5

Responses to competition effects of two anuran tadpoles according to life-history traits

Experimental manipulations of the densities of two larval anurans, *Pelodytes punctatus* and *Bufo bufo*, showed that these species compete asymmetrically in semi-natural conditions. Growth, mass at metamorphosis, date of metamorphosis, and survival were used as measures of response to interspecific competition. A mechanistic approach was used to collect information on the behaviour of the two species in different conditions. The competitive superiority of *Pelodytes* at individual level was correlated with a larger body, faster growth rate, increased per capita competitive impact on conspecifics, and greater reduction in the availability of trophic and spatial resources. In the presence of *Pelodytes*, *Bufo* showed slower growth, smaller size at metamorphosis and reduced survival. In the interspecific treatments *Bufo* individuals modified their behaviour by increasing activity and use of the water column while *Pelodytes* did not change their foraging activity or space use in the aquaria. However, the presence of *Bufo* resulted in a reduced larval period and smaller size at metamorphosis. We hypothesise that the presence of *Bufo* act as a signal of environmental degradation and shorten the larval period of *Pelodytes*, a typical temporal pond breeder. The smaller *Bufo* tadpoles are potentially stronger competitors at population level because they use relatively large amounts of energy (greater densities and higher metabolic rates). Consequently, they use larger proportions of the shared resources than their larger competitor. A possible evolutionary response for larger tadpoles is the development of interference mechanisms or ''escaping'' from ephemeral ponds where mortality by drying represent a high risk.

INTRODUCTION

A wide spectrum of interactions between species occurs in natural systems. Competition and predation are among the most significant forces that structure communities (Sih et al. 1985, Morin 1999, Gurevitch et al. 2000). The perceived role of competition in community organization is considerable (Connell 1983, Schoener 1983, Goldberg & Barton 1992, Gurevitch et al. 1992), especially asymmetric intra- and interspecific competition (Lawton & Hassell 1981, Persson 1985, Morin & Johnson 1988, Weiner 1990). Asymmetric competition

is of special interest because it does not necessarily lead to the evolution of weak interactions among species, as one might expect through divergent character displacement. Thus, natural selection generated by this kind of competition is likely to be a persistent and continuous phenomenon in communities. There are three kinds of studies on asymmetric competition, distinguished by the temporal scale used (Law et al. 1997). The microscopic scale deals with encounters between individuals that depend on behavioural mechanisms of competition between animals. The mesoscopic scale addresses population dynamics, and often involves the manipulation of densities of pairs of species in a reciprocal manner. Finally, the macroscopic study examines the largest temporal scale of phenotype evolution and attempts to account for phenotypic patterns across species as an outcome of evolution driven by asymmetric competition.

Anuran larvae have been used as experimental models to test the strengths of intra- and interspecific competition and other structural forces in communities, such as predation and environmental stochasticity (Morin 1983, Alford & Wilbur 1985, Wilbur 1987, Griffiths et al. 1991, Pearman 1995, Werner & Anholt 1996, Bardsley & Beebee 1998a, Alford 1999, Gurevitch et al. 2000). Experiments in the laboratory and in artificial ponds frequently show strong asymmetric intra- and interspecific effects of competitor density on the fitness-related parameters of these larvae (Morin & Johnson 1988, Gurevitch et al. 1992, Parris & Semlitsch 1998). The reasons for asymmetric competition are unclear. Traditionally, ecologists distinguished between exploitative competition, which operates indirectly by depleting a shared resource, and interference competition, which involves direct interactions between species. In tadpoles, the mechanism of competition is usually the former (Morin & Johnson 1988, Werner 1994, Alford 1999), although chemical interference competition also occurs (Licht 1967, Steinwascher 1979, Beebee & Wong 1992, Griffiths et al. 1993); however, chemical interference role in natural conditions is controversial (Wong et al. 1994, Beebee 1995, Griffiths 1995, Petranka 1995). Body size and activity are two of the main factors that affect interspecific interactions (Connell 1983, Schoener 1983, Persson 1985, Grill & Juliano 1996).

Most old studies on tadpole communities address species interactions and use simple density relations and measure fitness attributes at the end of experiments, essentially ignore or consider fixed behaviours, but during the last decades there are many studies looking at

competition and explaining it mechanistically (reviewed by Alford 1999, Hoff et al. 1999). Elucidation of competition requires mechanistic studies in which ecological factors shape behaviour and habitat choice, and the consequences of this behaviour on species interactions must be considered (Tilman 1987, Werner 1992). Several anuran species have been classified as temporary or permanent pond breeders depending on permanence of the freshwater habitat selected for breeding (Wilbur & Collins 1973, Denver 1997). Generally, tadpoles in temporary ponds are more active and have higher growth rates than those in permanent water bodies, thus they are considered more competitive and have a strong negative effect on permanent pond species (Woodward 1982, Morin 1983, Wilbur 1987). Here we examine the effects of intraespecific competition and different levels of interspecific interactions between two anuran species, *Pelodytes punctatus* (Daudin, 1803, hereafter *Pelodytes*) and *Bufo bufo* (Linnaeus, 1758, hereafter *Bufo*) in a laboratory experiment. The study links two time-scales (microscopic and mesoscopic) to explore competition dynamics, and discusses the possible phenotypic evolution (macroscopic timescale) of the species. To study the mechanisms of competition we focused two main classes of individual behaviours: activity and microhabitat use. We examined the growth and behaviour of the *Pelodytes* and *Bufo* tadpoles in experimental aquaria by manipulating their respective densities.

Pelodytes and *Bufo* are common anurans around the Parc Natural del Garraf (Natural Park of Garraf) near Barcelona (Spain), a typical karstic semi-arid Mediterranean shrubland area. However, they differ markedly in ecological traits. *Bufo* is an 'explosive' breeder and in Garraf reproduction occurs in late February to early March, whereas *Pelodytes* is a opportunistic breeder, with egg deposition throughout winter and spring after strong rainfalls (Toxopeus et al. 1993, Guyétant et al. 1999) and a second breeding period in autumn. *Pelodytes* reproduces in small ephemeral ponds (Sánchez-Herráiz & Barbadillo 1997, Guyétant et al. 1999) whereas *Bufo* is a more generalist species that uses preferentially permanent ponds (Stijbosch 1979, Augert & Guyétant 1995, Laurila 1998, 2000). However in the Parc Natural del Garraf these two species are found together in temporal ponds, the most common type of water body in this region (Montori 1997). Here we test the hypothesis that

Pelodytes, a temporary pond species, negatively affects *Bufo*, a commonly permanent pond species.

MATERIAL AND METHODS

In March 1999, two clutches of *Bufo* were collected from a pond in the Parc Natural del Garraf and added to an outdoor tank. Two *Pelodytes* spawns were collected from the same pond on the same day and hatched in outdoor buckets. The resulting tadpoles of these species were placed in the experimental buckets when they started to swim and feed actively at stage Gosner 24. Experiments were performed in glass aquaria (50x27x25 cm) filled with dechlorinated tap water. They were also inoculated with phytoplankton and zooplankton obtained from one natural pond. Aquaria were given two weeks to establish algae and a healthy zooplankton population before tadpoles in Gosner's stage 24-25, drawn from different spawns in equal proportions, were assigned randomly to each aquarium. All treatments were carried out outdoors under natural illumination and photoperiod, without thermal control. Tadpoles were fed ad libitum with boiled lettuce in relation to number of tadpoles and their body size. The aquaria did not contain macrophytes, thereby simulating the homogeneous breeding habitat of ephemeral ponds found in natural conditions.

Experimental design

Basic density manipulations were performed to measure the three types of competitive asymmetry, as proposed by Underwood (1986). In mixed treatments a replacement series was used (Harper 1977). Using 21 aquaria, a total of seven treatments were performed, each replicated three times. Four of the treatments were designed to identify interspecific differences in response to increases in intraspecific density, with a low (5 tadpoles) and a high (30 tadpoles) monospecific density treatment for each species. The other three treatments had a combined density of the two species equal to the high monospecific treatments (30 tadpoles), while the relative frequency of each varied following for species i the relation (i>j 25>5; to i=j 15=15; and to i<j 5<25). This design yields information about competition within and between populations of *Bufo* and *Pelodytes*. Responses used to measure competition The variables measured were as follows: tadpole body length (mm), mass at metamorphosis

(g), time to metamorphosis (days), and survival to metamorphosis (%). Tadpoles were measured at the start of the experiment and at weekly intervals throughout development. In the low density intraspecific treatment, and i<j interspecific treatment, all tadpoles of species i were measured, while in the others a sample of 12 were collected from each aquarium.

Metamorphosing individuals were collected daily as soon as the tail was fully resorbed, and then weighed to 0.001 g precision. The larval period was measured as the days between stocking and metamorphosing. These variables provided an estimation of the mean growth rate (mass/larval period; g/days).

Behavioural variables

Two types of behaviours were recorded independently: activity and microhabitat choice. We measured behaviours through a focal-animal sampling for two minutes per individual. Activity was recorded with help of EthoLog 2.2 software (Ottoni 1998) which allows measured accurate data on frequencies and duration of behaviour (Martin and Bateson 1991). Individuals from different aquaria and treatments were randomly selected. We made 40 behavioural samples per tank during the first three weeks when tadpoles compressing development between 25-30 Gosner's stages.

In the activity sampling, we measured the frequency and duration of distinct activities: foraging (we distinguished six distinct foraging techniques reported for Iberian tadpoles by Díaz-Paniagua (1987): 1) pumping detritus, 2) rasping bottom, 3) grazing on boiled lettuce, 4) filter midwater, 5) rasping aquaria walls, and 6) pumping from the surface film); time at rest; and time to spent swimming actively (judged by a moving tail). To sample the microhabitat choice, we divided the water column into three sections: bottom, mid, and surface waters. We then registered the time that tadpoles spend in different predefined spatial regions.

Statistical analysis

All statistical tests were conducted on tank means to avoid pseudoreplication. First, we compared treatment from the two species separately to learn how each species responded to different treatments. Measures of mass at metamorphosis, date of metamorphosis, and larval growth were log-transformed to improve normality. As survival was expressed as a

proportion, these data were arcsine-transformed before analysis. Because of the low number of individuals obtained in some treatments, not all the samples complied with homoscedasticity. Therefore, a non-parametric test (Kruskal-Wallis ANOVA) was used to assess the effects of intraspecific density on the two species and to evaluate the differences in datasets with competitors. When this value was significant, differences between individual treatments were compared with a Mann-Whitney U-test, applying a Bonferroni correction for the conservative multiple comparisons test (Rice 1989).

Second, to test the null hypothesis of symmetric response of species to intra- and interspecific treatments, we compared the response for each variable between species. Because *Bufo* and *Pelodytes* differed in baseline responses in the monospecific treatments, we standardised the absolute changes in responses for distinct variables to facilitate interspecific comparison (Morin & Johnson 1988). The model developed by Morin and Johnson (1988) was applied to standardise species response. We estimated the effect of increased
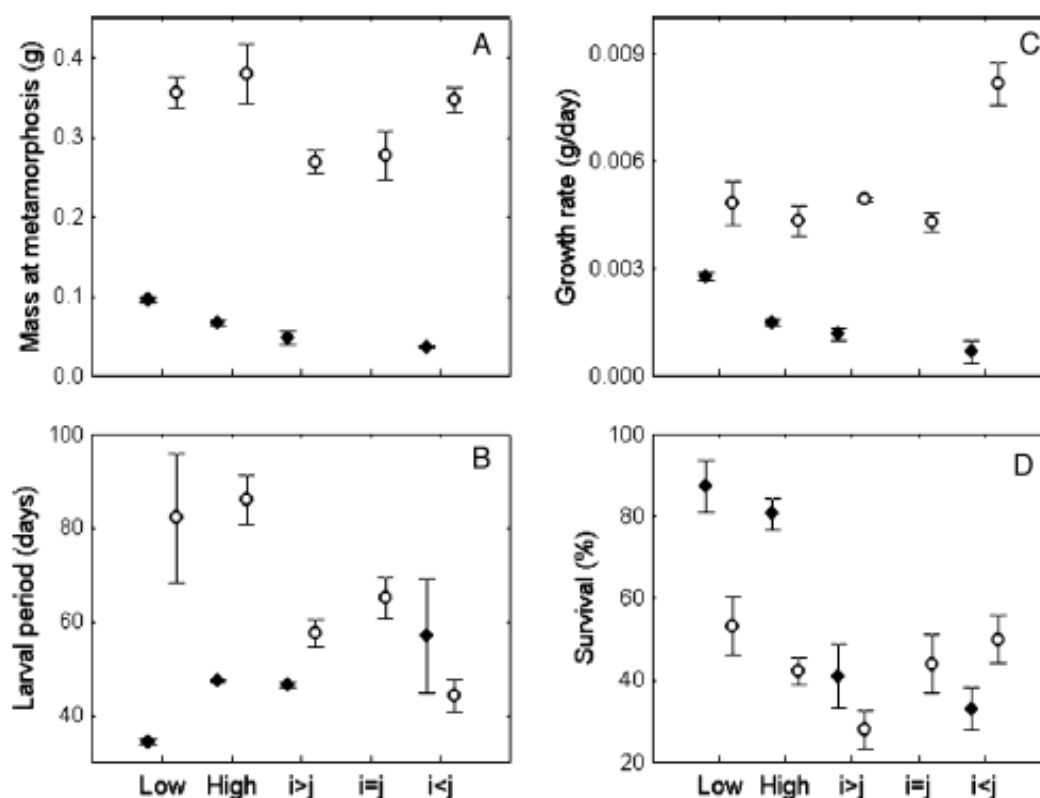

Fig. 1.- Mass at metamorphosis (A), larval period (B), growth rate (C) and survival (D) of *Bufo bufo* (black symbols) and *Pelodytes punctatus* (open circles). Error bars refer to standard error.

intraspecific density for each variable by subtracting the mean of the three low-density replicates from intraspecific treatments from the value for each individual in the highdensity monospecific experiment. We then used a variance analysis (Kolmogorov-Smirnov) for each variable to determine whether the absolute response differed between species. For interspecific effects, we estimated the influence of the competitor species (i) for each species (j) on each variable by subtracting the mean of the three highdensity replicates from monospecific treatments from the value for each individual in the distinct interspecific experiments (i, j). In both treatments, the absolute density of tadpoles remained the same (30 tadpoles), only the proportion of the species changed. This methodology allows the comparison of the effects of increasing the proportion of interspecific competitors. Also, we calculated relative standardised values by dividing the absolute change with the subtracting value (Morin & Johnson 1988). We then compared the species response by applying a Kolmogorov-Smirnov test.

A non-parametric Friedman ANOVA test was used to compare the weekly measurements of tadpole size between treatments of the same species. When differences between treatments were detected we then applied a Wilcoxon's test to compare individual treatments.

Behavioural effects of the treatments were first analysed as a multivariate analysis of variance (MANOVA) for each species, with subsequent separate univariate ANOVAs of each response. As behaviours were expressed as proportions, these data were arcsine transformed before analysis. We structured the analyses into two parts: 1) we tested changes in use of spatial regions (bottom, mid and surface waters), and changes in the three basic activities (foraging, swimming and resting) by compiling all data of different foraging techniques into a single category called foraging. If we detected changes in use of space and activity, especially in foraging activity, we then analysed whether treatments showed changes in foraging techniques because different techniques do change in different space regions. To compare behavioural traits of the two species we followed the same method described previously with standardised behavioural values applying another time (Morin & Johnson 1988) model (above). Standardised values were compared with Kruskal-Wallis ANOVA to absolute and relative values.

RESULTS

Effects on Bufo *tadpoles*

Mass of toadlets differed between treatments (t^2=12; df=3; p=0.0074, Fig. 1A). Mass at metamorphosis was lower in high intraspecific competition treatment than in low competition treatment. These monospecific treatments differed from interspecific treatments (pB/0.05). However, there were no differences among the interspecific treatments. Thus *Bufo* tadpoles raised with a high density of conspecifics and in the presence of *Pelodytes* were smaller at metamorphosis than those raised with low intraspecific densities. The larval period showed no significant differences among the treatments (t^2=4; df=3; p=0.2615, Fig. 1B). Growth rates
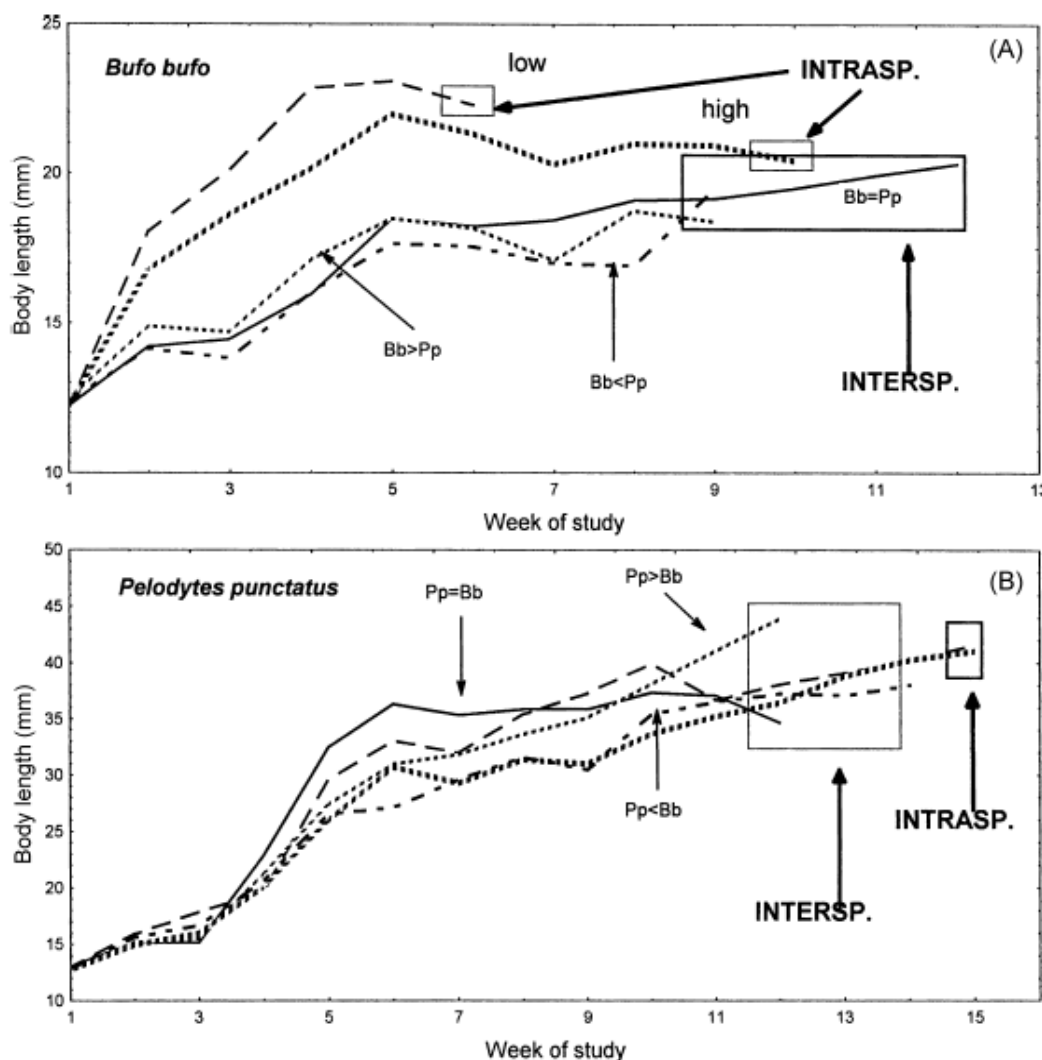

Fig. 2.- Growth curves of (A) *Bufo bufo* and (B) *Pelodytes punctatus* body length in distinct intra- and interspecific treatments to time of metamorphosis. From *Bufo* all treatments were incorporated, including Bb<Pp, because we had growth data of them before all individuals die. Only mean values of the three treatments were represented to facilitate their visualisation.

Intraspecific competition

Variable:	mass (g)-(%)	larval period (d)-(%)	growth rate (g/d) × 100-(%)	survival-(%)
Bufo:	− 0.02805 (− 29%)	12.96 (37.75%)	− 12.9164 (− 46.05%)	− 9 (− 10%)
Pelodytes:	0.02117 (5.9%)	6.62 (8.42%)	− 3.9279 (− 8.18%)	− 20 (− 40%)
K-Sp:	< 0.01 (< 0.01)	N.S.	< 0.05 (< 0.05)	N.S.

Interspecific competition

Bufo (BB > PP)	− 0.0175 (− 26.12%)	− 0.8987 (− 1.9%)	− 4.0955 (− 27.06%)	− 59 (− 72.83%)
Pelodytes (PP > BB)	− 0.1086 (− 29%)	− 28.472 (− 33.4%)	4.5485 (10.32%)	− 6 (− 20%)
K-Sp:	< 0.001 (< 0.01)	< 0.001 (< 0.01)	< 0.01 (< 0.05)	< 0.01 (0.05)
Bufo (BB < PP)	− 0.0306 (− 45.49%)	9.7021 (20.51%)	− 8.1421 (− 53.81%)	− 51 (− 62,96%)
Pelodytes (PP < BB)	− 0.0239 (− 6.4%)	− 42.2222 (− 49.54%)	39.1584 (88.9%)	20 (66,66%)
K-Sp:	< 0.05 (< 0.05)	< 0.05 (< 0.05)	< 0.05 (< 0.05)	< 0.05 (< 0.05)
Bufo (BB = PP)	*	*	*	− 81 (− 100%)
Pelodytes (PP = BB)	− 0.0971 (− 25.96%)	− 18.8888 (− 22.16%)	− 2.1058 (− .78%)	10 (33.33%)

Table 1.- Responses of the two anuran species to the treatments of intra- and inter-specific competition. Mean changes in mass, larval period, growth rate and survival. The brackets show these changes in relative terms, values obtained as a proportion of the low density treatment of the absolute change in intraspecific competition, and as a proportion of high density treatment in interspecific competition. Significance levels indicate results of the Kolmogorov-Smirnov test for absolute and relative (in brackets) changes for the effects of tadpole species responses. On interspecific competition, comparisons between species made between treatments with the same species conditions: response of *Bufo* in the Bb<Pp treatment was compared with the response of *Pelodytes* in the Pp<Bb treatment.

showed the same tendencies and the same significant differences ($t^2=12$; df=3; p=0.0074) between treatments as mass at metamorphosis (Fig. 1C). The growth of *Bufo* was strongly inhibited by the presence of *Pelodytes* (Fig. 2A). The mean size of *Bufo* tadpoles did not differ between treatments at the start of the experiment (Friedman ANOVA $t^2_{(10,4)}=7.7600$; p=0.1008). However, a significant interspecific effect was detected after only one week (Friedman ANOVA $t^2_{(9,4)}=17.1555$; p=0.0018) but no effect of intraspecific density was observed (Wilcoxon test between conspecific treatments: Z=1.1721; p=0.2411). The effect of conspecific density was apparent after three weeks (Friedman ANOVA $t^2_{(10,4)}=31.360$; p<0.0001; Wilcoxon test between conspecific treatments: Z=2.089; p=0.0366). The survival of tadpoles until metamorphosis can be divided into two groups: the monospecific treatments and the interspecific treatments. Low and high density treatments did not differ (post-Hoc Scheffé p=0.1213), and interspecific treatments did not show differences between them (p=0.0959), whereas between high intraspecific and Bb>Pp treatment differences did exist

(p=0.0261). An increase in conspecific density did not affect the mortality of *Bufo*, whereas the presence of *Pelodytes* did. The mortality of *Bufo* rose by 60% or more, reaching 100% in Bb<Pp treatments in the presence of *Pelodytes* (Table 1).

The synchronisation of metamorphosis for *Bufo* was measured as the coefficient of variation (CV) corrected for small samples (Sokal & Rohlf 1995) from the larval period. Treatments with *Pelodytes* showed a high CV, denoting a lower synchronisation than in intraspecific treatments.

Effects on Pelodytes *tadpoles*

Following the same statistic analysis as for *Bufo*, we found that in *Pelodytes* significant differences occurred only in mass at metamorphosis (t^2=9.64; df=4; p=0.0046) and time of larval period (t^2=9.63; df=4; p=0.0048). Similarly to *Bufo*, mass at metamorphosis of transformed *Pelodytes* was reduced by the presence of a competitor (Fig. 1A), while an increase in the density of conspecifics did not significantly alter this parameter. An increase in conspecific density had no effect on the time to metamorphosis (p=0,7911). However, time to metamorphosis of these tadpoles tended to be shorter in the presence of *Bufo* compared with conspecific treatments (Fig. 1B), statistical differences between intra- and inter-specific treatments being observed (p=0.0172). Friedman's test did not detect statistical differences between the growth curves of the treatments (Fig. 2B), and no differences in growth rate were observed between treatments (t^2=6.96; df=4; p=0.1378). Tadpole mortality did not differ between experiments and survival was low in all treatments (around 40-50%), being highest in low conspecific treatments (low monospecific treatment and Bb>Pp treatment). Mortality was constant throughout the larval period, which made it possible to maintain relative densities constant during the experiment duration. The survival of *Pelodytes* was apparently related to conspecific density and no effect of *Bufo* was detected for this variable. The synchronisation of metamorphosis did not show any tendency among treatments.

Differences between species

An increase in the density of conspecifics generated distinct responses between species. In the intraspecific treatments *Bufo* and *Pelodytes* differed in mass at metamorphosis and
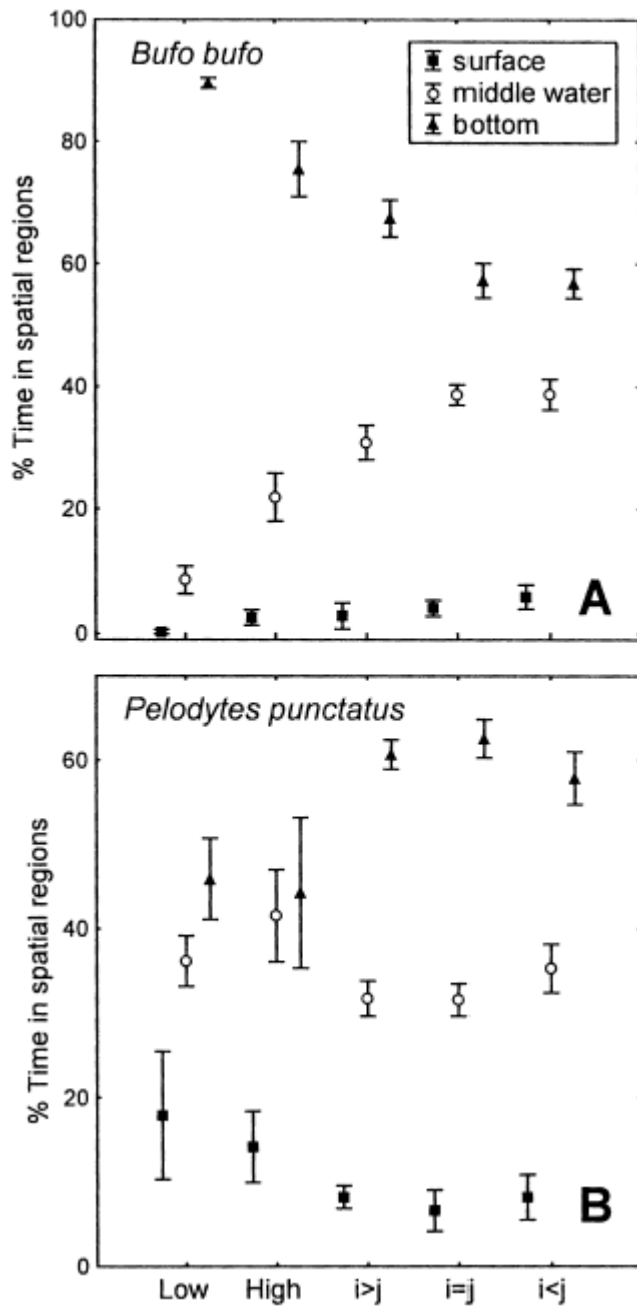
Fig. 3.- Percentage of time of occupancy of the distinct spatial regions of the water column by the two species: *Bufo bufo* (A) and *Pelodytes punctatus* (B) in distinct treatments. Error bars refer to standard error.
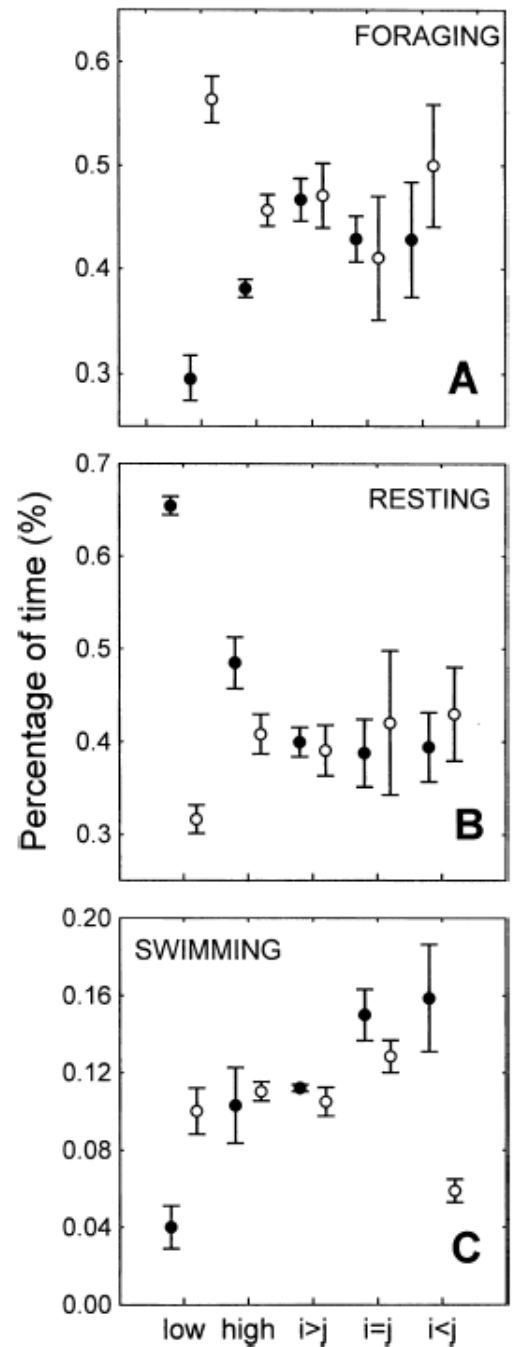

Fig. 4.- Mean values of proportion of time dedicated to foraging (A), resting (B) and swimming (C) of the two species in distinct treatments. Black circles correspond to *Bufo bufo*. Open circles correspond to *Pelodytes punctatus*. Error bars refer to standard error.

growth rate (Table 1). *Bufo* reduced its mean mass and growth rate. In contrast, a high density of conspecifics did not decrease the mean mass of *Pelodytes*, and growth rate decreased by only 8% compared with low-density treatment. *Pelodytes* mortality increased by 40% while

that of *Bufo* was 10%. From an individual perspective, intraspecific competition affected *Bufo* more than *Pelodytes*, but on a populational level these effects were stronger on *Pelodytes*, causing a considerable reduction in its populations via mortality. Significant differences were observed in the response of the two species to interspecific competition (Table 1). *Bufo* and *Pelodytes* showed a reduction in metamorphic mass in all the mixed treatments but this was greater for the former when *Pelodytes* was the more abundant species. The response to interspecific competition for the larval period differed between species. *Bufo* did not change its larval period whereas that of *Pelodytes* was shortened (Fig. 1B). Growth rates suffered a reduction in *Bufo* respect to monospecific treatments. In contrast to *Bufo* reaction, *Pelodytes* tadpoles increased their growth when exposed to the other species, but this change was not significant between treatments. Interspecific competition increased the mortality of *Bufo* (60% and 100% on Bb<Pp treatment). In general, the survival of *Pelodytes* maintained constant, with the exception of the treatment in which it was the most abundant species (Pp>Bb).
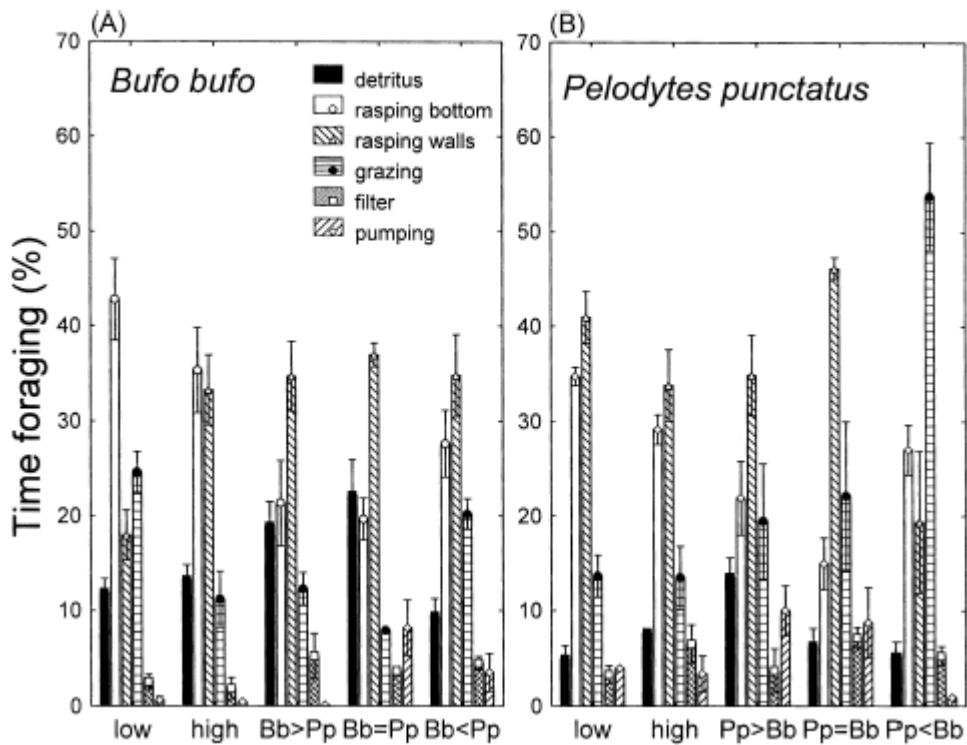

Fig. 5.- Percentage of foraging techniques for *Bufo bufo* and *Pelodytes punctatus* in their different treatments. Error bars refer to standard etrror.

Behavioural effects

MANOVA between *Bufo* treatments for time in distinct zones revealed that in presence of *Pelodytes*, *Bufo* altered its use of space (l=0.1143, $F_{3,12}$=2.3, p=0.047). ANOVA's for the different spatial zones revealed a reduction in use of bottom ($F_{4,10}$=4.89, p=0.019) with greater occupancy of mid ($F_{4,10}$=4.73, p=0.021) and surface waters ($F_{4,10}$=4.9, p=0.018, Fig. 3A). In contrast, *Pelodytes* maintained the same proportion of use of the three zones in all treatments (l=0.3970, $F_{3,12}$=0.75, p=0.6935). *Bufo* tadpole activity (foraging, swimming and resting) was affected by competition (l=0.037, $F_{3,12}$=4.41, p=0.0013). All the activity parameters changed between treatments: foraging ($F_{4,10}$=3.87, p=0.037), resting ($F_{4,10}$=13.59, p<0.001) and swimming ($F_{4,10}$=6.17, p=0.009). Changes occurred when conspecific competition increased. These changes were made in the same direction as interspecific effects, which with difficulty differentiated between density and interspecific effects. However some differences between high conspecific and interspecific treatments were detected in Scheffe´ posthoc test (Fig.

Intraspecific competition

Variable:	bottom-(%)	middle water-(%)	surface-(%)	foraging-(%)	resting-(%)	swimming-(%)
Bufo:	− 14.08 (− 0.16%)	11.76 (1.16%)	2.32 (7.73%)	0.08 (2.31%)	− 0.17 (− 2.07%)	0.06 (12.58%)
Pelodytes:	− 1.63 (− 0.04%)	5.38 (0.15%)	− 3.74 (− 0.21%)	− 0.17 (− 1.51%)	0.09 (2.31%)	0.01 (0.81%)
K-S p:	N.S.	N.S.	< 0.05 (< 0.5)	< 0.05 (< 0.05)	< 0.05 (< 0.05)	< 0.05 (< 0.05)

Interspecific competition

	bottom-(%)	middle water-(%)	surface-(%)	foraging-(%)	resting-(%)	swimming-(%)
Bufo (BB > PP)	− 8.07 (− 0.09%)	8.93 (0.89%)	− 0.86 (− 0.33%)	0.08 (1.81%)	− 0.08 (− 1.41%)	0.008 (0.69%)
Pelodytes (PP > BB)	16.42 (0.35%)	− 9.81 (− 0.27%)	− 6.61 (0.37%)	0.014 (0.25%)	− 0.017 (− 0.34%)	− 0.005 (− 0.38%)
K-S p:	N.S.	< 0.05 (< 0.05)	N.S.	< 0.05 (N.S.)	N.S.	< 0.05 (< 0.05)
Bufo (BB = PP)	− 18.22 (− 0.21%)	16.77 (1.65%)	1.45 (0.54%)	0.045 (1.01%)	− 0.09 (− 1.59%)	0.04 (3.63%)
Pelodytes (PP = BB)	18.33 (0.39%)	− 9.97 (− 0.27%)	− 8.36 (− 0.46%)	− 0.04 (0.89%)	0.012 (0.24%)	0.018 (1.31%)
K-S p:	N.S.	< 0.05 (< 0.05)	< 0.05 (< 0.05)	N.S.	N.S.	< 0.05 (N.S.)
Bufo (BB < PP)	− 18.71 (− 0.21%)	16.83 (1.66%)	1.88 (0.71%)	0.047 (0.99%)	− 0.09 (− 1.49%)	0.055 (4.31%)
Pelodytes (PP < BB)	13.62 (0.29%)	− 6.23 (− 0.17%)	− 7.38 (− 0.41%)	0.043 (0.75%)	0.021 (0.42%)	− 0.05 (− 3.72%)
K-S p:	N.S.	< 0.05 (N.S.)	< 0.05 (0.05)	N.S.	N.S.	< 0.05 (< 0.05)

Table 2.- Behavioural responses of the two species to the intra- and interspecific competition treatments. Brackets show changes in relative terms. Significance levels indicate results of the Kolmogorov-Smirnov test for absolute and relative (in brackets) changes for the effects of tadpole species responses. On interspecific competition, comparisons between species made between treatments with the same species conditions: response of *Bufo* in the Bb<Pp treatment was compared with the response of *Pelodytes* in the Pp<Bb treatment. MANOVA and univariate ANOVA for behavioural use of space of species to distinct treatments.

4A-C), especially in foraging (p<0.05 between high and Bb>Pp) and swimming (p<0.05 between high and Bb<Pp).

Pelodytes tadpoles showed differences in activity (l=0.091, $F_{3,12}$=2.62, p=0.0024), but these were only significant when compared to swimming activity ($F_{4,10}$=8.14, p=0.003), without statistical significance in foraging ($F_{4,10}$=1.48, p=0.278) and resting activity ($F_{4,10}$=0.82, p=0.536, Fig. 4A-C). Swimming activity increased with density (p<0.05), independently of intra or interspecific effects.

Differences in the six foraging techniques were analysed separately for the two species. *Bufo* displayed differences in their foraging techniques in the presence and absence of *Pelodytes* (l=0.003, $F_{6,24}$=3.1, p=0.0034). When *Bufo* was the only species present, they concentrated their feeding activity on bottom techniques like pumping detritus and rasping the bottom. However, in the presence of *Pelodytes*, *Bufo* reduced bottom rasping ($F_{4,10}$=3.61, p=0.045), and slightly increased the use of mid and surface water techniques: rasping walls ($F_{4,10}$=8.03, p=0.003) and suspension filtering and pumping on the surface (p>0.05) in presence of *Pelodytes* (Fig. 5A). Differences between treatments for all foraging techniques for *Pelodytes* were found (l=0.008; $F_{6,24}$=2.3; p=0.034) but posteriors ANOVA tests for any technique did not show statistical differences. *Pelodytes* did not concentrate their foraging activity in bottom substrates like *Bufo* did but rather used techniques linked to other regions of the water column like suspension filtering, grazing and pumping in mid water (Fig. 5B).

When we compared behavioural reactions to competition between species before standardised values, we could appreciate opposite reactions in some traits (Table 2). Both species reacted in a similar manner to conspecific competition in their use of space, whereas interspecific competition originated some differences. In general, *Bufo* increased the use of the water column and surface, however, *Pelodytes* reduced its use of them (no differences between treatments was found). Activity reactions were different between the two species in relation to conspecific competition. *Bufo* increased their activity at high densities whereas *Pelodytes* showed a reduced activity pattern (Table 2). Tendencies of activity change to interspecific competition were similar in the two species, which only differed in swimming parameters.

DISCUSSION

Usually, resource depletion and exploitation competition are the main arguments to explain weak competition between anuran larvae. However, interference competition mechanisms like chemical interference competition and physical displacement have been reported in laboratory experiments (Beebee & Wong 1992, Wong et al. 1994, Griffiths 1995). Our observations of individual interactions at the microscopic time-scale indicate that the two kinds of mechanisms may have been actuated in our system, which could explain the distinct responses of the species in the mesoscopic time-scale.

Bufo showed the greatest change in behaviour; some of them corresponded to density increment (intra and interspecific), and others correspond with theory and with previous laboratory studies, which indicate that large and more active tadpoles (in this case *Pelodytes* tadpoles) are competitively "superior" to smaller ones (Morin & Johnson 1988, Alford 1999). These smaller tadpoles were displaced from the bottom of the aquaria as a result of competition. *Bufo* were forced to abandon foraging or space resource in the presence of larger *Pelodytes* tadpoles. The consequences of this interaction were manifested in an increase in time spent foraging in other parts of the water column and the use of mid water as a refuge from competition. Similar results were observed in high intraspecific density, and it seems hard to differentiate between high conspecific density and asymmetric interspecific effects on behaviour. However, these reactions were more pronounced in the presence of *Pelodytes*. Given that *Bufo* species present low energy efficiency in swimming relative to other tadpole species, with an elevated number of tail impulses that manifests as high metabolic rates (Feder 1982, Wassersug & Hoff 1985), *Bufo* individuals may be forced to increase foraging activity to compensate for this expenditure. This corresponded with our observations; *Bufo bufo* are generalist tadpoles that readily scrape and filter detritus substrata (Campeny 2001) and show a low efficiency for filtering suspension in mid waters (Viertel 1995). As a result of all these behavioural changes the balance of cost and gain curves become skewed towards cost, despite an increase in activity. Consequently, the growth of *Bufo* tadpoles is limited and reduced, hence, individuals show a lower mass at metamorphosis. In natural conditions reduced growth rates also indirectly increase mortality rates by extending the time exposed to diseases, predators, and individual wear. In amphibians, a lengthened larval period

and smaller body at metamorphosis are factors that are negatively related to fitness (Berven & Gill 1983, Smith 1987, Semlitsch et al. 1988). Several studies show that mass at metamorphosis affects individual fitness in distinct manners, such as through parasite resistance (Goater 1994), risk of dehydration (Creusere & Whitford 1976), and age and size at maturation (Smith 1987, Crump 1989, Newman 1992, Kozlowski 1996). On the basis of our results we conclude that an interference mechanism of spatial displacement from *Pelodytes* to *Bufo* occurs together with a consumptive mechanism of optimum food resource as a consequence of this.

While interspecific competition on *Bufo* manifests the expected responses observed in other anuran species (Alford 1999, Gurevitch et al. 2000), the case with *Pelodytes* is more exceptional. Individuals did not show any behavioural change in the presence of the *Bufo bufo* competitor, and all arguments (large body and temporal pond breeder) support the competitive superiority of *Pelodytes* at individual level. Furthermore, in comparison with intraspecific treatments, in mixed treatments *Pelodytes* presented shorter larval periods and consequently lower mass at metamorphosis. Wilbur and Collins (1973) proposed that "superior" competitor individuals delay metamorphosis beyond the minimum size, and extend their larval period while capitalising on rich aquatic growth opportunity. On a microscopic time-scale *Bufo* apparently does not alter the foraging and spatial behaviour of *Pelodytes*, and growth rate did not show differences between treatments (Fig. 2B). The question then becomes not why *Pelodytes* tadpoles do not optimise their growth rates and metamorphose with larger bodies, as would be expected from a superior competitive species, but instead why *Pelodytes* tadpoles metamorphose soon after they reach the minimum size.

We hypothesise that the accelerated development of *Pelodytes* in the presence of bufonid tadpoles and their abandonment of water when they reach the minimum size is an adaptive response to the risk of prolonged larval period when there is a possible time constraint in the temporal pond and when competitive interference mechanisms are not enough to overcome the effects of exploitative competition. Species that breed in an unstable habitat allow prolongation of the larval period and maximum size at metamorphosis when conditions are favourable. In addition, tadpoles can avoid mortality in a drying pond by accelerating

metamorphosis, which implies a reduced size. We considered that the presence of *Bufo* acted as a signal of environmental degradation with declining growth opportunities due to resource depletion. This signal fixed larval period but not mass at metamorphosis, which explains why in Bb>Pp individuals showed higher values of mass than the other interspecific treatments. This is a consequence of their superiority over *Bufo* and exposure to low intraspecific competition permits capitalised resources. If at individual level Bufo was the "inferior" competitor, superior competitive effects of bufonids in natural conditions can occur at population level determined by their size (Persson 1985, Polis 1988). Smaller species present higher metabolic rates and support high population densities (Peters 1983, Peters & Wassenberg 1983, Blackburn & Gaston 1999). In our field area of study we founded ponds with a relationship between both species of 50-1 or twice in favour of *Bufo* (unpubl.). Under these conditions, and with a high resource overlap observed between *Pelodytes* and *Bufo* (Díaz-Paniagua 1985, 1989), at the population level small organisms can exploit relatively more energy than large competitors and can therefore potentially inflict harm (Peters 1983, Polis 1988). In homogeneous spatial structures like temporary ponds, large organisms cannot use the most common interference mechanisms, like territoriality and chemical warfare; consequently large species (like Pelodytes in our case) are unable to monopolise resources (Polis 1988, Blackburn & Gaston 1999). Physical displacement as we observed is an alternative strategy but does not impede potential exploitative competition of smaller individuals. In our case the increment of *Bufo* foraging activity in mid water could reduce the rain of phytoplankton to the bottom (Werner 1994), which, in turn, could be a main component of *Pelodytes* diet (Díaz-Paniagua 1987, 1989). We propose that under time constraints that limit ability to delay growth and develop, *Pelodytes*, a typical temporal pond breeder, has evolved mechanisms to accelerate development and to metamorphose earlier in the presence of a potential competitor. Morey and Reznick (2000) found a similar response in *Scaphiopus couchii* , an ephemeral breeder, to intraspecific competition in an induced decline growth opportunity environment via food suppression.

According to our hypothesis, smaller body mass is a cost of this adaptive phenotypic response as opposed to a benefit from reduced mortality in a deteriorating environment. In our study area, mortality is high in years with lengthy droughts, and a recent study showed the

loss of cohorts of *Pelodytes* that correspond to drought years (Esteban et al. 2002). Experimental results and field observations indicate a possible close relationship between different ecological traits: competition and time constraints for development in temporal ponds. Similar conclusions linking predation and pond drying in pool frogs have recently been reported by Altwegg (2002).

ACKNOWLEDGMENTS

REFERENCES

Alford, R. A. 1999. Ecology: resource use, competition, and predation. / In: McDiarmid, R. W. and Altig, R. (eds), Tadpoles: the biology of anuran larvae. The Univ. of Chicago Press, pp. 240-278.

Alford, R. A. and Wilbur, H. M. 1985. Priority effects in experimental communities: competition between Bufo and Rana. Ecology 66: 1097-1105.

Altwegg, R. 2002. Predator-induced life-history plasticity under time constraints in pool frogs. Ecology 83: 2542-2551.

Augert, D. and Guyétant, R. 1995. Space ocupation for egg deposition in amphibians living in plain woodland and pasture land (east of France). In: Llorente, G. A., Montori, A, Santos, X. et al. (eds), Scientia herpetologica. Asociación Herpetológica Española. Barcelona, pp. 165-169.

Bardsley, L. and Beebee, T. J. C. 1998a. Interspecific competition between *Bufo* larvae under condition of community transition. Ecology 79: 1751-1759.

Bardsley, L. and Beebee, T. J. C. 1998b. Interspecific competition between larvae is not an important structuring force in mixed communities of *Rana* and *Bufo* on an English sanddune system. Ecography 21: 449-456.

Beebee, T. J. C. 1995. Tadpole growth: is there an interference effect in nature? Herpetological Journal 5: 204-205.

Beebee, T. J. C. and Wong, A. L. 1992. Prototheca-mediated interference competition between anuran larvae operates by resource division. Physiological Zoology 65: 815-831.

Berven, K. A. and Gill, D. E. 1983. Interpreting geographic variation in life history traits. American Zoologist 23: 85-97.

Blackburn, T. M. and Gaston, K. J. 1999. The relationship between animal abundance and body size: a review of the mechanisms. Advances in Ecological Research 28: 181-210.

Campeny, R. 2001. Ecologia de les larves d'amfibis anurs al Montseny. PhD thesis. Universitat de Barcelona. Barcelona. Spain

Connell, J. H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. American Naturalist 122: 661-/696.

Creusere, F. M. and Whitford, W. G. 1976. Ecological relationships in a desert anuran community. Herpetologica 32: 7-18.

Crump, M. L. 1989. Life history consequences of feeding versus non-feeding in a facultatively non-feeding toad larva. Oecologia 78: 486-489.

Denver, R. J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. American Zoologist 37: 172-184.

Díaz-Paniagua, C. 1985. Larval diets related to morphological characters of five anuran species in the biological reserve of Doñana (Huelva, Spain). Amphibia-Reptilia 6: 307-322.

Díaz-Paniagua, C. 1987. Estudio en cautividad de la actividad alimenticia de las larvas de siete especies de anuros. Revista Española de Herpetología 2: 189-197.

Díaz-Paniagua, C. 1989. Larval diets of two anuran species *Pelodytes punctatus* and *Bufo bufo*, in SW Spain. Amphibia-Reptilia 10: 71-75.

Esteban, M., Barbadillo, L. J. and Sánchez-Herráiz, M. J. 2002. The effects of aquatic breeding environment availability on the population structure of amphibians: the *Pelodytes punctatus* case. In verbis comunication: VII Congresso Luso-Espanhol XI Congreso Español de Herpetologia, Evora, October 2002.

Feder, M. E. 1982. Effect of developmental stage and body size on oxygen consumption of anuran larvae: a reappraisal. Journal of Experimental Zoology. 220: 33-42.

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

Goldberg, D. E. and Barton, A. M. 1992. Patterns and consequences of interspecific competition in natural communities: a review of field experiments with plants. American Naturalist 139: 771-801.

Griffiths, R. A. 1995. Determining competition mechanisms in tadpoles. Herpetological Journal 5: 208-210.

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.

Griffiths, R. A., Denton, J. and Wong, A. L. C. 1993. The effect f food level on competition in tadpoles: interference mediated by protothecan algae? Journal of Animal Ecology 62: 274-279.

Grill, C. P. and Juliano, S. A. 1996. Predicting species interactions based on behaviour: predation and competition in container-dwelling mosquitoes. Journal of Animal Ecology 65: 63-76.

Gurevitch, J., Morrow, L. L., Wallace, A. et al. 1992. A metaanalysis of competition in field experiments. American Naturalist 140: 539-572.

Gurevitch, J., Morrison, J. A. and Hedges, L. V. 2000. The interaction between competition and predation: a metaanalysis of field experiments. American Naturalist 155: 435-453.

Guyétant, R., Temmerman, W. and Avrillier, J. N. 1999. Phénologie de la reproduction chez *Pelodytes punctatus* Daudin 1802 (Amphibia, Anoura). Amphibia-Reptilia 20: 149-160.

Harper, J. H. 1977. Populations biology of plants. Academic Press.

Hoff, K., Blaunstein, A. R., McDiarmid, R. W. et al. 1999. Behavior: interactions and their consequences. In: McDiarmid, R.W. and Altig, R. (eds), Tadpoles: the biology of anuran larvae. The Univ. of Chicago Press, pp. 215-239.

Kozlowski, J. 1996. Optimal size and adult size of animals: consequences for macroevolution and community structure. American Naturalist 147: 101-114.

Laurila, A. 1998. Breeding habitat selection and larval performance of two anurans in freshwater rock-pools. Ecography 21: 484-494.

Laurila, A. 2000. Competitive ability and the coexistence of anuran larvae in freshwater rock-pools. Freshwater Biology 43: 162-174.

Law, R., Marrow, P. and Dieckmann, U. 1997. On evolution under asymmetric competition. Evolutionary Ecology 11: 485-501.

Lawton, J. H. and Hassell, M. P. 1981. Asymmetrical competition in insects. Nature 289: 793-795.

Licht, L. E. 1967. Growth inhibition in crowded tadpoles: intraspecific and interspecific effects. Ecology 48: 736-745.

Martin, P. and Bateson, P. 1991. La medición del comportamiento. Alianza Universidad, Alianza editorial S.A. Madrid.

Montori, A. 1997. Els amfibis i rèptils dels Garraf, La Sentiu (ed.). Museu de Gava.

Morey, S. and Reznick, D. 2000. A comparative analysis of plasticity in larval development in three species of spade foot toad. Ecology 81: 1736-1749.

Morin, P. J. 1983. Predation, competition and the composition of larval anuran guilds. Ecological Monographs 53: 119-138.

Morin, P. J. 1999. Community ecology. Blackwell Science Inc.

Morin, P. J. and Johnson, E. A. 1988. Experimental studies of asymmetric competition among anurans. Oikos 53: 398-407.

Newman, R. A. 1992. Adaptative plasticity in amphibian metamorphosis. Biosciences 42: 671-678.

Ottoni, E. B. 1998. EthoLog 2.2. Toolpack. Software for field biologist.

Parris, M. J. and Semlitsch, R. D. 1998. Asymetric competition in larval amphibian communities: conservation implications for the northern crawfish frog, *Rana areolata circulosa* . Oecologia 116: 219-226.

Pearmann, P. B. 1995. Effects of pond size and consequent predatory density on two species of tadpoles. Oecologia 102: 1-/8.

Persson, L. 1985. Asymmetrical competition: are larger animals competitively superior? American Naturalist 126: 261-266.

Peters, R. H. 1983. The ecological implications of body size. Cambridge Univ. Press.

Peters, R. H. and Wassenberg, K. 1983. The effect of body size on animal abundance. Oecologia 60: 89-96.

Petranka, J. W. 1995. Interference competition in tadpoles: are multiple agents involved? Herpetological Journal 5: 206-207.

Polis, G. A. 1988. Exploitation competition and the evolution of interference, cannibalism, and intraguild predation in age/size-structured populations. In: Ebenman, B. and Persson, L. (eds), Size-structured populations: ecology and evolution. Springer-Verlang, pp. 185-202.

Rice, W. R. 1989. Analyzing tables of statistical test. Evolution 43: 223-225.

Sánchez-Herráiz, M. and Barbadillo, L. J. 1997. *Pelodytes punctatus*. In: Pleguezuelos, J. M. (ed.), Distribución y biogeografía de los anfibios y reptiles en España y Portugal. Universidad de Granada y Asociación Herpetológica Española, pp. 143-145.

Schoener, T. W. 1983. Field experiments on interspecific competition. American Naturalist 122: 240-285.

Semlitsch, R. D. and Wilbur, H. M. 1988. Effect of pond drying time on metamorphosis and survival in the salamander Ambyostoma talpoideum . Copeia 1988: 978-983.

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

Sih, A., Crowley, P., McPeek, M. et al. 1985. Predation, competition, and prey communities: a review of field experiments. Annual Review of Ecology and Systematics 16: 269-311.

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

Sokal, R. R. and Rohlf, F. J. 1995. Biometry. W. H. Freeman and Company.

Steinwascher, K. 1979. Competitive interactions among tadpoles: responses to resource level. Ecology 60: 1172-1183.

Stijbosch, H. 1979. Habitat selection of amphibians during their aquatic phase. Oikos 33: 363-372.

Tilman, D. 1987. The importance of the mechanisms of interspecific competition. American Naturalist 129: 769-774.

Toxopeus, A. G., Ohm, M. and Arntzen, J. W. 1993. Reproductive biology of the parsley frog, Pelodytes punctatus, at the northernmost part of its range. Amphibia-Reptilia 14: 131-147.

Underwood, A. J. 1986. The analysis of competition by field experiments. In: Kikkawa, J. and Anderson, D. J. (eds), Community ecology: pattern and process. Blackwell.

Viertel, B. 1995. The significance of suspension feeding for tadpoles survival in temporary ponds. In: Llorente, G. A., Montori, A., Santos,

X. et al. (eds), Scientia Herpetologica. Asociación Herpetológica Española. Barcelona, pp. 154-159.

Wassersug, R. J. and Hoff, K. 1985. The kinematics of swimming in anuran larvae. Journal of Experimental Biology 119: 1-30.

Weiner, J. 1990. Asymmetric competition in plant populations. Trends in Ecology and Evolution 5: 360-365.

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. 1994. Ontogenetic scaling of competitive relations: size-dependent and responses in two anuran larvae. Ecology 75: 197-213.

Werner, E. E. and Anholt, B. R. 1996. Predator induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. Ecology 77: 157-169.

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

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

Wong, A. L., Beebee, T. J. C. and Griffiths, R. A. 1994. Factors affecting the distribution and abundance of an unpigmented heterotrophic alga (*Prototheca richardsi*). Freshwater Biology 32: 33-38.

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