

Effects of pond drying on morphological and life-history traits in the anuran *Rhinella spinulosa* (Anura: Bufonidae)

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ABSTRACT

Question: How does the duration of temporary ponds affect the morphological attributes and life-history traits of post-metamorphic amphibians?

Organism: The anuran *Rhinella spinulosa* of the Andes range of central Chile. This species reproduces in temporary and permanent ponds and is subject to different desiccation regimes.

Time and place: Between October 2006 and January 2007 near Farellones (33°20'S, 70°18'W; 2331 m above sea level).

Methods: A natural experiment in ponds with three desiccation regimes: (1) high, (2) medium, and (3) low. We measured life-history traits (development rate, metamorph size, and time to metamorphosis) in 252 individuals collected from each desiccation regime. Nine morphometric measurements were made on each toadlet collected.

Results: Tadpoles from ponds with high and medium desiccation reached metamorphosis at an earlier age and at a smaller size than individuals in the low desiccation regime. Post-metamorphic individuals from ponds with low desiccation also had longer hindlimbs and larger values for head measurements.

Conclusion: According to these results, environmental heterogeneity promotes phenotypic variation in both morphology and life-history traits in *Rhinella spinulosa*.

Keywords: development time, hydroperiod, metamorphosis size, natural experiment, pond desiccation, *Rhinella spinulosa*.

INTRODUCTION

Theoretical models indicate that the degree of environmental heterogeneity is a major factor regulating the evolution of phenotypic plasticity (Moran, 1992; Sultan and Spencer, 2002). In spatially or temporally variable environments, natural selection may favour either a suite of specialized genotypes with fixed phenotypes, each adapted to a particular environment, or

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flexible genotypes that express different phenotypes in different environments, obtaining higher fitness over a range of environments (Via and Lande, 1985; Futuyma and Moreno, 1988; Whitlock, 1996). Thus, environmental heterogeneity provides a scenario for adaptation (Bradshaw, 1965; Levins, 1968; Schlichting and Pigliucci, 1998).

Temporary ponds are classic examples of heterogeneous environments and excellent systems for the study of phenotypic plasticity in amphibians (Newman, 1992). In these systems, making the transition from an aquatic to a terrestrial habitat at the right size and time is crucial for survival. Therefore, life-history traits such as larval growth rate, metamorph size, and time to metamorphosis are important fitness components (see Semlitsch, 2002, and references therein; Altwegg and Reyer, 2003; Johansson *et al.*, 2005). Recent models consider the influence of time constraints (e.g. habitat duration and seasonality) on development time and, consequently, on the optimal size and age at metamorphosis (Rowe and Ludwig, 1991; Abrams *et al.*, 1996; Rudolf and Rodel, 2007). When risk of mortality in the larval environment increases as a function of time, a younger age at metamorphosis may be favoured in spite of costs associated with a smaller body size (Newman, 1988, 1989; Laurila and Kujasalo, 1999; Merilä *et al.*, 2000; Laurila *et al.*, 2002; Loman and Claesson, 2003; Rudolf and Rodel, 2007).

On the other hand, the plasticity of morphological attributes in relation to pond desiccation has received little attention in the literature, except for a few recent papers. Richter-Boix *et al.* (2006) evaluated the effect of pond desiccation on life-history traits and the morphology of tadpoles and toadlets in *Pelodytes punctatus*. They found that tadpoles subjected to a drying treatment accelerated metamorphosis and reached this stage with a lower body mass. Furthermore, post-metamorphic individuals in the drying treatment showed shorter and less muscular hindlimbs, and reduced jump performance, compared with individuals in the constant water treatment. Similarly, Newman (1989) reported that post-metamorphic individuals of *Scaphiopus couchii* had shorter hindlimbs than individuals from less ephemeral habitats.

There is evidence of the effects of pond desiccation on life-history traits and morphology in different amphibian species. However, most of this evidence originates from experimental laboratory studies using vertical-sided containers (e.g. Semlitsch, 1987; Wilbur, 1987; Semlitsch and Reyer, 1992; Row and Dunson, 1995; Denver *et al.*, 1998). The disadvantage of such experiments is that the reduction in water volume is not accompanied by a recession of the shoreline, as occurs in natural ponds. Brady and Griffiths (2000) studied tadpole development by simulating desiccation in both conditions and found that tadpoles metamorphosed at a smaller size and had slower development in shoreline ponds. Therefore, it would be interesting to evaluate these responses in natural conditions incorporating the effects of pond topography.

In this study, we evaluated morphological and life-history trait variation in toadlets of the anuran *Rhinella spinulosa*, in a natural desiccation experiment. Specifically, we monitored a pond system in the Andes mountain range of central Chile. This zone provides an excellent opportunity to study the larval development of *R. spinulosa* as a function of different desiccation regimes. In post-metamorphic individuals, we measured development rate, metamorph size, and time to metamorphosis, as well as nine morphological measures.

We hypothesized that pond duration would affect the life-history traits of tadpole and toadlet morphology. Therefore, we predicted that development rate and time to metamorphosis would change as a function of time constraints associated with pond duration. Also, we predicted that toadlets from ponds with low desiccation levels would be larger and have longer hindlimbs at metamorphosis than individuals in ponds with higher desiccation levels.

MATERIALS AND METHODS

Study area

From October 2006 to January 2007, we conducted weekly sampling at a site located at Farellones, in the Andes range of Chile's Metropolitan Region (33°20'48.3"S, 70°18'51.2"W; 2331 m above sea level). This area is characterized by a warm temperate climate, with winter snow and a dry season of 4–5 months. The mean annual temperature is 8.8°C and the annual precipitation is 615 mm (Luebert and Pliscoff, 2006). At the beginning of the dry season (September–October), the increase in temperature melts the snow, flooding plain lands and creating pools simultaneously. These systems are filled continuously by streams and are usually dry before the season ends (M. Méndez, personal observation). At the study site, we identified ten ponds of different sizes, in which we followed egg masses of *R. spinulosa* laid in October 2006. We left one clutch of eggs in each pond; the other egg masses were transferred to pools that were not part of the experiment. We selected clutches of similar size and developmental stages. At the moment the experiment began, all clutches were of Gosner stage 15–16.

Since the selected ponds were of different shapes and had irregular depths, we did not consider these measures for comparison. Rather, as a comparative measure we used the maximum diameter of ponds (i.e. at the beginning of the experiment), which ranged from 1.9 to 14 m (see Table 1). As a proxy for the desiccation level of the pools, we estimated hydroperiod (i.e. the length of time that the pond holds water and therefore provides aquatic habitat for tadpoles). This criterion was based on the fact that all ponds were produced at the same time, and that we observed the simultaneous laying of egg masses after ponds were formed. A summary of the physical attributes of each pond is provided in Table 1.

Experimental procedure

During the experiment, we made weekly collections of five tadpoles and 10–15 post-metamorphic individuals. All individuals were anaesthetized with MS222 (tricaine)

Table 1. Physical characteristics of the ponds studied

Pond	Area (m ²)	Maximum diameter (m)	Hydroperiod (days)	Mean temperature (°C)*	Desiccation level
1	2.359	1.92	65	29.29	Medium
2	6.851	3.47	57	30.57	High
3	5.220	3.22	57	30.44	High
4	22.373	8.12	92	28.69	Low
5	17.434	10.86	92	28.23	Low
6	21.565	11.69	71	28.52	Medium
7	13.695	5.2	71	26.02	Medium
8	2.066	1.9	51	30.00	High
9	9.775	2.01	78	25.78	Medium
10	70.017	13.85	86	27.14	Low

* Values correspond to the mean temperature between 11:00 and 18:00 h.

methanesulphonate) before being fixed in 70% ethanol. We made weekly temperature measurements from November to January, every hour between 11:00 and 18:00 h, with a WTW digital thermometer.

Although previous studies have shown that predators have a strong impact on life-history traits and larval morphology (Skelly and Werner, 1990; McCollum and Leimberger, 1997; Van Buskirk and Schmidt, 2000; Relyea, 2002), we did not observe vertebrate (birds) or invertebrate (odonate larvae) predators in the study area during the experimental period. Since we have been following this pond system for 8 years, and we have never observed birds eating larvae, we ignore the possible effect of this kind of predator in our experimental area.

Development and time to metamorphosis

The developmental stage of each larva collected was determined using Gosner's (1960) table. Since we knew the date on which the egg masses were laid (16 October 2006), we estimated the age in days to reach: (a) the stage at which toes 4–5 began to separate (Gosner stage 35); (b) the stage at which the first forelimb emerged (Gosner stage 42); and (c) the stage when the tail was completely reabsorbed (Gosner stage 46, complete metamorphosis).

Morphological measurements in toadlets

We evaluated variation of external morphology in 252 toadlets from the study ponds. The following morphological traits were measured: snout-to-vent length (SVL), head width, head height, mandible width, nose-to-mouth length, distance between nostrils, eye diameter, inter-orbital width, forelimb length, and hindlimb length (Fig. 1). We included

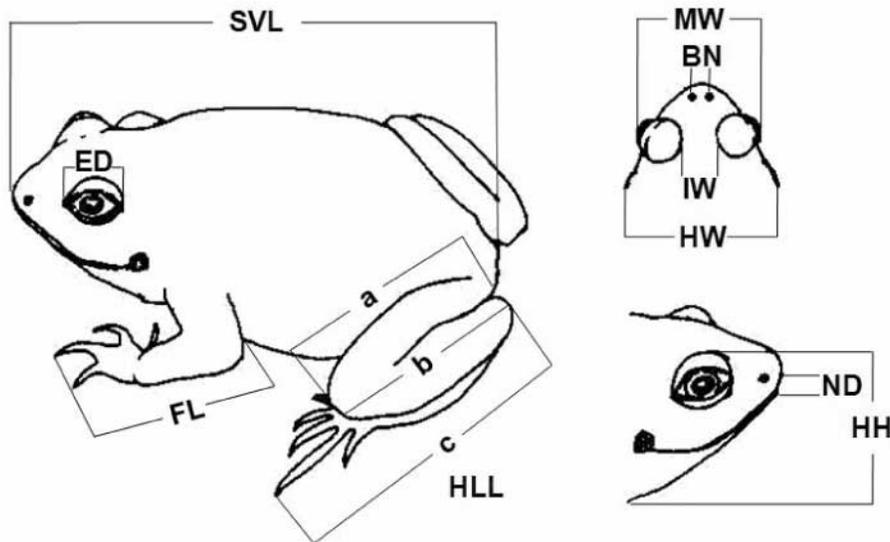


Fig. 1. Toadlet morphological measures. The ten measured traits were: SVL = snout-to-vent length; HW = head width; HH = head height; MW = mandible width; ND = nose-to-mouth distance; BN = distance between nostrils; ED = eye diameter; IW = inter-orbital width; FL = forelimb length; HLL = hindlimb length ($a + b + c$).

head measurements because it has been documented that larval development affects the rhythm and sequence of cranial ossification in anurans (Smirnov, 1992). Also, Emerson (1986) and Wassersug and Hoff (1982) found that the hormones that control metamorphosis also control limb and skull development in anurans. Additionally, Lutz and Rome (1994) suggested that some larval environments, such as high larval density, induce allometry in the muscular structure of the hindlimbs. So there is evidence to suspect that the relative head and limb sizes at metamorphosis are sensitive to development time as a function of desiccation risk. Measurements were made using a dissecting microscope incorporating an ocular micrometer (0.1 mm precision).

Statistical analyses

Size and age at metamorphosis

We used a linear mixed-model approach with hydroperiod length (in days) and pond area as continuous variables and pond number as the categorical variable. Since we detected convergence in size and age at metamorphosis, the data were \log_{10} -transformed, after which no convergence was detected. These analyses showed that only hydroperiod had an effect on size and age at metamorphosis (Table 2). Accordingly, we did not use pond number or pond area in posterior analyses. In a second statistical approach, we performed the analysis using only hydroperiod. This variable was categorized in terciles, classifying the ponds into three desiccation levels: high, medium, and low (Table 1). To compare the age of tadpoles at Gosner stages 35 and 42, and age at metamorphosis (Gosner stage 46) at different desiccation levels, we used Kruskal-Wallis tests. To evaluate the treatment effect on snout-to-vent length, we used one-way analysis of variance (ANOVA). For this analysis, we used \log_{10} transformation of the data to meet the assumptions of normality and homoscedasticity. The relationship between hydroperiod and snout-to-vent length was evaluated using regression analysis.

Table 2. Results of mixed-model analysis for (a) snout-to-vent length (SVL) and (b) age at metamorphosis

	Coefficient	Standard error	<i>z</i>	<i>P</i> > <i>z</i>	95% confidence interval	
(a) SVL						
Pond area	-0.007592	0.015836	-0.48	0.632	-0.038631	0.023446
Pond number	0.061084	0.090419	0.68	0.499	-0.116133	0.238302
Hydroperiod	0.036862	0.018975	1.94	0.052	-0.000328	0.074052
Const.	8.629061	1.357701	6.36	0.001	5.968015	11.29011
(b) Age at metamorphosis						
Pond area	0.001243	0.000869	1.43	0.153	-0.000460	0.002947
Pond number	0.005316	0.005008	1.06	0.289	-0.004500	0.015132
Hydroperiod	0.003873	0.000959	4.04	0.001	0.001992	0.005754
Const.	3.642578	0.075147	48.47	0.001	3.495291	3.789864

Note: All variables were \log_{10} -transformed.

Morphological variation

Variation in morphological attributes was determined by a multivariate analysis of covariance (MANCOVA), using snout-to-vent length as the covariate, followed by univariate analyses for each trait. All assumptions of MANCOVA were satisfied (i.e. normality, homoscedasticity, and parallelism).

All statistical analyses were performed using the software Statistica 6.0 (Statsoft, Inc., 2001) and Stata (Stata Corporation, 2008).

RESULTS

Size at metamorphosis

Desiccation level had a significant effect on size at metamorphosis (ANOVA, $F_{2,249} = 28.7$, $P = 0.001$). Toadlets from low desiccation ponds had longer periods of larval development and larger sizes at metamorphosis (snout-to-vent length) than toadlets that experienced a shorter hydroperiod. *A posteriori* comparisons (Bonferroni test) indicated no significant difference between individuals from high and medium desiccation ponds, but both of these differed significantly from individuals from the low desiccation ponds. Thus, the low desiccation treatment had a large effect on the snout-to-vent length of measured toadlets (Fig. 2). Additionally, regression analysis comparing size at metamorphosis and hydroperiod showed a significant, positive association ($r = 0.186$, $F_{1,250} = 57.61$, $P = 0.001$).

Field observations in the same population with marked toadlets show that individuals tend to settle in their pond after metamorphosis for 3 days (V. Vidal, personal communication). Since tadpoles were close to metamorphosis, we visited the experimental area three times a week, and are confident that the collected individuals belonged to the ponds where they were collected.

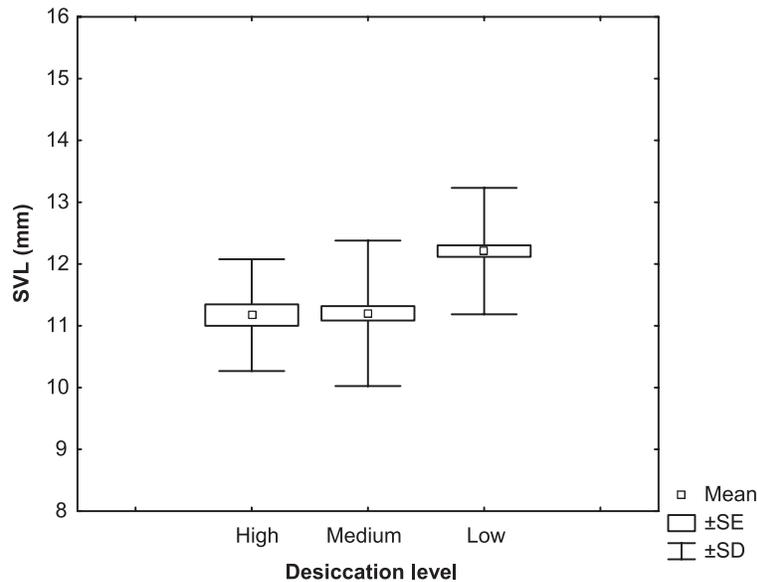


Fig. 2. Mean body length at metamorphosis (snout-to-vent length: SVL) for the three desiccation levels.

Development and age at metamorphosis

We observed high homogeneity in development rate within each pond, which was remarkably synchronous both within ponds and among desiccation treatments. The age of tadpoles at Gosner stage 35 was greater at low desiccation levels, although this difference was not statistically significant ($H = 3.908$, $P = 0.1417$; Kruskal-Wallis test). At Gosner stages 42 and 46 (metamorphosis), the mean age increased as desiccation level decreased (Fig. 3) ($H = 9.959$, $P = 0.007$ and $H = 28.366$, $P = 0.001$; Kruskal Wallis tests for Gosner stages 42 and 46, respectively).

Morphology of post-metamorphic individuals

Multivariate analysis of morphological variables showed significant differences as a function of desiccation level (MANCOVA, $\lambda = 0.8465$; $F_{18,480} = 2.3164$, $P = 0.001$). The univariate analyses and *a posteriori* comparisons (Bonferroni test) indicated that eye diameter, mouth-to-nose distance, and hind leg length were all greater in individuals from the low desiccation ponds. There was no significant difference between medium and high desiccation ponds (Table 3).

DISCUSSION

The temporary reduction of water volume in natural systems triggers a suite of environmental changes, such as an increase in water temperature (Newman, 1989), an increase in larval density, and changes in the concentrations of solutes (Denver, 1997). It has been documented that ephemeral and permanent ponds in the same region have similar mean temperatures (Semlitsch and Wilbur, 1988; Newman, 1989; Morey and Reznick, 2004). In the present experiment, no significant differences in mean temperature or daily temperature fluctuations were observed

Table 3. Results of multivariate (MANCOVA) and univariate (ANCOVA) analyses of covariance for morphological traits of toadlets at metamorphosis

Analysis	Factor	Dependent variable	Wilks' λ	d.f.	MS effect	F	P
MANCOVA	Treatment		0.846542	9,18		2.3164	<0.005
	Covariate		0.151372	9,18		149.50	<0.001
ANCOVA		Head width		2,248	0.000371	0.9548	0.386
		Head height		2,248	0.003573	1.9281	0.148
		Mandible width		2,248	0.000790	0.6856	0.505
		Nose-to-mouth distance		2,248	0.014208	7.0803	<0.005
		Distance between nostrils		2,248	0.001049	0.4614	0.631
		Eye diameter		2,248	0.005085	3.3506	<0.05
		Inter-orbital width		2,248	0.000722	0.3875	0.679
		Hindlimb length		2,248	0.002750	3.2446	<0.05

Note: Snout-to-vent length (SVL) was used as the covariate in all cases. All variables were \log_{10} -transformed.

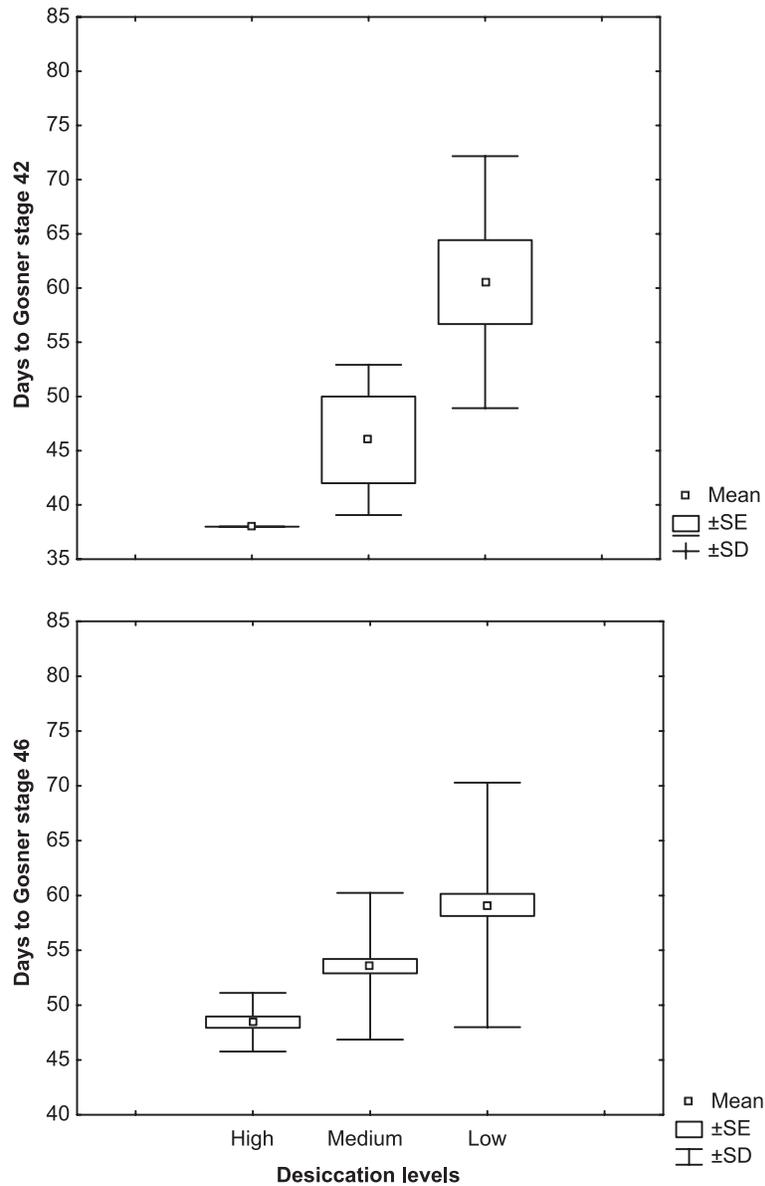


Fig. 3. Time to development at Gosner stage 42 and at metamorphosis (Gosner stage 46) for the three desiccation levels.

among desiccation levels. Thus, it appears that the effect of temperature in our experiment was not a relevant factor for the studied attributes.

Our data show that tadpole development time was influenced by hydroperiod (Table 2). Although the time to development at Gosner stage 35 did not vary among the three desiccation levels, the mean age at Gosner stages 42 and 46 was lower in ponds with high desiccation. This may be due to an acceleration of development during the last larval stages

(see Newman, 1992), when tadpoles probably detect specific cues associated with desiccation. Although these specific cues are still unknown, a potential candidate is water chemistry. Morey and Reznick (2004) found that concentrations of ammonia and nitrogen, and calcium carbonate hardness, tended to increase as larvae developed and ponds dried. Increases in hardness are probably a simple consequence of drying. Increases in the concentration of ammonia are caused by the excreta of tadpoles. The increases that Morey and Reznick observed were more pronounced in cases where drying was most extreme. Thus, it is possible that these changes represent chemical cues related to the imminent drying of the larval habitat. This is a plausible explanation for our results, especially given that we detected differences in larval development as a function of hydroperiod.

With respect to size at metamorphosis, our results indicate that there is a significant relationship between snout-to-vent length and hydroperiod. Although the mixed model showed marginally significant values ($P = 0.052$; Table 2), regression analysis showed a significant relationship. Also, our data showed that post-metamorphic individuals from low desiccation ponds had larger body sizes (snout-to-vent length). According to Morey (1998), larvae that inhabit longer-lived ponds develop more slowly, which delays metamorphosis, resulting in a larger body size. This difference was only found between the lowest desiccation level and the other two levels, suggesting that the development threshold of metamorphosis [defined by Day and Rowe (2002) as the minimum size that must be reached before the life-history transition can occur] was passed in this treatment, resulting in a substantial daily increase in size (Lind *et al.*, 2008). In spite of this, the variation in size at metamorphosis among ponds with different desiccation levels may be the result of multiple factors. One such factor that we did not evaluate in our study is egg size, which has been identified as a significant source of variation in hatchling, metamorphic, and even adult traits in amphibians (Kaplan, 1998) and other organisms (reviewed in Mousseau and Fox, 1998). In our experiment, it was not possible to measure this trait at the beginning of the experiment, because the clutches were at Gosner stage 15–16, which could introduce a bias in the interpretation of our results. However, we measured egg size in other clutches from Farellones and they showed a narrow range of variation for this population (2.08 ± 0.017 mm; $n = 157$ eggs, 12 clutches). Thus, if there was an effect mediated by egg size, we believe that it was similar in all of the clutches evaluated.

According to Loman (2002), the most important factor for size at metamorphosis is probably competition, which varies as a function of larval density. The field methodology used in our study does not allow us to establish precisely which factor is responsible for the positive relationship between hydroperiod and body size (e.g. density and/or competition). However, in a laboratory experiment performed in *R. spinulosa*, we observed that there were differences in size at metamorphosis at low densities of 5–15 individuals per 600 ml, but not at densities of 30–45 individuals per 600 ml (M. Méndez, unpublished data). Thus, because we did not observe such low densities (5–15 individuals per 600 ml) in our natural experiment, we suggest that hydroperiod had a major effect on the observed size at metamorphosis.

In terms of possible allometric changes associated with desiccation, our results show that desiccation had an effect on eye diameter, nose-to-mouth length, and hindlimb length, whereas no differences were detected in the other characters evaluated. These morphological traits varied independently of body size, and were larger in toadlets from low desiccation ponds. It has been documented that head width and leg length are functionally important traits in anurans (Emerson, 1978, 1985; Duellman and Trueb, 1986), and that differences in growth rate may produce changes in these characters (Emerson, 1986; Newman, 1989; Blouin and Brown,

2000). Therefore, the allometric relationship between shape and size is not constant, but rather is a function of development rate (Blouin and Loeb, 1991). Thus, environment might induce morphometric variation simply by controlling the overall rates of growth and differentiation (Blouin and Brown, 2000). Our data show that allometry could exist as a function of desiccation on hindlimb length and two head characters (eye diameter and nose-to-mouth length). For the salamander *Hynobius retardatus*, Kohmatsu *et al.* (2001) found lower head width at low densities, both under laboratory and field conditions. The authors associated this difference with density-dependent effects [for insects, see also Imasheva and Bublly (2003)]. Our data show a similar pattern of variation as a function of desiccation. Higher values of eye diameter and mouth-to-nose length were found in environments with low desiccation. Also we found longer hindlimbs in environments with low desiccation, apparently related to the extension of larval period. Blouin and Brown (2000) described a similar pattern of variation in *Rana cascadae*, but as a function of temperature. In their study, tadpoles with a longer larval period (lower temperature) had a longer tibia-to-fibula length, showing plasticity in this trait independent of body size.

There are a number of questions regarding the costs associated with plasticity in time and size at metamorphosis, as well as about the allometric variation of post-metamorphic individuals. For amphibians, it has been proposed that size at metamorphosis is one life-history trait directly related to survival in the first year of terrestrial life, and implies benefits in terms of fitness (Berven, 1990; Altwegg and Reyer, 2003; Gray and Smith, 2005). Thus, there appears to be a trade-off between size at metamorphosis and development time in amphibians that inhabit variable habitats (Abrams *et al.*, 1996). A positive correlation between size at metamorphosis and locomotor performance has been documented (Goater *et al.*, 1993; Beck and Congdon, 2000; Stevens *et al.*, 2004). Also, water loss during post-metamorphic life is directly related to body size (Newman and Dunham, 1994). Therefore, early metamorphosed individuals (from ponds with high desiccation rates) who have smaller sizes and shorter legs would have lower survival probabilities. Although our experiment provides no data on post-metamorphic fitness, lower survival in early metamorphosed individuals is a realistic scenario for our species, which lives and moves in a dry mountain environment with high solar radiation, with annual Andean vegetation that dries in summer and has complete snow coverage during the winter.

Although natural experiments provide a high degree of realism, they do not permit the regulation of independent variables that might confound the causes of the observed responses (Diamond, 1986). Therefore, the present study cannot evaluate the effect of desiccation *per se* on the variation of tadpole and toadlet traits. We only evaluate the effect of desiccation in the context of all of the changes that it implies. We consider that to determine precisely the effects of desiccation it will be necessary to measure other variables correlated with the decrease in water volume in natural conditions (e.g. chemistry), as well as to perform laboratory (common garden) experiments that allow manipulation and control of these variables. This approach will allow us to evaluate the effects associated with delaying or accelerating metamorphosis on toadlet performance. Our group is currently studying this type of question using a combination of laboratory and field experiments.

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