

Effects of phenotypic plasticity on post-metamorphic traits during pre-metamorphic stages in the anuran *Pelodytes punctatus*

Metamorphosis is assumed to be beneficial for organisms with complex life cycles because it breaks the developmental links between traits in the distinct phases. However, for many organisms and structures this does not occur. For those with developmental phenotypic plasticity, if metamorphosis does not break all developmental links, then changes in juvenile performance will take place as a cost of phenotypic plasticity. In a laboratory experiment (with a constant and a drying treatment), we assessed the effects of drought stress on the phenotypic plasticity (morphology and life history traits) of *Pelodytes punctatus* and studied post-metamorphic morphology and locomotor performance. Tadpoles in the drying treatment accelerated metamorphosis and reached this stage with a lower body mass. They also showed a reduction in tail fin during the larval phase. Toadlets from the drying treatment showed shorter and less muscular hind limbs and a reduced jump capacity compared with individuals in the constant water treatment, independently of time of development, which suggest trade-offs effects between phases.

INTRODUCTION

Amphibians have a complex life history strategy (Wilbur 1980). Organisms with a series of discrete, free-living states might be expected to have more morphological adaptations than taxa with simple life histories, particularly when successive stages occur in radically distinct environments (Hanken 1992). For anurans, a change in selective environment during the life cycle is accompanied by metamorphosis (Wilbur 1980). This process is commonly considered beneficial because it breaks the genetic and developmental relationships between traits expressed at distinct stages (Ebenman 1992; Hanken 1992; Moran 1994). Thus, the adaptive decoupling hypothesis (Moran 1994) may allow traits designed for a given function to evolve independently in different life stages (Moran 1994), thereby allowing pre- and post-metamorphic stages to adapt independently to their respective environments. This hypothesis is support by experimental studies on developmental compartmentalization (Alberch 1987;

Parichy 1998), which show that adult structures may arise *de novo* from embryonic cells that remain undifferentiated until metamorphosis and not as modifications of pre-existing larval structures. However, many post-metamorphic features arise from the re-patterning of larval precursors (Alley 1989; Alley & Omerza 1999).

In a recent study, Watkins (2001) examined genetic correlations between equivalent larval and adult characters (locomotor performance) in the Pacific tree frog *Hyla regilla*, and found greater phenotypic correlations between than within phases. These results do not support the adaptive decoupling hypothesis for the traits measured (Watkins 2001). In addition, correlations between larval tail phenotype and adult limb morphology have been reported (Van Buskirk & Saxer 2001). Other trade-offs between phases can occur between functionally unrelated traits as a result of developmental or physiological constraints (Álvarez & Nicieza 2002). For example, accelerated or retarded differentiation from anuran larvae as a consequence of water temperature or food availability affect juvenile morphology (Emerson 1986; Emerson et al. 1988; Tejedo et al. 2000, Tejedo, Sánchez-Herráiz & Pertoldi, unpublished), energy reserves and jump performance (Tejedo *et al.*, 2000; Álvarez & Nicieza, 2002). Studies on other organisms with complex life cycles (barnacles, copepods and insects) show similar effects of larval history on later life stages (reviewed by Pechenik et al. 1998). The correlations between phases indicate that the adaptive larval phenotype is constrained in order to preserve juvenile functionality but has a low impact on adult fitness. This fact has important implications for the study of adaptation in organisms with complex life cycles (Deban & Marks 2002).

Here we studied ontogenetic trade-offs in the development of anuran plasticity in response to drying ponds. In temperate areas, anuran reproduction frequently occurs in unpredictable freshwater habitats that vary in duration. An unpredictable habitat, and the fact that larvae cannot move to an alternative environment, provide the cues for plasticity during this stage (Doughty & Reznick, 2004).

In response to a drying pond, the larvae of some species exhibit accelerated developmental rates at the expense of growth in order to metamorphose before the water is lost (e.g. Newman 1989; Denver 1997; Morey & Reznick 2004). The benefit of accelerated development under these conditions is clear, but there must also be a trade-off otherwise

natural selection would presumably quickly render the larval phase redundant (Lane & Mahony 2002). Although anuran plasticity has been addressed in several studies, few have focussed on the potential costs of plastic responses because after several years in the adult form cost cannot be detected. Fitness reversal is normally interpreted from indirect support from other studies (Doughty & Reznick 2004). Smaller metamorphs that result from accelerated development show reduced survivorship to maturity (Goater 1994; Newman & Dunham 1994), and smaller size at maturity (Smith 1987). The direct costs of larval plasticity on tadpole growth rate (Van Buskirk 2000; Relyea 2002) or on metamorphic (froglet morphology and mortality in their first terrestrial period after metamorphosis) have been studied (e.g. Van Buskirk 2000; Van Buskirk & Saxer 2001; Lane & Mahoney 2002; Relyea & Hoverman 2003).

Because anuran larval plasticity is expressed in many other forms (behavioural plasticity and morphological modifications) and these changes could improve performance at metamorphosis (Tejedo et al. 2000; Van Buskirk & Saxer 2001; Álvarez & Nicieza 2002), it is of interest to examine the links between larval response to environment and their possible effects on froglet morphology. Morphology related to locomotor performance and the level of energy reserves are probably crucial for the determination of froglet survival and early growth (Pfennig 1992; Álvarez & Nicieza 2002).

We studied the plasticity response of *Pelodytes punctatus* (Daudin 1803) to habitat dehydration and the most direct cost after metamorphosis. We designed a laboratory experiment to evaluate whether pond drying promotes phenotypic plasticity in larval morphology and in life history traits. Secondly, we analyzed the effect of pond desiccation on juvenile morphology and jumping performance in order to establish whether there is a link between larval response to treatments and metamorphic consequences after metamorphosis.

MATERIALS AND METHODS

Experimental procedures

We used three clutches of *Pelodytes* collected from an ephemeral rain pond in Garraf (30 km south of Barcelona, Spain) on the same day in March 2002. Egg masses hatched in outdoor buckets and all experiments were started when tadpoles reached Gosner's stage 25. The effect of pond drying was analysed through two treatments: a constant treatment and

a drying treatment. The former simulated a permanent pond without changes in water volume during tadpole development, and had a larval density of 3 individuals (one individual from each clutch) per 2 liters (a density similar to that observed in natural ponds, Richter-Boix *unpublished data*). In contrast, the drying treatment simulated a temporal pond by reducing water volume during larvae development. The water level decrease following 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, the mean of temporal pond duration in our area of study), a is a shape parameter (0.4 in our treatment), and P is the depth at the start of the experiment. The two treatments were replicated 50 times, with a total of 100 experimental units (300 tadpoles) arranged in a random fashion.

Experimental units consisted of plastic boxes filled with 2 liters of dechlorinated tap water. To reduce the probability of infection and fouling, the water was changed every 12 days. In the drying treatment, we adjusted the water level every four days following the planned drying curves. The two treatments were performed under the same natural light and photoperiod, without thermal control, in laboratories at the University of Barcelona. Tadpoles were fed periodically (approximately every two days) with a mixture (4:1) of rabbit chow and fish food *ad libitum*.

Estimating response of larvae to drying

Larval phenotypic plasticity was measured at two levels: morphological and life history. To analyse morphology, at day 25, 12 randomly selected experimental units were taken from each treatment and all tadpoles were killed and preserved in formalin 4% for later morphological measurements. All the tadpoles in these selected experimental tubs were weighed at 0.001 g precision and their Gosner development stage was determined. We also made a total of six linear measures of traits that exhibit plasticity in other ecological contexts (e.g. Van Buskirk & Saxer 2001; Relyea 2002)(Fig. 1A): two related to body (body length and body depth), two to tail musculature (tail musculature length and tail musculature depth) and two to tail fin (tail fin length and tail fin depth).

The remaining experimental units (38 per treatment) were maintained until individuals completed metamorphosis. We measured mass at metamorphosis, time to metamorphosis

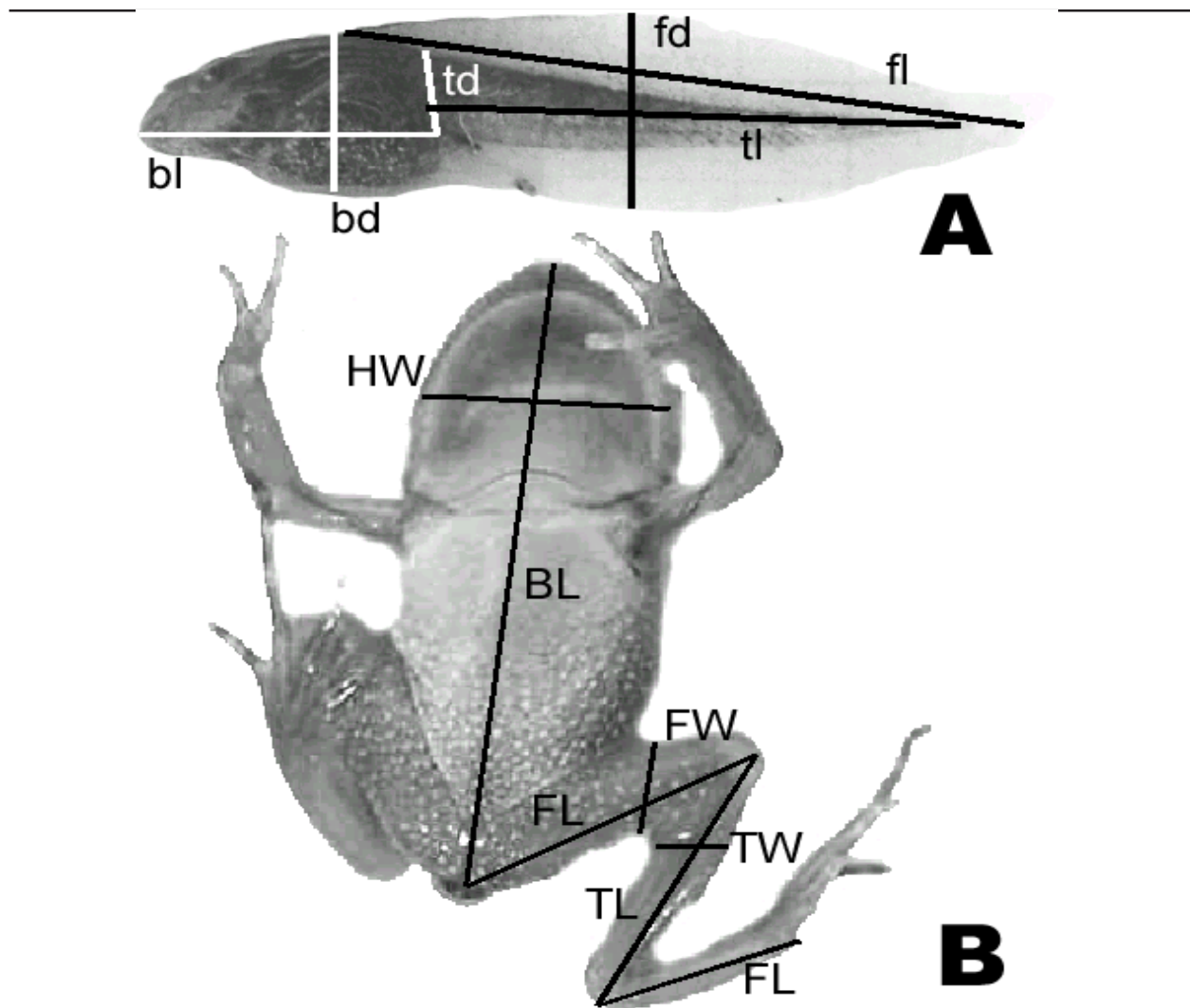


Fig. 1.- Tadpole (A) and toadlet (B) morphological measures. Measures considered for tadpole are: bl = body length; bd = body depth; tl = musculature tail length; td = musculature tail depth; fl = tail fin length; fd = tail fin depth. The seven traits for toadlets are as follow: BL = body length; BW = head body width; FL = femur length; FW = muscular femur width; TL = tibia-fibula length; TW = tibia-fibula width; FL = foot length.

and survival to metamorphosis. Time to metamorphosis was measured at Gosner's stage 42 at the time of collection. Survival was expressed as the proportion of larvae per tub that completed development.

Estimating metamorph response to drying

When metamorphosing tails were fully resorbed (stage 45), tadpoles were weighed to 0.001 g precision. We made seven measurements on the body and hind legs (Fig.1B). Measures related to legs were taken on both sides and the average was used for analysis.

All the limb measurements are associated with jumping performance (Peters, 1994), and thus may affect metamorphic survival.

Finally, we measured the hopping performance of toadlets one day after they reached stage 46. Toadlets were placed at the center of a table (0.8 x 0.6 meters) and allowed to hop repeatedly until they showed signs of fatigue. Generally, they jumped without any stimulation but when necessary they were prodded to induce an escape response. For each toadlet, we marked successive landing positions and later measured the distances between pairs of landmarks. We analysed total distance covered (sum of all distances), and length of the longest jump.

Statistical analyses

We studied the effects of the treatments on life history parameters, such as larval period, mass at metamorphosis and survival, by a multivariate analysis of variance (MANOVA), with subsequent separate univariate ANOVAs for each parameter. Measures of larval period, development stage and mass at metamorphosis were log-transformed to improve normality and homogeneity of variances. As survival was expressed as a proportion, these data were arcsine-transformed before analysis. In all analyses we used the box mean values to avoid pseudoreplication.

Before morphological analyses, tadpole and toadlet measures were corrected for variation in body size. To generate size-corrected measures, we used the residuals of the morphological measures of log-transformed traits after regressions against body size. Total tadpole length is not a good measure of body size because it is mostly a measure of tail length (Van Buskirk 2002), we therefore used centroid size, obtained from landmarks (Loy et al. 1993). Coordinates of these landmarks were collected using the TPSDIG computer program version 1.30 (Rohlf 2001). The centroid size, the square root of the sum of squared distances of a set of landmarks from their centroid (Bookstein 1991), was calculated for each specimen and used to represent size. After performing this correction, tadpole morphology was tested firstly with multivariate analysis for all traits together and secondly with a univariate analysis for each variable.

In the case of toadlets, we worked with the residuals of all morphometric measures with respect to their log-transformed individual body mass at metamorphosis. As the morphological

Analysis	Dependent variable	Wilks' λ	df	MS Effect	F	p
MANOVA		0.0187	6, 17		148.6842	<0.0001
ANOVA	Body length		1, 22	0.00032	6.0860	0.02188
	Body depth		1, 22	0.00052	2.5312	0.12588
	Tail length		1, 22	0.00001	0.3968	0.53520
	Tail depth		1, 22	0.00007	2.1230	0.15922
	Tail fin length		1, 22	0.0130	136.9564	<0.0001
	Tail fin depth		1, 22	0.0921	475.328	<0.0001

Table 1.- Summary of the multivariate and univariate analyses of variance for morphological traits of tadpoles at day 25 of experiment. All variables were log-transformed and regressed to their centroid size as a body size measure before analysis.

traits of toadlets could be influenced by development time, we used time to metamorphosis as covariate in all analyses. After MANCOVA, individual ANCOVAs for individual traits were performed.

Given our focus on absolute and size-independent hopping performance, this trait was analysed twice. One analysis was done with residuals with respect to body mass condition and the other without.

At the end of experiment we detected unequal replication because of 100% mortality in some experimental units, we therefore used type III sum of squares in all ANOVA and ANCOVA analyses.

RESULTS

Response of larvae to drying

The multivariate responses of developmental variables differed ($\lambda = 0.7563$; $F_{3, 60} = 6.442$; $p = 0.00745$). Drying had a significant effect on two metamorphic responses: larval period and mass at metamorphosis ($F_{1, 63} = 5.57$; $p = 0.0213$ and $F_{1, 62} = 13.926$; $p = 0.0004$

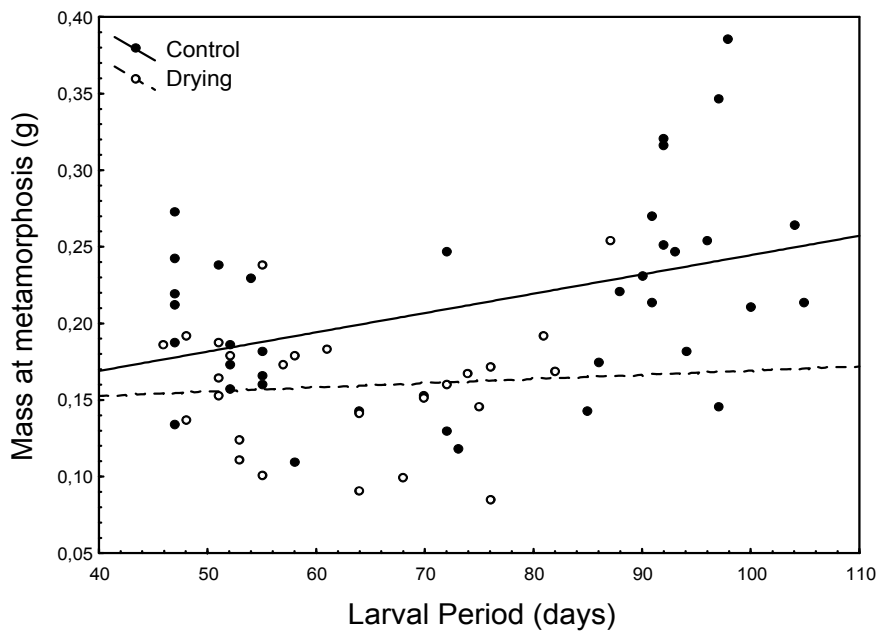


Fig. 2.- Relationship between larval period and mass at metamorphosis for the two experimental treatments.

respectively). Individuals in the drying treatment had shorter larval periods and smaller sizes at metamorphosis than those in the constant treatment. However, treatments did not differ in tadpole survival ($F_{1,74} = 2.243$; $p = 0.1380$) (61.12 ± 23.08 vs. 71.04 ± 19.19 in the constant and drying treatment, respectively). Correlation analysis between mass at metamorphosis and larval period showed a low but positive and significant relationship for the constant treatment ($F_{1,36} = 4.8781$; $p = 0.03365$; $R^2 = 0.1193$) whereas it was non-significant for the drying treatment ($F_{1,24} = 0.0027$; $p = 0.9871$; $R^2 = 0.00001$) (Fig. 2).

Tadpoles measured at day 25 did not differ in their development stage ($Z_{22} = 1.433$ U = 47; $p = 0.1489$ Mann-Whitney U-test), but differences were detected in their mass ($t_{22} = -2.6817$; $p = 0.0136$). Larvae in the constant treatment were larger than those in the drying treatment. In addition, the size-independent morphological traits of tadpoles differed in the two treatments (Table 1). The greatest changes were in the tail fin parameters. Tadpoles in the drying treatment showed shorter and shallower tail fins than controls, but no differences in measures related to tail musculature were detected between the two groups (Fig. 3).

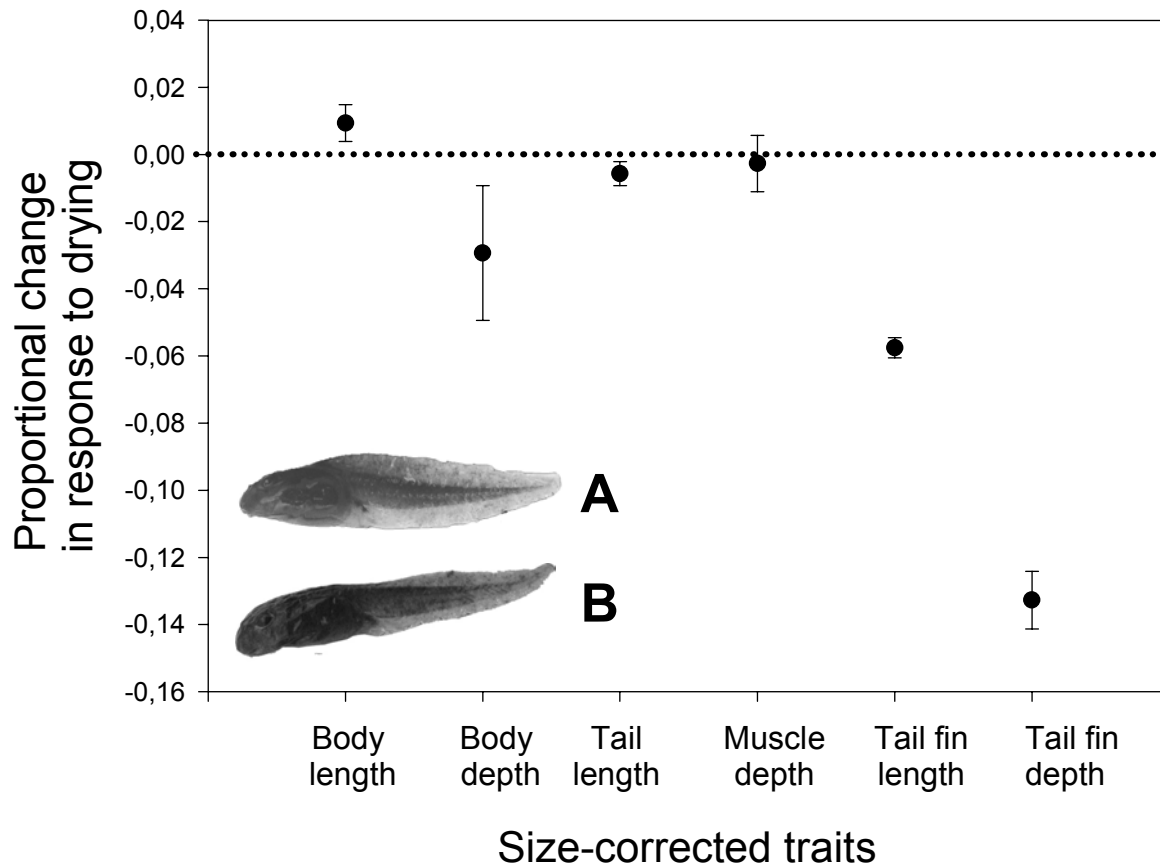


Fig. 3.- Proportional change in shape of tadpoles reared in the dry treatment, relative to tadpoles from the control. A) Constant treatment tadpole and B) drying treatment tadpole. The dashed line shows when the trait was identical to control treatment. All traits are residual after regression against centroid size. Bars show standard error of the mean.

Metamorph morphology and hopping performance

The morphological features of the toadlets that metamorphosed in the constant and drying treatments differed independently of larval period, with no effect of time to metamorphosis on toadlet morphology (Table 2). ANCOVAs showed that proximal leg traits (femur and tibio-fibula) were shorter in individuals in the drying treatment and that these had a relative lower width (Table 2, Fig. 4). Distal leg trait (foot) did not show differences between treatments. The magnitude of these differences was 0.90% for the femur and 1.12% for the tibia-fibula. In addition, toadlets in the drying treatment had greater head widths than those in the constant treatment.

Analysis	Factor	Dependent variable	Wilks' λ	df	MS Effect	F	p
MANCOVA	Treatment		0.2828	7, 54		19.5552	<0.0001
	Covariate		0.8214	7, 54		1.6768	0.1343
ANCOVA		Body length		1, 61	0.0176	3.8246	0.0551
		Head width		1, 61	0.1724	50.8041	<0.0001
		Femur length		1, 61	0.1443	26.6134	<0.0001
		Femur width		1, 61	0.2911	78.0455	<0.0001
		Tibio-Fibula length		1, 61	0.0524	13.3775	<0.0005
		Tibio-Fibula width		1, 61	0.3147	48.6931	<0.0001
		Foot length		1, 61	0.0129	1.1953	0.2786

Table 2.- Summary of the MANCOVA and ANCOVA analyses for morphological traits of toadlets at metamorphosis. Time to metamorphosis was used as covariate in all cases. All variables were log transformed and regressed to body mass at metamorphosis before analysis.

There were low significant differences in absolute jumping capacity between treatments ($\lambda = 0.9015$; $F_{2,62} = 3.384$; $p = 0.0403$). Toadlets in the constant treatment made the longest jumps (6% longer) and covered greater distances (22.5%) than those in the drying treatment (Fig. 5). This is explained because the former were larger. These differences disappeared after correction for body mass ($\lambda = 0.9735$; $F_{2,62} = 0.8281$; $p = 0.4416$).

DISCUSSION

Our results indicate that *Pelodytes punctatus* shows a plasticity response to drying conditions which affects toadlet morphology. However, few delayed consequences of these reactions were detected just after metamorphosis.

Life history phenotypic plasticity related to drying ponds is well documented (e.g. Newman 1992; Denver 1997; Denver et al. 1998). Several amphibian species, anura and urodela from distinct habitats, arid and non-arid, accelerate metamorphosis in response to habitat desiccation (Wilbur 1987; Newman 1989; Crump 1989; Denver 1997; Laurila & Kujasalo 1999; Morey & Reznick 2004). This accelerated development in dry conditions could be considered an adaptive trait that allows tadpoles to escape from a degrading environment

and avoid mortality caused by water scarcity (Doughty & Reznick 2004). Our results show that tadpole growth rate decreases and development rate increases as the pond dries, thereby resulting in smaller size at metamorphosis, as reported for other species (Newman 1989; Denver 1997).

However, although life history response to a drying environment appears to be a frequent event, to our knowledge, this is the first study to address the morphological plasticity of tadpoles in response to habitat desiccation. A field study with *Rana temporaria* showed that tadpoles in small temporary ponds had relatively smaller tail fins and narrower bodies than those in large ponds (Vences et al. 2002). In our case, the tadpoles in the drying treatment showed a particular phenotype, which is similar and in concordance with this previous study. The morphological differences between treatments, independently of developmental rate, indicate

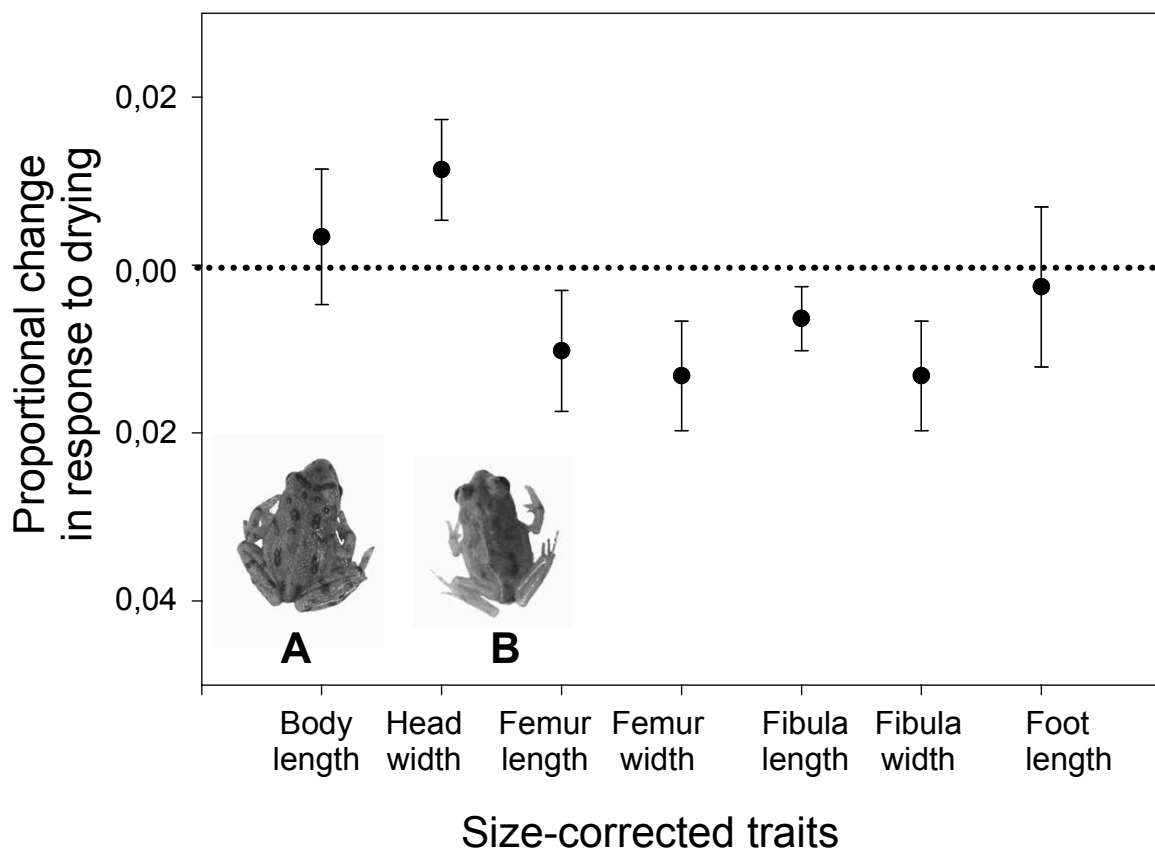


Fig. 4.- Proportional change in morphology of toadlets reared in the drying treatment, relative to toadlets from the control treatment. A) Constant treatment toadlet and B) drying treatment toadlet. The dashed line shows when the trait was identical to the control. All traits are residual after regression against mass at metamorphosis. Bars show standard error of the mean.

that these traits may be the result of drought stress, and not a consequence of a high development rate.

We propose that the tail fin becomes obsolete for tadpoles which maintain position in the mid-water column in shallow waters, like *Pelodytes* (Díaz-Paniagua 1987; Richter-Boix et al. 2004). In these organisms, the tail fin contributes mainly to maintaining anterior stability in the mid-water column, which disappears in drying ponds (Wassersug & Hoff 1985; Hoff & Wassersug 1986). Tadpoles of diverse species living in ephemeral pools show a similar morphology, with an elongated and finless tail and a depressed body (Altig & McDiarmid 1999).

An alternative explanation for the adaptive significance of this morphology is that a tail without a fin can be resorbed faster by tadpoles during metamorphosis, as proposed Van Buskirk and Saxer (2001). Downie et al. (2004) showed a significant correlation between metamorphic duration and tail length for several frog species. One can expect that the time it takes to occur is likely to be dependent on the amount of tissue to be transformed (Van Buskirk & Saxer 2001).

We detected morphological variations induced by drought not only during the larval phase but also as a carry-over effect in metamorphs. Differences in growth rate can produce changes in head width and leg length at metamorphosis (Emerson 1986; Newman 1989; Blouin & Brown 2000; Tejedo, Sánchez-Herráiz and Pertoldi, unpublished). Newman (1989) reported shorter legged toadlets in conditions of short pond duration compared to individuals in less ephemeral habitats. Correlation between shorter leg, low development rate and several stress factors during larval development (density effects, food availability, predation risk, pond duration) were showed in other study (Tejedo et al., unpublished). That study showed that *Pelodytes punctatus*, which shows a faster developmental rate, produces longer legged toadlets. A faster developmental rate corresponded to animals fed *ad libitum* with respect to those subjected to constrained food availability. In both studies *Pelodytes* raised under stress originated short legged toadlets. However, we observed differences in toadlet morphology in function of treatment but not larval period. Therefore, we hypothesise that toadlet constitution at metamorphosis depends, in part, directly on the tadpole morphology induced by the drying treatment.

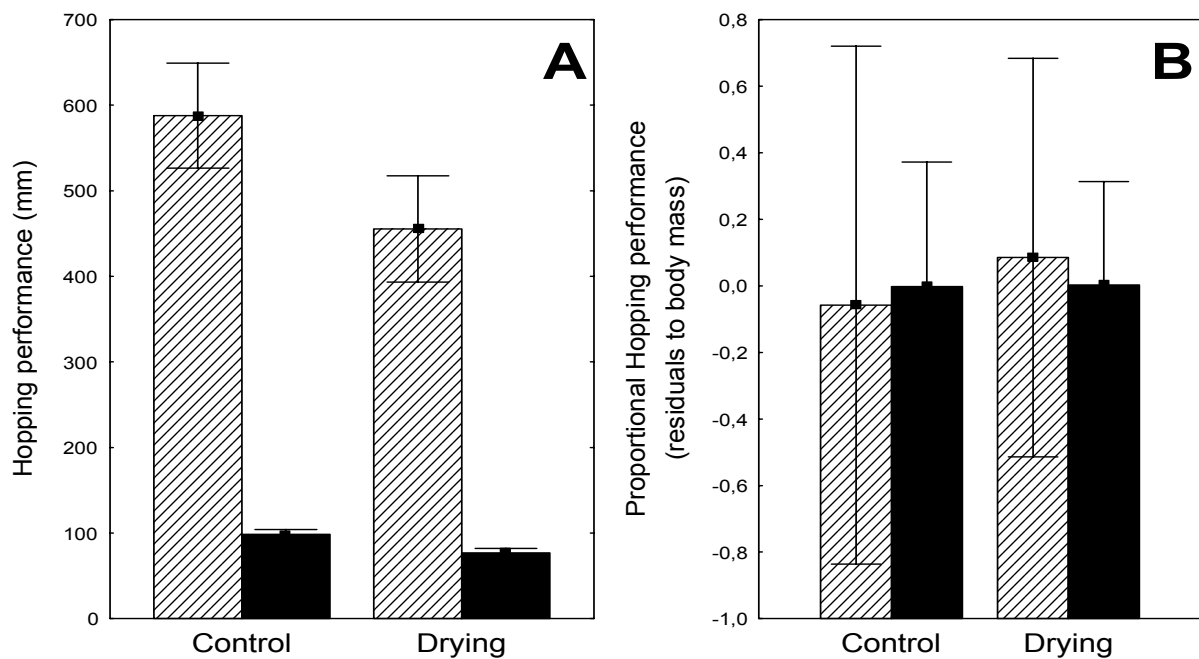


Fig 5.- A) Values absolute of the hopping performance of toadlets, the total distance jumping to fatigued and the longest hop. B) The proportional hopping performance after body mass correction. Mean and standard deviation are representing.

This argument was previously used by Van Buskirk and Saxer (2001) in which tadpole predator-induced morphology resulted in froglets with distinct morphological traits. In that case, tadpoles exposed to predators showed deeper and longer tail fins, a common tadpole response to predation (e.g. Van Buskirk 2002), and more muscular legs. Similarly, *Rana sylvatica* froglets exposed also to predator had longer legs (Relyea 2001). In our case, shorter and shallower finned tadpoles originated individuals with legs that were shorter and had a low muscular mass. In the three cases, the relationship between tail fin constitution and leg width is held. Part of the mass accumulated in the tail when resorbed during metamorphosis is reinvested in new structures and tissues (Hourdry & Beaumont 1985), possibly in leg tissues. Similarly, Watkins (2001) showed larger phenotypic correlations for locomotor traits between phases (tadpole-froglet) than within phases. These studies demonstrate that anuran metamorphosis does not break the developmental relationship between the traits evaluated at distinct stages, and that larval and adult traits are not entirely independent of evolution

(Pechenik et al. 1998). Thus, juvenile morphology may be partially constrained by tadpole morphology (Van Buskirk & Saxer 2001) and *vice versa*. Larval phenotypic plasticity may be constrained to originate a functional juvenile.

We observed low costs associated with jump performance, which is consistent with the findings of other studies (Tejedo et al. 2000; Van Buskirk & Saxer 2001). Changes in the length of the hind limb are not sufficient to generate measurable consequences (Emerson 1978). Small morphological differences lead to differences in absolute jumping performance, which could affect escape performance from predators. However, these distinct morphologies probably disappear over a few weeks during the juvenile stage by compensatory growth, a phenomenon observed in *Pelobates cultripes*, *Pelodytes punctatus* and *P. ibericus* (Tejedo and Gómez-Mestre, Tejedo and Marangoni, unpublished).

Our results show low direct costs associated with plastic response of tadpoles to pond desiccation. Fitness costs may appear later in the terrestrial stage and may be associated with juvenile size and survival. Accelerated metamorphosis or permanence in the larval habitat is a trade-off between size and shape at metamorphosis and the risk of mortality in the terrestrial habitat, especially when size at transformation is positively correlated with toadlet and adult fitness (Smith 1987; Goater 1994; Lane & Mahoney 2002; Relyea & Hoverman 2003). The assumption that it is beneficial to remain in the aquatic habitat for as long as possible was put forward by Wilbur (1980), although it was questioned by Werner (1986). However, if growth rate is similar in the terrestrial and aquatic habitats, then accelerated metamorphosis does not compromise fitness, because the small early metamorphs remain longer in the terrestrial habitat and reach a similar size to later metamorphs (Loman & Claesson 2003).

ACKNOWLEDGMENTS

We thank N. Garriga for her help in the laboratory, M. Tejedo for providing important unpublished data and helpful comments, and two anonymous reviewers for their comments on the manuscript. Thanks also go to the *Departament de Medi Ambient de la Generalitat de Catalunya*, and especially to S. Llacuna at the Parc Natural del Garraf for providing the corresponding permits and facilities.

REFERENCES

- Alberch, P. 1987. Evolution of a developmental process: irreversibility and redundancy in amphibian metamorphosis. In *Development as an evolutionary process* (R. Raff and E. Raff, eds), pp. 23-46. New York: Alan Liss.
- Alley, K.E. 1989. Myofiber turnover is used to retrofit frog jaw muscle during metamorphosis. *American Journal of Anatomy* 184: 1-12.
- Alley, K.E. and Omerza, F.F. 1999. Neuromuscular remodeling and myofiber turnover in *Rana pipiens*' jaw muscles. *Cells Tissues Organs* 164: 46-58.
- Altig, R. and McDiarmid, R.W. 1999. Diversity: Familial and generic characterizations. In *Tadpoles: the biology of anuran larvae* (R.W. McDiarmid and R. Altig, eds), pp. 295-338. Chicago: The University of Chicago Press.
- Álvarez, D. and Nicieza, A.G. 2002. Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. *Oecologia* 131: 186-195.
- Blouin, M.S. and Brown, S.T. 2000. Effects of temperature-induced variation in anuran larval growth rate on head width and leg length at metamorphosis. *Oecologia* 125: 358-361.
- Brady, L.D. and Griffiths, R.A. 2000. Developmental responses to pond desiccation in tadpoles of the British anuran amphibians (*Bufo bufo*, *B. calamita* and *Rana temporaria*). *Journal of Zoology* 252: 61-69.
- Bookstein, F.L. 1991. *Morphometrics tool for landmark data: geometry and biology*. Cambridge: Cambridge University Press..
- Crump, M.L. 1989. Effects of habitat drying on developmental time and size at metamorphosis in *Hyla pseudopuma*. *Copeia* 1989: 794-797.
- Deban, S.M. and Marks, S.B. 2002. Metamorphosis and evolution of feeding behaviour in salamanders of the family *Plethodontidae*. *Zoological Journal Linnean Society* 134: 375-400.
- Denver, R.J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. *American Zoologist* 37: 172-184.
- Denver, R.J., Mirhadi, N. and Phillips, M. 1998. An experimental analysis of adaptive plasticity in amphibian metamorphosis: developmental response of *Scaphiopus hammondi* tadpoles to habitat desiccation. *Ecology* 79: 1859-1872.
- Díaz-Paniagua, C. 1987. Estudio en cautividad de la actividad alimenticia de las larvas de siete especies de anuros. *Revista Española de Herpetología* 2:189-197.
- 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. New York: Oxford University Press.
- Downie, JR, Bryce, R. and Smith, J. 2004. Metamorphic duration: an under-studied variable in frog life histories. *Biological Journal of the Linnean Society* 83: 261-272.
- Ebenman, B. 1992. Evolution in organisms that change their niches during the life-cycle. *American Naturalist* 139: 990-1021.
- Emerson, S.B. 1978. Allometry and jumping in frogs: helping the twain to meet. *Evolution* 32: 551-564.
- Emerson, S.B. 1986. Heterochrony and frogs: the relations of a life history trait to morphological form. *American Naturalist* 127: 167-183.
- Emerson, S.B., Travis, J. and Blouin, M. 1988. Evaluating a hypothesis about heterochrony: larval life-history traits and juvenile hind-limb morphology in *Hyla crucifer*. *Evolution* 42: 68-78.
- Goater, C.P. 1994. Growth and survival of postmetamorphic toads: interactions among larval history, density, and parasitism. *Ecology* 75: 2264-2274.

- Hanken, J. 1992. Life history and morphological evolution. *Journal of Evolutionary Biology* 5: 549-557.
- Hoff, K.V. and Wassersug, R.J. 1986. The kinematics of swimming in larvae of the clawed frog, *Xenopus laevis*. *Journal of Experimental Biology* 122:1-12.
- Hourdy, J. and Beaumont, A. 1985. *Les métamorphoses des amphibiens*. Paris: Masson.
- Lane, S.J. and Mahony, M.J. 2002. Larval anurans with synchronous and asynchronous development periods: contrasting responses to water reduction and predator presence. *Journal of Animal Ecology* 71: 780-792.
- Laurila, A. and Kujasalo, J. 1999. Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *Journal of Animal Ecology* 68: 1123-1132.
- 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.
- Loy, A., Corti, M. and Marcus, L.F. 1993. Landmarks data: size and shape analysis in systematics. A case study on old world talpidae (Mammalia, Insectivora). In *Contributions to morphometrics* (L.F. Marcus, E. Bello and A. García-Valdecasas eds), pp. 215-239, Madrid: Monografías del Museo Nacional de Ciencias Naturales.
- Moran, N.A. 1994. Adaptation and constraint in the complex life-cycles of animals. *Annual Review of Ecology and Systematics* 25: 573-600.
- 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. 1989. Developmental plasticity of *Scaphiopus couchii* tadpoles in desert ponds. *Ecology* 70: 1775-1787.
- 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.
- Parichy, D.M. 1998. Experimental analysis of character coupling across a complex life cycle: pigment pattern metamorphosis in the tiger salamander, *Ambystoma tigrinum*. *Journal of Morphology* 237: 53-67.
- Pechenik, J.A., Wendt, D.E. and Jarrett, J.N. 1998. Metamorphosis is not a new beginning: larval experience influences juvenile performance. *BioScience* 48: 901-910.
- Peters, S.E. 1994. Properties of twitch motor units of the ankle extensor muscles in the bullfrog *Rana castebiana*. *Journal of Morphology* 221: 121-131.
- Pfennig, D.W. 1992. Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionary stable strategy. *Evolution*, 46: 1408-1420.
- Relyea, R.A. 2001. The lasting effects of adaptive plasticity: predator-induced tadpoles become long-legged frogs. *Ecology*, 82: 1947-1955.
- Relyea, R.A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs* 72: 523-540.
- Relyea, R.A. and Hoverman, J.T. 2003. The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. *Oecologia* 134: 596-604.
- Richter-Boix, A., Llorente, G.A., and Montori, A. 2004. Responses to competition effects of two anuran tadpoles according to life-history traits. *Oikos* 106: 39-50.
- Rohlf, F.J. 2001. *Thin-Plate Spline (TPS) Software*. Free available on: <http://life.bio.sunysb.edu/morp/> (last modified abril 2004).
- Smith, D.C. 1987. Adult recruitment in chorus frog: effects of size and date at metamorphosis. *Ecology* 68: 344-350.

- Tejedo, M., Semlitsch, R.D. and Hotz, H. 2000. Covariation of morphology and jumping performance in newly metamorphosed water frogs: effects of larval growth history. *Copeia* 2000: 448-458.
- Tejedo, M., Sánchez-Herráiz, M.J. and Pertoldi, C. 2005. Effects of food level induced changes in larval developmental time on hindlimbs and head shape at the metamorphosis in the parsley frog, *Pelodytes punctatus*. (Submitted)
- Van Buskirk, J. 2000. The cost of an inducible defense in anuran larvae. *Ecology* 81: 2813-2821.
- Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: relationship between habitat and phenotype in anuran larvae. *American Naturalist* 160:87-102.
- Van Buskirk, J. and McCollum, S.A. 2000. Functional mechanisms of an inducible defense in tadpoles: morphology and behaviour influence mortality risk from predation. *Journal of Evolutionary Biology* 13:336-347.
- Van Buskirk, J. and Saxer, G. 2001. Delayed costs of an induced defense in tadpoles? Morphology, hopping, and development rate at metamorphosis. *Evolution* 55: 821-829.
- Vences, M., Puentes, M., Nieto, S. and Vieites, D.R. 2002. Phenotypic plasticity of anuran larvae: environmental variables influence body shape and oral morphology in *Rana temporaria* tadpoles. *Journal of Zoology London.*, 257: 155-162.
- Wassersug, R.J. and Hoff, K. 1985. The kinematics of swimming in anuran larvae. *Journal of Experimental Biology* 119:1-30.
- Watkins, T.B. 2001. A quantitative genetic test of adaptive decoupling across metamorphosis for locomotor and life-history traits in the Pacific tree frog, *Hyla regilla*. *Evolution* 55: 1668-1677.
- Werner, E.E. 1986. Amphibian metamorphosis: growth rate, predation risk; and the optimal size at transformation. *American Naturalist* 128:319-341.
- Wilbur, H.M. 1980. Complex life cycles. *Annual Review of Ecology and Systematics* 11: 67-93.
- Wilbur, H.M. 1987. Regulation of structure in complex systems: experimental temporary pond communities. *Ecology* 68:1437-1452.

