

Anuran larvae species distribution across a predation risk gradient and anti-predator phenotypic plasticity

In a field survey, we studied the distribution of several pond-breeding anuran species and their potential large predators along a freshwater habitat gradient ranging from ephemeral pools to permanent ponds. In a laboratory experiment, we examined predator-induced plasticity for these anuran species, to test whether the plastic response of species that breed in ephemeral and temporary ponds differs from that of species that reproduce in permanent water bodies. Desiccation and predation face conflicting demand; reduced activity lowers the risk of death by predation but causes an increased risk of death by desiccation in temporary ponds. We expected species from time-constrained habitats to show a morphotype to reduce vulnerability to invertebrate predators, and thereby maintain a high activity. In contrast, in most permanent ponds without time constraints, morphological changes can be accompanied by behavioural changes in order to reduce encounters with predators.

Species distribution and predator composition along the hydroperiod gradient differed. The first transition between ephemeral and temporary ponds can be attributed to differences in the hydroperiod and the presence of large invertebrate predators in the latter, while the second transition between temporary and permanent ponds can be attributed only to hydroperiod because top predators and their abundance in these two types of habitats were similar. Here we also discuss other traits of amphibian life history in order to explain the absence of temporary pond breeders in permanent fishless ponds.

With the exception of bufonids, all species showed predator-induced plasticity, which is consistent with previous studies. Species from ephemeral habitats showed a low reduction in activity with respect to the other species, but no clear pattern was observed between species that bred in temporary and permanent ponds. Possible explanations for this result are discussed.

INTRODUCTION

Models of community structure in lentic systems indicate that interactions between the abiotic constraints of hydroperiod length, predation, and life history characteristics of individual species will produce predictable patterns of community structure along a hydroperiod gradient that ranges from ephemeral pools to permanent waters (Schneider & Frost 1996; Wellborn et al. 1996). This lentic community model is particularly applicable to larval amphibian

assemblages (Snodgrass et al. 2000; Babbitt et al. 2003; Van Buskirk 2003, 2005). Also, potential tadpole predator community varies along the hydroperiod. Ephemeral habitats that dry in a few weeks or months are generally free of large predators (Gunzburger & Travis 2004) while temporary habitats that dry every year are fishless but have large invertebrate predator communities. Permanent habitats may have the largest invertebrate predators or large fish predators in function of the connectivity and origin of water bodies (Werner & McPeck 1994; Gunzburger & Travis 2004). Predators are a major biotic cause of mortality for amphibian larvae and have a strong impact on larvae assemblage (e.g. Woodward 1983; Werner & McPeck 1994; Wilbur 1997).

Amphibian assemblages are based on trade-offs in life history that maximise fitness in one section of the hydroperiod gradient while decreasing this parameter in others (Wellborn et al. 1996). Species that persist in ephemeral or temporary ponds have the capacity to complete the aquatic phase of their life cycle before ponds dry. This explains why temporary pond species show rapid development and metamorphosis in a short time, while species in more permanent ponds have defensive mechanisms to prevent predation by large invertebrates and normally show longer larval periods. Predation prevention is, in part, mediated behaviourally through a reduction of activity levels because these are positively correlated. Increased activity leads to increased predation risk (Anholt & Werner 1995). Thus, resource acquisition in amphibian larvae may be affected by the need for timely development and the need to avoid predators across the hydroperiod gradient (Richardson 2002). Tadpoles exposed to the dual risk of predation and desiccation face conflicting demands; reduced foraging lowers the risk of death by predation but increases the risk of death by desiccation (Laurila & Kujasalo 1999; Bridges 2002).

However, because species distribution across the hydroperiod gradient does not show perfect segregation between defined transitions (Snodgrass et al. 2000; Babbitt et al. 2003; Van Buskirk 2003, 2005), the plasticity of several traits may have evolved to adjust phenotypes to the distinct environments inhabited by species (DeWitt & Scheiner 2004). In recent years, many studies have focused on a number of aspects of amphibian anti-predator phenotypic plasticity. These studies showed that phenotypic plasticity is widespread in nature and includes variation in foraging behaviour, morphology and life history parameters (Skelly & Werner

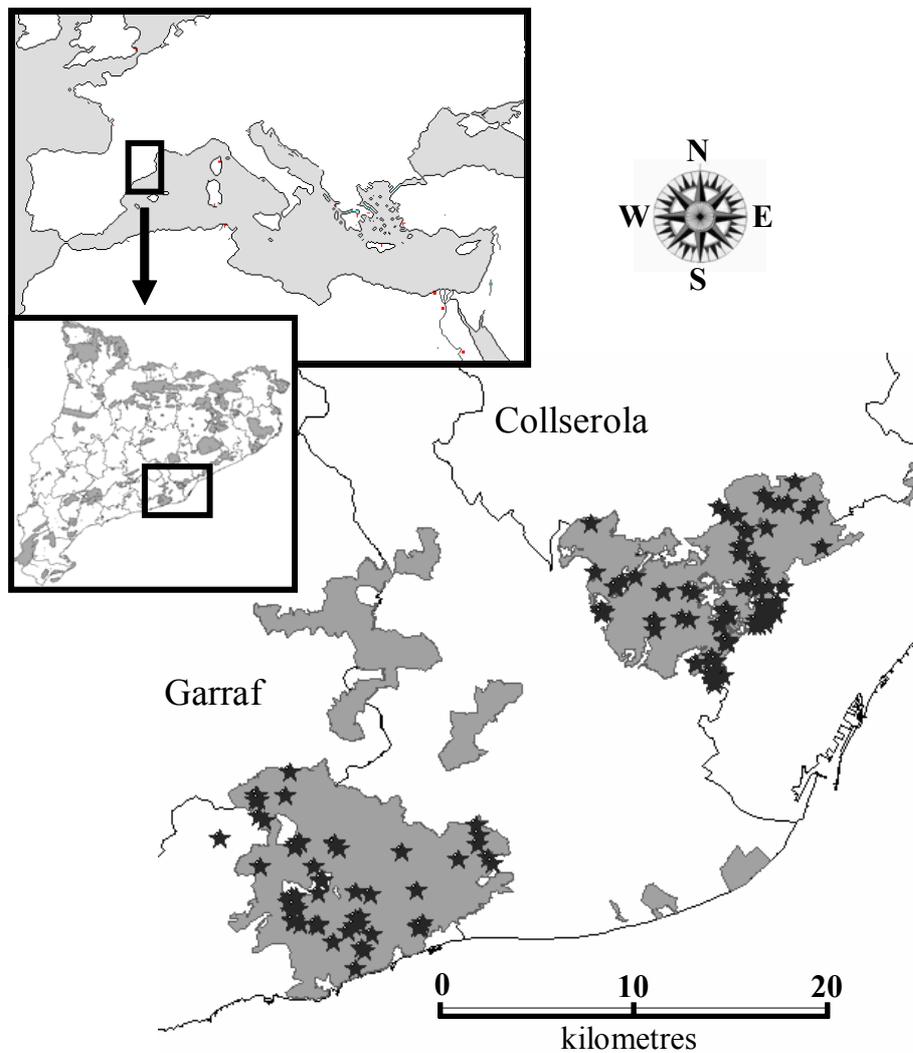


Fig. 1.- Location of the two Natural Parks studied (shaded areas) and the reproductive pond habitats surveyed during the field study (black stars).

1990; Chovanec 1992; Anholt et al. 1996; McCollum & Leimberger 1997; Lardner 2000; Van Buskirk 2002; Relyea 2004). Predator-induced changes in behaviour and morphology are common in tadpole (e.g. Lardner 2000; Van Buskirk & Relyea 1998; Relyea 2002; Van Buskirk 2002) and newt larvae (Schmidt & Van Buskirk 2005). Since amphibian larvae as a group occupy a wide variety of aquatic habitats, they provide an ideal model to test predator-induced plasticity among species in function of the habitat occupied (Richardson 2001; Van Buskirk 2002).

Because of the trade-offs between rapid development or minimisation of predation risk across the hydroperiod gradient (Laurila & Kujasalo 1999; Bridges 2002), one can expect phenotypic plasticity to predation pressure to differ between species in distinct habitats. In species that breed in ephemeral and temporary ponds, predator-induced plasticity is mainly morphological in order to allow the species to maintain high levels of activity to escape a high risk environment as quickly as possible and at the same time reduce the risk of predation (Relyea & Werner 1999; Anholt et al. 2000). In contrast, in most permanent ponds without time constraints, morphological changes can be accompanied by behavioural changes in order to reduce encounters with and detection by predators (Chovanec 1992; Anholt et al. 2000). On the basis of this assumption, we expect a distinct integration of the various traits (if any genetic or developmental constraints prevent traits from being correlated) to fine-tune the plastic phenotype response in function of species ecology and history (Relyea 2004).

Here we studied whether the division of amphibian species and their large invertebrate predators across the hydroperiod gradient in a system of isolated ponds in a Mediterranean region follows the same pattern as in temperate regions (e.g. Babbitt et al. 2003). In addition, in a laboratory experimental procedure, we tested whether predator-induced plasticity (morphology and activity level) of amphibian larvae differs in function of the kind of habitat occupied along the hydroperiod gradient.

MATERIAL AND METHODS

Site

The field study was carried out in protected natural areas surrounding Barcelona (NE Iberian Peninsula), including the Natural Park of Garraf and the Metropolitan Park of Collserola (Figure 1). At an altitude of 70-100 m and 2-10 km wide, the coastal plateau runs North-South for about 50 km. It is bordered by a chain of small mountains (maximum 600 m altitude) called the Prelitoral Sierra and holds the Llobregat delta in the middle of the area. The climate in this zone is Mediterranean, with hot, dry summers, mild winters and two rain periods, one in spring and the other in autumn. The amount of precipitation varies considerably between years in this region. The amphibian community in this zone comprises seven anuran species (*Alytes obstetricans*, *Pelobates cultripipes*, *Pelodytes punctatus*, *Bufo calamita*, *Bufo bufo*,

Hyla meridionalis and *Rana perezii*) and *Salamandra salamandra*, with an introduced and isolated population of *Triturus marmoratus* and *Triturus helveticus* in one locality. We focused our study on the anuran community but excluded *Pelobates* because it is a rare species in the area and few data are available.

Field sampling methods

We evaluated amphibian larvae and their potential invertebrate predators in four sampling periods during the spring and summer, from March to August 2002, in a total of 193 isolated ponds. These localities span the range of aquatic breeding habitats of the species studied, including ephemeral pools, and temporary and permanent ponds. Sampling time periods were dictated by preliminary sampling and accounted for temporal differences in breeding activity between species (unpublished data) and ensured that all species breeding were captured. Due to variation in hydroperiod, not all sites were surveyed in all the sampling periods, thus sample sizes of ponds were not uniform. Amphibian larvae and predacious invertebrates were sampled with dip-net sweeps (30 cm x 40 cm) to obtain relative species densities. This is a standardised technique used to sample these two groups (e.g. Heyer et al. 1994; Babbitt et al. 2003). A minimum of 5-10 dip-net sweeps were taken in each possible tadpole microhabitat following standard techniques in function of pond size (Heyer et al. 1994). All tadpoles were identified in field, and photographed with a grid background. The number of individuals of each species was counted and tadpoles were then returned to water. Predacious invertebrates, demonstrated in previous studies to prey on tadpoles, were identified (e.g. Woodward 1983; Travis et al. 1985; Cronin & Travis 1986), counted and photographed with a grid background. Three types of insects were considered potential predators: dragonfly larvae (considering aeshnid and libellulid odonate naiads as predators), heteroptera (notonectids and *Nepa* spp.) and diving beetles (Coleoptera). Fish presence was determined through visual surveys in addition to dip-net captures. We divided the hydroperiod into 3 categories: (1) ephemeral or rain pools which dry within weeks; (2) temporary ponds which dry every year during the summer; and (3) permanent ponds, which contain water year round. We did not include a category for permanent ponds with or without fish as in previous studies (Babbitt et al. 2003; Stocks & McPeck 2003; Van Buskirk 2003)

because in the study area only 6 of the 193 ponds contained introduced fish that prey on tadpoles and were not included in the study, remaining 187 localities for analysis.

Total counts for each amphibian species and predacious invertebrate captured in each pond were divided by the number of dip-net sweeps taken in each pond, following the procedures described in previous studies (Babbitt et al. 2003). This yielded an abundance on the basis of catch per unit effort, which could be compared across the three habitats considered. A computer was used to measure the body lengths of predacious invertebrates from pictures and invertebrates were classified in two groups: (1) small, above 5 mm until 15 mm, and (2) large predators over 15 mm body length. We conducted ANOVA analyses of variance to determine whether amphibian species and predator group abundances and relative densities varied between the hydroperiod categories. Ponds were considered as the units in all analyses and variables were log-transformed before analyses.

Experimental procedure: effect of predation risk on larval morphology and activity.

Morphological and behavioural anti-predator responses of the six species to a common predator were tested in a short-term laboratory experiment. Clutches of the six species were collected from natural ponds. In all cases, egg masses were taken during the *amplexus* period in order to reduce the time of embryo exposure to predators and thereby avoid the induction of phenotypic plasticity on hatchlings. For *Pelodytes*, egg samples were taken from 5 egg masses and were hatched separately. For *Hyla*, we collected 15 egg masses from three ponds. In the case of bufonids, fragments of three distinct egg strings were taken for each species (*B. bufo* and *B. calamita*). In the case of *Rana*, tadpoles were obtained from 5 clutches from two ponds. The *Alytes* larvae had been recently deposited in the pond by males and had recently hatched when we collected them. As males of *Alytes* protect eggs, negligible predation on hatchlings should occur during the egg period and no defence response is expected to develop.

The experiment was conducted in laboratory tanks (30 l) under similar conditions to the natural photoperiod and around 22 °C, during the spring of 2001. Containers assigned for each species were filled with dechlorinated water. 30 tadpoles were subsequently randomly drawn from a mixture of all the clutches and transferred from hatching aquariums to these

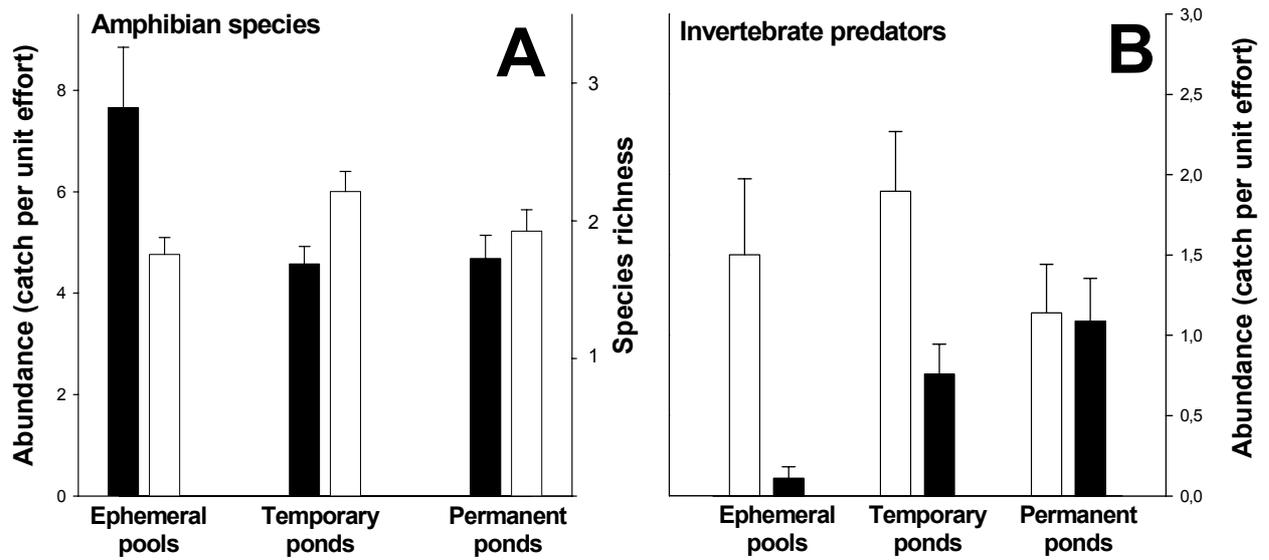


Fig. 2.- (A) Larval amphibian abundances (catch per unit effort) and larval amphibian species richness in the three hydroperiods. **(B)** Potential small invertebrate predators (white boxes) and large invertebrate predators (black boxes) abundances (catch per unit effort) in the three hydroperiods. Mean and standard error is shown.

tanks when they reached Gosner 25 stage. During all the experiment, tadpoles fed on rabbit pellets *ad libitum*. Two transparent cylindrical predator cages were placed in both sides of each tank to prevent predators from capturing tadpoles but allowing chemical signals to flow. The two treatments (predator presence and predator absence) were each replicated several times (6 for *Alytes*, 10 for *Pelodytes*, 10 for *Hyla*, 7 for *B. calamita*, 6 for *B. bufo* and 6 for *Rana*). In experiments to test predator presence, aeshnid odonate naiads larvae were used. Each predator was fed one tadpole per day. Supplementary tadpoles from the six amphibian species were raised in separate containers to be used as food for predators.

At week two of the experiment, tadpole activity was measured. We sampled activity behaviour by counting the number of tadpoles moving in each tub the instant the tub was first viewed (Skelly 1995). Each tub was observed every half hour for a total of 60 replicates during two weeks. This protocol was repeated for all six species.

At week four of each species of the experiment, in order to obtain data on tadpole morphology, all tadpoles were individually photographed with a grid background. We

measured six traits which show plasticity (Van Buskirk & Relyea 1998; Van Buskirk 2002): body length, body depth, tail fin length, tail fin depth, tail musculature length and tail musculature depth.

Statistical analyses

For behavioural response of tadpoles to the predator presence, the mean proportion of active tadpoles per tub was calculated. These proportional data were arc-sine square root transformed before testing the hypothesis that the mean number of active tadpoles differs between treatments (presence or absence of predator) using ANOVA analyses.

Before analysing morphological plasticity response, tadpole measurements 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. We used centroid size as a measure of body 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 for each species in the two treatments was tested first with multivariate analysis for all traits together and second with a univariate analysis for each variable.

We measured differences in plasticity magnitude among species (activity and morphological traits) by examining the changes in traits that occurred between treatments divided by the mean value of the trait in the absence of predators ($(\text{presence of predator} - \text{absence of predator}) / \text{absence of predator}$) (Van Buskirk 2002). In predator presence, positive values of plasticity reflect an increase in the value of the trait, whereas negative values show a decrease. The magnitude of plasticity in traits can be compared among species because they are all represented in unitless measures of proportional change. We used univariate and multivariate analyses to determine whether the phenotypes of the species were affected by the presence of a predator. Univariate tests were performed on activity and on morphological traits.

As species cannot be considered independent data points because trait values could be affected by shared common ancestry (Felsenstein 1985; Richardson 2001), we tested whether the distribution of a particular species in phenotypic plasticity space is correlated with its phylogeny. We compare proportional change in activity for each species and the proportional change in morphology in function of phylogenetic topology of the six species. Proportional change in morphology was calculated as the mean of the absolute values of proportional changes ($[\text{presence of predator} - \text{absence of predator}] / \text{absence of predator}$) in the six size-corrected traits considered (Van Buskirk 2002). The phylogenetic relationships between the six species were reconstructed using the combined data set of three genes: 12S, 16S and *cyt b*. Sequences were obtained from specimens in a personal collection (collected and sequenced by S. Carranza) and from the GenBank database. All sequences were compiled, aligned and refined manually using Sequence Navigator. Observed distances in pair-wise comparisons were obtained using the PAUP software. We tested phylogenetic independence of larval traits with the computer programme “Phylogenetic Independence 2.0” (Reeve and Abouheif 2003). The Test For Serial Independence (TFSI) was performed on continuous data using the phylogenetic topology and node distances obtained from molecular reconstruction. Topology was randomly rotated 10000 times to build a null hypothesis.

RESULTS

Quantification of species abundance along the gradient

During spring-summer 2002, we surveyed: 49 ephemeral pools, 85 temporary ponds and 53 permanent ponds. Species richness ranged from 0 to 6 species per locality, with a total count of 24,938 tadpoles during field sampling. Amphibian species richness did not show statistical differences in the three habitats considered ($F_{2, 184} = 2.537$; $p = 0.0818$). Temporary ponds showed relatively higher richness than that ephemeral or permanent ones (Figure 2A). In addition, total tadpole abundance did not vary significantly between hydroperiod categories ($F_{2, 184} = 1.227$; $p = 0.2954$), although it was highest in ephemeral pools (Figure 2A), perhaps because of the small size of these localities. Relative abundances of larval amphibians and the two predator classes varied between hydroperiod categories (Figures 3A, 3B). The abundance of most amphibian species was determined by the hydroperiod

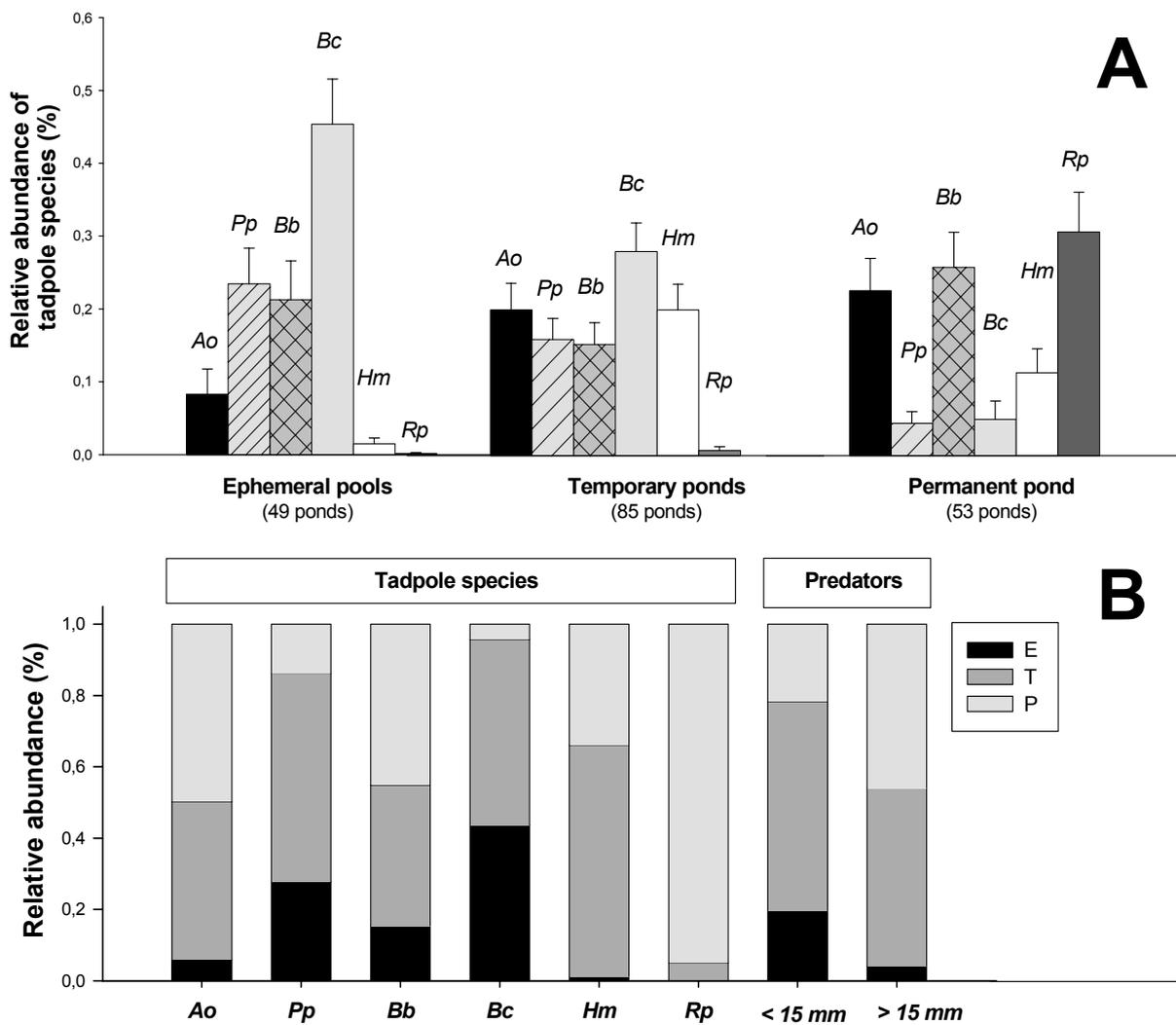


Fig. 3.- (A) Relative abundance of tadpole species for each hydroperiod category. Mean and standard error is shown. **(B)** Relative abundance of amphibian species and invertebrate predators in ephemeral (E), temporary (T) and permanent ponds (P). Ao = *Alytes obstetricans*, Pp = *Pelodytes punctatus*, Bb = *Bufo bufo*, Bc = *Bufo calamita*, Hm = *Hyla meridionalis*, Rp = *Rana perezi*.

category, showing a clear segregation of species along the gradient. *Pelodytes* and *B. calamita* were significantly related to ephemeral and temporary ponds ($F_{2, 184} = 4.69$; $p = 0.0102$ and $F_{2, 184} = 12.42$; $p < 0.001$ respectively), *Alytes*, *Hyla* and *B. bufo* were more abundant in temporary and permanent ponds ($F_{2, 184} = 12.44$; $p < 0.001$; $F_{2, 184} = 9.81$; $p < 0.001$ and $F_{2, 184} = 4.35$; $p = 0.014$ respectively), while *Rana* occupied mainly permanent ponds ($F_{2, 184} = 34.10$; $p < 0.001$). The two groups of predacious invertebrates also differed along the gradient; small predators were more abundant in temporary ponds ($F_{2, 184} = 13.02$; $p < 0.001$) while large predators increased along the gradient, showing greater values in

permanent ponds ($F_{2,184} = 34.10$; $p < 0.001$). Ephemeral pools showed the lowest variability in abundance (Figure 2B); however, small predacious invertebrates were more variable in ephemeral and temporary ponds.

Larval abundance of amphibian species was correlated with predacious invertebrates abundance. *Pelodytes* and *B. calamita* were negatively correlated with large predators ($r = -0.168$; $p = 0.021$ and $r = -0.271$; $p < 0.001$ respectively), whereas *Hyla* and *Rana* were positively correlated with large predators ($r = 0.188$; $p = 0.01$ and $r = 0.229$; $p = 0.002$ respectively).

Effects of predation risk on larval morphology and activity

For only two of the six species (both bufonids) activity was not altered in the presence of predators. In contrast, the activity of the other four species was significantly reduced (Table 1, Figure 4D). The MANOVAs for each species indicated that only two species (*Pelodytes* and *Hyla*) showed a response in morphological traits. The individual morphological traits showed differences between treatments for each species, with the exception of the two bufonids where no morphological change was detected. Morphological plasticity was detected in several traits in function of the species considered, with major changes in tail fin and body traits (Table 1, Figures 4A, 4B, 4C). The experiment illustrated numerous phenotypic

		Univariate ANOVA on Activity level							
Source of variation	d.f.	F	P						
Alytes	1, 9	27.69	0.0005						
Pelodytes	1, 17	17.79	0.0005						
B. bufo	1, 10	0.72	0.4136						
B. calamita	1, 12	2.18	0.1655						
Hyla	1, 17	14.31	0.0014						
Rana	1, 9	29.37	0.0004						
		MANOVA on morphology		Univariate ANOVA on					
Source of variation	d.f.	F	P	Body length	Body depth	Tail fin length	Tail fin depth	Tail musculature length	Tail musculature depth
Alytes	6, 4	2.08	0.2485	8.15*	2.14	1.19	0.49	1.61	12.97*
Pelodytes	6, 12	39.46	<0.0001	19.13*	23.99*	38.61*	76.58*	14.99*	48.97*
B. bufo	6, 5	3.27	0.1068	0.86	10.41*	0.88	1.39	0.87	0.81
B. calamita	6, 7	1.63	0.2663	4.32	0.41	0.24	0.59	2.93	0.02
Hyla	6, 12	20.48	<0.0001	2.27	0.01	1.47	97.87*	0.03	0.45
Rana	6, 4	3.83	0.1069	0.43	0.78	14.77*	33.88*	0.30	0.91

Table 1.- Summary of univariate and multivariate ANOVA on plasticity in activity and morphology in tadpoles of the six species. Entries for individual morphological traits are F-values in univariate ANOVA, asterisk indicates statistical significance at $\alpha < 0.05$.

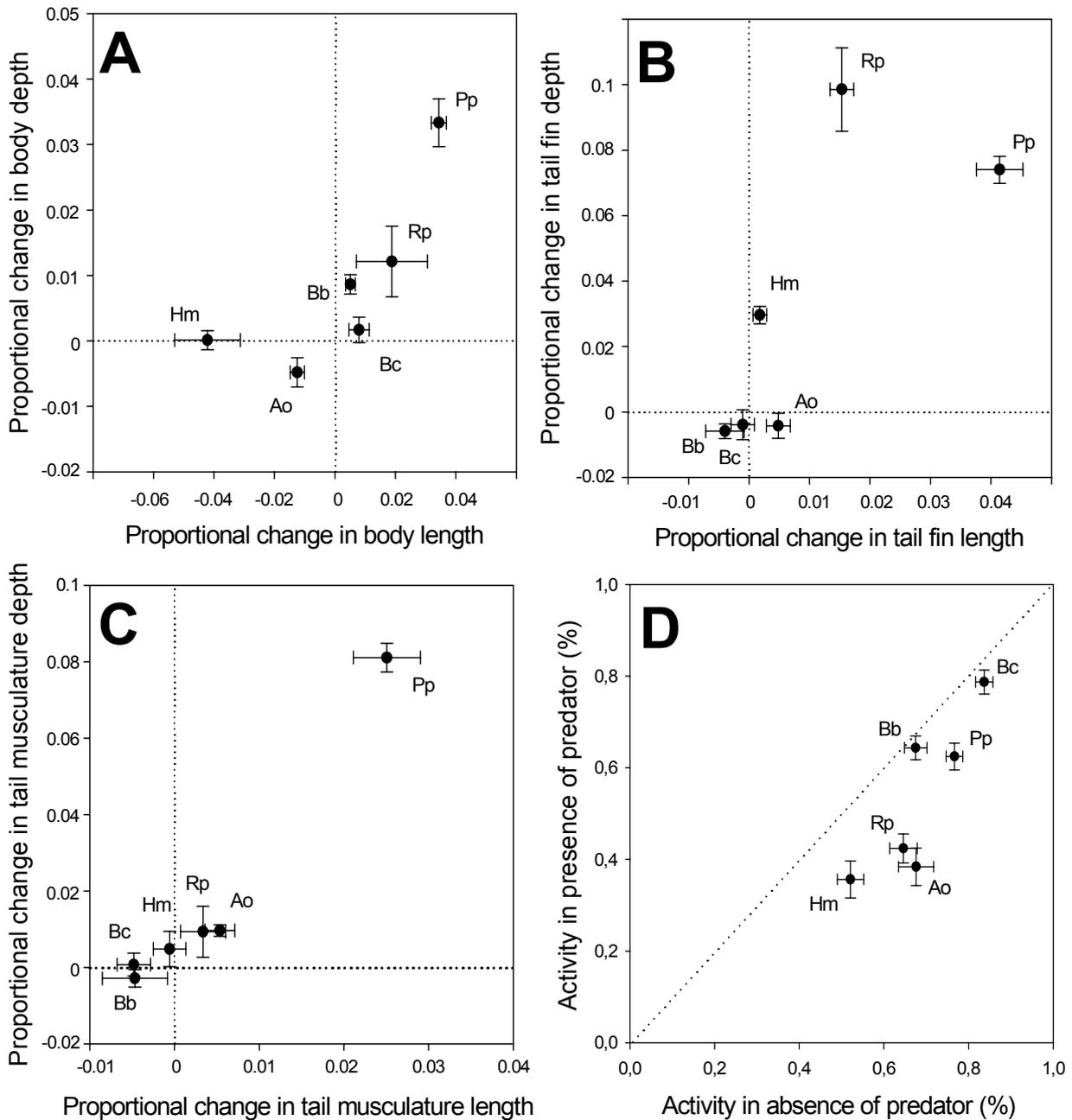


Fig. 4.- Predator-induced plasticity in the body (A), tail fin (B) and tail muscle (C) shape of amphibian species. Figure shows changes in shape caused by the presence of predator (mean and standard error). Dashed lines indicate the case in which there was no plasticity. Figure (D) shows proportion of time active in the presence and absence of predator. Species near the dashed line showed little behavioural response to predator. Ao = *Alytes obstetricans*, Pp = *Pelodytes punctatus*, Bb = *Bufo bufo*, Bc = *Bufo calamita*, Hm = *Hyla meridionalis*, Rp = *Rana perezi*.

differences among species for all traits considered: body length ($F_{5,36} = 17.07$; $p < 0.001$), body depth ($F_{5,36} = 23.27$; $p < 0.001$), tail fin length ($F_{5,36} = 46.13$; $p < 0.001$), tail fin depth ($F_{5,36} = 61.71$; $p < 0.001$), tail musculature length ($F_{5,36} = 16.41$; $p < 0.001$), tail musculature depth

($F_{5,36} = 73.98$; $p < 0.001$) and activity ($F_{5,36} = 9.98$; $p < 0.001$) (Figures 4A, 4B, 4C, 4D and 5A). A general response in the presence of predator caused an increase in tail fin length and depth, while body traits showed a range of responses in function of species. In some species (*Pelodytes* and *Rana*) an increase in body length and body depth was observed, whereas in others (*Alytes* and *Hyla*), the response was the opposite with shorter and shallower bodies. With the exception of *Pelodytes*, tail musculature did not show important changes in the presence of predator with respect to the absence of this factor. Species were highly variable in the predator-induced morphological phenotypic plasticity, but showed a common response in activity, which varied in magnitude in function of species (Figure 5A).

The TFSI showed no significant phylogenetic autocorrelation between species for the morphological data set (C -statistics = 0.0930; $p = 0.2820$) (Figure 5B), or for proportional change in activity (C -statistics = 0.1451; $p = 0.2882$) (Figure 5C). These results indicate little correlation between these traits and its phylogenetic story for the six species studied.

DISCUSSION

Our quantitative field data show a turnover in anuran larvae species along the hydroperiod habitat gradient, as documented in previous studies (Wellborn et al. 1996; Snodgrass et al. 2000; Babbitt et al. 2003, 2005). Our results have an important difference respect the mechanisms shaping community structure along freshwater gradient proposed by Wellborn et al. (1996). They assumed that permanence transition (between temporary habitats and permanent habitats) also coincides with the shift from communities without predators to communities with large invertebrate top predators. In our case, invertebrate predation and hydroperiod transition did not coincide. The relative abundance of large invertebrate predators was higher in permanent ponds, but temporary ponds could not be considered a predator-free habitat. The transition towards communities dominated by large invertebrate predators occurs between ephemeral pools and temporary ponds (Stoks & McPeck 2003). Previous studies have shown that large invertebrate predators are abundant and important top predators in temporary ponds (Skelly 1996; Wilbur 1997; Stoks & McPeck 2003).

Species assemblages differ at both ends of the permanence transitions (from ephemeral pools to temporary ponds, and from temporary ponds to permanent ponds). Tadpole

assemblages may differ in the first transition in ephemeral pools because of the high risk of habitat drying. Moreover, these assemblages may also vary as a result of distinct predator communities in ephemeral and temporary ponds. In the second transition, from temporary to permanent ponds, hydroperiod was the most important factor, because few differences were observed between large invertebrate predators. Strong asymmetric competition between species or differences in phenological events, such as breeding activity, could shape assemblages in the second transition where top predators show similar abundance and density. These two transitions allow the identification of three species assemblages along the freshwater gradient studied. *B. calamita* and *Pelodytes* occurred between the ephemeral and temporary pond transition. *Hyla* bred mainly in temporary ponds, whereas *B. bufo* and

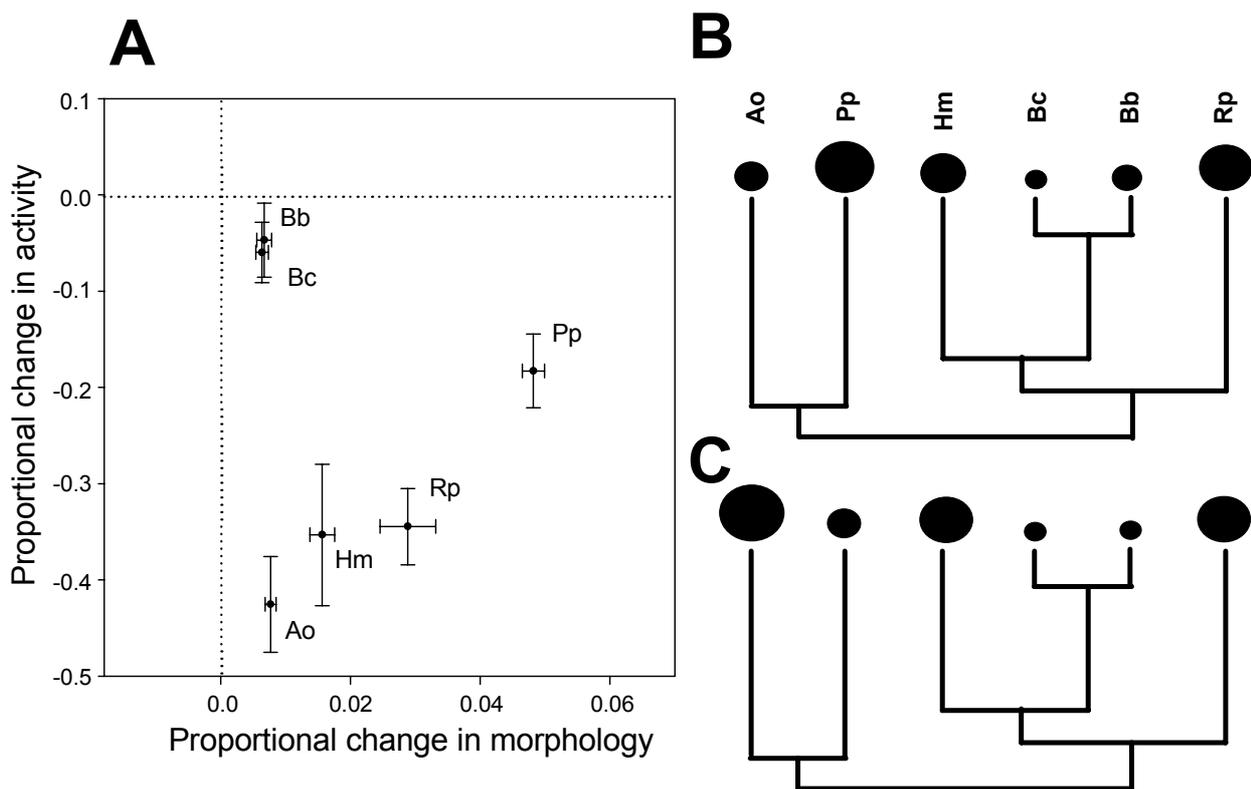


Fig. 5.- (A) Relationship between behavioural plasticity and morphological plasticity. All proportions are calculated relative to the predator-free treatment, bars indicate standard error. Hypothesised phylogenetic relationships between the 6 anuran species, where branch lengths do not correspond to the true molecular data estimated. Sizes of full circles represent magnitude of plasticity for morphology (**B**) and for behaviour (**C**). Ao = *Alytes obstetricans*, Pp = *Pelodytes punctatus*, Bb = *Bufo bufo*, Bc = *Bufo calamita*, Hm = *Hyla meridionalis*, Rp = *Rana perezi*.

Alytes were found in temporary and permanent ponds. And in the permanent extreme of the gradient, we detected *Rana*, which reproduce only in permanent ponds. Species do not show a perfect distribution along the hydroperiod, and many use two or three of the habitats considered (Babbitt et al. 2003; Van Buskirk 2003). 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 (Marsh & Trenham 2001). Theory proposes that in these circumstances, plasticity for several environmental variable traits evolve to allow species to successfully colonise a wide range of habitat types (DeWitt & Scheiner 2004). Plasticity has a primary ecological significance as it permits a widening of the niche breadth of species with a metapopulation structure.

In our study, plasticity and life history played a key role in shaping species turnover along the gradient. Trade-offs between rapid growth rates and predation risk is well documented (Woodward 1983; Werner & McPeck 1994; Anholt et al. 2000). As expected, we detected differences in behaviour plasticity between species in ephemeral pools and tadpoles in other habitats. Our experimental observations reveal that predator presence reduced activity levels in all species; however, those in ephemeral pools (*B. calamita* and *Pelodytes*) showed a small decrease in activity compared with the most permanent pond species (with the exception of *B. bufo* perhaps as a result of phylogeny). Presumably these ephemeral and short temporary pond species must maintain activity levels to achieve a growth rate adequate to metamorphose before ponds dry (Laurila & Kujasalo 1999; Anholt et al. 2000). Species in temporary and permanent ponds were normally exposed to increased abundance and density of large predators. These species may weight the balance more to predation risk because they have the option of remaining in the aquatic habitat as larvae for more time before the metamorphosis (Anholt et al. 2000), and however showed a major plasticity in activity than ephemeral pool species.

Behavioural and morphological plasticity to predators show extensive variation among species not ever related with habitat (Van Buskirk 2002). A number of anti-predator strategies used by species reflect phylogenetic lineages selected in distinct branches of the anuran phylogenetic tree (Lardner 2000; Richardson 2001; Van Buskirk 2002). Our results did not show any phylogenetic correlation between the characters studied; however, the small number

of species included in the study and their great phylogenetic distance may obscure the identification of a possible relationship. The similar response observed in activity and morphology between the two bufonid species clearly reveals phylogenetic constraints. In general terms, the morphological reactions in response to predator presence are consistent with previous studies (e.g. Van Buskirk 2002 and references therein). Relyea and Werner (1999) documented that temporary pond species responded morphologically to invertebrate predators by increasing tail fin depth and decreasing body size. This morphotype is an adaptation designed to reduce vulnerability to invertebrate predators (Van Buskirk et al. 1997; Van Buskirk & Relyea 1998), thereby allowing these species to maintain high activity while responding morphologically rather than behaviourally to the presence of predators. We observed the same morphology for *Hyla*, a temporary pond breeder, but not in *Pelodytes* an ephemeral and temporary pond breeder which also shows an increased tail fin depth but also an increased body size. Several predator-induced morphological changes observed may be constrained by functional or design limitations (Van Buskirk 2002). The low morphological plasticity detected in other species like *Alytes* or bufonids could be attributed to historical and phylogenetic events. The differences in morphological plasticity shown by distinct species in the presence of a predator have been extensively discussed by Lardner (2000) and Van Buskirk (2002). Our data reveal a clear pattern only in predator response between a number of species that inhabit ephemeral pools and other species which inhabit other habitats. The ephemeral pools species show low behavioural plasticity to avoid risk of death before the pool dries, and in the case of *Pelodytes*, a morphology that reduces predation risk without renouncing high levels of activity. Without genetic or developmental constraints that prevent traits from being correlated, tadpoles should use correlated traits that help individuals to reduce mortality in the distinct conditions in an integrative mode (Relyea 2004).

In the Mediterranean system studied here, hydroperiod and associated changes in predator composition are crucial in shaping tadpole assemblages. This importance is stronger in the first transition between ephemeral and temporary ponds, which determines a hydroperiod transition and a predator assemblage transition. Tadpole assemblages can be explained in part by the interplay of differences in the life histories of species and their vulnerability to predation. In the context of an ecological community, the adaptive variation in

anti-predator plasticity may affect many interactions other than the immediate predator-prey link, for example competition (e.g. Morin 1983; Werner & McPeck 1994; Werner & Anholt 1996). However, differences observed in the second transition could be attributed to factors other than an increase in predator abundance, such as phenological breeding differences between species, strong asymmetric competition, physiological adaptations to abiotic factors (dissolved oxygen, temperature of water) or feeding strategies related to resources availability. For example, *Rana* are largely excluded from temporary ponds because their breeding period starts in late spring when most of these ponds are completely dry. However, it is more difficult to explain the absence or poor use of permanent ponds by “typical” temporary pond species, and why these species use high risk temporary ponds if predation risk is similar to that in permanent ponds. This difficulty increase when theoretical models question the assumption of linearly increasing effects of predator density on prey mortality when prey exhibit plastic and costly anti-predator behaviour (Abrams 1993).

Other life history parameters not considered in this study may also contribute to shaping species distribution along the gradient by inducing asymmetric interactions between temporary pond and permanent pond tadpoles. The availability of alternative habitat types allows more anuran species to coexist on a regional scale. Strong habitat-specific selection regimes along the gradient decrease local species richness (two species per habitat in our study), while at the same time increasing regional species richness (Stoks & McPeck 2003).

ACKNOWLEDGMENTS

We sincerely thank Nuria Garriga for her help with the laboratory experiments and for her useful comments. We thank Laura Pérez for assisting with field surveys, and for S. Llacuna and F. Llimona for providing the corresponding permits and fieldwork facilities in the Natural Parks. Permission to capture anurans was granted by the *Departament de Medi Ambient de la Generalitat de Catalunya*. We also thank S. Carranza for assistance with the phylogenetic topology reconstruction of the species covered in this study and for providing personal data for this purpose.

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