2

A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient.

Is developmental phenotypic plasticity an adaptive trait and therefore more flexible in variable and unpredictable environments? To answer this question we analysed developmental phenotypic plasticity of anuran larvae in function of their ecological breadth.
In the field, we examined the ecological breadth (spatial and temporal variability) of six anuran species (*Alytes obstetricans*, *Pelodytes punctatus*, *Bufo bufo*, *B. calamita*, *Hyla meridionalis* and *Rana perezi*) along a pond permanency gradient in a total of 240 ponds. Also, we designed a laboratory experiment to measure developmental plasticity (time to and size at metamorphosis) of each species using two treatments: (1) constant water level and (2) drying treatment. A comparative analysis of developmental plasticity in function of species ecological breadth and their phylogenetic relationship was made.
Species that use a wide variety of habitats or unpredictable environments showed a greater plasticity of responses than those occurring in predicable habitats. At the two extremes of the hydroperiod (ephemeral and permanent ponds) occur specialist developmental phenotypes with limited plasticity, whereas species from variable habitats (temporary ponds) can be considered plastic strategists with asymmetric bet-hedging. Results support the hypothesis that interspecific differences in developmental phenotypic plasticity are adaptive and are related to ecological breadth and unpredictability.

INTRODUCTION

The role of phenotypic plasticity in adapting to natural variable environments has been the focus of considerable studies (Schlichting & Pigliucci 1998; DeWitt & Scheiner 2004). To understand the evolution and adaptive nature of plasticity, it is necessary to study how plasticity is optimised and integrated with other strategies developed for dealing with variable and unpredictable environments. A comparative phylogenetic study among related or distant taxa can provide evidence of whether plasticity is correlated with differences in the environment in which species occur (Doughty 1995). Strong evidence of the adaptive significance of a trait

is obtained from comparisons among populations and species (Endler 1986). Several studies have compared the plasticity of species (e.g. Schlichting & Levin 1986; Bell and Sultan 1999;Leips et al. 2000), but most of these have limited their focus to two closely related taxa, with some exceptions (e.g. Pigliucci et al. 1999; Richardson 2002; Van Buskirk 2002). Furthermore, few studies contrast the plasticity of species included in a guild or community in the same region (e.g. Lardner 2000), and the contribution of plasticity and other strategies to community structure. To interpret phenotypic plasticity as an adaptive trait and to establish its contribution to species distribution, the environmental heterogeneity of species must be examined (Doughty & Reznick 2004). Here we studied the life-history response of tadpoles to desiccation in a anuran larvae community in the Mediterranean region in function of habitat breadth and temporal variability.

Plasticity is often thought to be adaptive, enabling tadpoles to develop a suitable life-history phenotype in order to respond to habitat desiccation (Newman 1992). Amphibian larvae exhibit plasticity in the timing of metamorphosis and capitalise on favourable conditions for growth while these conditions last. This plasticity may allow these larvae to match their phenotype to prevailing environmental conditions (Wilbur &Collins 1973). Species that show phenotypic plasticity may have a higher probability of survival in unpredictable habitats than those with canalised development (Newman 1992) and may occur in a wide range of habitats along the pond permanence gradient. Species do not show a random distribution and predictable assemblages are usually found along this gradient (Jeffries 1994; Skelly 1996; Babbitt et al. 2003). Ponds with different permanency periods exert selective pressures on organisms, which, in response, develop a range of adaptive strategies (Brock et al. 2003; Lake 2003; Johansson & Suhling 2004).

Here we addressed the following questions. (i) Does the magnitude of response of development time to pond drying differ among species in relation to habitat variability? (ii) Is the evolution of phenotypic plasticity in response to habitat desiccation constrained by historical events (phylogenetic perspective)?

MATERIAL AND METHODS

Study area and habitat characteristics

To characterise the ecological breadth of the species, we surveyed in the range of conditions and their frequency distribution of environment states in nature. The field study was confined to a littoral Mediterranean region covering 22645 ha around Barcelona in the north east of the Iberian Peninsula and containing isolated ponds that vary in hydroperiod. The Prelitoral Sierra climate has an annual average rainfall of around 600 mm and an annual average temperature above 18ºC. During the spring and summer of 2003, we surveyed a total of 246 isolated ponds as potential larval habitats of anura. Localities surveyed span the range of aquatic breeding habitats of the species studied, including ephemeral pools, and temporary and permanent ponds. The temporary ponds flood after strong autumn storms (September). The shallowest temporary ponds often dry out from winter onwards (December), whereas the deepest temporary ponds remain flooded until summer when dry. Many ephemeral and temporary ponds were flooded by rainfall in late February or early March and then dried up from mid-May to mid-July. The amphibian community of the area is comprised of 6 anuran species and 1 native urodela (*Salamandra salamandra*). The anuran species are: *Alytes obstetricans* (Discoglossidae), *Pelodytes punctatus* (Pelodytidae), *Bufo bufo* (Bufonidae), *Bufo calamita* (Bufonidae), *Hyla meridionalis* (Hylidae) and *Rana perezi* (Ranidae).

We assessed amphibian presence and successful reproduction in the ponds by dipnetting and egg searches. For all ponds, sampling periods for amphibians were determined by preliminary surveys and accounted for differences in breeding activity between species and ensured that all species were detected. In the spring and early summer (a minimum of four visits, covering the breeding period of all species), we used a dip-net to sample tadpoles and predacious invertebrates. A minimum of 5-10 dip-net sweeps were taken in potential tadpole microhabitats following standard techniques (Heyer et al. 1994). All amphibian specimens were identified in the field and returned to water. Predacious invertebrates were identified to order only (except Odonate larvae, which were identified to family level). Because the water at the study site was generally clear, we determined fish presence through visual surveys in addition to dip-net captures. Egg searches were conducted throughout the same period as dipnetting and consisted of searching water and submerged vegetation within 3

meters of the pond shore. We considered ponds successful breeding sites only when eggs and larvae were found. Eggs and larvae rather than adults were used to judge presence, so the data included actual breeding attempts.

Tadpoles inhabit ponds that vary along a gradient of permanency; this gradient has been extensively studied, and although it is continuous, two transitions have been identified (Wellborn et al. 1996): (1) the "permanence transition" between temporary and permanent ponds, and (2) the "predator transition" between permanent ponds without fish and permanent ponds with fish. We did not make the latter distinction because all the ponds studied here were isolated and did not hold native fish populations. We found only 6 ponds with fish, and these were excluded from our analyses. We limited our study to the "permanence transition", and we adjusted the freshwater gradient to 3 categories: (1) ephemeral ponds that dry within weeks (less than 60 days of duration); (2) temporal ponds that dry every year during spring or summer (until 180 days of duration); and (3) permanent ponds, defined as containing water all year (360 days). The remaining 240 ponds (excluding ponds with fish) were included in one of these categories. We visited ponds approximately every two weeks throughout the year to establish the date of drying.

From 2001 to 2003, we periodically monitored 73 of these ponds that represented all three categories. To establish the initiation of the hydroperiod, ponds were visited especially before heavy rain periods and were subsequently visited every week to establish the duration for each year. Thus, we established the variation of pond duration between years.

Laboratory experiments

Development phenotypic plasticity was measured in laboratory experiments during spring 2001 and spring 2002. In 2001 we conducted experiments with *Alytes obstetricans* and *Bufo bufo*. In 2002 we repeated the same experiments with *Pelodytes punctatus*, *Hyla meridionalis*, *Bufo calamita* and *Rana perezi*. All experiments were conducted in the same environmental chamber at the University of Barcelona, in a constant water temperature of 21ºC. Larvae from the six species were obtained from clutches collected in natural ponds from the study area (6 egg masses from *Alytes obstetricans*, 3 from *Pelodytes punctatus*, 6 from *Hyla meridionalis*, 3 from *Bufo bufo*, 3 from *Bufo calamita* and 6 from *Rana perezi*).

We collected clutches from temporary and permanent ponds to have a representative sample of possible differences between populations, except clutches of *Rana* all from permanent ponds. Egg masses hatched in buckets and all experiments were started when tadpoles had reached Gosner's stage 25.

We designed an experiment to analyse plastic response to drying using two treatments: a constant and a drying treatment. The former, which simulated a permanent pond without changes in water volume during tadpole development, had a larvae density of 3 individuals (each from distinct clutches to avoid population differences in phenotypic plasticity) per 2 liters. In contrast, the drying treatment simulated a temporary pond by reducing water volume during larvae development and had the same larval density. The water level decrease followed the curve $D_j = 1 - (j/t)^a P$ defined by Wilbur (1987), where D_j is the desired depth on day j, t is the target day for depth = 0 (110 in our case, approximately the mean duration of temporary ponds in our study area), a is a shape parameter (0.4 in our treatment), and P is the water depth at the start of the experiment. Each treatment was replicated 20 times, with the exception of the *Pelodytes* treatments, which were replicated 38 times.

Experimental units consisted of plastic tubs (27 cm diameter) filled with 2 liters of dechlorinated tap water. To reduce the probability of infection and fouling, the water was changed approximately every 12 days. In the drying treatment, we adjusted the water level every four days following the planned drying curves. Tadpoles were fed periodically with a mixture (4:1) of rabbit chow and fish food *ad libitum* in relation to the number of tadpoles and their body size in order to avoid food accumulation and problems derived from water fouling. After the first metamorph was observed, the tubs were checked daily and all metamorphs were collected and kept in plastic boxes with 5 mm of water until tail resorption was complete. For all the individuals, we measured: time to metamorphosis, or period elapsed since the start of the experiment until forelimb protusion at Gosner stage 42 (potential plastic variable response to drying by accelerated development), and mass at metamorphosis at tail resorption at Gosner stage 46 to 0.001 g precision (we used differences of mass at metamorphosis between treatments as a measure of cost of plasticity. Mass at metamorphosis is crucial for post-metamorphic fitness in amphibians (Altwegg & Reyer 2003)). Survival to metamorphosis was expressed as the proportion of larvae per tub that completed development.

Species ecological breadth

We calculated the mean and variability in habitat use for each species after assigning numerical values to each pond category, as listed above: 1 to ephemeral ponds, 2 to temporary ponds and 3 to permanent ponds. Variation in habitat use by species has two important components: spatial (among ponds) and temporal (within ponds but between years or seasons). Habitat variability can be determined by a generalist behaviour (those that breed along the entire freshwater gradient) or by the intrinsic temporal variability of the breeding habitat. Values of temporal variation within ponds were calculated from field data. We used data from the field surveys of 73 ponds in which we established the date of drying and duration over three years (2001-2003). Change in duration between years was used to examine variability in each pond category. For each species, we calculated an index of habitat variability, developed previously by Van Buskirk (2002). This index incorporates contributions from both sources. Spatial and temporal variation was calculated as ($p_e + 2p_t + p_p$), where p_e is the occurrence score in ephemeral ponds, p_t in temporary ponds, and p_p in permanent ponds. Temporary ponds (p_t) have the highest temporal variation (Figure 2A), and the weightings in this equation ensured that temporary ponds contributed most strongly to habitat variability.

Magnitude of phenotypic plasticity and statistical analyses

The response of tadpoles to the two experimental treatments was studied by analysis of variance for each species. We used the mean individual response for each experimental unit to avoid the lack of independence of individual measures and thus pseudoreplication. Mass at and time to metamorphosis were $\log_e$ transformed because of heterogeneity of variances between treatments. As survival data is not a continuous trait (we only have four categories) we used non-parametric Mann-Whitney U-test to compare between treatments. We also examined correlations between larval period and mass at metamorphosis for each species.

We conducted analyses with all data, and then repeated the same analyses but using only the early 40% tubs per treatment that had reached metamorphosis. This second analysis was performed to reduce bias promoted by the truncated distribution of those in the drying treatment wherein time to metamorphosis was limited and survival could be reduced by this

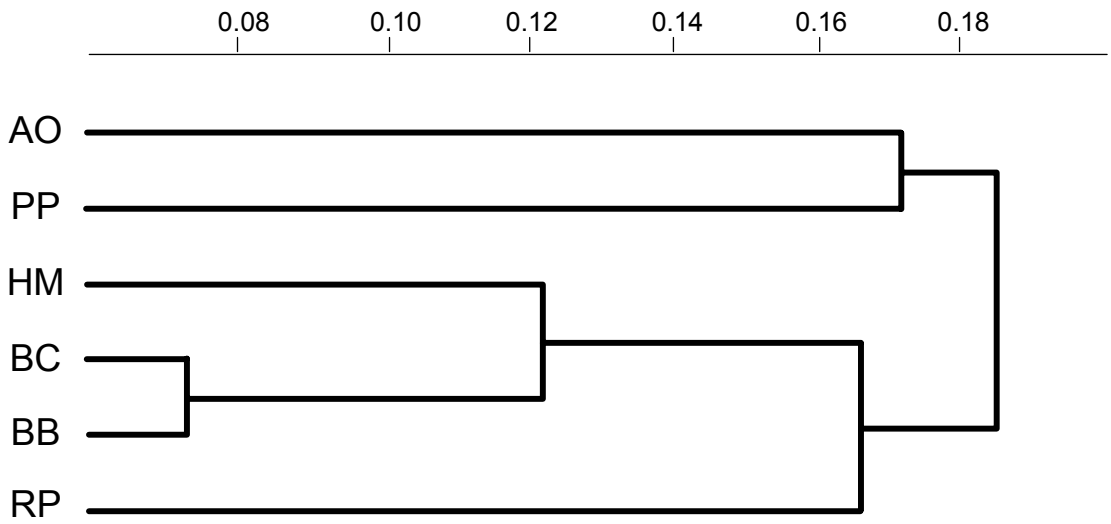


Fig. 1.- Phylogenetic hypothesis depicting relationships between the six species on the basis of genetic distances for three genes: 12S, 16S and cyt *b.* AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

time limitations. This is a common problem in studies that use time horizons. Consequently, some authors readjust the data series (e.g. Tejedo and Reques 1994) whereas others work with complete data sets. We performed both analyses to examine whether the use of complete data or truncated data series alters the interpretation of results.

To compare magnitude of phenotypic plasticity among species we need a unitless proportional measure of plasticity. For this reason we measured the plasticity of life-history traits by examining changes in traits that occurred between treatments divided by the mean value of the trait in the constant treatment ([drying – constant]/constant). In the drying treatment, negative values of plasticity reflect a decrease in the value of the trait (e.g. larval period), whereas positive values show an increase in this value.

If the traits measured affect species performance within a habitat type, then species from distinct habitats should differ in trait values. Therefore, we first tested for differences among the six species, regardless of habitat variability. To consider the effects of spatial and temporal variation in habitat, we ran analyses of variance using habitat as a fixed factor and species nested within habitat as a random factor. Species were nested within habitats

following the index of habitat variability (see above). As we expected that the magnitude of plasticity would be in function of habitat variation and predictability, we considered two groups of species in function of their index of habitat variability: constant habitats (predictable) and

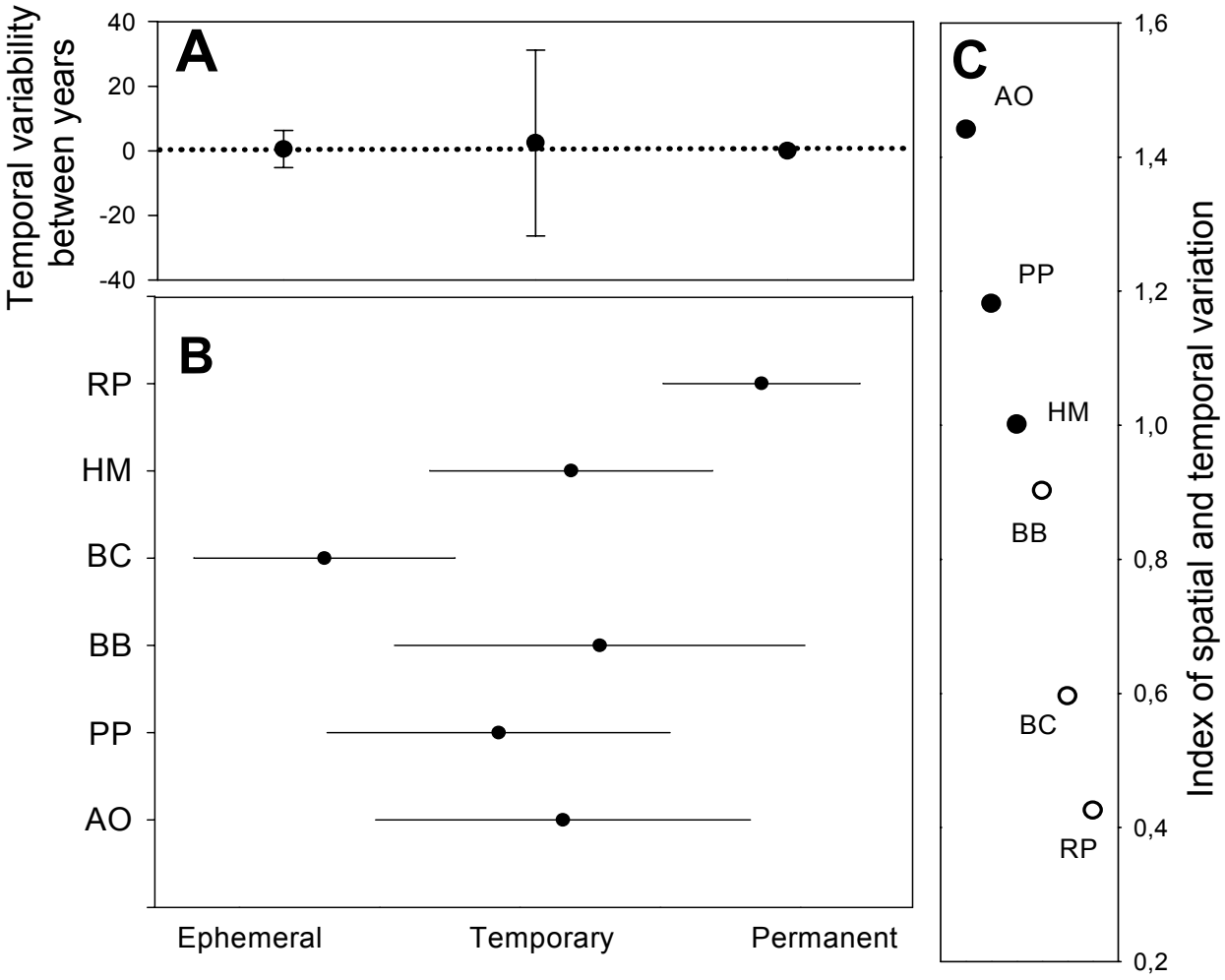


Fig. 2.- (**A**) Temporal variability from each pond category (X-axis: ephemeral, temporary and permanent) between years (2001-2003) using data from 73 ponds. Open circles represent habitats with high temporal variability and black circles low temporal variability. Graphics represent mean and standard error deviation. (**B**) Spatial use and variability of pond categories for each species. Graphics represent mean and standard error deviation. (**C**) Value of index of spatial and temporal variability for each species after applying the model explained in the Material and Methods section. Open circles represent species exposed to a high spatial and temporal variability (values over 1.0), whereas filled circles represent those from more predicable environments (values inferior to 1.0). AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

variable habitats (unpredictable). The six species were classified into one of these categories. Species with values ranging from 0.0 to 1.0 were considered from constant habitats while those between 1.0 and 2.0 were considered from variable habitats. As in previous analyses, we calculated the magnitude of plasticity in order to perform a comparison between species using complete and truncated data series (see above).

The trait values of species are influenced by shared common ancestry and thus species cannot be considered independent data points (Felsenstein 1985). To test whether the distribution of a particular species in phenotypic plasticity space is correlated with its phylogeny, we calculated Euclidean distances between species using standardised plastic traits values. The phylogenetic relationships between the six species were reconstructed. Phylogenetic distance analyses were performed using the combined data set, which included three genes: 12S, 16S and cyt *b*. Sequences were obtained from specimens in a personal collection (individuals collected and sequenced by Carranza) and from the GenBank database. All sequences were compiled, aligned and refined manually using Sequence Navigator. Observed distances in pairwise comparison were obtained using the software PAUP. We calculated correlation between the two matrices: distance for plasticity values between species (Mahalanobis distances from a discriminant analysis) and phylogenetic distances between species. To test the correlation between matrices, we applied a Mantel test. Correlation between the resulting evolutionary contrasts was repeated 5000 times and 95% confidence intervals were determined. Alternatively we tested phylogenetic independence to larval development with the computer program "Phylogenetic Independence 2.0" (Reeve & Abouheif 2003). Test For Serial Independence (TFSI) on continuous data were performed using the phylogenetic topology and node distances obtained from molecular reconstruction (Figure 1). Topology was randomly rotated 2000 times to build the null hypothesis.

RESULTS

Species' ecological breadth

All pond categories were present in a similar proportion in the study area (60 ephemeral ponds, 100 temporary ponds and 80 permanent ponds). Most of the species used the three categories of ponds. We tested if species differed in the frequency of use of the three ponds

categories with a Kruskal-Wallis ANOVA by ranks. Species don't use the three habitats with the same frequency (*Alytes* $H_{2, 31} = 30.0$, $p < 0.001$; *Pelodytes* $H_{2, 66} = 65.0$, $p < 0.001$; *B. bufo* $H_{2, 63} = 62.0$, $p < 0.001$; *B. calamita* $H_{2, 81} = 80.0$, $p < 0.001$; *Hyla* $H_{2, 56} = 55$ $p < 0.001$; *Rana* $H_{2, 31} = 30$, $p < 0.001$). Two species occupied habitats from the two extremes of the hydroperiod range (*B. calamita* the ephemeral end and *Rana* the permanent end) whereas the remaining species showed a preference for temporary ponds or occupied two categories indifferently (ephemeral and temporary, or temporary and permanent) (Figure 2B). For each species, we calculated the index of habitat variability, which incorporates contributions from temporal (Figure 2A) and spatial variation (Figure 2B). *Rana*, *B. calamita* and *B. bufo* were considered species from constant habitats (index values under 1.0), and *Hyla*, *Pelodytes* and *Alytes* from variable habitats (index values over 1.0) (Figure 2C).

Response to experimental treatments

Survival to metamorphosis did not differ between treatments, with the exception of *Rana*, which showed a higher mortality in the drying treatment (Table 1, Figure 3C). In general, species tended to reduce their larval period and reached metamorphosis with a lower body mass in drying treatment (Figures 3A-B, Table 1). However, the differences between treatments were not statistically significant for all species (Table 1). The two bufonids did not show a shorter larval period in the drying treatment, whereas the rest of species showed a shorter period. Smaller size at metamorphosis in drying treatment was detected in all species except in *B. calamita* and *Rana*. A positive correlation between larval period and mass at metamorphosis was observed in practically all species except *B. calamita* (*Alytes*: $R = 0.471$, $p = 0.007$; *Pelodytes*: $R = 0.326$, $p = 0.008$; *B. bufo*: $R = 0.540$, $p = 0.001$; *B. calamita*: $R = 0.198$, $p = 0.277$; *Hyla*: $R = 0.711$, $p = 0.001$; *Rana*: $R = 0.558$, $p = 0.002$).

The levels of statistical significance did not change greatly in the analysis of variance between treatments using the truncated data series (last two columns of Tables 1A-1B). However, truncation of data for the upper distribution tail (tubs with longer larval periods, because we only use the early 40% tubs that had reached metamorphosis) change the relationship between larval period and mass at metamorphosis in two species: *Pelodytes* and *Rana*, which don't showed the positive correlation observed when we used the complete

A) Larval period (days):

Species	Treatment	N	Mean	Variance	Standard error	Min.	Max.	F	*P*	Truncate F	Truncate *P*
Ao	Constant	18	135.16	270.73	3.87	106	163	26.37	**<.001**	23.10	**<.001**
	Drying	14	109.35	116.39	2.88	97	110				
Pp	Constant	38	73.89	429.77	3.36	47	105	4.73	**<.05**	8.67	**<.01**
	Drying	27	62.01	152.61	2.37	46	87				
Bb	Constant	17	92.17	37.91	1.49	83	101	0.6	.453	2.49	.135
	Drying	16	90.50	47.20	1.71	79	103				
Bc	Constant	16	61.18	96.29	2.45	48	77	2.69	.113	0.29	.594
	Drying	16	56.12	42.65	1.63	47	72				
Hm	Constant	19	144.47	366.71	4.39	108	171	60.75	**<.001**	45.82	**<.001**
	Drying	15	102.80	113.88	2.75	85	110				
Rp	Constant	18	129.16	242.03	3.66	104	153	21.79	**<.001**	7.39	**<.05**
	Drying	11	107.09	15.49	1.18	99	110				

B) Mass at metamorphosis (g):

Species	Treatment	N	Mean	Variance	Standard error	Min.	Max.	F	*P*	Truncate F	Truncate *P*
Ao	Constant	18	1.26	0.023	0.036	1.007	1.588	34.58	**<.001**	42.56	**<.001**
	Drying	14	0.99	0.012	0.029	0.856	1.274				
Pp	Constant	38	0.21	0.004	0.011	0.108	0.385	1.18	**<.001**	2.02	0.166
	Drying	27	0.16	0.002	0.008	0.084	0.253				
Bb	Constant	17	0.12	0.001	0.005	0.089	0.157	4.82	**<.05**	5.57	**<.05**
	Drying	16	0.10	0.001	0.005	0.078	0.158				
Bc	Constant	16	0.11	0.001	0.006	0.085	0.168	0.11	.736	0.10	.756
	Drying	16	0.10	0.001	0.005	0.086	0.148				
Hm	Constant	19	0.80	0.004	0.015	0.687	0.976	22.23	**<.001**	31.38	**<.001**
	Drying	15	0.68	0.006	0.021	0.572	0.827				
Rp	Constant	18	0.73	0.006	0.018	0.649	0.894	1.08	.309	0.16	.694
	Drying	11	0.71	0.005	0.021	0.594	0.801				

C) Survival to metamorphosis (%) Mann-Withney U test:

Species	Treatment	N	Median	Lower quartile	Upper quartile	Min.	Max.	Z value	*P*
Ao	Constant	20	83	66	100	0	100	1.82	.083
	Drying	20	64	0	66	0	100		
Pp	Constant	38	66	66	100	33	100	0.69	.488
	Drying	36	66	0	100	0	100		
Bb	Constant	20	80	73	100	0	100	0.14	.882
	Drying	19	80	66	100	0	100		
Bc	Constant	20	90	66	100	0	100	0.02	.978
	Drying	20	80	80	100	0	100		
Hm	Constant	20	66	66	100	0	100	1.31	.191
	Drying	20	66	33	100	0	100		
Rp	Constant	20	83	66	100	0	100	3.55	**<.001**
	Drying	19	33	0	66	0	66		

Table 1.- Descriptive statistics and ANOVA and Mann-Withney U test between treatments for each species. Results of ANOVA for time to metamorphosis and mass at metamorphosis with the two data series: complete and truncated data series. AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

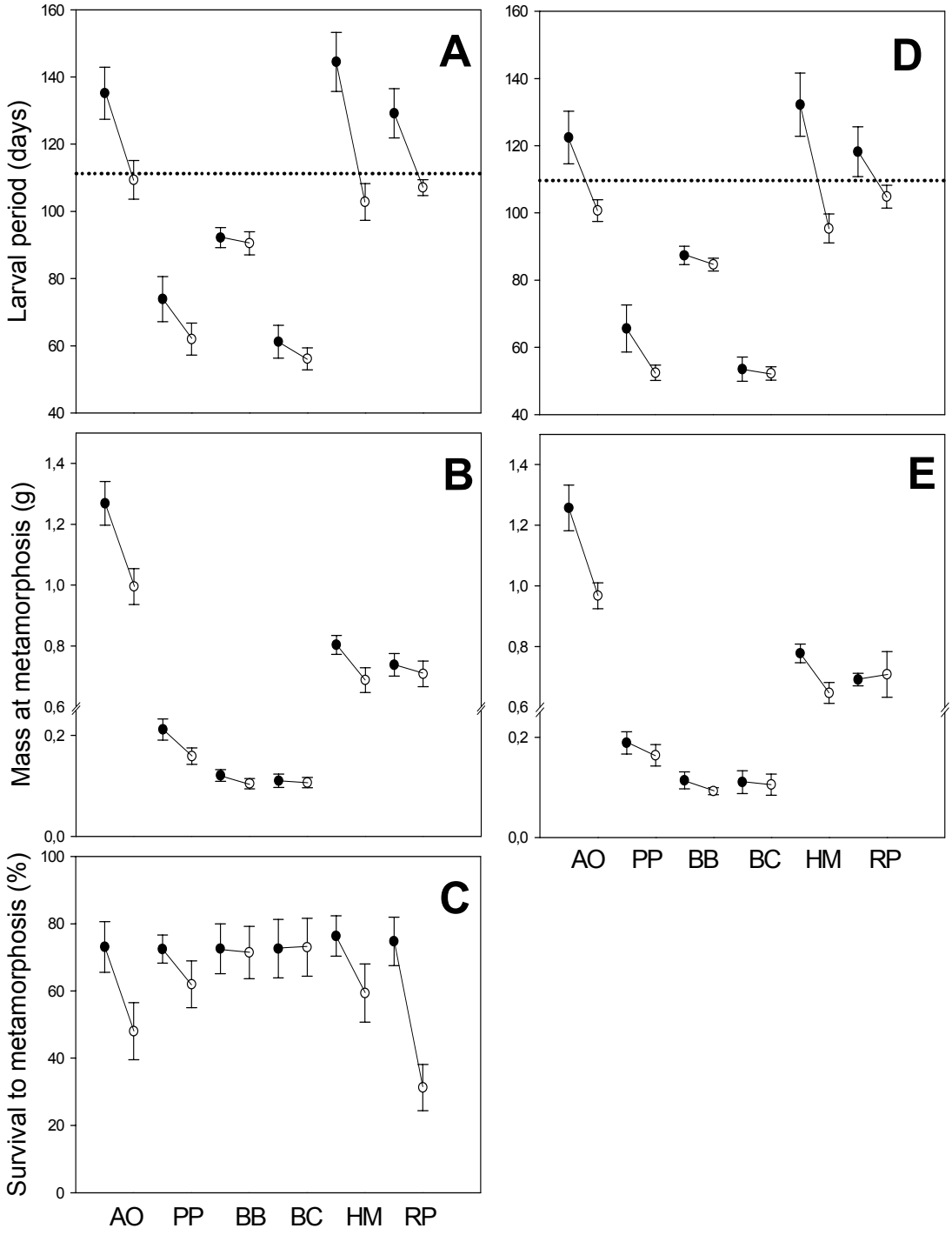


Fig. 3.- Results expressed as mean and standard error of the traits measured for each species in the laboratory experiments. Filled circles correspond to permanent treatment and open circles to drying treatment. Graphics from the first column were elaborated using all data, whereas those from the second column were constructed with the truncated data series. (**A**) and (**D**) show changes in larval period, (**B**) and (**E**) mass at metamorphosis, and (**C**) survival to metamorphosis. Dashed line in A and D indicates temporal horizon to drying treatment (110 days) when tubs were completely dry. Survival to metamorphosis was not estimated with truncated data. AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

data (*Alytes*: R = 0.633, p = 0.008; *Pelodytes*: R = 0.062, p = 0.729; *B. bufo*: R = 0.235, p = 0.362; *B. calamita*: R = 0.341, p = 0.196; *Hyla*: R = 0.655, p = 0.002; *Rana*: R = 0.173, p = 0.520). Loss of individuals with a longer larval period by truncation of data also implied loss of larger froglets and toadlets.

Magnitude of phenotypic plasticity and habitat use

Species and the two groups considered in function of habitat (constant habitats and variable habitats) differed in larval period phenotypic plasticity (Table 2). Species from variable habitats showed a higher magnitude of phenotypic plasticity, especially for larval period (Figure 4A). Although the change of mass at metamorphosis differ among species, differences for species nested within the appropriate habitat affiliation were not significant. The truncated data series did not modify the results for larval period whereas differences in mass at metamorphosis between species disappeared (Table 2, Figure 4B).

Position in phenotypic space (using all variables measured) was closely linked to habitat type, but not to lineage. The Mantel test did not show any matrix correlation between the phenotypic matrix and the phylogenetic matrix after 5000 random permutations ($p_{(\text{random } Z <= \text{ observed } Z)} = 0.5349$ and $p_{(\text{random } Z >= \text{ observed } Z)} = 0.4659$). Also magnitude of developmental plasticity were phylogenetically independent (C-statistics = 0.0471 with p = 0.4052) according to the test for serial independence.

	Source of variation		
Variable	Species (SP) (df = 5)	Habitat (HAB) (df = 1)	Species (SP[HAB]) (df = 4)
Larval period	**10.8333****	**28.1025****	**6.2980****
Larval period “truncate”	**36.3371****	**136.37****	**8.3920****
Mass at metamorphosis	**5.7779****	**16.1107****	2.3163
Mass at metamorphosis “truncate”	2.3547	**5.1098****	2.0325

Notes: Six species, and constant vs. variable habitat species are compared (Habitat). Table entries are *F* ratios; df for the error = 93 for larval period and mass at metamorphosis, df for the error = 44 for larval period and mass at metamorphosis in truncate data series.
** $P < 0.001$.

Table 2. -ANOVA of the two standardised variables (unitless magnitude of the phenotypic plasticity) with species nested within habitat.

DISCUSSION

Magnitude of plasticity and habitat unpredictability

The species showed significant differences in ecological distribution along the freshwater gradient described in other amphibian communities (Skelly 1996; Babbitt et al. 2003; Van Buskirk 2003). The documentation of environmental heterogeneity of species (in space and time) is an early step by which to recognise and interpret phenotypic plasticity as adaptation (Doughty & Reznick 2004). Variability in desiccation risk is predicted to vary more within and between years in temporary ponds of intermediate duration than in permanent ponds and ephemeral pools (Leips et al. 2000). The magnitude of response follows the pattern predicted by models (Moran 1992; Van Tienderen 1997). Tadpoles of species that use a wide variety of habitats, while typically breeding in temporary ponds (*Alytes*, *Pelodytes* and *Hyla*), showed major plastic responses in life history traits and a tendency towards a reduced larval period than those occurring in constant or predicable habitats. A positive relationship between plasticity and environmental heterogeneity is expected when divergent selection promotes the evolution of plasticity within a species and when species differ in the extent to which they experience this selection. At the two extremes of the hydroperiod range, evolution may favour the development of specialist phenotypes with limited plasticity. Fast development rates were selected in predictable ephemeral ponds to escape the risk of drying (e.g. *B. calamita*). On the contrary in predictably permanent water bodies slower development was evolved to optimise larval growth opportunities (e.g. *Rana*).

The acceleration of metamorphosis is clearly advantageous when the pond is at risk of drying. This response has an associated cost as there is a trade-off between development rate and size at metamorphosis (Wilbur & Collins 1973; Newman 1992). Individuals that develop faster are typically smaller than those that develop more slowly. Small size at metamorphosis may reduce resistance to parasites (Goater 1994), may lead to a major risk of water loss during postmetamorphic life (Newman & Dunham 1994), may affect locomotor performance and the metabolic rates of metamorphs (Beck & Congdon 2000; Richter-Boix et al. 2005), and, finally, may reduce reproductive fitness (Smith 1987; Semlitsch et al. 1988). Consequently, these studies indirectly support that larval period plasticity implies as associated cost (but see Loman & Claesson 2003 for a discussion of cost models). Species with higher

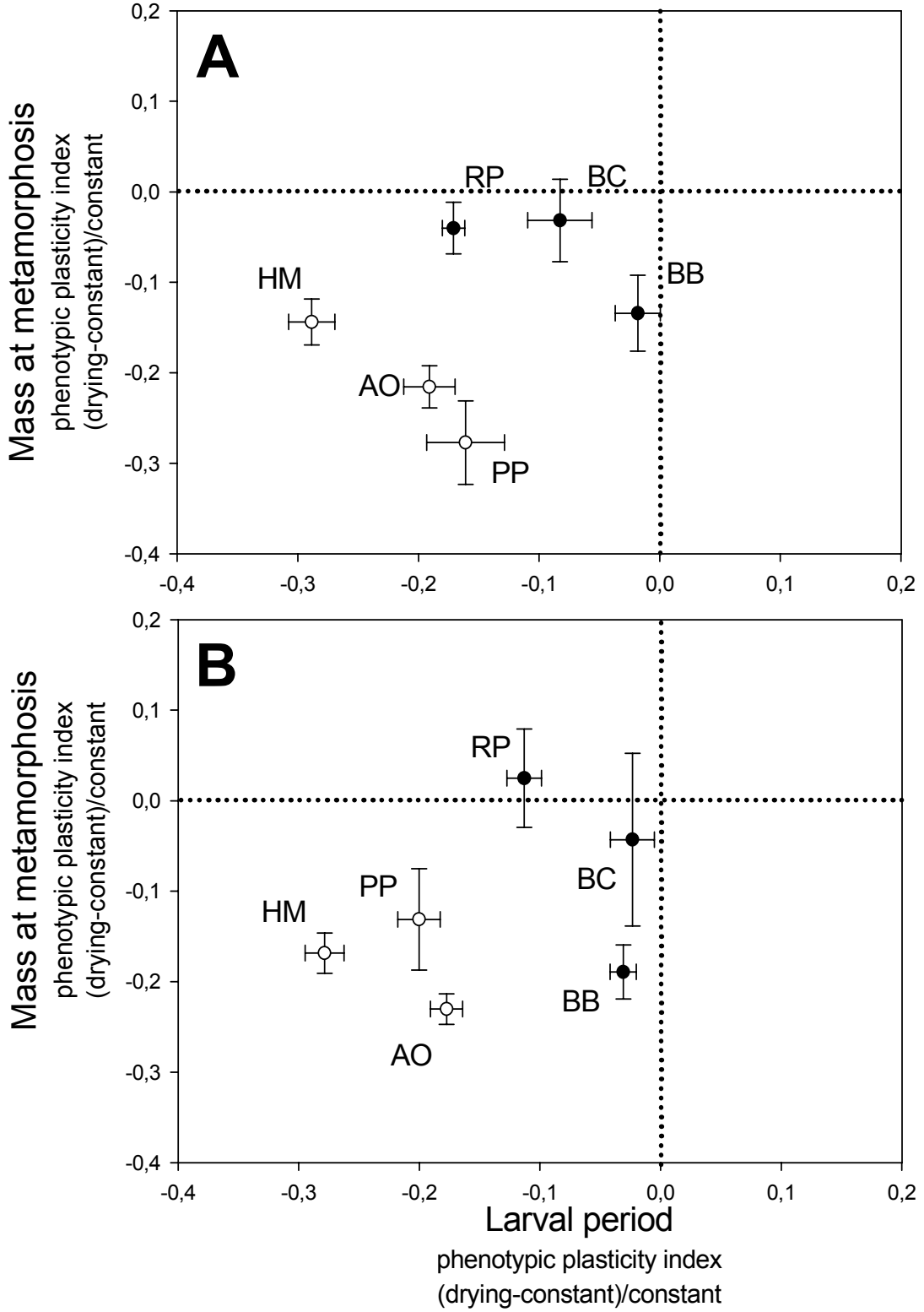


Fig. 4.- Figure shows phenotypic plasticity index (proportional changes between treatments) in larval period and mass at metamorphosis for each species (mean and SE). (**A**) Graphic constructed with complete data series, and (**B**) with truncated data series. Dashed lines indicate the case in which there was no plasticity. Open circles represent species exposed to a high spatial and temporal variability and filled circles species from more predicable environments. AO = *Alytes obstetricans*, PP = *Pelodytes punctatus*, BB = *Bufo bufo*, BC = *Bufo calamita*, HM = *Hyla meridionalis*, RP = *Rana perezi*.

values of larval plasticity also don't showed higher cost associated, with higher values of reduction on mass at metamorphosis. In the future will be necessary to measure others variables to search possible costs associate to developmental plasticity (e.g. morphological changes independently of size (Newman 1992, Richter-Boix et al. 2005)).

As in previous studies (Richardson 2001; Van Buskirk 2002), in our study, phylogeny seems to be not correlated with phenotypic plasticity and did not contribute to identifying a phylogenetic pattern of plasticity evolution. However we must be cautious with this result because can be a reflection of limited power of statistics. In some statistical tests the phylogenetic signal is significant only if the number of species is large, which obviously is not our case. However differences in developmental plasticity among species from the same genera have been reported (Morey & Reznick 2000, 2004). These studies suggested that variability of response within families implies that plasticity evolves over relatively short time-scales. The present study support the hypothesis that interspecific differences in phenotypic plasticity to pond desiccation are adaptive and are related to temporal unpredictability of habitat and niche breadth, however recognise the role of phylogeny in the evolution of plasticity requires a great number of species. In our study we assume no geographic variation within species among populations from temporary and permanent ponds, but recent studies with tadpoles describe geographic variations in plasticity within species (Gómez-Mestre & Tejedo 2003; Van Buskirk & Arioli 2005).

A potential weakness of this study is that the same slow decrease in water level, which is typical of a temporary pond, was applied for all species. Consequently, this treatment may not have been sufficient to stimulate a response in species with short larval periods like bufonids. However, Brady and Griffiths (2000) obtained similar results with the two bufonid species and reported that the timing of metamorphosis was unaffected, whereas Tejedo and Reques (1994) found a positive response of *B. calamita*. Several variables are informative cues of environment drying: increment of conspecific density, decrease in swimming volume, decrease in food, and changes in chemical and physical properties of water (reviewed in Denver et al. 2002). The six species studied here may not have responded in the same manner to these factors, and for example, in the case of bufonids, the density of treatments may have been insufficient to generate a stress response in species that normally develop in

high density cohorts in nature. Bufonids may have responded to desiccation through detecting a great increase in density. For example, *B. americanus* and *B. bufo* accelerate development rate at high density but not at low density (Wilbur 1987; Tejedo & Reques *unpublished data*).

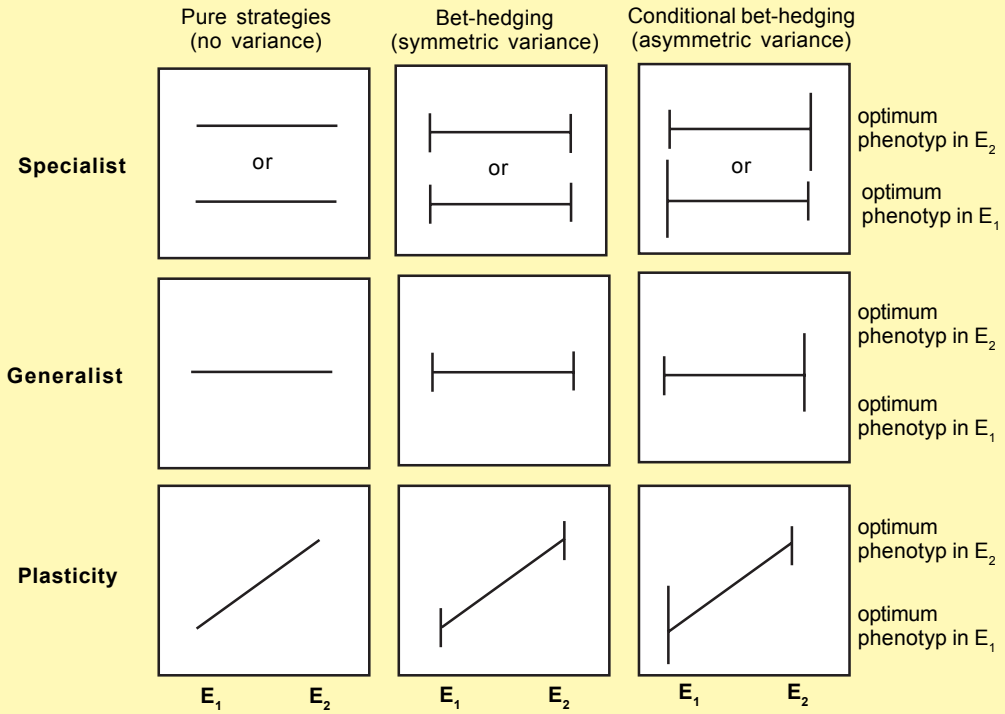
An additional problem encountered was the difficulty to measure plasticity in species in which the larval period was truncated in the drying treatment by their longer larval periods. In the case of *Alytes* and *Hyla,* we hypothesize an acceleration of metamorphosis and, as a result, survival to metamorphosis was unaffected between treatments. However, in the case of *Rana,* where the drying treatment showed a high mortality, time-horizon originated a truncation of data, which may overestimate plasticity (observe position change of *Rana* and *Alytes* between Figures 3A and 3B). The use of the truncated data series (the early 40% replicas per treatment) in analyses contributes to minimising this problem by working with the same proportion of early tubs that reach metamorphosis in the two treatments. Nevertheless, this data series underestimates the cost of plasticity. Removing from analyses individuals with longer larval periods also implies remove bigger individuals and consequently modified positive correlation between time of and size at metamorphosis. The use of both data series can help us to interpret results in a correct form.

Habitat breadth and strategies for environmental heterogeneity

Following the categories of possible strategies for environmental heterogeneity, described by DeWitt and Langerhans (2004)(Box 1), species from variable habitats can be consider plastic strategists with asymmetric bet-hedging. In this strategy, the terrestrial environment is continually supplied with metamorphs provided the water body does not dry out and, consequently, there is large variance in larval period. If desiccation takes place in a very short time, therefore not allowing tadpoles to react, as occurs in early summer in the Mediterranean region (where ponds can dry in less than one week, *personal observation*), the faster developing individuals of the cohort will have reached the terrestrial phase (Lane & Mahoney 2002; Thumm & Mahoney 2002). Alternatively, in ponds that dry during spring at a lower and more constant velocity, all individuals react in the same manner by increasing development rate, as in our drying treatment, and metamorphosing more synchronously (low variance of larval period with respect to the constant water treatment).

BOX 1 Strategies to environmental heterogeneity (DeWitt & Langerhans 2004)

Among the many adaptations organisms have to cope with environmental variability, traditionally evolutionary ecologist define four strategies: specialization, generalization, bet-hedging and phenotypic plasticity. The distinction between these strategies comes down to whether an organism adopts a single phenotype (specialists and generalists) or variable phenotypes (bet-hedging and plasticity). The specialist produced one phenotype that is optimal for a given environment, even through the specialist may find itself sometimes in alternative environments. The generalist produced an intermediate or otherwise general-purpose phenotype, which is at least moderately successful in most environments. Plastic strategists produce variant phenotypes based on the nature of the environment, whereas bet-hedging strategists produce phenotypic variation within single environments, producing several phenotypes, diversified offspring or single phenotypes probabilistically. What is strategic about bet-hedging is that it reduces variance in fitness across generations, and hence increases geometric mean fitness.



DeWitt and Langerhans (2004) redefined the four strategies in three basic strategies: specialist, generalist and plastic. The specialist genotype produces only the optimum environment phenotype in one environment; the generalist genotype produces phenotypes intermediate between the fitness peaks of the two environments, whereas a plastic genotype produces phenotypes near the two fitness peaks depending on its interpretation of the environmental cues. The fourth strategy, bet-hedging, adding variance around a phenotypic mean, which could be added to any of the first three strategies. So the three core strategies with bet-hedging being a possible attribute of each. Furthermore, the degree of bet-hedging (phenotypic variance) can differ across environments generating a “conditional bet-hedging” (asymmetric variance) where variance differs by environment in a similar manner that plasticity produces different mean phenotypes across environments. As a consequence this point of view defines nine possible strategies represented in the figure above.

Specialised species, such as *B. calamita,* did not show a change in mean larval period but also that variance values differ across environments. *B. calamita* showed low variance in its specialised environment (drying treatment) and some optimal level of variance in its non-specialised environment (permanent waters). This strategy allows optimise growth opportunities for some individuals. This integrated solution increases the fitness of specialists across environments (DeWitt & Langerhans 2004). As breeding amphibian populations occur as networks of sub-divided populations connected by migration of long-lived and mobile adults, which can breed in patches of distinct variability, maintenance of variance in these traits is expected to persist at a range of magnitudes in all species. These strategies may provide that individuals with some magnitude of plasticity can successfully colonise a wide range of habitat types. Developmental plasticity has a primary ecological significance in relation to the extent that it permits a widening of the niche breadth of species with a metapopulation structure.

ACKNOWLEDGMENTS

We extend our gratitude to S. Carranza for his advice on the phylogenetic analysis. N. Garriga and L. Pérez are acknowledged for their invaluable help on laboratory experiments and field assistance. M. Tejedo for providing comments and facilities to unpublished data. R. Huey suggested significant improvements concerning the content and clarity of this manuscript. Also we thank F. Llimona and S. Llacuna for providing the facilities for field research at the field camp of Collserola Metropolitan Park and Garraf Natural Park respectively.

REFERENCES

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

Babbitt, K.J., Baber, M.J. and Tarr, T.L. 2003. Patterns of larval amphibian distribution along a wetland hydroperiod gradient. Canadian Journal of Zoology 81: 1539-1552.

Beck, C.W. and Congdon, J.D. 2000. Effects of age and size at metamorphosis on performance and metabolic rates of Southern Toad, *Bufo terrestris*, metamorphs. Functional Ecology 14: 32-38.

Bell, D.L. and Sultan, S.E. 1999. Dynamic phenotypic plasticity for root growth in *Polygonum*: a comparative study. American Journal of Botany 86: 807-819.

Brady, L.D. and Griffiths, R.A. 2000. Developmental responses to pond dessication in tadpoles of the British anuran amphibians (*Bufo bufo*, *Bufo calamita* and *Rana temporaria*). Journal of Zoology 252: 61-69.

Brock, M.A., Nielsen, D.L., Shiel, R.J. Green, J.D. and Langley, J.D. 2003. Drought and aquatic community resilience: the role of eggs and

seeds in sediments of temporary wetlands. Freshwater Biology 48: 1207-1218.

Denver, R.J., Mirhadi, N. and Phillips, M. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology 79: 1859-1872.

Denver, R.J., Glennemeier, K.A. and Boorse, G.C. 2002. Endocrinology of complex life cycles: amphibians. In *Hormones, Brain and Behavior Rough Toc*. vol. II (D. Pfaff, A. Arnold, A. Etgen, S. Fahrbach and R. Rubin, eds), pp. 469-513. USA: Elsevier Science.

DeWitt, T. and Langerhans, R.B. 2004. Integrated solutions to environmental heterogeneity. Theory of multimoment reaction norms. In *Phenotypic plasticity. Functional and Conceptual approaches* (T.J. DeWitt and S.M. Scheiner, eds), pp. 98-111. Oxford: Oxford University Press.

Doughty, P. 1995. Testing the ecological correlates of phenotypically plastic traits within a phylogenetic frameworks. Acta Oecologica 16: 519-524.

Doughty, P. and Reznick, D.N. 2004. Patterns and analysis of adaptive phenotypic plasticity in animals. In *Phenotypic plasticity. Functional and Conceptual approaches* (T.J. DeWitt and S.M. Scheiner, eds), pp. 126-150. Oxford: Oxford University Press.

Endler, J.A. 1986. *Natural selection in the wild*. Princeton, NJ.: Princeton University Press.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist 125: 1-13.

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

Gómez-Mestre, I. and Tejedo, M. 2003. Local adaptation of an anuran amphibian to osmotic stressful environments. Evolution 57:1889-1899.

Jeffries, M. 1994. Invertebrate communities and turnover in wetland ponds affected by drought. Freshwater Biology 32: 603-612.

Johansson, F. and Suhling, F. 2004. Behaviour and growth of dragonfly larvae along a permanent to temporary water habitat gradient. Ecological Entomology 29: 196-202.

Lake, P.S. 2003. Ecological effects of perturbation by drought in flowing waters. Freshwater Biology 48: 1161-1172.

Lane, S.J. and Mahoney, M.J. 2002. Larval anurans with synchronous and asynchronous development periods: contrasting responses to water reduction and predator presence. Journal Animal Ecology 71: 780-792.

Lardner, B. 2000. Morphological and life history responses to predators in larvae of seven anurans. Oikos 88: 169-180.

Leips, J., McManus, M.G. and Travis, J. 2000. Response of treefrog larvae to drying ponds: comparing temporary and permanent pond breeders. Ecology 81: 2997-3008.

Loman, J. and Claesson, D. 2003. Plastic response to pond drying in tadpoles *Rana temporaria*: test of cost models. Evolutionary Ecology Research, **5**: 179-194.

Moran, N.A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist 139: 971-989.

Morey, S.R. and Reznick, D.N. 2000. A comparative analysis of plasticity in larval development in three species of spadefoot toads. Ecology 81: 1736-1749.

Morey, S.R. and Reznick, D.N. 2004. The relationship between habitat permanence and larval development in California spadefoot toads: field and laboratory comparisons of developmental plasticity. Oikos 104: 172-190.

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.

Pigliucci, M., Cammell, K. and Schmitt, J. 1999. Evolution of phenotypic plasticity a comparative approach in the phylogenetic neighbourhood of *Arabidopsis thaliana.* Journal of Evolutionary Biology 12: 779-791.

Reeve, J. and Abouheif, E. 2003. *Phylogenetic Independence*. Version 2.0, Department of Biology, McGill University. Distributed freely by the authors on request.

Richter-Boix, A., Llorente, G.A., Montori, A. 2005. Effects of phenotypic plasticity on post-metamorphic traits during pre-metamorphic stages in the anuran *Pelodytes punctatus*. Evolutionary Ecology Research, in press (available on-line).

Schlichting, C.D. and Levin, D.A. 1986. Phenotypic plasticity: an evolving plant character. Biological Journal of the Linnean Society 29: 37-47.

Schlichting, C.D. and Pigliucci, M. 1998. *Phenotypic evolution: a reaction norm perspective.* Massachusetts: Sinauer Associates, Inc., Publishers.

Semlitsch, R.D., Scott, D.G. and Pechmann, J.H.K. 1988. Time and size at metamorphosis related to adult fitness in *Ambyostoma talpoideum*. Ecology 69: 184-192.

Skelly, D.K. 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. Copeia 1996: 599-605.

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

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

Thumm, K. and Mahoney, M. 2002. Hatching dynamics and bet-hedging in a temperate frog, *Pseudophryne australis* (Anura: Myobatrachidae). Amphibia-Reptilia 23: 433-444.

Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. American Naturalist 160: 87-102.

Van Buskirk, J. 2003. Habitat partitioning in European and North American pond-breeding frogs and toads. Diversity and Distributions 9: 399-410.

Van Buskirk, J. and Arioli, M. 2005. Habitat specialization and adaptive phenotypic divergence of anuran populations. Journal of Evolutionary Biology 18: 596-608.

Van Tienderen, P.H. 1997. Generalist, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. Evolution 51: 1372-1380.

Wellborn, G.A., Skelly, D.K. and Werner, E.E. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27: 337-363.

Wilbur, H.M. 1987. Regulation of structure in complex systems: experimental temporary pond communities. Ecology 68: 1437-1452.

Wilbur, H.M. and Collins, J.P. 1973. Ecological aspects of amphibian metamorphosis. Science 182: 1305-1314.