among biotic predictors of recruitment of *Melanophryniscus montevidensis*. The influence of larval density directly on metamorphosis success was evident. Also, the ability to respond to pond desiccation depended on reaching a threshold BL (Wilbur and Collins, 1973), as larger median sizes inside the enclosures allowed higher metamorphosis success. This ability to respond to desiccation (i.e., developmental plasticity) was more evident in the time to complete development than in the size attained at the end of metamorphosis. No direct effect of density on growth was observed in the present study, so either the effect is indirect (e.g., by increasing the level of corticosterone secretions typical of confinement situations; see Belden et al., 2005) or other constraints to growth at larval and metamorphosing stages may be acting, such as local physicochemical conditions (Denver et al., 2002). In spite of these findings, some caveats in our models should be mentioned. To determine metamorphosis success, we only analyzed those enclosures with surviving individuals beyond day 20 of development. For this reason, the interrelation and importance of bi- otic predictors on survival in previous stages would not be evident. Thus, the effects of predator density on larval survivorship or larval density on growth were not directly acting in later stages, but cannot be discarded for early stages. Moreover, as we analyzed biotic variables close to the metamorphosing stage, data of unsuccessful hatching in pond 2 were not included in the models, so no predictions based on the biotic variables measured were available to explain this phenomenon.

The results of this study support general patterns of growth and development times reported for Bufonidae and other species that breed in ephemeral ponds (Richter-Boix et al., 2011). However, questions about the role of density on growth and the role of abiotic variables are still open. These questions are also relevant for conservation. Although a potential response of the species to pond desiccation was reported, ponds that dry in less than 20 d might not yield any offspring. Pond drying might intensify under global warming scenarios that would make more difficult for ponds to retain water for long periods of time (increasing densities, among other effects); this was previously suggested as an explanation for projected losses in distribution areas for several threatened *Melanophryniscus* species, especially *M. montevidensis* (Toranza and Maneyro, 2013; Zank et al., 2014). The in situ data provided by this study, combined with demographic data for terrestrial stages (Bardier et al., 2019), could be applied to population stage-structured models (e.g., Biek et al., 2002). These models could be constructed not only for *M. montevidensis,* but for ecologically similar congenerics, helping to understand the processes behind their declines and establish proper conservation actions for the populations and the ponds where they breed (Conroy and Brook, 2003).

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Online supporting information

The following Supporting Information is available for this article online:

Figure S1. Water depth inside enclosures with indications when the cages were moved.

Table S1. Main features of the water bodies surveyed and additional biotic information of the experimental design.

Table S2. Weather conditions before and during the experiment.