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Adaptive plasticity of life-history traits to pond drying in three species of Patagonian anurans

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ABSTRACT

Question: Does pond permanency affect life-history traits of endemic anurans from Patagonia?

Background: Amphibians inhabiting North Patagonia breed at ponds that differ strongly in their water permanency. The role of phenotypic plasticity in adapting to variable environments could be fundamental in these Patagonian anurans.

Prediction: In comparisons between populations and between species, we predicted that size at metamorphosis and developmental time of three endemic anurans from Patagonia are adaptive traits when these species are exposed to the selective pressure of pond drying.

Organisms: *Pleurodema thaul*, *Pleurodema bufoninum*, and *Rhinella spinulosa*, the most common species of amphibians in North Patagonia, breed and develop in a gradient of environmental scenarios that include ponds with highly heterogeneous hydroperiods.

Methods: We recorded age and size at metamorphosis of larvae and metamorphs in 15 natural ponds. To analyse the effect of hydroperiod on these traits, we performed common garden experiments by simulating two different water volumes (decreasing vs. constant).

Results and conclusion: The anurans responded to the selective pressure of drying environments by reducing size and time to metamorphosis. These responses were also evident in laboratory experiments. Wetlands with shorter hydroperiods resulted in shorter larval periods and smaller metamorphs. *Rhinella spinulosa* developed faster in the decreasing water treatment, but its metamorphs did not differ in size between treatments. The two *Pleurodema* species also developed faster in the decreasing water treatment, and metamorphs reached smaller sizes in the decreasing water treatment. *Pleurodema* species showed strong evidence of adaptive significance of these traits based on comparisons between populations.

Keywords: adaptive plasticity, development, Patagonian anurans, pond drying,
size at metamorphosis.

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INTRODUCTION

Species that live in temporary wetlands have developed different responses to habitat desiccation, and those with complex life cycles such as insects (Blakley, 1981; Buns and Ratte, 1991), freshwater invertebrates (Pechenik, 1990; Twombly, 1996), and amphibians (Wilbur, 1980) can exhibit plasticity in timing and size to metamorphosis.

Many amphibian species breed in temporary ponds where the volume of water can fluctuate between periods of desiccation and refilling. The species inhabiting these variable environments are subject to trade-offs between mortality and size at metamorphosis. Thus, variability of the hydroperiod may play a fundamental role in variability of size at metamorphosis. Several studies have documented that tadpoles develop faster and metamorphose earlier and at a smaller size when ponds dry up. However, this reduction in size can involve costs that may be evidenced at later stages (juvenile and adult). Several studies have shown that smaller metamorphs have a lower capacity for locomotion (Goater *et al.*, 1993; Richter-Boix *et al.*, 2006), lower tolerance to dehydration (Newman and Dunham, 1994), reduced resistance to parasites (Goater, 1994), a weaker immune system (Gervasi and Foufopoulos, 2008), lower juvenile survivorship (Reques and Tejedo, 1997; Morey and Reznick, 2001; Altwegg and Reyer, 2003), and lower reproductive success later on (Smith, 1987; Howard, 1988; Scott, 1994). Moreover, recent studies have documented that global warming can have profound consequences for the plastic responses in many anurans, ultimately influencing their fitness (Tejedo *et al.*, 2010).

When considering gradients of pond drying, we need to differentiate between ephemerality and variability. But we cannot assume that species breeding in ephemeral habitats will exhibit adaptive plasticity. It is likely that species breeding in more predictably ephemeral habitats could be selected to favour fast development rates and limited plasticity. In general, such species have evolved short larval periods (e.g. *Scaphiopus couchii*) compared with species that breed more frequently in temporary ponds and exhibit higher levels of plasticity (Dayton and Fitzgerald, 2005).

In Northwestern Patagonia, Argentina, the most common spring-breeding anurans are *Pleurodema thaul*, *Pleurodema bufoninum* (Leiuperidae), and *Rhinella spinulosa* (Bufonidae). These species breed primarily in ephemeral and temporary habitats, although they also occasionally reproduce in permanent habitats (Jara and Perotti, 2010). The reproductive season starts during the austral spring and extends until early summer. *Pleurodema thaul* and *P. bufoninum* are closely related species and are only found in Argentina and Chile (Úbeda, 1998; Ferraro and Casagranda, 2009). In nature, these species exploit larval habitats that are distinct in terms of pond permanence, from ephemeral to temporary ponds. It is possible to find tadpoles living both in ephemeral ponds of less than 3 months' duration and in temporary ponds of 8–9 months' duration, depending on the rain regime and the year. In dry years, ponds are of short duration, whereas in rainy years they may remain for longer, sometimes turning into permanent ponds. *Pleurodema thaul* inhabits mostly large vernal ponds and flood meadows in the forest, which vary in permanence from year to year. *Pleurodema bufoninum* is a typical steppe species, inhabiting temporary and ephemeral Patagonian flood meadows called 'mallines'. The tadpoles of *R. spinulosa* typically develop in ephemeral/temporary ponds originating in the flood plains of mountain rivers, sometimes sharing these water bodies with *P. bufoninum* (Jara *et al.*, 2007). *Pleurodema bufoninum* was also found sharing ponds with *P. thaul*; however, there are no reports of *P. thaul* sharing ponds with *R. spinulosa*. The distinctive distribution of these species and their larvae due to habitat choice, and the habitat variability imposed on them each year represent an excellent system to study adaptive phenotypic plasticity by comparing populations and species.

Our main objective was to determine whether three Patagonian anurans, *Pleurodema thaul*, *Pleurodema bufoninum*, and *Rhinella spinulosa*, modify their size at metamorphosis and time to metamorphosis (larval period) in natural environments in response to differences in hydroperiod permanence, by comparing species and populations. In addition, we undertook interspecific comparisons of the reaction norms of size at metamorphosis and larval period over two levels of simulated hydroperiod duration (decreasing water volume and constant water volume). These comparisons allowed us to generate hypotheses about how these Patagonian species optimize the selective trade-off between size at metamorphosis and larval period in changing environments.

MATERIALS AND METHODS

Field studies

A total of 15 wetlands in five localities in Nahuel Huapi National Park and its surroundings, Patagonia, Argentina, were sampled each week during the breeding seasons of 2006–2007. Localities were situated along a transect oriented along a west–east longitudinal gradient of 20 km: Bernal, six wetlands; Ñirihuau, four wetlands; Ñireco, three wetlands; Fantasma lagoon, one wetland; and Pinar de Festa, one wetland. Permanence of wetlands varied from 71 days to 365 days (Table 1). We sampled larval anurans by dip-net sweeps (net dimensions 36 × 25 cm; 5-mm mesh) at different microhabitats in each wetland (Schaffer *et al.*, 1994). The most frequent anuran species in our surveys was *Pleurodema bufoninum* (12 wetlands), followed by *Rhinella spinulosa* (3 wetlands) and *Pleurodema thaul* (2 wetlands). *Pleurodema bufoninum* and *P. thaul* differ in the time they take to develop and in the synchrony of the reproductive events during a single reproductive season. We found that *P. bufoninum* has a synchronized period of development with only one cohort/season of tadpoles in the ponds. However, *P. thaul*, which is more common in temporary than in ephemeral ponds, has a long period of development and several cohorts during the same season. *Rhinella spinulosa* showed one or several larval cohorts depending on the population and spring rains (Sympson *et al.*, 2006). In *P. thaul*, we assessed size at metamorphosis in response to different hydroperiods and different cohorts at each locality where it was found (Fantasma lagoon and Pinar de Festa pond). We also assessed size at metamorphosis and larval period in *Pleurodema bufoninum* and *Rhinella spinulosa* by comparing different localities (populations) (Ñirihuau and Bernal rivers) and ponds with different hydroperiods at two developmental stages [stage 41 = larvae and stage 46 = until they reach metamorphosis (Gosner, 1960)].

In the field, we measured four response variables in all individuals: size at metamorphosis (SM-42) and length of larval period at metamorphosis (LP-42), and size and larval period until reaching metamorphic climax (SM-46, LP-46). We measured size as the wet weight of each individual (to the nearest 0.001 g).

Laboratory experiment

In a common garden experiment, we tested whether a reduction in water volume influences the development time and size of tadpoles of *Pleurodema thaul*, *Pleurodema bufoninum*, and *Rhinella spinulosa*.

Table 1. Anuran species, localities, wetlands, and permanency of wetlands sampled, 2006–2007: mean sizes (± s.e.) at stages 41 (larvae) and 46 (metamorphs)

Species	Locality	Wetland	Permanency (days)	Larvae (g)	Metamorphs (g)	*P*
P. thaul	Fantasma lagoon		107	1.87 ± 0.05	0.62 ± 0.03	<0.001**
	Pinar de Festa pond		71	0.45 ± 0.05	0.27 ± 0.007	
P. bufoninum	Bernal river	1	138	1.60 ± 0.09	0.72 ± 0.09	<0.005**
		2	110	1.82 ± 0.1	0.72 ± 0.05	
		3	110	1.47 ± 0.15	0.57 ± 0.04	
		4	110	1.15 ± 0.06	0.70 ± 0.05	
		5	91	1.23 ± 0.09	0.42 ± 0.01	
		6	91	1.42 ± 0.08	0.64 ± 0.05	
	Ñirihuau river	1	125	1.96 ± 0.14	0.71 ± 0.05	<0.001**
		2	135	2.38 ± 0.07	1.15 ± 0.07	
		3	135	1.63 ± 0.05	1.32 ± 0.07	
		4	135	2.21 ± 0.12	1.28 ± 0.9	
	Ñireco river	1	365	n.a.	n.a.	
		3	81			
R. spinulosa	Ñireco river	1	360	n.a.	0.32 ± 0.01	<0.001**
		2	100	n.a.	0.13 ± 0.003	
		3	81	n.a.	0.20 ± 0.008	

Note: *P*-values for comparisons are shown. n.a. = data not available. Comparison of metamorphs between: * localities; ** wetlands.

For the experiment, we used a stock of tadpoles reared from eggs collected in the field. Tadpoles came from four clutches of *Pleurodema bufoninum* and four clutches of *Rhinella spinulosa* from Ñireco locality (wetland 1; Table 1), as well as four clutches of *Pleurodema thaul* collected from Fantasma lagoon. Clutches of each species were mixed, transferred, and placed in outdoor 500-L tanks (one tank per species) to allow eggs to hatch. Tadpoles of each species were fed with a mix of algal culture and fish food (VitaFish®) until they reached stage 25–26. For the experiments, they were haphazardly selected from a mixture of four clutches (Gosner, 1960).

We ran a '*Pleurodema* experiment' in spring 2006. The experiment consisted of a factorial design with two factors, anuran species (two levels: *P. bufoninum* and *P. thaul*) and water volume (two levels: constant or decreasing water level) arranged into six blocks (resulting in six replicate/treatment combinations). We also performed a '*Rhinella spinulosa* experiment', which was run in spring 2007, to examine water volume as a single factor with two levels of water volume (constant or decreasing water level). Each treatment was replicated 10 times.

The experimental units consisted of circular plastic tanks (38 cm diameter). The tanks were filled with 500 g of mud from wetlands as substrate and 9 L of tap water, and inoculated with a mix of algal culture (*Scenedesmus* sp. + *Clamidomonas* sp.). One week later, we introduced five tadpoles into each tank that were fed every 3 days with fish food (VitaFish®). *Pleurodema thaul* tadpoles were of clutches from Fantasma lagoon, and *Pleurodema bufoninum* and *Rhinella spinulosa* tadpoles were of clutches from the Ñireco river. For each experiment, tanks were displayed in randomized arrays to homogenize temperature and light. Tadpoles in the constant water treatment were maintained in a constant volume of 9 L, whereas in the decreasing water treatment, water was allowed to evaporate constantly throughout the experiment until the volume reached a minimum of 3 L, at which time this 'minimum' was maintained. To determine whether hydroperiod affects time to complete metamorphosis and size at metamorphosis, all tadpoles were allowed to complete larval development (stage 46). During the experiment, we recorded water temperature in all the tanks every hour using data loggers (HOBO) to determine differences in temperature between treatments. Because no differences in temperature were observed between treatments, this variable was not included in the statistical analyses.

Statistical analyses

Statistical analyses were conducted for both data from field samples and data from tank means (experiments). For all individuals we measured two response variables: size (SM) and length of larval period (LP) at metamorphic climax (stage 41) or metamorphosis (stage 46). Variables were computed as the mean per tank for forward analysis. One-way and two-way analyses of variance (ANOVA) to test for differences between size at metamorphosis and larval period between species and localities were performed using SPSS software (SPSS, Inc., Chicago, IL). To test the species × environment interaction, we performed separate univariate analyses for each trait in each experiment. *Post-hoc* tests were performed when necessary and for specific cases.

To homogenize variances between treatments, all the variables recorded at stage 41 were transformed as follows: SM to natural logarithmic transformation and LP to inverse transformation (1/LP). This transformation followed Smith-Gill and Berven (1979), which interprets inverse LP as the average developmental rate over the larval period.

Since the distribution of observations within each treatment was approximately bivariate normal, the regression of one variable on the other passes close to the bivariate mean of the variables (SM and LP). In that case, we described the reaction norm of each species as the linear regression of the natural logarithm of SM on 1/LP, and tested for differences between species throughout the slopes of these regressions (Blouin, 1992).

Quantitative estimation of phenotypic plasticity

To quantify the magnitude of phenotypic plasticity, we measured life-history traits (size at metamorphosis and larval period) by assessing changes in these traits that occurred between treatments and divided by the mean value of the trait in the constant treatment (Richter-Boix *et al.*, 2006). Negative and positive values indicate a decrease and an increase in the trait value, respectively.

RESULTS

Plasticity of size in natural environments

The three species studied showed similar responses to the drying ponds when comparing metamorph sizes.

Pleurodema bufoninum was present in ponds associated with three river localities (Bernal, Ñirihuau, and Ñireco rivers; Table 1). Each of these localities has several ponds with

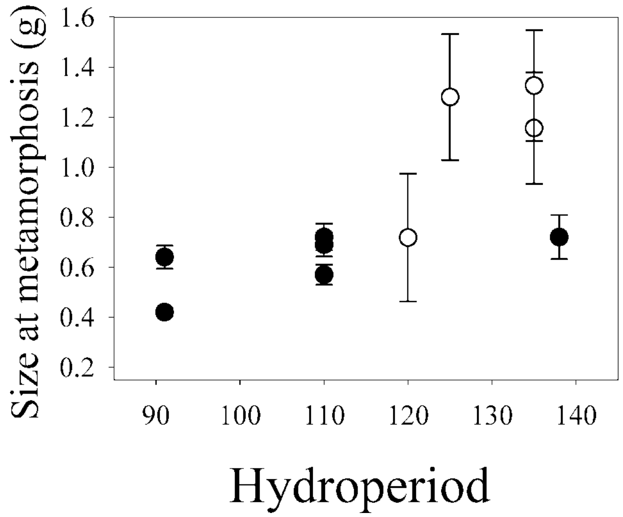

Fig. 1. Size at metamorphosis and length of hydroperiod of two *Pleurodema bufoninum* populations. ○, Ñirihuau; ●, Bernal.

variable hydroperiods (from ephemeral to permanent). Comparisons of size at metamorphosis in two of the localities (Bernal and Ñirihuau) showed significant differences between them and among wetlands at each locality (Table 1, Fig. 1). Larvae and metamorphs in Ñirihuau were larger than individuals in Bernal locality (mean size of larvae ± s.d.: Ñirihuau = 2.04 ± 0.06 g, $n = 53$; Bernal = 1.51 ± 0.05 g, $n = 54$; mean size of metamorphs ± s.d.: Ñirihuau = 0.97 ± 0.36 g, $n = 59$; Bernal = 0.65 ± 0.22 g, $n = 47$; details of each wetland in Table 1). When exploring size of metamorphs at each locality, we found that at Ñirihuau sizes were significantly different between wetlands (ANOVA: $F_{3,46} = 19.35$, $P < 0.001$). *Post-hoc* comparisons showed that metamorphs at wetlands with short hydroperiods were smaller than those at wetlands with prolonged hydroperiods (wetland 1 ≠ wetlands 2, 3, and 4, $P < 0.001$; Fig. 1, Table 1). This pattern was also observed in Bernal, with larvae showing significant differences in their sizes among wetlands (ANOVA: $F_{5,53} = 5.35$, $P < 0.001$; Table 1). Metamorphs from wetlands with short hydroperiods were significantly smaller than those from wetlands with long hydroperiods (wetland 5 ≠ wetlands 1, 2, 4, and 6, $P < 0.005$; Fig. 1, Table 1).

In *Pleurodema thaul*, metamorphs at two localities with different hydroperiod were significantly different. At Fantasma lagoon, metamorphs were significantly larger than metamorphs at Pinar de Festa pond (Mann-Whitney $U = 698.5$, $P < 0.001$; Table 1). We also tested for differences between cohorts at each locality. In Fantasma lagoon, we observed five different cohorts throughout the breeding season, with significant differences between cohort sizes (Fantasma lagoon: $F_{4,31} = 39.31$, $P < 0.001$, $n = 36$; cohorts 1, 2, and 3 ≠ 4 and 5 and cohort 4 ≠ 5, $P < 0.001$; Fig. 2A). A different pattern was observed at Pinar de Festa pond, which had only two cohorts during the breeding season, with no significant differences between cohort sizes (Pinar de Festa pond: $F_{2,17} = 2.21$, $P = 0.14$, $n = 20$; Fig. 2B).

Rhinella spinulosa was present at the Ñireco river locality, in several ephemeral ponds associated with river shores. Comparisons of metamorphs between three of these ponds

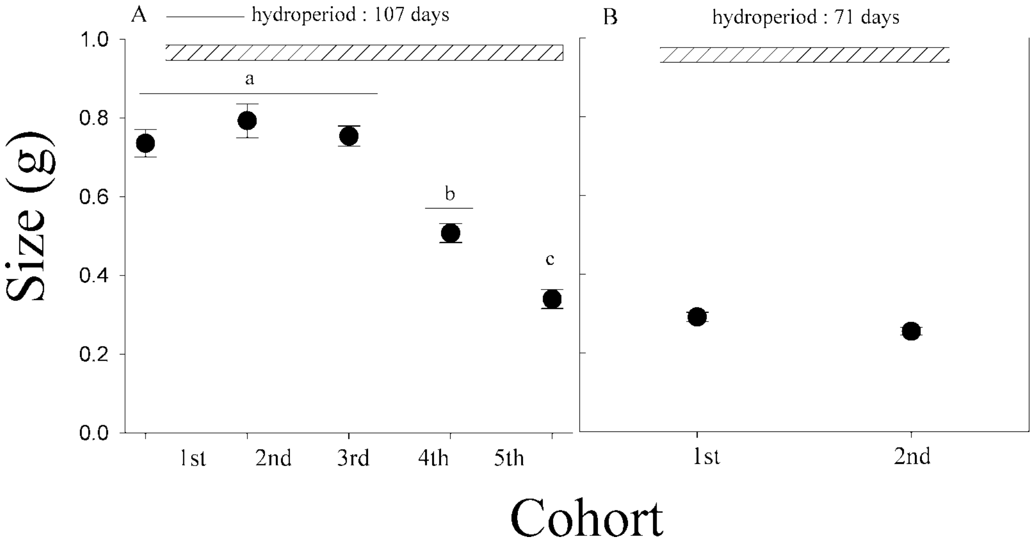

Fig. 2. Size of metamorphs (bars) and length of hydroperiod of *Pleurodema thaul* populations: (A) Fantasma, (B) Pinar de Festa. The x-axis indicates cohorts observed across a breeding season. Letters indicate significant differences in size ($P < 0.005$).

revealed that they were significantly different (Kruskall-Wallis $H_2 = 18.37$, $P < 0.001$, $n = 23$). Metamorphs at pond 1, closely associated with the river with a long hydroperiod (permanent pond, 360-day permanence), reached significantly larger sizes than those from the other two ephemeral ponds (Dunn's test: $P < 0.001$; Fig. 3).

Plasticity in experimental mesocosms

Results of untransformed SM and LP reached by each species and the hydroperiod treatment combination are shown in Table 2. The two-way ANOVA comparing size at metamorphosis between species and hydroperiod treatments showed significant differences between hydroperiod treatments ($F_{1,18} = 13.61$, $P = 0.002$), but no differences in size between *Pleurodema* species ($F_{1,18} = 1.61$, $P < 0.22$). In general, *P. thaul* reached larger sizes than *P. bufoninum* with the same hydroperiod treatments (Table 2). The interaction was not significant ($F_{1,18} = 0.27$, $P = 0.60$). When comparing hydroperiod treatments for each individual species, larvae of *P. thaul* reached metamorphic climax (stage 41) at a larger size in the constant water treatment than in the decreasing water treatment (*P. thaul*, $t = 4.128$, $P = 0.003$; Table 2), but *P. bufoninum* did not show differences between treatments ($t = 0.920$, $P = 0.379$; Table 2). *Rhinella spinulosa* showed significant differences in size with the different hydroperiod treatments. Larvae reached metamorphic climax at a larger size in the constant water treatment than in the decreasing water treatment ($t = 2.411$, $P = 0.027$; Table 2).

Length of larval period was significantly different between *Pleurodema* species and between hydroperiod treatments (species: $F_{1,20} = 7.84$, $P = 0.015$; hydroperiod treatment: $F_{1,20} = 17.27$, $P = 0.001$). Larval period was longer in *P. thaul* than in *P. bufoninum* metamorphs in both treatments. In both species, time to metamorphosis was shorter with the

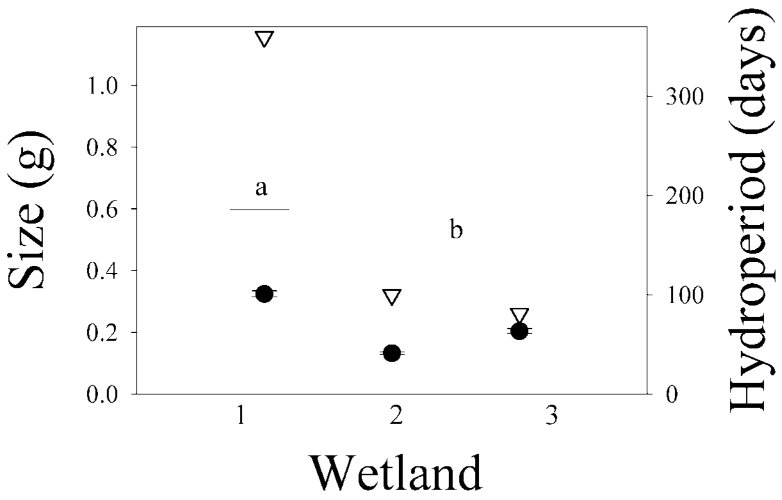

Fig. 3. Size of metamorphs (●) and length of hydroperiod (▽) of *Rhinella spinulosa* at Ñireco. Letters indicate significant differences in size ($P < 0.001$).

Table 2. Size at metamorphosis and length of larval period: mean (± S.E.) of untransformed data of larvae (stage 41) and metamorphs (stage 46) in each species for each treatment

Species	Hydroperiod regimen	Size at metamorphosis (g)		Larval period (days)	
		Larvae	Metamorphs	Larvae	Metamorphs
2006					
P. bufoninum	Constant	0.38 ± 0.03 ($n = 6$)	0.25 ± 0.009 ($n = 6$)	63.16 ± 5.9 ($n = 6$)	68.58 ± 3.01 ($n = 6$)
	Decreasing	0.34 ± 0.01 ($n = 6$)	0.21 ± 0.008 ($n = 6$)	47.25 ± 5.8 ($n = 6$)	55.08 ± 4.33 ($n = 6$)
P. thaul	Constant	0.63 ± 0.03 ($n = 5$)	0.27 ± 0.01 ($n = 5$)	59.1 ± 5.3 ($n = 5$)	77.2 ± 2.68 ($n = 5$)
	Decreasing	0.38 ± 0.04 ($n = 5$)	0.23 ± 0.007 ($n = 5$)	59.2 ± 5.6 ($n = 5$)	62.7 ± 4.36 ($n = 5$)
2007					
R. spinulosa	Constant	0.26 ± 0.01 ($n = 5$)	0.10 ± 0.006 ($n = 5$)	52.5 ± 3.2 ($n = 5$)	59.61 ± 1.11 ($n = 5$)
	Decreasing	0.2 ± 0.01 ($n = 5$)	0.11 ± 0.009 ($n = 5$)	49.5 ± 2.6 ($n = 5$)	53.02 ± 2.58 ($n = 5$)

decreasing water treatment (Table 2). Size of metamorphs showed significant differences between *Pleurodema* species but no differences between hydroperiod treatments (species: $F_{1,20} = 9.55$, $P = 0.008$; hydroperiod treatment: $F_{1,20} = 6.98$, $P = 0.02$). Also, individuals reached smaller sizes in the decreasing water treatment than in the constant water treatment.

The univariate analysis to test for differences between hydroperiod treatments in *Rhinella spinulosa* showed that they reached metamorphosis in a shorter time in the decreasing water treatment than in the constant treatment (mean ± S.E.: constant water treatment = 59.61 ± 1.1 days, decreasing water treatment = 53.02 ± 2.58 days; $t = 2.51$, $P = 0.02$) (Table 2). However, no differences in metamorph sizes were observed between treatments (mean ± S.E.: constant water treatment = 0.102 ± 0.006 g, decreasing water treatment = 0.113 ± 0.01 g; $t = 0.984$, $P = 0.34$) (Table 2).

Pleurodema bufoninum and *P. thaul* had parallel norms of reaction at SM, changing proportionately in the two species. In contrast, the norms of reaction of 1/LP were not parallel in the two *Pleurodema* species. *Pleurodema bufoninum* appeared to have a shorter larval period than *P. thaul* in the decreasing water treatment (Fig. 4). However, this was not obvious from the results of the ANOVA. Changes were not significant between *Pleurodema* species ($F_{1,18} = 1.18$, $P = 0.29$) and treatments ($F_{1,18} = 2.14$, $P = 0.16$), and there was no significant species × treatment interaction ($F_{1,20} = 2.41$, $P = 0.13$; Fig. 5). In addition, no differences were observed for 1/LP in *R. spinulosa* ($t = 0.065$, $P = 0.94$; Fig. 4).

The slope of the bivariate change in SM and 1/LP across the water treatments was statistically different between species (test for parallel slopes: $F_{2,30} = 6.84$, $P < 0.005$). This result indicates non-parallel norms of reaction (Fig. 5) as observed in the species-specific analysis of norms of reaction (Fig. 4). This non-parallel or significant interaction results because *Pleurodema thaul* and *Rhinella spinulosa* show steeper slopes in their norms of

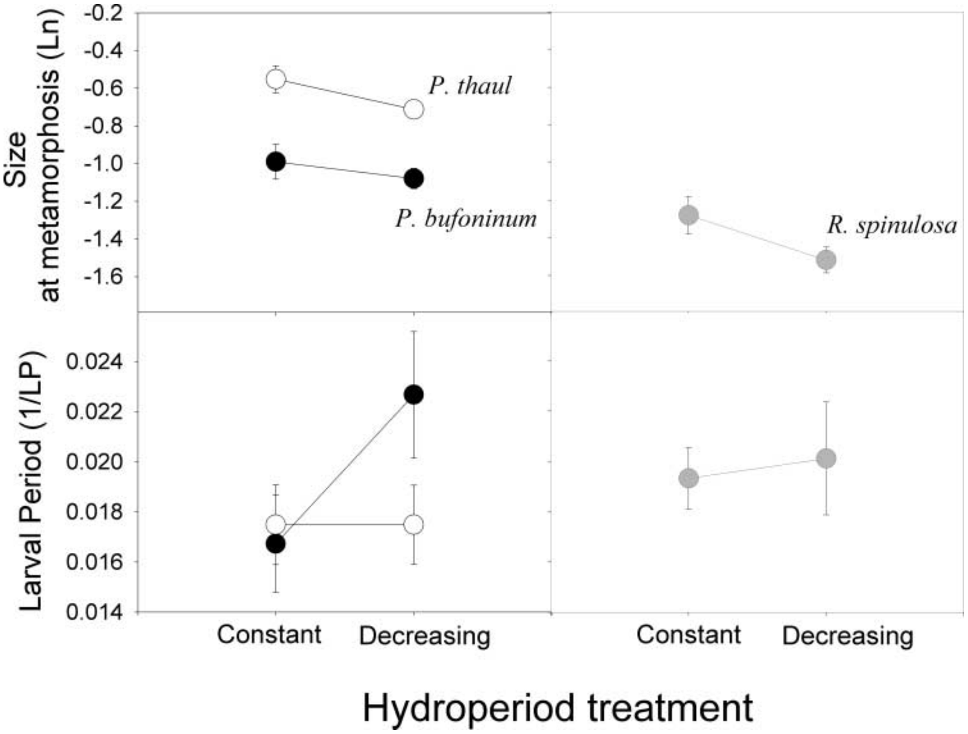

Fig. 4. Reaction norms across hydroperiod regimens of size at metamorphosis (SM) and larval period at metamorphosis (1/LP) for each species in each treatment (mean ± 1 S.E.). Long periods until metamorphosis = smaller values of 1/LP.

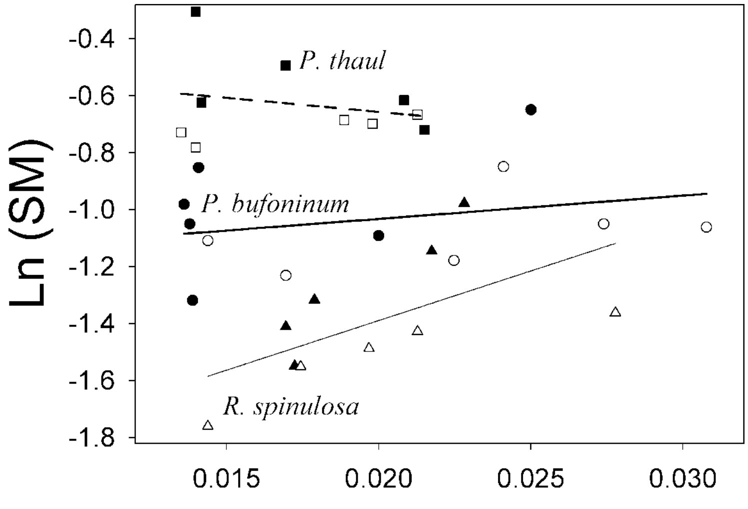

Fig. 5. Reaction norms for size at metamorphosis and larval period across hydroperiod treatments in *Pleurodema bufoninum* (circles), *Pleurodema thaul* (squares), and *Rhinella spinulosa* (triangles). Solid symbols represent larvae in the constant hydroperiod treatment; empty symbols represent larvae in the decreasing hydroperiod treatment.

reaction than *Pleurodema bufoninum* (Fig. 4). Although *P. bufoninum* showed the largest absolute change in 1/LP between the two water treatments (Fig. 4), the univariate test comparing the treatments was not significant ($t = 1.65$, $P = 0.13$) and no differences were observed at size at metamorphosis ($t = 0.83$, $P = 0.42$). There was a marginal difference in size at metamorphosis in the univariate comparison of *P. thaul* ($t = 2.16$, $P = 0.06$) and no differences in size at metamorphosis between the treatments in *R. spinulosa* ($t = 1.94$, $P = 0.8$) (Fig. 4).

Estimates of phenotypic plasticity of larval period and mass at metamorphosis

Species showed different patterns of phenotypic plasticity in the experiments, expressed as the difference observed in the magnitude of the variables measured (size and larval period to metamorphosis). Although changes between species were not significant (larval period: $F_{2,13} = 1.13$, $P = 0.351$; size at metamorphosis: $F_{2,13} = 2.19$, $P = 0.151$) (Fig. 6), a trend was observed. For the unrelated *Pleurodema thaul and Rhinella spinulosa*, the larval period was similar but not plastic, while *Pleurodema bufoninum* showed a higher magnitude of phenotypic plasticity in the larval period. In contrast, *P. bufoninum* showed no plasticity in size at metamorphosis, while *P. thaul* and *R. spinulosa* showed a high plasticity for this trait.

DISCUSSION

We compared the phenotypic plasticity of size and development time in different species and different populations of three Patagonian anurans, with pond drying as a selective factor operating on populations. We found that tadpoles of the Patagonian *Pleurodema*

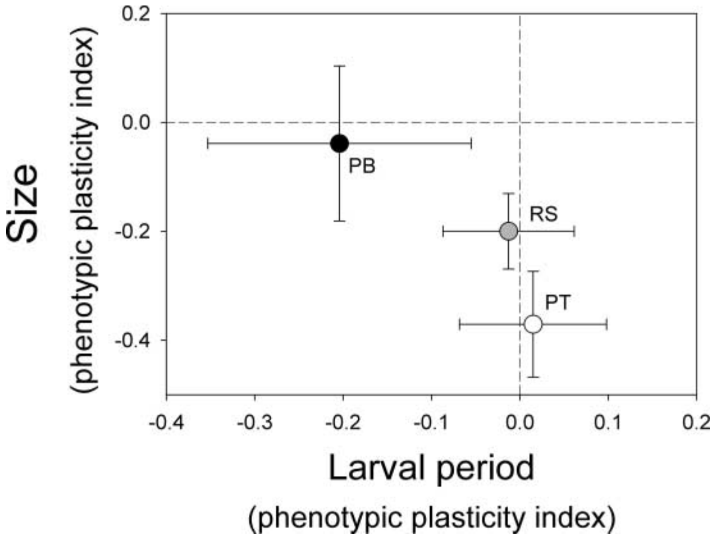

Fig. 6. Changes in phenotypic plasticity between treatments (index) for larval period and size at metamorphosis (mean and standard error are shown). PB = *Pleurodema bufoninum*, PT = *Pleurodema thaul*, RS = *Rhinella spinulosa*.

bufoninum, *Pleurodema thaul*, and *Rhinella spinulosa* face unpredictable environments, which show high spatiotemporal variation of desiccation risk among localities (populations) and ponds at each locality (Jara, 2010). Local populations of these species have access to temporary ponds as well as to permanent ponds, being exposed to different environmental states in a localized area, allowing phenotypic plasticity to evolve in size and age to metamorphosis.

In our study sites, *Pleurodema* species were present in ephemeral, temporary and, occasionally, permanent ponds, while *Rhinella spinulosa* was present mostly at temporary ponds, sometimes connected to permanent wetlands. According to Morey (1998), larvae that inhabit longer-lived ponds develop more slowly, which delays metamorphosis, resulting in a larger body size. As predicted, these Patagonian species responded to the selective pressure of drying environments by reducing their size. These responses were evident when comparing populations exposed to wetlands with different rates of desiccation, and also when comparing different cohorts of the same population. To our knowledge, this is the first study to report results comparing different cohorts, i.e. looking for adaptive responses when the risk (desiccation) is considered in a single season and constitutes a short-term selective pressure.

Based on our experimental results, *Pleurodema bufoninum* reached a threshold in size at metamorphosis, with significant differences between hydroperiod treatments. However, this result was proportional to acceleration in development time after the threshold was matched by a trade-off in reduced body size at metamorphosis in drying environments as observed in nature. In natural environments, *P. bufoninum* was shown to be consistent with the general prediction about reducing size as a response to drying conditions. This response was observed when we compared wetlands with different hydroperiods, and also when comparing ponds with different permanence for the same population (locality). Our comparisons were carried out in ponds that ranged between 90 and 140 days in duration, and a reduction in size was evident when ponds differed by almost 2 months in hydroperiod. Tadpoles that face different drying conditions in the same locality and throughout the breeding season (variation at spatial scale) might be able to respond to the selective force imposed by the differential drying risks, resulting in individuals with smaller sizes. Furthermore, individuals with rapid larval growth often reach metamorphosis sooner, which might improve fitness by minimizing exposure to sources of mortality in the aquatic environment (Wilbur, 1984, 1988; Werner, 1986). These observed responses (variability in developmental expressions) to the drying conditions experienced by *P. bufoninum* could be explained as adaptive phenotypic plasticity, rather than local specialization. However, this variation in size in natural environments (among ponds with different desiccation rates) might also be the result of such factors as temperature and food availability that are linked with the wetland hydroperiod (Tejedo and Reques, 1994; Denver *et al.*, 1998; Loman, 1999). In addition, maternal effects (egg size) (Márquez-García *et al.*, 2009) could also explain trait variability, but these effects were not evaluated in this study. Time to metamorphosis (larval period) in *P. bufoninum* was not calculated in natural populations, but was shown to have a strong response in our experimental treatments.

Pleurodema thaul was studied at two different localities that differed in their hydroperiod characteristics. This species was shown to have a reproductive strategy with a partially continuous reproductive cycle (Díaz-Péz and Ortiz, 2001) different from that of *P. bufoninum*, with several cohorts present in the wetlands throughout the reproductive season. Since we observed that earlier cohorts reached larger sizes than later cohorts, we can assume that early breeding is more competitive (Loman, 1984; Enoksson, 1993)). Also, this reasoning carries the assumption that early breeding (egg laying) also means early metamorphosis (Loman, 2009). As observed in high latitudes in the northern hemisphere, in Patagonia wetlands have quite low temperatures during early spring, sometimes with ice covering the ponds. These environments are particularly enclosed in dry mountain landscapes with high solar radiation, dry summers, and long periods of snow cover in the winter (Perotti *et al.*, 2005; Márquez-García *et al.*, 2009). At our study sites, water temperature tended to increase as spring progressed. Consequently, late eggs experience, on average, higher temperatures, which allow tadpoles to develop faster, as observed in several studies (Beattie, 1987; Laugen *et al.*, 2002). Late cohorts of *P. thaul* at Fantasma lagoon (December–January) experienced higher water temperatures as spring advanced (water temperatures ranging from 9.58°C in September to 14.11°C in December). Hence, these stressful conditions operating during the larval stages (e.g. lower water volume, lower resource levels) appear to promote a faster whole-body developmental rate that allows larvae to escape from a poor growing environment at the cost of smaller sizes, which could affect fitness. Also, it has been proposed that the breeding date or the genetic differences in developmental and growth rates can profoundly affect size and development time (Loman, 2009). Then, eggs laid late and differing genetically can show different developmental patterns as a function of different temperature and desiccation patterns. This has been suggested in a study at a regional level (Sparks *et al.*, 2007), but there are very few studies at the local level (Loman, 2009). Also, Laugen *et al.* (2002) found that egg development time has a genetic component. However, further studies are required to test for this.

Other research has attempted to explain the mechanisms that are responsible for the accelerated development in ponds of short duration, with contradictory results (Székely *et al.*, 2010). Some authors have suggested that larvae respond to temperature, crowding populations, and the increase in solute concentration (Newman, 1992 and references therein). As regards temperature, there were no differences between our experimental treatments. Thus, size and developmental differences observed between treatments could be responses to the water level or the associated increase in solute concentration. However, further experiments are required to evaluate the variables associated with the desiccation process (water chemistry and food resources) and the effects related to delaying or accelerating metamorphosis.

In contrast, developmental responses of *P. thaul* cohorts at Pinar de Festa pond did not show any differences. At this locality, only two cohorts were observed, and metamorphs did not show differences in size at metamorphosis between the two cohorts, although the hydroperiod was shorter than at Fantasma lagoon. The shorter duration of this pond could have led to the absence of changes between cohort sizes, with these two cohorts comparable to the late cohorts at Fantasma lagoon (Figs. 2A and B). It is also possible that the characteristics of the pond at Pinar de Festa are the cause of the quite different cohort responses observed.

The analysis of phenotypic plasticity showed different patterns as a consequence of the different magnitudes reached by the variables measured (larval period and size at metamorphosis). In our experiment, *P. thaul* and *R. spinulosa*, which are unrelated species, showed no plasticity for larval period, while *P. bufoninum* showed a strong plasticity for this trait. The opposite trend was observed for size at metamorphosis, which was much more plastic in *P. thaul* and *R. spinulosa* than in *P. bufoninum*. Position in the phenotypic space of these bivariate changes could be related to habitat type rather than to a lineage relationship. Thus, our results show that Patagonian anuran responses could be explained by phenotypic plasticity when environmental changes become a strong selective force (in our case, drying ponds). This is supported by the changes observed in size at metamorphosis, both in natural environments and in controlled experiments, and by changes in the larval period in two of the species (*P. thaul* and *R. spinulosa*). Based on comparisons of these three anuran species and their populations, we conclude that our results show strong evidence of the adaptive significance of size and time to metamorphosis when pond drying is a powerful selective force.

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REFERENCES

Altwegg, R. and Reyer, H.-U. 2003. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*, **57**: 872–882.

Beattie, R.C. 1987. The reproductive biology of common frog (*Rana temporaria*) populations from different altitudes in northern England. *J. Zool. (Lond.)*, **211**: 387–398.

Blakley, N. 1981. Life history significance of size-triggered metamorphosis in milkweed bugs *(Oncopeltus)*. *Ecology*, **62**: 57–64.

Blouin, M.S. 1992. Comparing bivariate reaction norms among species: time and size at metamorphosis in three species of Hyla (Anura: Hylidae). *Oecologia*, **90**: 288–293.

Buns, M. and Ratte, H.T. 1991. The combined effects of temperature and food consumption on body weight, egg production and development time in *Chaoborus crystallinus* DeGeer (Diptera: Chaoboridae). *Oecologia*, **88**: 470–476.

Dayton, G. and Fitzgerald, L.A. 2005. Priority effects and desert anuran communities. *Can. J. Zool.*, **83**: 1112–1116.

Denver, R.J., Mirhadi, N. and Phillips, M. 1998. An experimental analysis of adaptive plasticity in amphibian metamorphosis: developmental response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology*, **79**: 1859–1872.

Díaz-Páez, H. and Ortiz, J.C. 2001. The reproductive cycle of *Pleurodema thaul* (Anura, Leptodactylidae) in Central Chile. *Amphibia-Reptilia*, **22**: 431–445.

Enoksson, B. 1993. Effects of female age on reproductive success in European nuthatches breeding in natural cavities. *Auk*, **110**: 215–221.

Ferraro, D.P. and Casagranda, M.D. 2009. Geographic distribution of the genus *Pleurodema* in Argentina (Anura: Leiuperidae). *Zootaxa*, **2024**: 33–55.

Gervasi, S.S. and Foufopoulos, J. 2008. Costs of plasticity: responses to desiccation decrease post-metamorphic immune function in a pond-breeding amphibian. *Funct. Ecol.*, **22**: 100–108.

Goater, C.P. 1994. Growth and survival of postmetamorphic toads: interactions among larval history, density and parasitism. *Ecology*, **75**: 2264–2274.

Goater, C.P., Semlitsch, R.D. and Bernasconi, M.V. 1993. Effects of body size and parasite infection on the locomotory performance of juvenile toads, *Bufo bufo. Oikos*, **66**: 129–136.

Gosner, N. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**: 183–190.

Howard, R.D. 1988. Reproductive success in two species of anurans. In *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems* (T.H. Clutton-Brock, ed.), p. 99–113. Chicago, IL: University of Chicago Press.

Jara, F.G. 2010. Plasticidad fenotípica en anuros Patagónicos de los géneros *Pleurodema y Rhinella*: respuestas al hidroperíodo y a los depredadores. Doctoral thesis, Universidad Nacional del Comahue, Centro Regional Universitario Bariloche.

Jara, F.G. and Perotti, M.G. 2010. Risk of predation and behavioral response in three anuran species: influence of tadpole size and predator type. *Hydrobiologia*, **644**: 313–324.

Jara, F.G., Ubeda, C.A. and Lozada, M. 2007. Effect of larval density on development and metamorphosis in two anuran species (Bufonidae and Leptodactylidae) of the Patagonian steppe. *Cuad. Herpetología*, **21**: 53–58.

Laugen, A.T., Laurila, A. and Merilä, A. 2002. Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life-history traits. *Biol. J. Linn. Soc.*, **76**: 61–70.

Loman, J. 1984. Breeding success in relation to parent size and experience in a population of the hooded crow *Corvus corone cornix. Ornis Scand.*, **15**: 183–187.

Loman, J. 1999. Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration. *Amphibia-Reptilia*, **20**: 421–430.

Loman, J. 2009. Primary and secondary phenology: does it pay a frog to spawn early? *J. Zool.*, **279**: 64–70.

Márquez-García, M., Correa-Solis, M., Sallaberry, M. and Méndez, M.A. 2009. Effects of pond drying on morphological and life-history traits in the anuran *Rhinella spinulosa* (Anura: Bufonidae). *Evol. Ecol. Res.*, 2009, **11**: 803–815.

Morey, S.R. 1998. Pool duration influences age and body mass at metamorphosis in the western spadefoot toad: implications for vernal pool conservation. In *Ecology, Conservation, and Management of Vernal Pool Ecosystems – Proceedings from a 1996 Conference* (C.W. Witham, E.T. Bauder, D. Belk, W.R. Ferren, Jr. and R. Ornduff, eds.), pp. 86–91. Sacramento, CA: California Native Plant Society.

Morey, S. and Reznick, D. 2001. Effects of larval density on postmetamorphic spadefoot toads (*Spea hammondii*). *Ecology*, **82**: 510–522.

Newman, R.A. 1992. Adaptive plasticity in amphibian metamorphosis. *Bioscience*, **42**: 671–678.

Newman, R.A. and Dunham, A.E. 1994. Size at metamorphosis and water loss in a desert anuran (*Scaphiopus couchii*). *Copeia*, **1994**: 372–381.

Pechenik, J.A. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? *Ophelia*, **32**: 63–94.

Perotti, M.G., Diéguez, M.C. and Jara, F.G. 2005. State of the knowledge of north Patagonian wetlands (Argentina): major aspects and importance for regional biodiversity conservation. *Rev. Chil. Hist. Nat.*, **78**: 723–737.

Reques, R. and Tejedo, M. 1997. Reaction norms for metamorphic traits in natterjack toads to larval density and pond duration. *J. Evol. Biol.*, **10**: 829–851.

Richter-Boix, A., Llorente, G.A. and Montori, A. 2006. Effects of phenotypic plasticity on post-metamorphic traits during pre-metamorphic stages in the anuran *Pelodytes punctatus*. *Evol. Ecol. Res.*, **8**: 309–320.

Schaffer, H.B., Alford, R.A., Woodward, B.D., Richards, S.J., Altig, R.G. and Gascon, C. 1994. Quantitative sampling of amphibian larvae. In *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians* (W.R. Heyer, M.A. Donnelly, R.W. McDiarmid, L.C. Hayek and M.S. Foster, eds.), pp. 130–141. Washington, DC: Smithsonian Institution Press.

Scott, D.E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum. Ecology*, **75**: 1383–1396.

Smith, D.C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. *Ecology*, **68**: 344–350.

Smith-Gill, S.J. and Berven, K.A. 1979. Predicting amphibian metamorphosis. *Am. Nat.*, **113**: 563–585.

Sparks, T., Tryjanowski, P., Cookje, A., Crick, H. and Kuzniak, S. 2007. Vertebrate phenology at similar latitudes: temperature response differs between Poland and the United Kingdom. *Climate Res.*, **34**: 93–98.

Sympson, V., Jara, F.G. and Úbeda, C.A. 2006. *Bufo spinulosus papillosus* reproduction. *Herpetol. Rev.*, **37**: 200–201.

Székely, P., Tudor, M. and Cogălniceanu, D. 2010. Effect of habitat drying on the development of Eastern spadefoot toad (*Pelobates syriacus*) tadpoles. *Amphibia-Reptilia*, **31**: 425–434.

Tejedo, M. and Reques, R. 1994. Plasticity in metamorphic traits of natterjack tadpoles: the interactive effect of density and pond duration. *Oikos*, **71**: 295–304.

Tejedo, M., Marangoni, F., Pertoldi, C., Richter-Boix, A., Laurila, A., Orizaola, G. *et al.* 2010. Contrasting effects of environmental factors during larval stage on morphological plasticity in post-metamorphic frogs. *Climate Res.*, **43**: 31–39.

Twombly, S. 1996. Timing of metamorphosis in a freshwater crustacean: comparison with anuran models. *Ecology*, **77**: 1855–1866.

Úbeda, C.A. 1998. Batracofauna de los bosques templados patagónicos: un enfoque ecobiogeográfico. Doctoral thesis, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Buenos Aires.

Werner, E.E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am. Nat.*, **128**: 319–341.

Wilbur, H.M. 1980. Complex life cycles. *Annu. Rev. Ecol. Syst.*, **11**: 67–93.

Wilbur, H.M. 1984. Complex life cycles and community organization in amphibians. In *A New Ecology: Novel Approaches to Interactive Systems* (P.W. Price, C.N. Slobodchikoff and W.S. Gaud, eds.), pp. 114–131. New York: Wiley, New York.

Wilbur, H.M. 1988. Interactions between growing predators and growing prey. In *Interactions in Size-structured Populations: From Individual Behavior to Ecosystem Dynamics* (B. Ebenmann and L. Pensson, eds.), pp. 157–172. Berlin: Springer.