

Pond breeding amphibian metacommunity structure: the importance of trade-offs

Amphibian species living in lentic freshwater habitats exist along a gradient from ephemeral to permanent ponds. Community structure and distributions of species across the gradient have been explained by three principal ecological models, all them based on different trade-offs and inherent properties of the species. One model assumes that patterns of occurrence can be understood in terms of metapopulation models, closely connected to ideas about fugitive or pioneer species and trade-offs between colonization and competition abilities. The other two models assert that a gradient in pond permanence is the main cause of community structure. One argues for a pond permanence-competition trade-off, whereas the other opts for species tolerance to predators across the hydroperiod (permanence-predation trade-off). We measured in-field distribution of species across the gradient and tadpole and predator abundances in different habitats. Using field surveys and laboratory experiments we examined the different traits suggested by the models in order to assess the relative role of each model in explaining community structure. Species distribution, tadpole abundance and predation risk across the hydroperiod gradient were in concordance with previous studies. Correlations among traits reflected trade-offs suggested by the models, whereas a test of equality of correlation coefficients does not favour any single trade-off model over the others. These results suggest that this community can be interpreted as a metacommunity in which local interactions (pond permanence, predation risk and competition) and regional processes (colonization-extinction rates) are important and related, and they emphasize the importance of habitat heterogeneity for both local and regional diversity maintenance.

INTRODUCTION

Understanding broad-scale patterns of species richness is a central concern of the sciences of ecology and biogeography. A variety of mechanisms have been posited to cause environmental gradients of species richness (Scheiner & Willig 2005). Diversity changes along a variety of types of gradients (Ricklefs & Schluter 1993), some primarily spatial (e.g., latitude, depth, elevation; Willig et al. 2003) and some primarily ecological (e.g., disturbance, salinity, succession, predation risk; Grace 1999). Many of the ecological gradient models are based on trade-offs and inflexion points, which leads to a hump-shaped pattern of species richness along the gradient (Scheiner & Willig 2005). These models propose that two

mechanisms acting in concert but in different directions originate an inflexion point. This trade-off can be conceptualized as gradients for each of two factors that run in opposite directions. Along one portion of the combined gradient, the first factor limits the number of individuals or species. At some other point, the second factor becomes limiting. This switch results in the number of individuals and species increasing along one portion of a gradient and decreasing along another. This switch in importance is controlled by inherent properties of species (Tilman 1982).

Trade-offs are not only important for the regulation of communities at a local scale, but also at a regional scale, from a metacommunity perspective (Leibold & Miller 2004, Leibold et al. 2004). A metacommunity perspective would represent a great improvement in the understanding of how communities are structured by the joint action of processes operating at both local and regional scales and of what regulates the coexistence of multiple species. From a metacommunity point of view long-term coexistence of species requires that they show some trade-offs with regard to important aspects of their biology at some scale, whether local (abiotic or biotic specialization of species to different habitats) or regional (different migration and colonization rates of species) (Chesson 2000).

The aim of the present study is focused explicitly on trade-offs in species traits of an anuran larvae assemblage across a well-known freshwater habitat gradient (Wellborn et al. 1996), which ranges from ephemeral pools to permanent ponds, and looks at how these trade-offs may affect species persistence and coexistence at local and regional levels. We explored the role of different possible trade-offs suggested by the literature that might regulate pond amphibian metacommunities. Three different mechanisms or points of view based on trade-offs are used to try to explain patterns of occurrence at local and regional level for amphibians and other freshwater organisms.

The first is based on a “classic” metapopulation concept of isolated ponds as patches, in which extinction and recolonization of ponds explain patterns of occurrence (Hecnar & M'Closkey 1996; Marsh & Trenham 2001). This patch-dynamic perspective is closely connected to ideas about fugitive or pioneer species and trade-offs between colonization and competition abilities (Leibold & Miller 2004; Leibold et al. 2004).

The two remaining models hold that a gradient in pond permanence is the main cause of tadpole assemblage structure, recognizing that ponds as patches are heterogeneous in their characteristics, and that different species are favored in different habitats. These models are more related to a species-sorting perspective (Tilman & Pacala 1993; Leibold et al. 2004) or a “mass effects” (source-sink dynamic) perspective (Mouquet & Loreau 2002, 2003; Leibold et al. 2004). One of these trade-off models states that species sort along the gradient according to whether they are tolerant of pond drying in temporary ponds or strong competitors in more permanent ponds, creating a permanence-competitive ability trade-off (Wiggins et al. 1980; Smith 1983). On the other hand Wellborn et al. (1996) proposed a model for trade-offs along

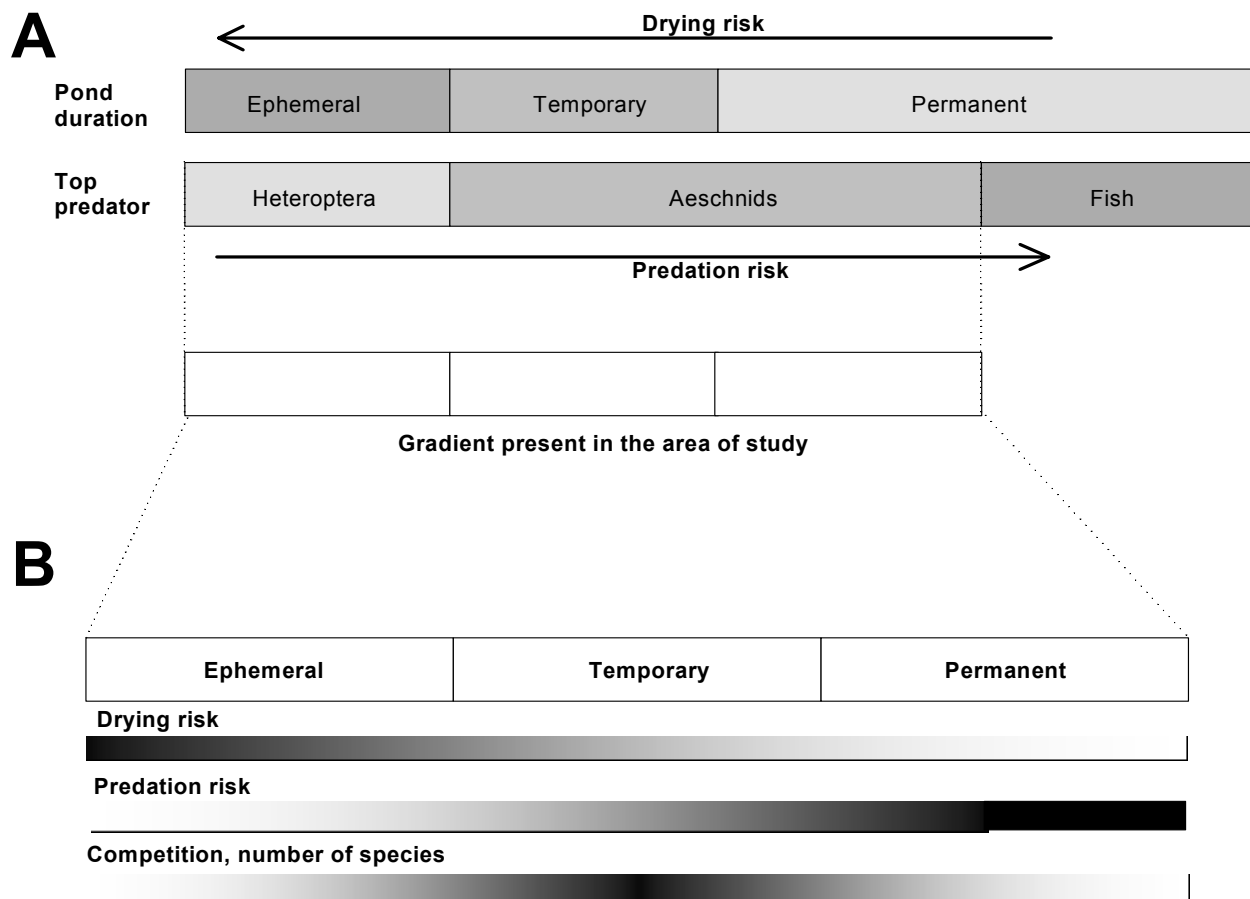


Fig. 1. – (A) Diagram showing the freshwater gradient and the permanence transitions (ephemeral to temporary to permanent) and predator transition. Arrows indicate direction of negative effects of drying risk and predation risk. Dashed lines restrict the portion of freshwater gradient present in the area of study. In (B) the freshwater gradient studied and the three principal abiotic and biotic effects proposed by the models are indicated, along with their respective directions (grey intensity indicates direction of negative effects).

predator-permanence gradients across a freshwater habitat gradient. The model, based on studies conducted principally with larval anurans, suggests that the behavioral, developmental, and morphological characteristics that promote rapid development and timely metamorphosis in temporary habitats are disadvantageous in permanent habitats, where these same traits lead to increased vulnerability to predation. Conversely, the low activity rates and risk-sensitive foraging behaviors exhibited by anurans that coexist with predators are disadvantageous in temporary habitats, where selection is for rapid growth and metamorphosis (Wilbur 1987; Skelly 1992, 1994, 1996; Werner & Anholt 1993; Werner & McPeck 1994; Anholt & Werner 1995).

The majority of empirical studies test distribution and trade-offs of two closely related species (but see Richardson 2001), to define mechanisms contributing to the local and regional coexistence of similar species. Here we used a combination of field observations and laboratory experiments to evaluate the applicability of divergent models in amphibians inhabiting a portion of the freshwater gradient (Fig. 1) in a Mediterranean region.

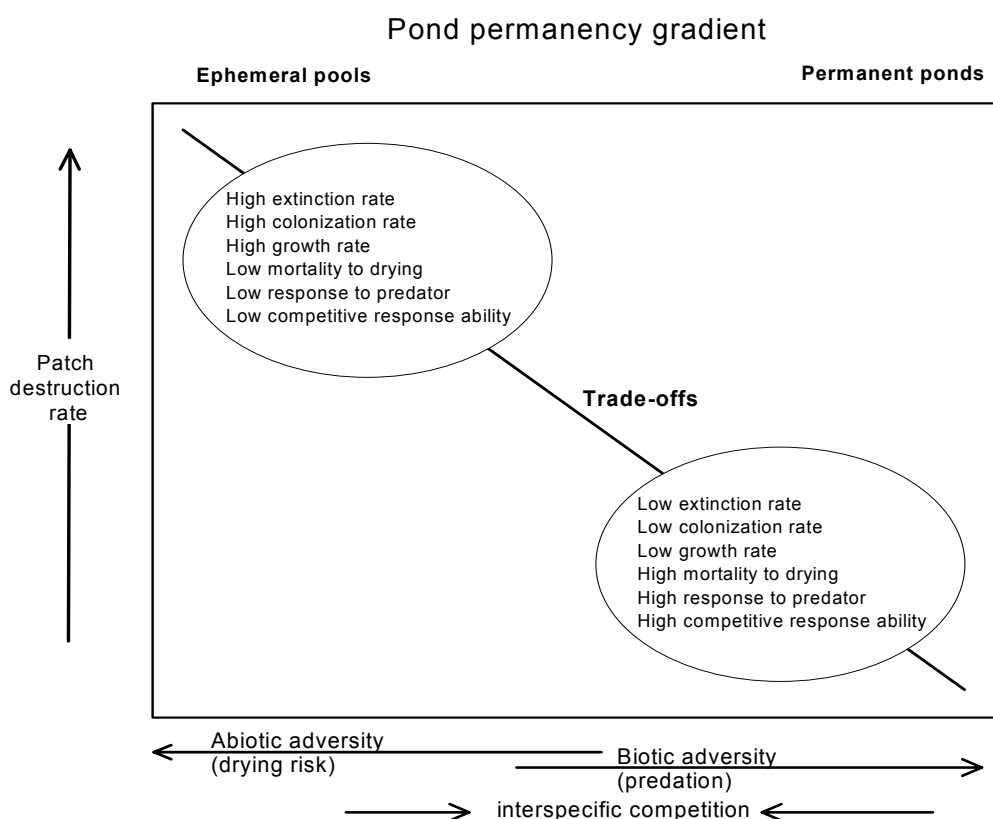


Fig. 2.- Diagram of the trade-off traits to be expected according to the literature across the freshwater gradient for organisms distributed at both ends of the permanence gradient (and the biotic gradients associated to it).

We used field data from a four-year study to evaluate the wide range of habitats across the freshwater gradient of each species and their colonization and extinction rates. We chose traits that the literature suggests are the result of selective pressures caused by trade-offs among the different habitat types and measured in laboratory experiments. In Figure 2 we summarized expected different traits at both extremes of pond permanence (ephemeral to permanent pond).

MATERIAL AND METHODS

From 2000 to 2003 we conducted field observations of six species in protected areas near Barcelona and experiments to measure phenotype traits of the species in the laboratories of the University of Barcelona. The six species studied are from 5 different families: Discoglossidae (*Alytes obstetricans*), Pelodytidae (*Pelodytes punctatus*), Bufonidae (*Bufo bufo* and *Bufo calamita*), Hylidae (*Hyla meridionalis*) and Ranidae (*Rana perezi*).

Estimating species' ecological breadth in nature

During the four years we sampled 87 isolated ponds every year which span the range of aquatic habitats of the species studied, from ephemeral pools to fishless permanent ponds (Fig. 1). We assessed successful reproduction in all of these ponds every year by conducting a standardized test (Heyer et al. 1994) using dipnets in all the microhabitats present in the ponds and an active search of clutches during spring breeding periods. All tadpoles were identified in the field and returned to the pond. We only considered ponds to be successful breeding sites if eggs and larvae were found. With these data we constructed a presence-absence matrix for species and locality for the four years. We used these data to calculate extinction and colonization rates and co-occurrence among species. The probability of extinction and colonization between each pair of consecutive years was calculated. We used the models developed by Gotelli and Taylor (1999) as follows: (1) the probability of extinction is the number of sites occupied in year (t) that were unoccupied in year ($t+1$)/number of sites occupied in year (t). (2) The probability of colonization is the number of unoccupied sites in year (t) that were occupied in year ($t+1$)/the number of sites that were monitored in year (t).

Independently, for tadpole and clutch surveys from 2001 to 2003 we visited ponds approximately every four weeks throughout the year to establish the date of drying and determine the position of the pond across the hydroperiod gradient. In this way we can evaluate the number of days (in 30 by 30 days) that ponds retain water. Based on these data of pond permanence we classified the 87 ponds in just three categories: (1) ephemeral ponds that dry within weeks (less than 60 days of duration), (2) temporary ponds that dry every year during spring or summer, and (3) permanent ponds which contained water all year during the three years of monitoring.

Characterizing predation risk in nature

From March to August, 2002, in addition to estimating presence-absence of species in the 87 ponds, we evaluated amphibian larvae and potential invertebrate predator abundance in four sampling periods during the spring and summer. Due to variation in the 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 dipnet sweeps (25 cm x 35 cm) to obtain relative species densities. This is a standardized technique used to sample these two groups (Heyer et al. 1994; Babbitt et al. 2003). A minimum of 5-10 dipnet sweeps were taken in each possible tadpole microhabitat following standard techniques according to pond size. 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; Van Buskirk & Arioli 2005), counted and measured. Three types of insects were considered potential predators: dragonfly larvae (considering aeshnid and libellulid odonate naiads as predators), heteroptera (notonectids and *Nepa* sp.) and diving beetles (Coleoptera). We classified potential predators in two groups: (1) small, from 5 to 15 mm, and (2) large predators, over 15 mm in body length. A body length cut-off of 15 mm was used to consider the dangerous size classes of notonectids, dytiscids and dragonfly larvae (Van Buskirk & Arioli 2005). Abundance distribution of predators across the permanence gradient was used to characterize the predation risk gradient. Some authors considered that since all predators are not equally dangerous the predation risk could not be the sum of all predators without their having been previously weighted by the threat

Species trait	Definition	Trade-off models
Colonization	Probability of colonize an unoccupied pond, estimated from field surveys.	Colonization-competition trade-off Colonization-extinction trade-off
Extinction	Probability of disappear from an occupied pond, estimated from field surveys.	Colonization-extinction trade-off
Intraspecific (mass)	Effects of intraspecific competition on mass at metamorphosis, estimated from laboratory experiments.	Colonization-competition trade-off Permanence-competition trade-off
Intraspecific (survival)	Effects of intraspecific competition on survival to metamorphosis, estimated from laboratory experiments.	Colonization-competition trade-off Permanence-competition trade-off
Interspecific (mass)	Effects of interspecific competition on mass at metamorphosis, estimated from laboratory experiments.	Colonization-competition trade-off Permanence-competition trade-off
Interspecific (survival)	Effects of interspecific competition on survival to metamorphosis, estimated from laboratory experiments.	Colonization-competition trade-off Permanence-competition trade-off
Survival to drying	Survival to a simulated drying pond as a measure of developmental plasticity. Estimated from laboratory experiments.	Permanence-competition trade-off Permanence-predation trade-off
Predator (growth rate)	Effects of predator presence on tadpole growth rate. Estimated from laboratory experiments.	Permanence-predation trade-off
Predator (activity)	Effects of predator presence on tadpole activity as a measure of the risk to be predate. Estimated from laboratory experiments.	Permanence-predation trade-off
Activity	Activity level of tadpoles without any stress factor as a measure of their growth capacity. Estimated from laboratory experiments.	Permanence-predation trade-off Permanence-competition trade-off

Table 1.- Definitions of traits used in the correlation analyses and their implication at different trade-off models tested.

they represent (Van Buskirk & Arioli 2005). However, estimating predation risk for different prey species and different habitats turned out to be more complicated than a sum of predator densities weighted by their respective dangerousness, in part because the dangerousness of the predator probably changes for each prey species. Also the danger a given predator represents for its prey could change with habitat type (Griffen & Byers 2005). Furthermore some studies have demonstrated that effects of multiple predator species on prey are not cumulative, in part because of complex negative interactions between predators (Sih et al. 1998; Vance-Chalcraft & Soluk 2005). As we do not have data on the dangerousness of the different predator taxa for prey species, we opted to express predation risk simply in terms of unweighted abundance of predators across habitats, like some previous studies (e.g. Babbitt et al. 2003).

Total counts for tadpoles and predacious invertebrates captured in each pond were divided by the number of dipnet sweeps taken in each pond, following the procedures described in previous studies (Babbitt et al. 2003). This yielded abundance on the basis of catch per unit effort, which could be compared across localities. Abundances were log transformed prior to statistical analyses.

Experimental response to drying

During the spring of 2001 and spring 2002 we conducted laboratory experiments with the six species to estimate their capacity to escape from drying environments. All species were submitted to the same treatments under similar conditions. The experiment design included two treatments: a constant water level treatment (simulating a permanent pond), and a drying treatment simulating a temporary pond. In the drying treatment the water level decreased following the curve defined by Wilbur (1987), with a time-horizon of 110 days. Each treatment was replicated 20 times, except the *P. punctatus* treatments, which were replicated 38 times. Replicates consisted of plastic tubs with 2 liters of dechlorinated tap water and 3 tadpoles, which were fed *ad libitum* with a mixture (4:1) of rabbit and fish food. For the present study we used only the data on survival to metamorphosis, which were expressed as the proportion of larvae per tub that completed development. All individuals that did not reach metamorphosis before day 110 from the beginning of the experiment were considered dead. Survival to metamorphosis was standardized by examining changes that occurred between treatments divided by the mean value of the trait in the constant treatment ($[(\text{drying} - \text{constant})/\text{constant}]$). In this way we obtained a unitless proportional measure of response to drying environment. Large negative values indicate a high mortality in drying environment as compared to the constant treatment and were interpreted as a low capacity of the species to develop successfully in temporary ponds.

Experimental competitive ability

During the same period as the drying experiment we conducted laboratory experiments to measure effects of intra- and interspecific competition between the six species. Competitive ability was determined from pair-wise competition experiments by estimating the effect of one species upon another by comparing the performance of each species when reared in the same (mixed) environment with performance when reared alone (single treatment). An additive experimental design was used: the tadpole density for each species in the mixed and in the corresponding single treatment was the same. Therefore, with this additive design when species (A) has no competitive effect on species (B), the mass of species (B) at

metamorphosis in the mixed environment (A,B) will be the same as in the single treatment. The experimental design consisted of 27 treatments, each replicated 12 times: (1) a low density single species treatment for each species, with 15 tadpoles per experimental unit (6 treatments). (2) A high density single species treatment for each species, with 30 tadpoles per unit (6 treatments), and (3) all possible two-way mixtures of the six species with 15 tadpoles of each species (15 treatments). Experimental units consisted of plastic tanks filled with 30 liters of dechlorinated tap water. Tadpoles were fed twice a week with a mixture of ground rabbit chow and fish food (4:1). All treatments used the same amount of food, which was gradually increased throughout the experiment in accordance with tadpole growth and food requirements.

For the present study we used mass at metamorphosis and survival to metamorphosis as variables to measure response to intra- and interspecific competition. To establish whether competition between species was symmetric or asymmetric, and because species differed in baseline responses in the single treatments, we standardized the absolute changes in responses to facilitate interspecific comparison. The model developed by Morin and Johnson (1988) was applied to standardize species response. We estimated the absolute effect of increased intraspecific density for mass at metamorphosis and the other variables by subtracting the value of the mean of the low-density single treatment from the value for each individual in the high-density single treatment. We calculated relative standardized values by dividing the absolute change by the subtracting value (Morin & Johnson 1988). For interspecific effects we followed the same model. We estimated the absolute influence of the competitor species (B) on (A) for each variable by subtracting the mean of the 12 replicates from (A)

Kruskal-Wallis ANOVA by ranks. N=87				
	2000	2001	2002	2003
<i>Alytes obstetricans</i>	H=2.27; p=0.3251	H=1.36; p=0.5042	H=0.54; p=0.7599	H=2.39; p=0.3020
<i>Pelodytes punctatus</i>	H=9.11; p=0.0105	H=5.61; p=0.0604	H=10.48; p=0.0053	H=8.24; p=0.0162
<i>Bufo bufo</i>	H=3.79; p=0.1503	H=7.01; p=0.0300	H=6.74; p=0.0343	H=5.23; p=0.0728
<i>Bufo calamita</i>	H=2.10; p=0.3498	H=11.41; p=0.0033	H=8.75; p=0.0126	H=9.80; p=0.0074
<i>Hyla meridionalis</i>	H=2.47; p=0.2895	H=2.13; p=0.3431	H=0.84; p=0.6538	H=3.85; p=0.1456
<i>Rana perezi</i>	H=10.48; p=0.0053	H=8.64; p=0.0133	H=6.93; p=0.0313	H=6.97; p=0.0306

Table 2.- Results for the Kruskal-Wallis ANOVA on the use by species of the three kinds of habitats (ephemeral, temporary and permanent ponds) during the four years of study (2000-2003).

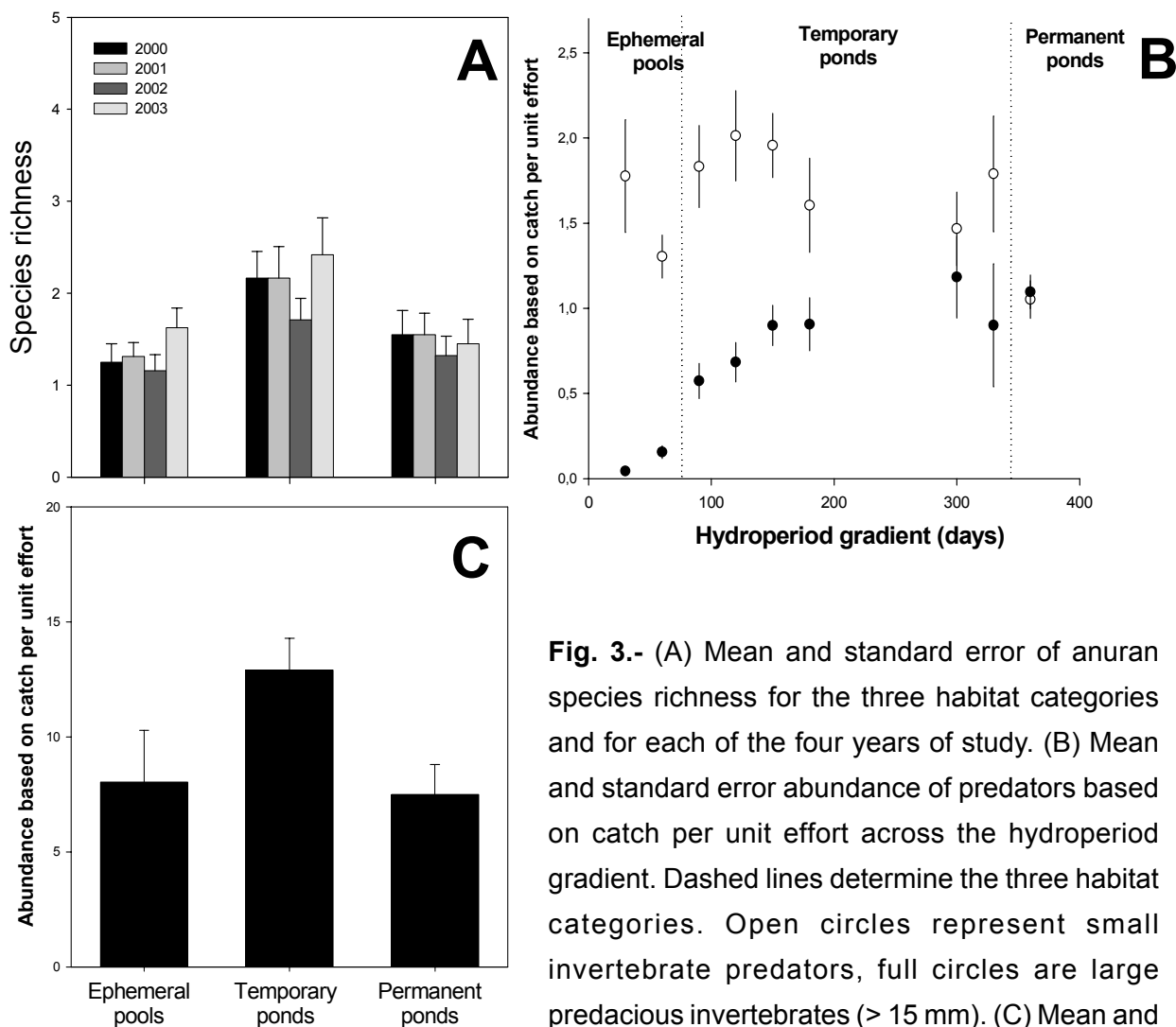


Fig. 3.- (A) Mean and standard error of anuran species richness for the three habitat categories and for each of the four years of study. (B) Mean and standard error abundance of predators based on catch per unit effort across the hydroperiod gradient. Dashed lines determine the three habitat categories. Open circles represent small invertebrate predators, full circles are large predacious invertebrates (> 15 mm). (C) Mean and standard error abundance of tadpoles of all species across the hydroperiod gradient. Figures B and C correspond to field abundance surveys of 2002.

high density from the values for each (A) individual in the interspecific treatment (A, B). We also calculated relative standardized values by dividing the absolute change by the mean subtracting value. For the actual analyses we used the mean overall response observed in a species to others, excluding intraspecific effects, as a measure of interspecific effects. In this way a negative value in mass at metamorphosis or survival to metamorphosis indicated that on average the other species decreased the performance of the target species. Species with positive or low negative values were considered good competitors, whereas species with high negative values were poor competitors.

Experimental activity traits and behavioral response to predators

Percentage of active individuals was recorded for each species under two different treatments: with no predator present and in the nonlethal presence of a *Aeschna* sp. larva. Each treatment was replicated several times for each species (6 for *A. obstetricans*, 10 for *P. punctatus*, 10 for *H. meridionalis*, 6 for *B. bufo*, 7 for *B. calamita* and 6 for *R. perezii*). Each replica consisted of a container (30 liters) filled with dechlorinated water and 30 tadpoles, which were fed rabbit pellets *ad libitum*. Two transparent cylindrical cages were placed in either side of each tank with one dragonfly larva in each in the predator-presence treatment. These cages prevented predators from capturing tadpoles but allowed chemical signals to flow. Each predator was fed one tadpole (raised in separate containers to be used as food for predators) per day. We sampled activity behaviour by counting the number of tadpoles moving in each tub the instant the tub was first viewed. We made a minimum of 30 measurements for each replica. For the present study we used activity level in absence of predator as an indicator of the activity level of the species. We established the behavioural plasticity in reaction to predation by examining changes in activity between treatments ($[\text{presence of predator} - \text{absence of predator}] / \text{absence of predator}$). Negative values indicate a reduction of activity in presence of predator. Thus species with low negative values were interpreted as species with a poor response to the presence of the predator, and therefore more susceptible to predation either because they are more easily detected by predators or because of their higher encounter rates with predators (Woodward 1983).

Statistical analyses

We tested whether species differed in the frequency of use of the three pond categories (ephemeral, temporary and permanent ponds) with a non-parametric Kruskal-Wallis ANOVA by ranks for each year of study. To test for the co-occurrence of species, we used a null model analysis of the presence-absence matrix, using data from each of the four years. We applied Stone and Roberts' (1990) checkerboard score (*C*-score). This index also measures the degree to which species co-occur but is not as stringent as others because it does not require perfect segregation among species (Gotelli 2000). For a community structured by species interactions, the *C*-score should be significantly larger than what might be expected if it were

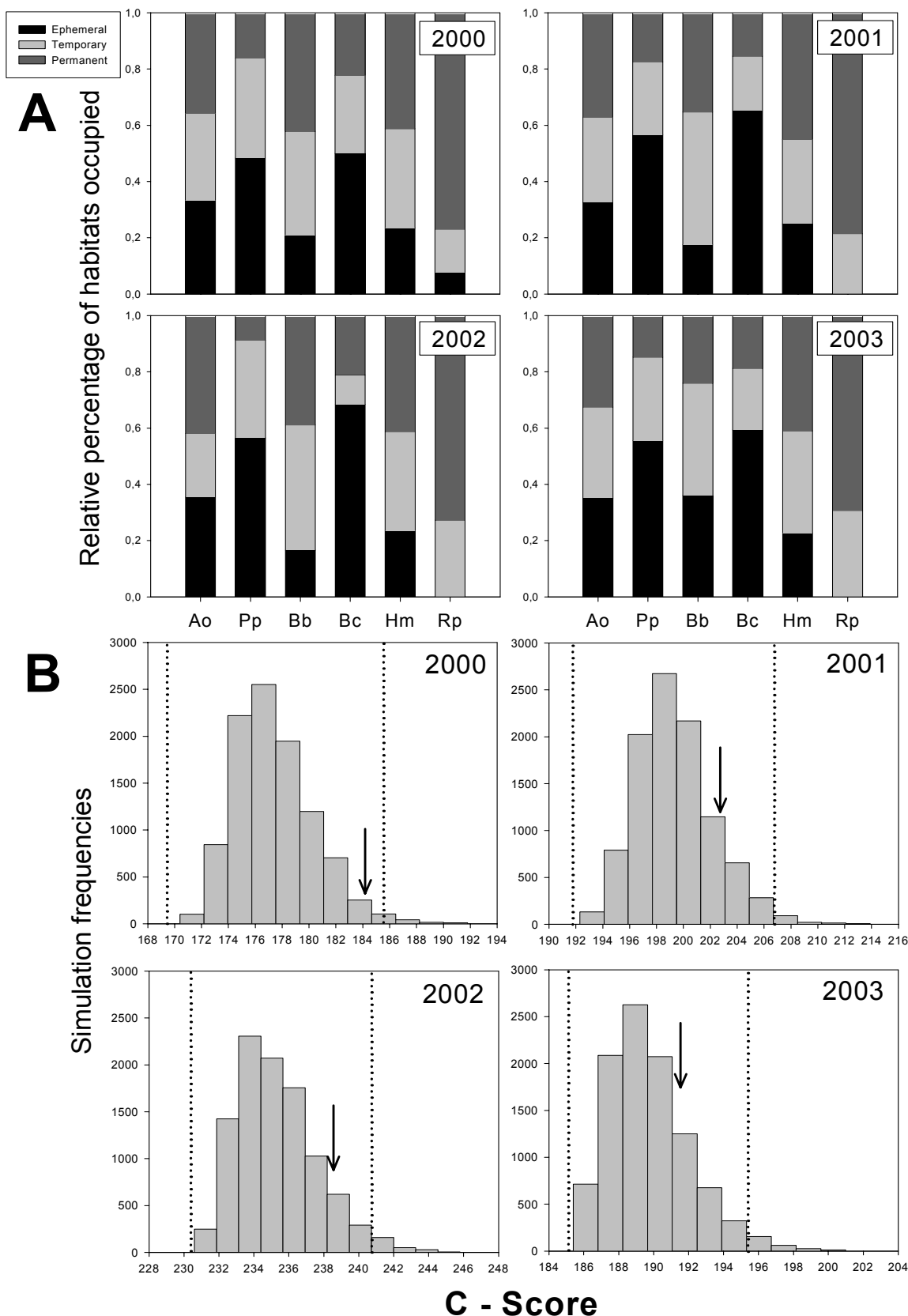


Fig. 4.- (A) Relative percentage of the three habitats occupied by the six species each year. (B) Representation of the C-score of co-occurrence analyses. Arrow indicates the value of co-occurrence observed; dashed lines showed variance of simulated indices after 10,000 bootstrap matrix simulations. Ao=*A. obstetricans*, Pp=*P. punctatus*, Bb=*B. bufo*, Bc=*B. calamita*, Hm=*H. meridionalis*, Rp=*R. perezii*.

Year	Observed index	Mean of simulated indices	Variance of simulated indices	P(obs≤exp)	P(obs>=exp)
2000	184.1	177.39	8.45	0.9775	0.0234
2001	202.8	199.41	8.07	0.8766	0.1287
2002	238.3	235.34	5.23	0.8912	0.1158
2003	191.1	189.77	5.35	0.7609	0.2500

Table 3.- Values of the C-score observed for the presence-absence matrix for the different years, and the mean and variance of the simulated indices after 10,000 null matrices. The significance of the observed matrix was calculated as the frequency of simulated matrices that had indices that were equal to, or greater than, the index of the matrix observed (one-tailed test).

determined by chance. We used a Monte Carlo “null model” simulation to randomize each matrix in the data set. Gotelli’s procedure with fixed sum row and column constraints, and the sequential swap algorithm for randomization was followed. We constructed 10,000 null matrices using EcoSim version 7.6 simulation software (Gotelli & Entsminger 2002). The software calculated the C-score for each null matrix, and the significance of the observed matrix was calculated as the frequency of simulated matrices that had indices that were equal to, or greater than, the index of the matrix observed (one-tailed test).

Prior to test correlation among species traits we tested if these traits could be considered statistically independent without any phylogenetic inertia due to the historical non-independence among taxa (Felsenstein 1985). We tested the assumption of phylogenetic independence with a test for serial independence (TFSI) in our reconstructed phylogenetic topology (Appendix 1). We used the program “Phylogenetic Independence” (Reeve & Abouheif 2003), which calculated the observed C-statistics for each trait and randomized the mean by randomly rotating the nodes within our reconstructed phylogenetic topology 2000 times. None of the traits showed phylogenetic autocorrelation; however, we must be cautious with these results because we were working with a small phylogeny and there could be a lack of statistical power. As traits are not significantly correlated to phylogeny, we used traditional (ahistorical) statistical analyses (Abouheif 1999).

To test the existence of trade-offs between traits we calculated correlations among species’ trait means from different studies (Table 1). First we estimated the complete correlation matrix for all the traits in the six species. Significance tests were corrected for multiple testing

using the sequential Dunn-Šidák method. We also tested the three conceptual models with a correlation hypothesis test. We evaluated the equality of two correlations among the three models: (1) colonization-competition trade-off (probability of colonization-competition ability), (2) pond permanence-competition trade-off (survival to drying-competition ability correlation), and (3) pond permanence-predation risk trade-off (survival to drying-activity plasticity in presence of predator correlation).

RESULTS

Amphibian species richness varies for habitats but not for years (Two-way ANOVA: Habitat ($F_{2, 336} = 10.36$; $p < 0.001$), Year ($F_{3, 336} = 1.58$; $p = 0.193$), Habitat x Year ($F_{6, 336} = 0.35$; $p = 0.912$)). Temporary pond habitats showed higher values compared to ephemeral and permanent ponds (Fig. 3A), and the highest values for tadpole abundance were observed in temporary ponds ($F_{2, 83} = 3.23$; $p = 0.04$) (Fig. 3C). The abundance of large predators did not show this hump-shaped pattern and increased along the permanence gradient (ANOVA $F_{2, 64} = 12.89$; $p < 0.001$), with higher predation risk in permanent ponds and low predation risk in ephemeral and short-term temporary ponds, but without statistical differences between temporary and permanent ponds (Bonferroni post-hoc $p = 0.15$) (Fig. 3B).

The Kruskal-Wallis ANOVA test showed that the frequency of use of the three categories of ponds varies for the six species of amphibians (Table 2). Throughout the four years *R. perezii* showed a clear preference for the permanent end of the gradient with high predation risk. *B. bufo* also used this habitat, but their preference was not so clear as in the case of *R. perezii*. In the other extreme of the gradient we found *B. calamita*, and *P. punctatus*, which showed preference for ephemeral and temporary ponds with low predation risk during three of the four years (Fig. 4A). *A. obstetricans* and *H. meridionalis* were distributed along the entire gradient, principally in temporary and permanent ponds; however, they did not show a significantly different use of the habitats during the period of study. These results are in concordance with abundance distributions obtained from the 2002 sampling period (Appendix 2). The variation detected in the frequency of use of the different categories over years and species was in concordance with the results obtained from the co-occurrence analyses. The C-score observed did not differ from values of simulated matrices for three of the four years

	Colonization	Extinction	Intrasp. (mass)	Intrasp. survival	Intersp. (mass)	Intersp. survival	Drying survival	Predator (growth rate)	Predator (activity)	Activity
Colonization	**** R=.958 p=.003		R=.207 p=.693	R=.701 p=.120	R=-.901 p=.014	R=-.714 p=.111	R=.786 p=.064	R=-.090 p=.865	R=.714 p=.111	R=.715 p=.110
Extinction		****	R=.276 p=.596	R=.761 p=.078	R=-.869 p=.024	R=-.711 p=.113	R=.832 p=.040	R=-.047 p=.929	R=.721 p=.106	R=.555 p=.253
Intrasp. (mass)			****	R=-.573 p=.234	R=-.587 p=.220	R=-.672 p=.144	R=-.210 p=.689	R=.410 p=.419	R=.717 p=.109	R=.277 p=.593
Intrasp. (survival)				****	R=-.768 p=.074	R=-.947 p=.004	R=.653 p=.159	R=.454 p=.365	R=.901 p=.014	R=.643 p=.168
Intersp. (mass)					****	R=.863 p=.027	R=-.754 p=.083	R=-.004 p=.993	R=-.897 p=.015	R=-.759 p=.080
Intersp. (survival)						****	R=-.669 p=.146	R=-.338 p=.511	R=-.983*** p<.001	R=-.801 p=.055
Drying survival							****	R=-.371 p=.469	R=.734 p=.096	R=.601 p=.207
Predator (growth rate)								****	R=.202 p=.701	R=.018 p=.974
Predator (activity)									****	R=.782 p=.066
Activity										****

Table 4. - Correlation coefficient between species' mean trait values (above the diagonal) and their P value. Bold values indicate those correlation coefficients which are significantly different from zero before the Šidák correction. Bordered cells preserve correlation coefficients significantly different from zero after Šidák correction. In all cases $n = 6$.

(Table 3), indicating that species were not well segregated. Only in the first year of study did the test detect a segregation pattern among the six species with a larger C -score than would be expected to be produced by chance (Table 3, Fig. 4B).

In the test of correlations between species trait values, 24 of 45 possible correlations had $R > 0.7$ (Table 4). After applying a Dunn-Šidák correction method, only the correlation between activity in presence of predator and survival in interspecific competition was statistically significant. Other strong but non-significant correlations included negative correlations between colonization and response to interspecific competition (mass at metamorphosis and survival). These correlations suggested the existence of a trade-off between colonization-competition ability. Strong correlations were also found between response to drying and interspecific competition (trade-off: pond permanence-competition ability), and between response to drying and activity in presence of predator (trade-off: pond permanence-predation risk) (Fig. 5).

The test of equality of correlation coefficients between the colonization-competition trade-off (colonization rate and survival in competition) and between pond permanence-competition trade-off (survival in drying and survival in competition) showed that the differences between both correlations were not statistically significant ($z = -0.106$; $p = 0.916$). Also the colonization-competition trade-off and permanence-predator trade-off ($z = 0.053$; $p = 0.958$), and permanence-competition and permanence-predator trade-offs ($z = 0.159$; $p = 0.874$) did not differ. These results do not favour any single trade-off model over the others.

DISCUSSION

Our field presence-absence data at different freshwater habitats show a turnover in anuran larvae species along the hydroperiod habitat gradient, as documented in previous studies (Woodward 1983; Wellborn et al. 1996; Skelly et al. 1999; Snodgrass et al. 2000a; Babbitt et al. 2003; Van Buskirk 2003, 2005). We observed the same unimodal pattern of species richness with a peak in habitats of intermediate hydroperiods as in these previous works. However, in the section of the hydroperiod studied, the transition towards communities

dominated by large invertebrate predators occurs between ephemeral pools and temporary ponds and not between temporary and permanent ponds as suggested in Wellborn et al. (1996). This observation is in agreement with previous studies which show that large invertebrate predators can be abundant and important top predators in temporary ponds (Skelly 1996; Wilbur 1997; Stoks & McPeck 2003). Abundance of invertebrate predators increases from short temporary ponds (of two-three months of life) to large temporary ponds, which only dry for one or two months during summer. Abundance of predators between large temporary ponds and permanent ponds seems to be the same.

Anuran species do not show a perfect distribution among habitats, and many of the species use two or three of the habitats considered. However, species show preferences for some proportion of the gradient and are distributed nonrandomly among ponds categorized by their tendency to dry and associated biotic characteristics (predation and competition). The pattern of distribution observed agrees with previous knowledge of species (e.g. Duguet & Melki 2003; García-París 2004). Under these circumstances, with preferences for certain types of habitats, it is to be expected that divergent selection pressures may ecologically canalize the anuran community by selecting for the tadpole characteristics that are successful in one habitat type but unsuccessful in other habitats (Richardson 2001), originating trade-offs between tadpole traits.

Results of correlations among species traits reveal patterns in trait evolution and ecological functional relationship between traits within species, which correspond to theories of species distribution determined by trade-offs between habitats (Tilman 1982; Scheiner & Willig 2005). In general, species from one kind of habitat show a set of traits which help them to successfully exploit different habitats. Trait correlations among species provide the appropriate measurements with which to determine whether selection has favoured certain combinations of trait values (Price 1997; Richardson 2001).

In general terms trait combinations predicted from models were observed. Species from ephemeral and short temporary ponds such as *B. calamita* and *P. punctatus* presented the traits expected, listed in Figure 2, while species from more stable habitats (large temporary and permanent ponds), like *R. perezi*, *H. meridionalis* and *A. obstetricans*, show just the opposite set of traits. These combinations of traits are in accord with the three principal

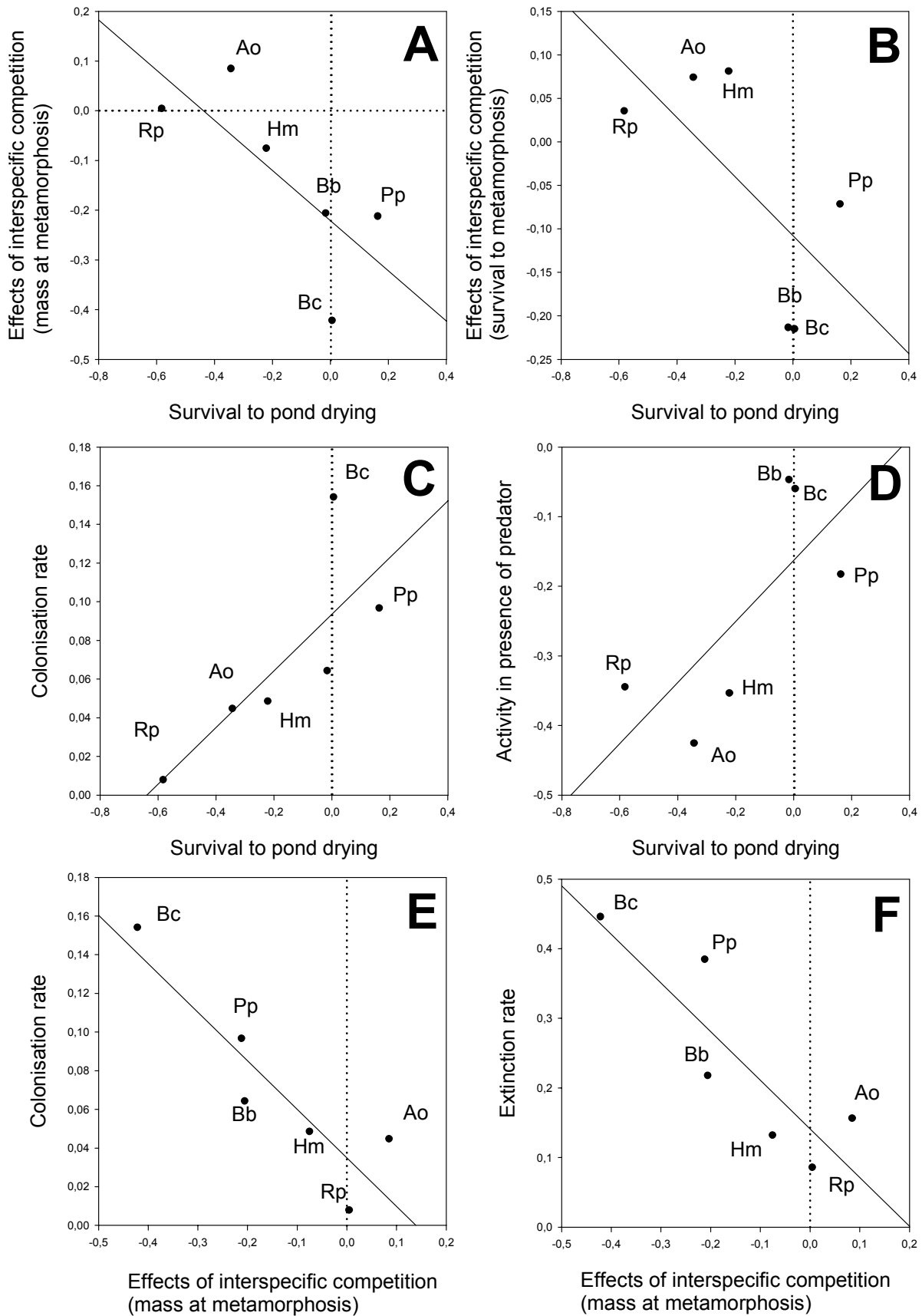


Fig. 5.- Some of the 45 correlations performed, representing the three trade-off models: permanence-competition trade-off (A-B), permanence-predation risk trade-off (D) and colonization-competition trade-off (E-F). Ao=*A. obstetricans*, Pp=*P. punctatus*, Bb=*B. bufo*, Bc=*B. calamita*, Hm=*H. meridionalis*, Rp=*R. perezii*.

trade-off models proposed (Wiggins et al. 1980; Smith 1983; Hecnar & M'Closkey 1996; Wellborn et al. 1996; Marsh & Trenham 2001) to explain the structure of amphibian communities. Their respective trade-offs are supported by our analyses.

The negative relationship between colonization rate and competition abilities is connected to ideas about fugitive species (Leibold & Miller 2004), and also with the concept of pioneer species. This concept suggests a positive correlation between extinction and colonization rates, which we observed. Indeed, colonization potential is scaled by the rate of patch dynamics, and reductions in occupancy are due to the cumulative effects of extinction and loss of habitat suitability. Some theoretical models suggest that sensitivity to colonization and dispersal ability increases as the frequency of habitat destruction increases (Johst et al. 2002; DeWoody et al. 2005). Ephemeral and small temporary ponds are habitats with a higher rate of destruction within and between years, and as a consequence species related to these habitats (*B. calamita* and *P. punctatus*) have higher extinction and colonization rates compared to species from more stable habitats. The negative relationship between survival to metamorphosis in drying ponds and competition effects support the trade-off proposed by Wiggins et al. (1980) and Smith (1983) but contrast with the widespread idea (based on empirical studies) that species from temporary ponds are superior competitors due to their higher activity levels which are the result of their need to grow quickly and escape from drying habitats (Woodward 1982; Wilbur 1987; Werner 1992; Dayton & Fitzgerald 2001, but see Griffiths et al. 1991, and Laurila 2000 for results similar to ours). Finally, the relationship observed between susceptibility to predation and survival in drying habitats confirms the existence of a trade-off between both traits as proposed by Wellborn et al. (1996) and supported by numerous observations and empirical studies (e.g. Woodward 1983; Werner & McPeck 1994; Van Buskirk 2005). The test of equality between correlations for the three models did not favour any of the models. These results suggest that this community can be interpreted as a metacommunity in which local interactions and regional process are important and related (Chesson 2000; Leibold & Miller 2004, Leibold et al. 2004).

The results show that at local scale (ponds) population control of the tadpoles occurs through an interaction of pond persistence, predation, and competition (Fig. 1B and 2 representing the direction of the three factors). Species from short temporary ponds (*P.*

punctatus and *B. calamita*) are eliminated from permanent ponds by abundance of predation, and from intermediate temporary ponds by density effects, because it is in these intermediate ponds where all species breed and interspecific competition is expected to be higher. In this way *P. punctatus* and *B. calamita* are “forced” to act as fugitive species and use risky habitats such as ephemeral pools free of predators and competitors, where mortality of all tadpoles is common (*personal observation*).

Changes in species co-occurrence over a period of years indicate that amphibian distribution and communities can be highly dynamic, so that local extinctions may be attributed to local process and species traits and thus may be deterministic rather than stochastic (Skelly et al. 1999). This perception is closer to a source-sink dynamic and the mass-effect framework (Mouquet & Loreau 2002) than to other metacommunity models, in which species fitness varies among habitats according to their traits, but dispersal rates exceed the rate at which habitat conditions exclude taxa. For this reason species showed an imperfect segregation and high levels of co-occurrence. The spatial structure inherent to many freshwater habitats, and the life histories of many taxa that inhabit them, suggest many organisms with complex-life cycles exist as metapopulations, with a source-sink dynamic (Hecnar & M'Closkey 1996; Marsh & Trenham 2001; Caudill 2003, 2005). Many amphibian species can occur in different habitats (source or sink) across the gradients with relative success by adjusted their larval phenotype to the local environment. Phenotypic plasticity to pond drying, predator presence and competition is well documented (e.g. Newman 1992; Relyea 2002; Van Buskirk 2002; Morey & Reznick 2004; Relyea & Auld 2005) and enables species to increase habitat breadth. Phenotypic plasticity might have important ecological consequences affecting the stability of populations and also species coexistence within communities (Miner et al. 2005). At the moment consequences of plasticity for whole-community and dynamics remain largely unknown.

This study indicates the importance of habitat heterogeneity for maintaining amphibian community diversity and supports idea from a regional approach to wetlands regulation, focusing in part on conserving a diversity of ponds that represent the entire hydroperiod gradient (Snodgrass et al. 2000b). Empirical studies demonstrated that pond characteristics differed from year to year (Semlitsch et al. 1996; Van Buskirk 2005) and consequently a

source habitat in one year may become a sink habitat in the next year, or vice versa. Spatial differences along the hydroperiod gradient at a regional level and local temporal uncertainty of resource availability offer many opportunities for such differentiation in species' responses and their coexistence. This shifting mosaic of different pond habitats over space and time, along with species phenotypic plasticity, suggest that amphibians and other freshwater organisms may be resilient to local or spatially synchronous disturbances as long as inter-pond connectivity and habitat heterogeneity are maintained (Urban 2004). However, the loss of a specific habitat type, as for example ephemeral or temporary ponds in many Mediterranean areas (Gallego-Fernández et al. 1999), or landscape fragmentation could lead to a significant impoverishment of communities at local and regional scale.

REFERENCES

- Abouheif, E. 1999. A method for testing the assumption of phylogenetic independence in comparative data. *Evolutionary Ecology Research* 1: 895-909.
- Anholt, B.R. and Werner, E.E. 1995. Interactions between food availability and predator mortality mediated by adaptive behavior. *Ecology* 76: 2230-2334.
- 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.
- Caudill, C.C. 2003. Empirical evidence for nonselective recruitment and a source-sink dynamic in a mayfly metapopulation. *Ecology* 84: 2119-2132.
- Caudillo, C.C. 2005. Trout predators and demographic sources and sinks in a mayfly metapopulation. *Ecology* 86: 935-946.
- Chesson, P.L. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematic* 31: 343-366.
- Dayton, G.H. and Fitzgerald, L.A. 2001. Competition, predation, and the distribution of four desert anurans. *Oecologia* 129: 430-435.
- DeWoody, Y.D., Feng, Z. and Swihart, R.K. 2005. Merging spatial and temporal structure within a metapopulation model. *American Naturalist* 166: 42-55.
- Duguet, R. and Melki, F. 2003. Les amphibiens de France, Belgique et Luxembourg. Collection Parthénope, éditions Biotope, Mèze (France). 480 pp.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1-15.
- Gallego-Fernández, J., García-Mora, M.R. and Gracia-Novo, F. 1999. Small wetlands lost: a biological conservation hazard in Mediterranean landscapes. *Environmental Conservation* 26: 190-199.
- García-París, M. 2004. Anura. In: *Amphibia, Lissamphibia*. García-París, M., Montori, A. and Herrero, P. Fauna Ibérica, vol. 24. (Ramos, M.A. et al. eds.). Museo Nacional de Ciencias Naturales. CSIC. Madrid: 275-480.
- Gotelli, N.J. 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81: 2606-2621.
- Gotelli, N.J. and Taylor, C.M. 1999. Testing metapopulation models with stream-fish

- assemblages. *Evolutionary Ecology Research* 1: 835-845.
- Gotelli, N.J. and Entsminger, G.L. 2002. EcoSim: Null models software for ecology. Version 7.0. Acquired Intelligence Inc. and Kesey-Bear. <http://homepages.together.net/~gentsmin/ecosim.htm>.
- Grace, J.B. 1999. The factors controlling species density in herbaceous plant communities: an assessment. *Perspectives in Plant Ecology Evolution and Systematics* 2: 1-28.
- Griffen, B.D. and Byers, J.E. 2005. Partitioning mechanisms of predator interference in different habitats. On-line. 000-000.
- Griffiths, R.A., Edgar, P.W. and Wong, A.L.C. 1991. Interspecific competition in tadpoles: growth inhibition and growth retrieval in natterjack toads, *Bufo calamita*. *Journal of Animal Ecology* 60: 1065-1076.
- Hecnar, S.J. and M'Closkey, R.T. 1996. Regional dynamics and the status of amphibians. *Ecology* 77: 2091-2097.
- Johst, K., Brandl, R. and Eber, S. 2002. Metapopulation persistence in dynamic landscape: the role of dispersal distance. *Oikos* 98: 263-270.
- Laurila, A. 2000. Competitive ability and the coexistence of anuran larvae in freshwater rock-pools. *Freshwater Biology* 43: 161-174.
- Leibold, M.A. and Miller, T.E. 2004. From metapopulations to metacommunities. In: *Ecology, genetics, and evolution of metapopulations*. (eds Hanski, I. and Gaggiotti, O.E.) Elsevier Academic Press, UK.
- Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M. and Gonzalez, A. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7: 601-613.
- Marsh, D.M. and Trenham, P.C. 2001. Metapopulation dynamics and amphibian conservation. *Conservation Biology* 15: 40-49.
- Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. and Relyea, R.A. 2005. Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution*: on-line (in press)
- 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.
- Morin, P.J. and Johnson, E.A. 1988. Experimental studies of asymmetric competition among anurans. *Oikos* 53: 398-407.
- Mouquet, N. and Loreau, M. 2002. Coexistence in metacommunities: the regional similarity hypothesis. *American Naturalist* 159: 420-426.
- Mouquet, N. and Loreau, M. 2003. Community patterns in source-sink metacommunities. *American Naturalist* 162: 544-557.
- Newman, R.A. 1992. Adaptive plasticity in amphibian metamorphosis. *Bioscience* 42: 671-678.
- Price, T. 1997. Correlated evolution and independent contrast. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* 352: 519-529.
- 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 Auld, J. 2005. Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotype trade-offs. *Ecology* 86: 1723-1729.
- Reeve, J. and Abouheif, E. 2003. *Phylogenetic Independence*. Version 2.0, Department of Biology, McGill University. Distributed free by the authors on request.
- Richardson, J.M.L. 2001. The relative roles of adaptation and phylogeny in determination of larval traits in diversifying anuran lineages. *American Naturalist* 157: 282-299.
- Ricklefs, R.E. and Schluter, D. 1993. *Species diversity in ecological communities: historical and geographical perspectives*. University of Chicago Press, Chicago.
- Scheiner, S.M. and Willig, M.R. 2005. Developing unified theories in ecology as exemplified with diversity gradients. *American Naturalist* 166: 000-000 (on-line)
- Semlitsch, R.D., Scott, D.E., Pechmann, J.H.K and Gibbons, J.W. (1996) Structure and dynamics of amphibian community. Evidence from a 16-

- year study of a natural pond. In: Long-term studies of vertebrate communities (Cody, M.L. and Smallwood, J.A. eds.) Academic Press Inc. San Diego.
- Sih, A., Englund, G. and Wooster, D. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution* 13: 350-355.
- Skelly, D.K. 1992. Field evidence for a behavioral antipredator response in a larval amphibian. *Ecology* 73: 704-708.
- Skelly, D.K. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. *Ecology* 76: 150-164.
- Skelly, D.K. 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. *Copeia* 1996: 599-605.
- Skelly, D.K., Werner, E.E. and Cortwright, S.A. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology* 80: 2326-2337.
- Smith, D.C. 1983. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. *Ecology* 64: 501-510.
- Snodgrass, J.W., Bryan, A.L and Burger, J. 2000a. Development of expectations of larval amphibian assemblage structure in southeastern depression wetlands. *Ecological Applications* 10: 1219-1229.
- Snodgrass, J.W., Komoroski, M.J., Bryan, A.L. and Burger, J. 2000b. Relationships among isolated wetland size, hydroperiod, and amphibian species richness: implications for wetland regulations. *Conservation Biology* 14: 414-419.
- Stocks, R. and McPeck, M.A. 2003. Predators and life histories shape *Lestes* damselfly assemblages along a freshwater habitat gradient. *Ecology* 84: 1576-1587.
- Stone, L. and Roberts, A. 1990. The checkerboard score and species distribution. *Oecologia* 85: 74-79.
- Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, NJ.
- Tilman, D. and Pacala, S. 1993. The maintenance of species richness in plant communities. In: Species diversity in ecological communities: historical and geographical perspectives. (eds Ricklefs, R.E. and Schluter, D.) University of Chicago Press, Chicago.
- Urban, M.C. 2005. Disturbance heterogeneity determines freshwater metacommunity structure. *Ecology* 85: 2971-2978.
- 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. 2003. Habitat partitioning in European and North American pond-breeding frogs and toads. *Diversity and Distributions* 9: 399-410.
- Van Buskirk, J. 2005. Local and landscape influence on amphibian occurrence and abundance. *Ecology* 86: 1936-1947.
- Van Buskirk, J. and Arioli, M. 2005. Habitat specialization and adaptive phenotypic divergence of anuran populations. *Journal of Evolutionary Biology* 18: 596-608.
- Vance-Chalcraft, H.D. and Soluk, D.A. 2005. Multiple predator effects result in risk reduction for prey across multiple prey densities. On-line. 000-000.
- Wellborn, G.A., Skelly, D.K. and Werner, E.E. 1996. Mechanism creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*. 27: 337-363.
- Werner, E.E. 1992. Competitive interactions between wood frog and northern leopard frog larvae: the influence of size and activity. *Copeia* 1992: 26-35.
- Werner, E.E. and Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* 142: 242-272.
- Werner, E.E. and McPeck, M.A. 1994. Direct and indirect effects of predators on two anuran species along an environmental gradient. *Ecology* 75: 1368-1382.
- Wiggins, G.B., Mackay, R.J. and Smith, I.M. 1980. Evolutionary and ecological strategies of animals in annual temporary ponds. *Archiv für Hydrobiologie, Supplement* 58: 97-206.
- Wilbur, H.M. 1987. Regulation of structure in complex systems: experimental temporary pond communities. *Ecology* 68: 1437-1452.

Wilbur, H.M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. *Ecology* 78: 2279-2302.

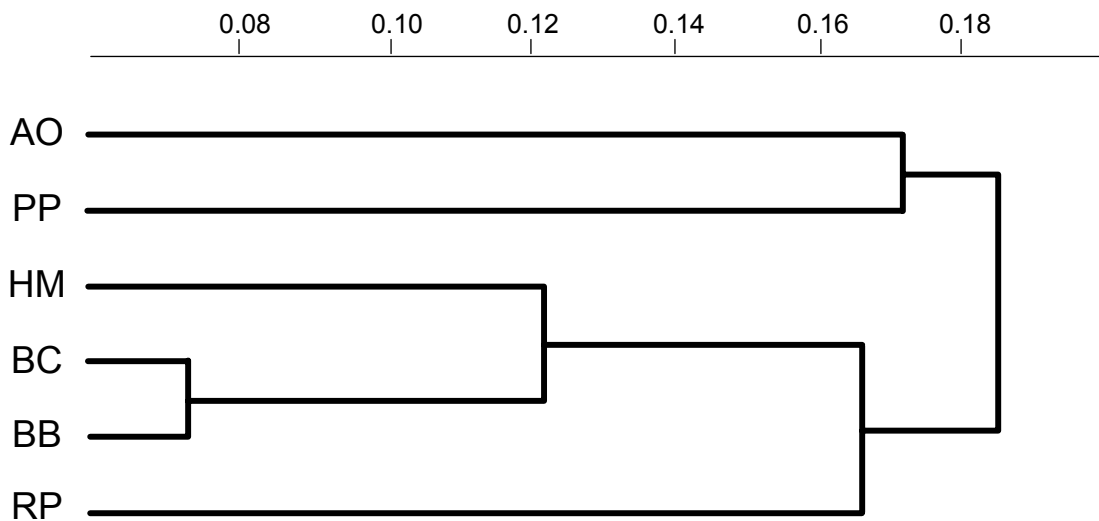
Willig, M.R., Kaufman, D.M. and Stevens, R.D. 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics* 34: 273-309.

Woodward, B.D. 1982. Tadpole competition in a desert anuran community. - *Oecologia* 54: 96-100.

Woodward, B.D. 1983. Predator-prey interactions and breeding-pond use of temporary-pond species in a desert anuran community. *Ecology* 64: 1549-1555.

Appendix 1

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 a Sequence Navigator. Observed distances in pair-wise comparisons were obtained using the PAUP software. We tested phylogenetic independence of species 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 2000 times to build a null hypothesis.



Appendix 2

Representation of abundances based on catch per unit effort for each species across the hydroperiod gradient. Values show mean and standard error of abundances.

