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Effects of the non-native amphibian species *Discoglossus pictus* on the recipient amphibian community: niche overlap, competition and community organization

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Abstract The painted frog, *Discoglossus pictus*, was introduced into the Iberian Peninsula 100 years ago and its distribution has steadily increased since then. We studied the effects of this non-native amphibian species on the native ones in the northeastern area of the peninsula. We compared amphibian assemblages in regions with and without *D. pictus* to estimate niche overlap between species. Additionally, we carried out a laboratory evaluation of the effects of competition between the non-native and the two native species with which it overlaps most commonly: *Bufo calamita* and *Pelodytes punctatus*. The presence of *D. pictus* larvae reduced the survival, body mass and activity of *B. calamita*, and increased time to metamorphosis. Furthermore, *D. pictus* showed the highest consumption rate while *P. punctatus* showed the lowest. One possible consequence of these competitive interactions is an alteration of species co-occurrence patterns in the recipient community on a regional scale. In the non-invaded area, the checkerboard score (C-score) of co-occurrence indicated that the community was structured, whereas the standardized C-score in the invaded area indicated that the community did not differ significantly from having a random structure. These results suggest that competition between native and non-native species can cause recipient communities to become less structured.

Keywords Invasive species · Competition · Community response · Co-occurrence

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Introduction

The effects of invasions by non-native species are often complex, which makes their impact on a recipient community difficult to predict (Williamson 1999; Ficetola et al. 2007; Osunkoya et al. 2011). Several ecological interactions between non-native

and native species may be involved, and their effects may be apparent to a greater or lesser degree (Kiesecker et al. 2001; Blaustein and Kiesecker 2002; Kats and Ferrer 2003). The various levels of impact a non-native species may have on a native community are related to the capacity of the non-native species to invade and the invasion stage it has reached (Blackburn et al. 2011). Many studies have assessed the determinants of invasiveness (Van Kleunen et al. 2010) to elucidate why some species succeed and others fail in establishing themselves and spreading over a range into which they have been introduced, but quantifying the consequences of invasion on native communities is much more difficult. However, the negative impact of biological invasions on communities and ecosystem dynamics is considered one of the primary threats to global biodiversity (IUCN 2000; Mack et al. 2000; Clavero and García-Berthou 2005; D'Amore et al. 2010). Amphibians are the most threatened animal group under IUCN Red List criteria (IUCN 2010). 29 % of the world's amphibian species are threatened with extinction (IUCN 2010) and at least 43.2 % of all described species are currently experiencing population decline (Stuart et al. 2004). In the Mediterranean basin, there is a high level of endemism (64 %) and 25.5 % of the amphibian species are threatened (Cox et al. 2006). Habitat loss, pollution and invasive alien species have the largest impact on Mediterranean amphibian communities (Cox et al. 2006). Mediterranean ecosystems have a long history of biological invasions (Di Castri 1990; Rebelo et al. 2010) and their temporary wetlands are especially threatened due to invasions and habitat destruction (Geiger et al. 2005; Ficetola et al. 2011; Lillo et al. 2011), which have an impact on amphibian populations.

Although there are a few exceptions (e.g. *Rhinella marina*, *Lithobates catesbeianus* and *Eleutherodactylus coqui*), the invasion dynamics of amphibian species and the effects on recipient amphibian communities have been little studied (Kupferberg 1997; Beard and Pitt 2005; Ficetola et al. 2007). This is presumably because of the perception that most of these species do not pose severe ecological or economic threats (Kraus and Campbell 2002). Recent studies show that high-impact invaders among amphibians and reptiles tend to show lower establishment success rates in native communities than mammals or fish (Ricciardi and Cohen 2007). Although the

mechanisms of invasion and impact are not necessarily linked (Hayes and Barry 2008), a non-native species that spreads widely is more likely to affect multiple native species and lead to a reduction in their populations (Ortega and Pearson 2005).

Because of the widely recognized importance of competition in amphibian communities during their larval phase (e.g. Alford 1999), several studies have evaluated the possible impact of competition from non-native tadpoles on native tadpoles (Kupferberg 1997; Crossland 1998; Kiesecker and Blaustein 1998; Smith 2005; Knight et al. 2009; Crossland and Shine 2010; Rebelo et al. 2010) and their effects on community structure; although only a few of these studies were carried out on arid or Mediterranean amphibian communities (Woodward 1982; Richter-Boix et al. 2007a; Escoriza and Boix 2012). The competitiveness of the different species can be quantified using tadpole performance measures such as larval period, size at metamorphosis and mortality. Pre-metamorphic traits such as timing of and size at metamorphosis influence post-metamorphic performance and future fitness of amphibians (Altwegg and Reyer 2003). If non-native species substantially outperform co-occurring natives, then we can expect serious widespread consequences for the community structure (Daehler 2003). On the other hand, if non-native species rarely outperform co-occurring natives, or if their fitness is only marginally superior to that of the natives, then the effects on the community are likely to be minor. Nevertheless, even if non-native species are not very competitive, competition in the community may persist due to other factors, such as the ability of a species to colonize unoccupied niches rapidly (Lodge 1993; Rejmanek and Richardson 1996).

The aim of the present study was to examine the effects of competition between the non-native amphibian species *Discoglossus pictus* and native amphibians in the northeastern Iberian Peninsula. The specific objectives were: (1) to quantify the ecological breadth during the larval phase of each species in the community in order to estimate niche overlap between species; (2) to evaluate, in laboratory experiments, the competitive interactions and mechanisms between *D. pictus* and the native species with which it overlaps most frequently in nature; and (3) to evaluate whether the presence of *D. pictus* alters species co-occurrence patterns on a regional scale by comparing community

organization in a non-invaded region with that of an invaded region. We hypothesized that if the invasive species overlaps with some native species in breeding sites and competes with them, then these interactions could lead to changes in the community structure.

Materials and methods

Study system

Discoglossus pictus is native to North Africa and Sicily (Zangari et al. 2006) and is thought to have been introduced from Algeria to Banyuls-sur-Mer (Pyrénées-Orientales department, France) in 1906 (Wintrebert 1908), from where it colonized suitable areas of northeastern Spain and southeastern France (Martens and Veith 1987). In the invaded areas, amphibian species richness is similar to that of non-invaded areas (Cheylan et al. 1987; Llorente et al. 1995; Pascal et al. 2003), but with greater overlap (Montori et al. 2007), which could modify the amphibian community structure.

Since *Discoglossus pictus* was introduced in Banyuls-sur-Mer, it has expanded to a continuous range of over 7,000 km², from southeastern France to northeastern Spain. It has expanded about 140 km towards the south and north, and about 60 km to the west, in favorable areas (Fig. 1). The introduced species appears to be abundant, both in terms of populations and individuals, and continues to expand its range in both France and Spain (Fradet and Geniez 2004; Montori et al. 2007). An overall dispersion rate of 1.53 km/year ± 0.8 km (Montori et al. 2007) has been calculated, which is lower than other invasive amphibian species such as *R. marina* (Phillips et al. 2007; Urban et al. 2008). Recently, new human-mediated introductions outside its range of expansion have been described along the Spanish Mediterranean coast (Fradet and Geniez 2004; Franch et al. 2007).

The area of northeastern Spain occupied by *D. pictus* is a typical Mediterranean region, with hot and dry summers, mild winters and two rainy seasons: one in spring and one in autumn (Martín Vide and Olcina 2001). The combination of climate, ground composition, and pond size and depth gives rise to a range of potential breeding areas for amphibians, from ephemeral rain pools to permanent water bodies. The native amphibian community in this zone comprises a total of 10 species: three urodela (*Salamandra salamandra*, *Triturus marmoratus* and *Lissotriton helveticus*) and seven anuran species (*Alytes obstetricans*,

Fig. 1 Distribution of *Discoglossus pictus*: native range (*dark grey*) and introduced range (*square*). The higher resolution map represents the region of northeastern Spain with both study areas (*black spots*). The *arrow* indicates where the species was introduced (Banyuls-sur-Mer)
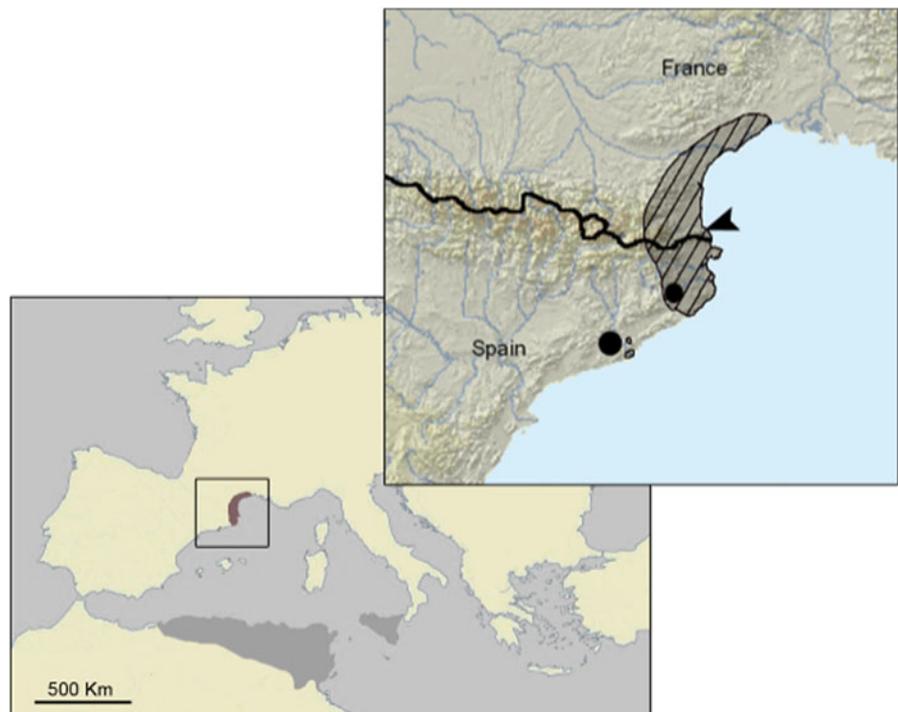
Pelodytes punctatus, *Pelobates cultripes*, *Bufo cala-mita*, *Bufo bufo*, *Hyla meridionalis* and *Pelophylax perezi*).

Field survey methods: ecological breadth
in breeding habitats

In the spring of 2005 and 2006, we searched for water body habitats in a region within the current range of *D. pictus*, which is well documented (Montori et al. 2007), and in regions outside its range (Fig. 1). The first citations of *D. pictus* in the former region were from 1995, and this entire region was fully colonized by 1997 (SIARE 2008). The regions present the same species richness and cover similar areas. Breeding sites were identified by the presence of eggs, tadpoles or metamorphic individuals, which were detected by conducting dip-netting and egg searches (Richter-Boix et al. 2007a). The sites surveyed spanned the range of aquatic breeding habitats in the area. Sampling time periods were established on the basis of preliminary data and took account of temporal differences in breeding activity between species (Richter-Boix et al. 2006), thereby ensuring that all species were detected. All the sites were surveyed once or twice each year during the breeding season, and the surveys were performed by two expert observers. The duration of active sampling and number of dip-nets varied depending on pond size following standard techniques (Heyer et al. 1994). All amphibian species were identified in the field and returned to the water. Several studies performed in northeastern Spain provide us with good information about the distribution and pond breeding habits of the amphibian communities (Llorente et al. 1995; Pleguezuelos 1997; Pleguezuelos et al. 2002; SIARE 2008). Although the gradient of permanence is continuous, we categorized the ponds by hydroperiod and top predator (detected by visual survey and dip-netting). Based on previous studies (Wellborn et al. 1996; Stocks and McPeek 2003; Van Buskirk 2003; Richter-Boix et al. 2007a) we described four categories: (1) ephemeral pools that dry up within weeks, containing water for less than 2 months, and with few or no predators; (2) temporary ponds that flood in the spring and autumn rainfall, and hold water until the end of spring or early summer, containing water for more than 2 months, usually with small invertebrate predators; (3) permanent ponds containing water all year

round, with many invertebrate predators but without fish; and (4) permanent ponds where fish are the top predators. Using this data, we constructed a presence–absence matrix for the different species at each site, which were first assigned to one of the four habitat categories described.

For the analysis of niche overlap, the similarity of habitat use (calculated using the pond categories previously explained) between two species was calculated using the symmetric niche overlap coefficient (Pianka 1973). Pianka's coefficient was measured using percentage use of each pond category by each pair of species following the formula:

$$O_{jk} = O_{kj} = \frac{\sum_i^n p_{ij}p_{ik}}{\sqrt{\sum_i^n p_{ij}^2 \sum_i^n p_{ik}^2}}$$

where p_{ij} and p_{ik} represent the proportions of the *ith* pond used by the *jth* and *kth* species. The mean niche overlap of the communities was tested statistically by performing 10,000 Monte Carlo randomizations of the original data to create pseudo-communities, using EcoSim v7.72 (Gotelli and Entsminger 2007). We applied the randomization algorithm RA2 (Lawlor 1980) to the data, with niche breadth relaxed but with the zero structure of the matrix retained (Gotelli and Entsminger 2007). This algorithm is more realistic for our data because the larval life-history traits of the species studied impose constraints on their resource utilization. For example, some species never use ephemeral pools because their larval periods are longer than the water permanence of the habitat. Since not all breeding-pond habitats are equiprobable (some habitat categories are very common and others very rare), the observed utilization matrix and simulated matrices were scaled relative to the number of ponds in each category we sampled, with the user-defined function available in EcoSim (Gotelli and Entsminger 2007).

Competition measured in laboratory experiments

Experiment I: intraspecific and interspecific effects on larval life-history traits

In 2006, we conducted laboratory experiments to measure the effects of competition on the non-native species and the two native species with the highest estimated niche overlap in nature: *B. calamita* and

P. punctatus. The experiments were performed in the laboratory at the University of Barcelona under the same photoperiod conditions (12D:12L) and temperatures of 21–23 °C. The competitiveness of the species was determined from pairwise experiments, with the effects of *D. pictus* on the native species calculated by comparing the performance of each species when reared alone (intraspecific competition) with their performance when reared together (interspecific competition). The experimental design consisted of eight treatments, each replicated four times: (1) a low-density, single-species treatment for each species, with 15 tadpoles per experimental unit (three low-density treatments: *D. pictus*, *B. calamita* and *P. punctatus*, with four replicates per species and therefore 12 tanks); (2) a high-density, single-species treatment for each species, with 30 tadpoles per unit (three high-density treatments: *D. pictus*, *B. calamita* and *P. punctatus*, with four replicates per species and 12 tanks); and (3) interspecific treatments between the invasive *D. pictus* and the two native species, with 15 tadpoles of each species (two treatments or combinations: [*D. pictus* + *B. calamita*; *D. pictus* + *P. punctatus*], four replicates and eight tanks).

The experimental units consisted of plastic tanks filled with 30 L of dechlorinated tap water. The water in each tank was changed every week to prevent the accumulation of lethal levels of metabolic products. The tadpoles were fed three times a week with commercial rabbit pellets (SANKY PIBAR. S.A., Barcelona, Spain). All the treatments consisted of the same amount of food per week, which was gradually increased throughout the experiment from approximately 4–5 g of pellets at the beginning to 15 g at the end of the experiment, in accordance with tadpole growth and food requirements. We used a variable number of clutches from the area containing the invaded community for each species (four for *D. pictus*, four for *B. calamita* and five for *P. punctatus*). We mixed tadpoles from different clutches in a tank and used a sample of this mixture in all experimental units in order to reduce the chance of any genetic bias. The tadpoles were added to the experimental tanks when they reached the developmental Gosner stage 25 (Gosner 1960).

During the experiment, tadpole behavior was monitored by recording the number of individuals still alive in the tank and the proportion of active tadpoles (swimming or feeding). Activity was recorded three times a day by conducting focal observations during the second and third weeks of the experiment, with a total of 40 records for each tank. The timing of metamorphosis, defined as the stage when the tail has been completely absorbed, was determined by daily checks at the beginning of the experiment and twice-daily checks during metamorphosis climax. At metamorphosis, wet body mass was measured (to the nearest 0.1 mg) and the growth rate estimated as the mass gained each day during the larval period [mass at metamorphosis divided by larval period (g/days)]. Survival was expressed as the proportion of larvae that completed metamorphosis in each experimental tank. In the analysis, tank means were used in order to avoid pseudoreplication. Before analysis, larval period, mass at metamorphosis and growth rate were log transformed, and survival to metamorphosis and tadpole activity were arcsin transformed. To analyze the intraspecific and interspecific effects of each species, we used multivariate analyses of variance (MANOVA) for the three traits together (larval period, mass at metamorphosis and growth rate). When statistically significant effects were detected, successive ANOVAs were conducted for each variable. Since we wanted to test whether competition between species was symmetric or asymmetric, and because the baseline responses of the species differed in the intraspecific treatments, we standardized the absolute changes in responses to facilitate interspecific comparisons. We applied the standardized model developed by Morin and Johnson (1988). Standardized intraspecific effects were calculated by subtracting the mean of the four replicates of the low-density treatment from the values for each individual in the high-density treatment, divided by the subtracted value. Standardized interspecific effects for each species were calculated by subtracting the mean of the four replicates of the species at high density from the individual values for the same species in the interspecific treatment, divided by the mean subtracted value (Morin and Johnson 1988). Differences between the species for intraspecific and interspecific effects were tested with MANOVAs and consecutive ANOVAs for the three continuous traits and with generalized linear models (GLZ) for survivorship and activity proportions. All statistical tests were carried out using Statistica software.

Experiment II: species consumption rate estimation

In spring 2007, we conducted an experiment to measure the consumption rate of the species used in the previous experiment. Three spawns from each species were collected and transported to the laboratory in Barcelona. In the laboratory the clutches were mixed. When embryo development was complete at Gosner stage 25, a total of 25 tadpoles were taken at random and raised individually in 1-L plastic containers. We added a rigid mesh 2–3 cm from the bottom of the container to prevent tadpoles from eating their own feces. For 2 weeks, each tadpole was fed with small pieces of Sera® Spirulina tabs placed in a small Petri dish on the mesh. After 2 weeks, individuals were removed from the water with a glass screen and positioned on an absorbent cloth to remove excess water. Their wet mass was then weighed to 0.1 mg accuracy. The procedure took less than 30 s and did not affect the tadpoles adversely. The day before this procedure was carried out, the tadpole food was removed to ensure that their guts were empty before being weighed (Savage 1952). After weighing, the tadpoles were replaced individually in the same containers for a further five days. During this period, the tadpoles were fed on Spirulina Tabs that had previously been dried and weighed. Every 2 days, any uneaten food was removed carefully and placed on a small pre-weighed glass Petri dish, then replaced by a new weighed piece of Spirulina Tab. When tadpole feces were detected in the Petri dish, they were carefully pipetted out to make sure that the tadpoles only fed on the pieces of Spirulina Tab during these 5 days. After the fifth day, any uneaten food was removed and the tadpoles were starved for 1 day before being weighed. Uneaten food was dried and weighed. Using these data, we calculated the consumption rate ("Food ingested"/time (days)) for each tadpole (Richardson 2002), where: "Food ingested" = (amount of food given) − (amount of food uneaten). The consumption rate for each tadpole was divided by its initial body mass (measured at the beginning of the experiment). Differences in the consumption rate between species were analyzed using ANOVA. Previously, the consumption rate corrected for body mass was log transformed in order to conform to the assumptions of normality.

Analysis of the community structure in the invaded and non-invaded areas

We compared the structure of invaded and intact amphibian communities to evaluate whether the presence of *D. pictus* alters the co-occurrence patterns of species. For this analysis, we created a presence–absence matrix using the survey data. In the matrix, each column represents a breeding pond, and each row represents a different native amphibian species. Newts and anurans can compete with each other in the terrestrial phase (juveniles and adults), but the study only focused on the larval overlap where the biotic interaction between the groups is a predator–prey interaction and not a competition interaction as presupposed in co-occurrence studies. As larval newts and tadpoles do not compete for the same food resources within the pond (Anderson 1967; Wassersug 1980), we excluded the three urodela from this analysis. We used the Guild Structure Module in EcoSim (Gotelli and Entsminger 2007) to compare patterns of anuran co-occurrence between the invaded and the non-invaded regions. We used the distribution range of *D. pictus* in northeastern Spain (Montori et al. 2007) to classify breeding ponds as invaded or non-invaded. *D. pictus* itself was not included in this analysis of species occurrence because it was used to define the two regions (Sanders et al. 2003). We applied Stone and Roberts' (1990) checkerboard score (C-score) as a quantitative index of co-occurrence. This index measures the degree to which species co-occur, but is not as stringent as other indices because it does not require perfect segregation between species (Gotelli 2000). We constructed two presence–absence submatrices, for the invaded and non-invaded communities, and we calculated the C-score for each submatrix and the variance in these two C-scores. The larger this variance, the more the two communities may differ in their pattern of species co-occurrence. The observed variance in C-scores between the two presence–absence submatrices was compared with the 10,000 variances created through random partitions of the data. If the observed variance was larger than expected by chance, the co-occurrence patterns in the two submatrices could be considered statistically distinct (Sanders et al. 2003). To compare C-scores from the randomized matrices, we calculated the standardized C-score for each matrix as $(\chi_{obs} - \chi_{sim})/SD_{sim}$, where χ_{obs} is the observed index,

χ_{sim} is the mean index of the 10,000 randomized matrices, and SD_{sim} is the standard deviation of the randomized data (Gotelli and Arnett 2000).

We also repeated the niche overlap analysis independently for each region, including only anuran species and excluding *D. pictus*, to study whether niche overlap of native species was similar in both invaded and non-invaded regions. The parameters for the niche overlap test were the same as those explained above. We computed the mean niche overlap coefficients for each species and the standard error for the invaded and non-invaded region.

Results

Niche overlap

From 2005 to 2006, a total of 333 ponds were surveyed: 138 in the range of *Discoglossus pictus* and 195 outside the range of distribution of this non-native species. Of these 333 potential breeding sites, most were ephemeral and temporary ponds (Fig. 2). The non-native species *D. pictus* was detected in 64.3 % of the sites surveyed inside its range of distribution and showed a clear preference for ephemeral and temporary ponds. Like *D. pictus*, the native species were not uniformly distributed in all available water bodies and showed a preference for certain categories of water bodies (Fig. 2). In the pairwise niche overlap calculations, the index was greater than 0.5 for most pairs of species (Table 1). *D. pictus* overlaps most with the native *B. calamita* and *P. punctatus* in breeding habitats. The values of the niche overlap index between *D. pictus* and these two native species were higher than 0.99 (the index ranges from 0 to 1). The three species were commonly syntopic in ephemeral and temporary ponds (Fig. 2), and co-occurred at 19 % of the sites where *D. pictus* was found. At the community level, the simulations indicated that neither the observed mean niche overlap [mean observed = 0.699; simulated indices = 0.681; *P* (obs > expected) = 0.363] nor the observed variance [observed variance = 0.053; simulated variances = 0.042; *P* (obs > expected) = 0.181] were statistically significant. The null model suggests that niche overlap in this community in the category of breeding pond is random, with the detection of all pairwise species combinations possible within the area.

Competition laboratory experiments

Effects of non-native species on native species

Discoglossus pictus had a different effect on the two native species (Fig. 3). The presence of *D. pictus* reduced the survivorship of *B. calamita* tadpoles ($F_{2,9} = 28.659$; $P < 0.001$), but not of *P. punctatus* ($F_{2,9} = 3.339$; $P = 0.082$) (Fig. 3a). In addition, the presence of *D. pictus* reduced the activity of *B. calamita* ($F_{2,9} = 6.135$; $P = 0.021$) (Fig. 4a). Consequently, *B. calamita* tadpoles transformed later and were significantly smaller than those under the intraspecific treatment (Fig. 3b–d). Meanwhile, the presence of non-native tadpoles did not affect the activity ($F_{2,9} = 2.715$; $P = 0.119$) (Fig. 4a), growth or developmental rates of *P. punctatus*. The presence of *D. pictus* did not significantly affect body mass or the other traits analyzed which were similar to the results for *P. punctatus* under the intraspecific treatment (Fig. 3b–d).

The presence of native tadpoles also reduced the survivorship of *D. pictus* ($F_{3,12} = 10.780$; $P = 0.001$) (Fig. 3a). However, their presence had no effect on either activity ($F_{3,12} = 2.455$; $P = 0.113$) (Fig. 4a) or life-history traits (Table 2a).

Differences between species

An increase in the density of conspecifics generated similar responses in the species. In all cases, mortality increased with intraspecific competition but without significant differences between species ($F_{2,9} = 0.944$; $P = 0.424$) (Fig. 5a). Both *D. pictus* and *B. calamita* increased activity, while *P. punctatus* reduced it (Fig. 4a), even though there were no significant differences in the activity response compared to intraspecific competition either ($F_{2,9} = 2.117$; $P = 0.176$). With regards to the life-history traits, there were intraspecific effects, but the only significant differences were in mass at metamorphosis (Table 2b). The mass at metamorphosis of *P. punctatus* was lower than the conspecific effects recorded for *D. pictus* and *B. calamita* (Fig. 5c), suggesting strong density effects for *P. punctatus*.

When we compared behavioral and life-history reactions to interspecific competition between species after calculating standardized values, we could see that interspecific competition was asymmetric. The
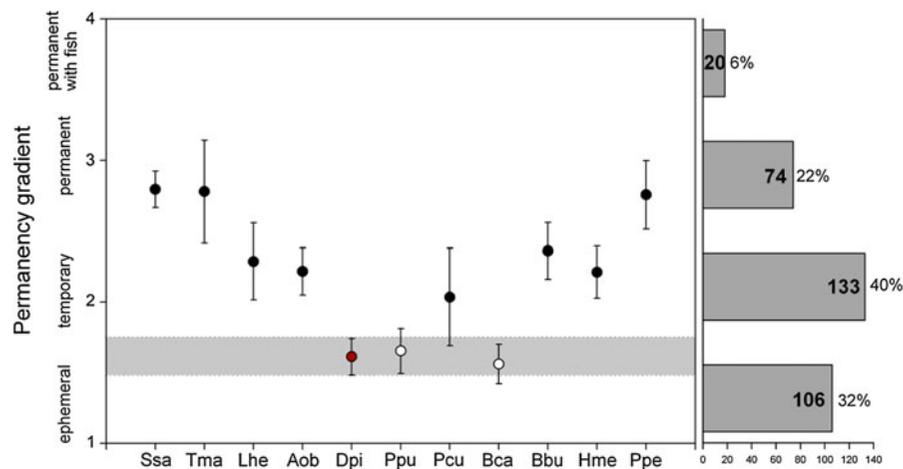
Fig. 2 Species pond preference (mean ± SE) and number and percentage of ponds of the different categories. *Dashed lines* limit the overlap zone with the non-native species. Species which overlap more with *D. pictus* are shown as *white circles*. Ssa: *Salamandra salamandra*; Tma: *Triturus marmoratus*; Lhe:

Lissotriton helveticus; Aob: *Alytes obstetricans*; Dpi: *Discoglossus pictus*; Ppu: *Pelodytes punctatus*; Pcu: *Pelobates cultripes*; Bca: *Bufo calamita*; Bbu: *Bufo bufo*; Hme: *Hyla meridionalis*; Ppe: *Pelophylax perezi*

Table 1 Niche overlap index based on breeding habitats of amphibian species in the study area

Species	Tma	Lhe	Aob	**Dpi**	Ppu	Pcu	Bca	Bbu	Hme	Ppe
Ssa	0.819	0.712	0.790	*0.327*	0.353	0.691	0.305	0.863	0.779	0.796
Tma		0.567	0.947	*0.288*	0.312	0.825	0.261	0.931	0.874	0.989
Lhe			0.715	*0.581*	0.634	0.685	0.506	0.792	0.820	0.466
Aob				*0.568*	0.593	0.955	0.533	0.984	0.981	0.924
Dpi					**0.997**	*0.775*	**0.994**	*0.568*	*0.674*	*0.285*
Ppu						0.788	0.984	0.598	0.704	0.299
Pcu							0.754	0.935	0.965	0.818
Bca								0.529	0.631	0.272
Bbu									0.984	0.898
Hme										0.835

The italicized values indicate the overlap index of *D. pictus* with all the other species. The highest values in bold

Ssa: *Salamandra salamandra*; Tma: *Triturus marmoratus*; Lhe: *Lissotriton helveticus*; Aob: *Alytes obstetricans*; Dpi: *Discoglossus pictus*; Ppu: *Pelodytes punctatus*; Pcu: *Pelobates cultripes*; Bca: *Bufo calamita*; Bbu: *Bufo bufo*; Hme: *Hyla meridionalis*; Ppe: *Pelophylax perezi*

interspecific differences in survivorship were almost significant. *Bufo calamita* suffered high mortality in the presence of *D. pictus*, whereas *P. punctatus* and *D. pictus* had a lower reduction in survivorship in the presence of other species ($F_{3,12} = 3.472$; $P = 0.051$) (Fig. 5a). A similar pattern was observed in the other traits measured: *B. calamita* reduced its activity when competing with the non-native species, whereas the activity of the other species was affected less by the

presence of individuals of other species ($F_{3,12} = 28.712$; $P < 0.001$) (Fig. 4b). Other negative effects of interspecific competition on *B. calamita* were a longer larval period, a smaller size at metamorphosis and slower growth (Table 2b; Fig. 5b–d). *B. calamita* suffered stronger effects than the other two species, demonstrating the poor competitiveness of this species and the asymmetry of competition in this system.
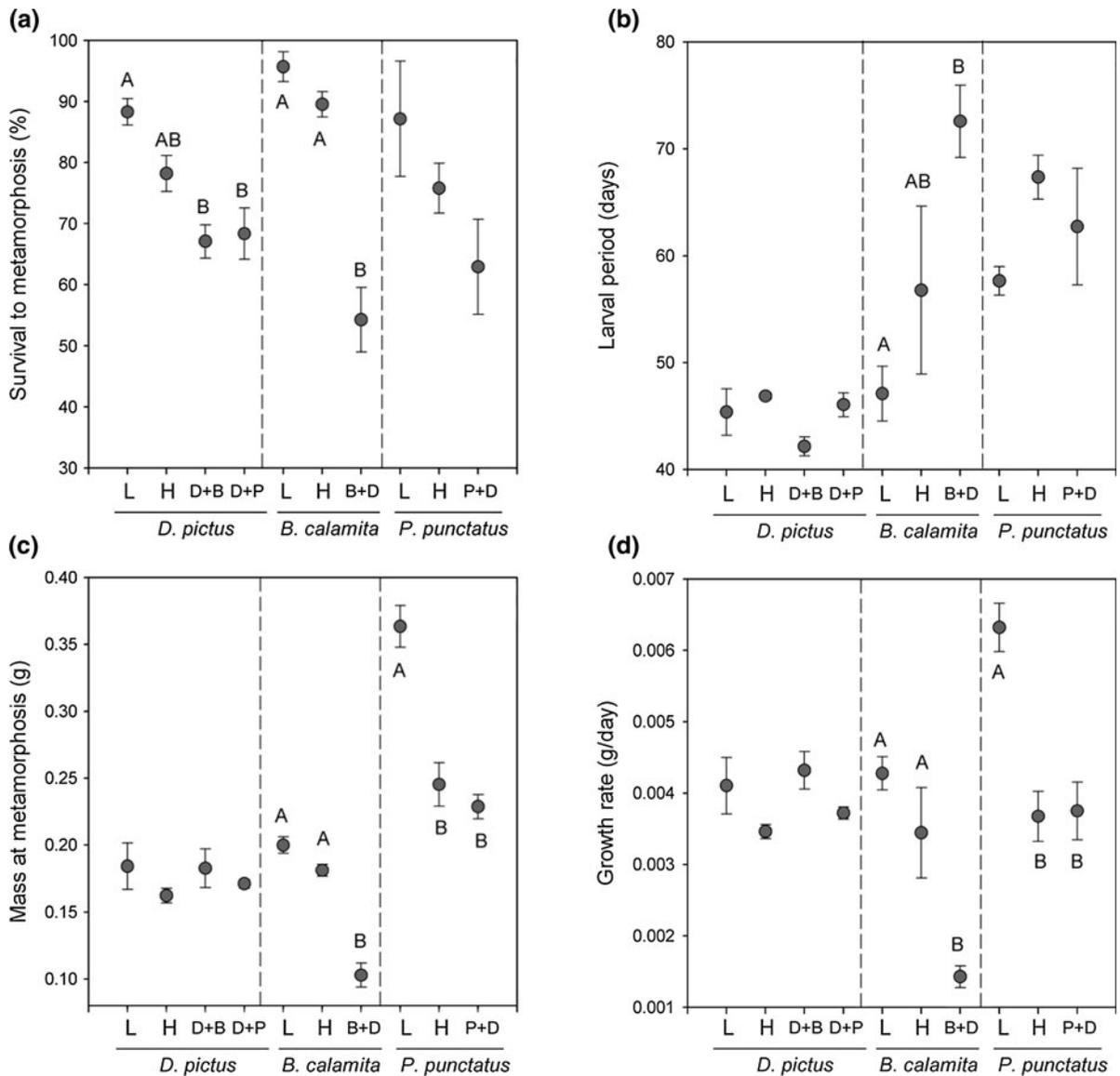
Fig. 3 Mean response and standard error for each species to intra- and interspecific competition: **a** survival to metamorphosis, **b** larval period, **c** mass at metamorphosis, **d** growth rate. Intraspecific interactions: *L* low density, *H* high density; Interspecific interactions: D + B = *Discoglossus pictus* with *Bufo calamita*, D + P = *D. pictus* with *Pelodytes punctatus*,

B + D = *B. calamita* with *D. pictus*, P + D = *P. punctatus* with *D. pictus*. *Labels A* and *B* indicate Scheffé post hoc differences between treatments. The post hoc differences are between treatments with *different letters*. The *absence of labels* indicates no differences in the post hoc analysis

Species consumption rate estimation

The results of the second experiment showed that the species differ in their consumption rates ($F_{2, 62}$ = 180.52; $P > 0.001$). The consumption rate of *D. pictus* was significantly higher than that of *P. punctatus*. The consumption rate of *B. calamita* was intermediate between the other two species (Fig. 6).

Analysis of the community structure in the invaded and non-invaded areas

The community co-occurrence patterns differed between the non-invaded area and the invaded area. In the non-invaded area, the C-score was significantly higher than expected by chance, with a standardized C-score of 3.43, whereas in the invaded area it fell to
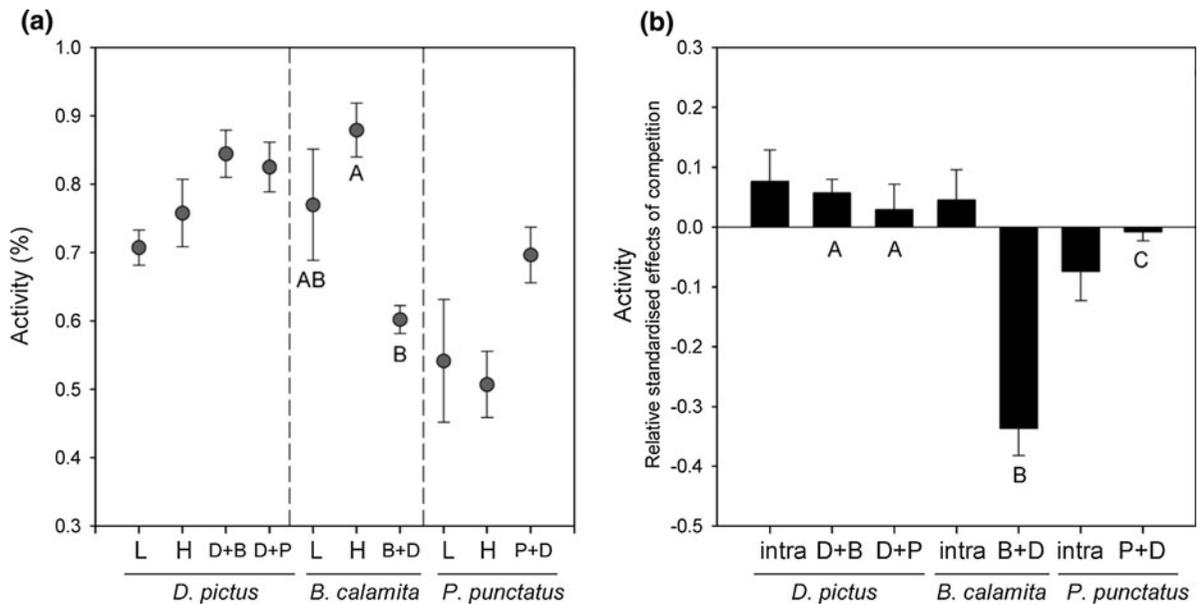
Fig. 4 Mean and standard error of the relative effect of intra- and interspecific competition for each species: **a** activity as proportion of tadpoles swimming or feeding. *Labels A* and *B* indicate Sheffé post hoc differences between treatments,

b relative effect of competition to activity. *Labels A*, *B* and *C* indicate Scheffé post hoc inter-specific differences. *Intra* intraspecific interaction. See Fig. 3 for abbreviations

1.18 and did not differ significantly from random. The analysis of variance of the co-occurrence index between the two areas showed an observed variance index larger than expected by chance (observed variance index = 154,903.10; simulated variances = 102,780.30; P (obs > expected) = 0.036) (see "Materials and methods" section). Species segregation was larger in the non-invaded area than in the invaded area for almost all the species, although the overlap differences between areas were less than the standard error for each area. The results indicate that the two regions are not significantly different, but there is a tendency for species segregation to decrease in the presence of the non-native species (Table 3).

These differences in species segregation between the invaded and non-invaded area were also reflected in a change of pond use by species, especially *B. calamita* and *P. punctatus*. Both species showed a reduction in use of ephemeral ponds in the invaded area with respect the non-invaded area (from 58.97 to 50 % for *B. calamita* and from 51.35 to 36.36 % for *P. punctatus*). Otherwise, use of temporary ponds increased in the invaded region (from 28.21 to 42.31 % for *B. calamita*, and from 32.43 to 57.58 % for *P. punctatus*). Both species showed significant

differences in habitat preferences between regions (*B. calamita*: $\chi^2 = 11.591$; $P = 0.003$; *P. punctatus*: $\chi^2 = 9.329$; $P = 0.009$).

Discussion

The invasion process has been divided into several stages that the non-native species needs to complete to succeed in its invasion (Van Kleunen et al. 2010; Blackburn et al. 2011). According to the unified framework described by Blackburn et al. (2011), *D. pictus* has reached the spread stage by overcoming all the barriers and should be referred to as an invasive species. The present study demonstrates that the establishment of *D. pictus* in its introduced range alters the native amphibian communities, possibly by increasing competition at the larval stage.

Larval amphibian communities are structured by an ecological gradient of habitats defined by water duration, competition and predation risk (Semlitsch et al. 1996; Wellborn et al. 1996; Pechmann et al. 1989, 2001). These traits of breeding-ponds, which determine the success of several species across the hydroperiod gradient, affect species in different ways

Table 2 Multivariate analyses (MANOVA and ANOVA tests) of the effects of competition using period, mass and growth as explanatory variable (Experiment I)

Species	Source	MANOVA			ANOVA		
		Wilks' λ	df	P	F	df	P
a.							
Discoglossus pictus		0.2905	9	0.1188			
	Period				2.69	3,12	0.0934
	Mass				0.78	3,12	0.5272
	Growth				2.44	3,12	0.1149
Bufo calamita		0.0651	6	**0.0015**			
	Period				5.23	2,9	**0.0311**
	Mass				56.54	2,9	**<0.0001**
	Growth				13.45	2,9	**0.0020**
Pelodytes punctatus		0.0793	6	**0.0029**			
	Period				2.12	2,9	0.1759
	Mass				27.39	2,9	**0.0001**
	Growth				16.99	2,9	**0.0009**
b.							
Intraspecific effects		0.0929	6	**0.0048**			
	Period				0.85	2,9	0.4578
	Mass				14.59	2,9	**0.0015**
	Growth				2.35	2,9	0.1507
Interspecific effects		0.0877	9	**0.0011**			
	Period				10.93	3,12	**0.0010**
	Mass				20.40	3,12	**<0.0001**
	Growth				25.78	3,12	**<0.0001**

(a) effects of competition on each species, (b) intra and interspecific effects. In bold there are the P values of the significant tests

in a trade-off (Smith 1983; Wellborn et al. 1996; Snodgrass et al. 2000; Van Buskirk 2005; Richter-Boix et al. 2007b). Our results indicate that each species shows a preference for a certain habitat for breeding, even though they do not breed exclusively in a given pond type. Moreover, the species show high niche overlap indices and the community's use of breeding ponds follows a random pattern. The highest niche overlap indices were between *D. pictus* and the two native species *B. calamita* and *P. punctatus*; all three species showing a preference to breed in ephemeral ponds. These results agree with those of previous studies performed in the invaded region (Montori et al. 2007; Escoriza and Boix 2012).

Several empirical studies demonstrate that increased activity leads to greater risk of predation (Skelly 1994; Anholt and Werner 1995; Eklöv and Halvarsson 2000). The high activity levels and foraging rates of *D. pictus* observed in the laboratory experiments fit with its preferential occupation of ephemeral and short-term temporary ponds, which usually have very few aquatic predators. Activity levels reflect the differences in habitat type across the hydroperiod gradient (Woodward 1983; Skelly 1996; Richardson 2002; Richter-Boix et al. 2007b). Other studies show that ephemeral-pond-breeding species are not only more susceptible to predators, but are less competitive than temporary or permanent pond species (Heusser 1972a, b; Griffiths 1991; Laurila 2000; Richter-Boix et al. 2007c). In contrast, experimental studies found that species such as *Bufo terrestris* and *Scaphiopus holbrookii*, which breed in habitats with very short hydroperiods, are extremely active and competitively dominant species in the absence of predators (Morin 1983). In a competitive hierarchical scenario such as the system studied (Richter-Boix et al. 2007c), inferior competitors rely on the space left empty by superior competitors (Wu and Levin 1994;
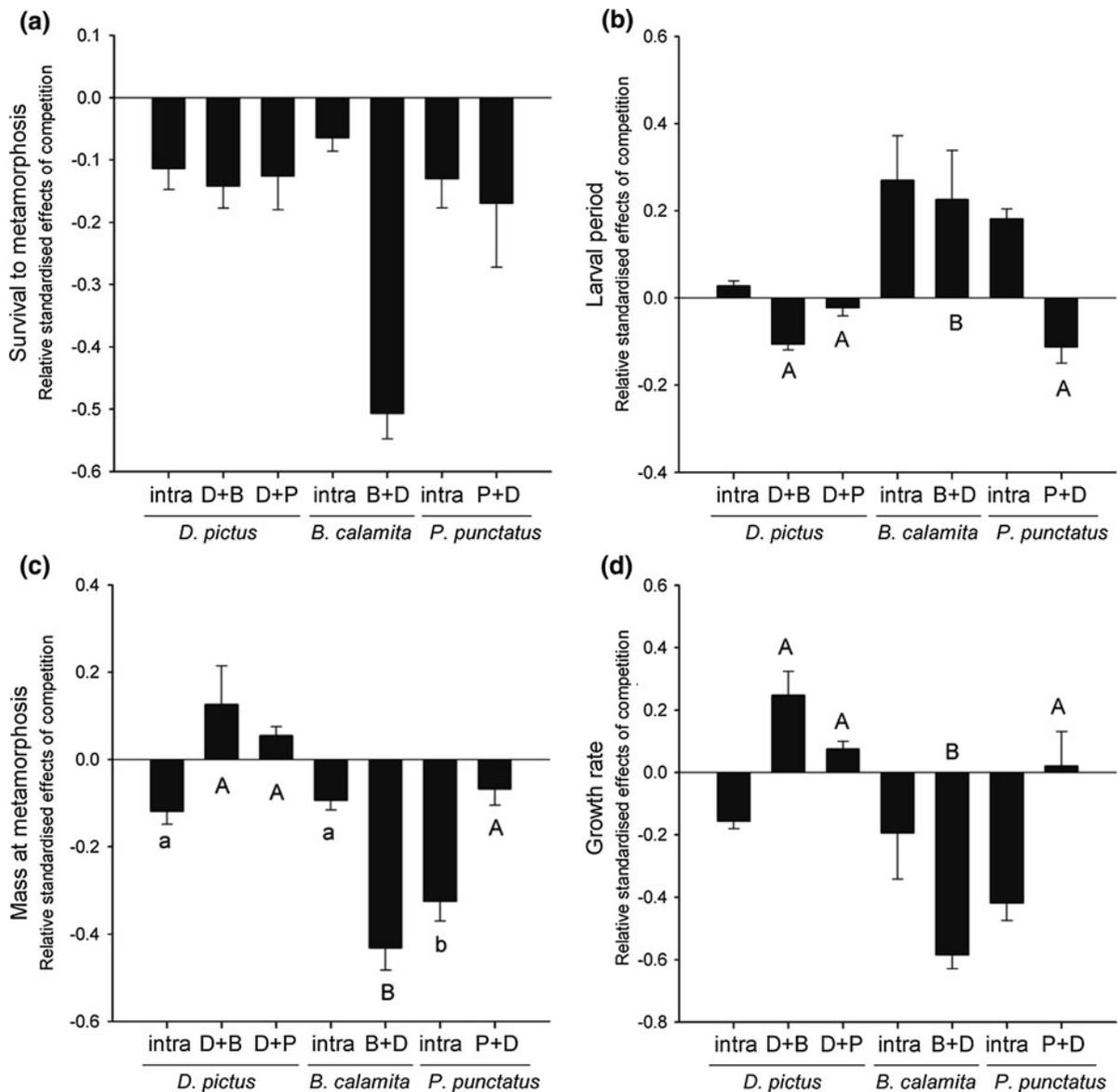
Fig. 5 Standardized mean response and standard error of each species to intra- and interspecific competition: **a** survival to metamorphosis, **b** larval period, **c** mass at metamorphosis, **d** growth rate. *Intra* intraspecific interaction. See Fig. 3 for abbreviations. *Labels A*, *B*, *a* and *b* indicate differences in the

Scheffé post hoc analysis between species. *Different capital letters* indicate differences in inter-specific response. *Different lower case letters* indicate differences in intra-specific response. The *absence of labels* indicates no differences in the post hoc analysis

Keymer et al. 2000). Species that breed in ephemeral ponds avoid the predators and high levels of competition found in ponds with longer hydroperiods (Smith 1983; Richter-Boix et al. 2007a, c), as is the case with *B. calamita* and *P. punctatus*, which are less competitive than species that breed in temporary and permanent ponds (Richter-Boix et al. 2007a). The findings of

our competition experiments demonstrate that competition between *D. pictus* and *B. calamita* is strongly asymmetric in fitness-related parameters, as observed in other competition studies (e.g. Morin and Johnson 1988; Griffiths 1991; Gurevich et al. 2000). However, competition between *D. pictus* and *P. punctatus* is symmetric, and if the competition hierarchy remains
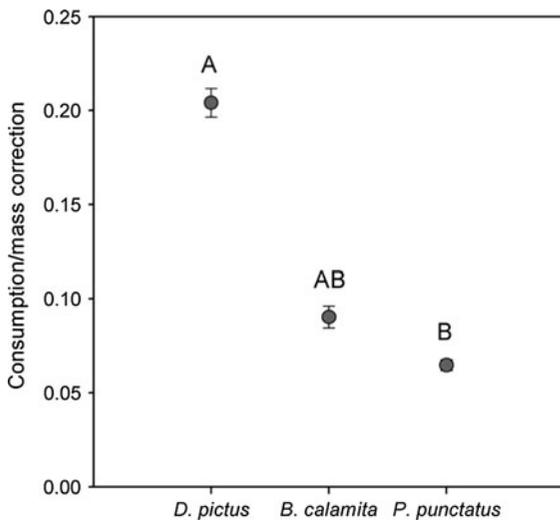
Fig. 6 Mean and standard error of intra- and interspecific competition for each species to differential consumption rate for each species. *Labels A and B* indicate differences in the post hoc analysis between species

unchanged within the community, we expect *D. pictus* to be less competitive than the other species in the community studied (Richter-Boix et al. 2007a). Its lower competitiveness compared to species that breed in temporary and permanent ponds could be one of the reasons why it is restricted to ephemeral ponds.

Amphibian species within each pond where they co-occur may well be able to select different micro-habitats and thus minimize the resource use overlap. Therefore, the spatial overlap observed by the authors could be completely different at the within-pond scale. Despite the evidence that community assembly rules can be scale-dependent (Gotelli and Ellison 2002; Sanders et al. 2003), null model community tests are typically assessed at a single scale (Burns 2007). A study including two spatial scales, such as species co-occurrence among ponds and species co-occurrence

among micro-habitats within ponds, would be a perfect complement to develop in the future and to improve our understanding of how communities are organized and the possible consequences of species introduction, which is the focus of the present study.

A recent theoretical model based on metacommunity concepts suggests that environmental heterogeneity increases invasiveness and reduces the impact on native species in the community, because it promotes invasion and coexistence mechanisms that are not possible in homogeneous environments (Melbourne et al. 2007). Similarly, the fluctuating resource hypothesis developed by Davis et al. (2000) predicts that a community's susceptibility to invasion is not a static attribute but a condition that fluctuates over time depending on pronounced fluctuations in resource availability. In our study areas, the availability of breeding ponds varies from year to year, especially the ephemeral and temporary rain pools whose creation and permanence depend on the weather. In particularly wet years, temporary environments experience a burst of resource supply because of the increase in hydroperiod and the number of aquatic habitats. During such wet years, *D. pictus* may have access to more resources and have greater success invading and persisting in the community, especially if it does not encounter intense competition for those resources from native species. Temporal fluctuation of breeding and larvae environments, high efficiency in resource consumption and asymmetric competition with its direct competitor, *B. calamita*, allow *D. pictus* to invade and persist in the new community and may prevent competitive exclusion from occurring.

Although the presence of non-native species may alter the properties of the system, the invasion and exploitation of "competitor-free" ephemeral ponds by *B. calamita* and, to a lesser degree, by *P. punctatus*,

Table 3 Mean overlap index and Standard Error by species, for both regions compared and the difference between them. Almost all the species show an increase of the overlap in the invaded area

Species	Non-invaded area	Invaded area	Niche overlap difference
Alytes obstetricans	0.784 ± 0.067	0.751 ± 0.110	−0.033
Pelodytes punctatus	0.470 ± 0.125	0.544 ± 0.083	0.074
Pelobates cultripes	0.668 ± 0.165	0.822 ± 0.077	0.153
Bufo calamita	0.434 ± 0.130	0.528 ± 0.086	0.094
Bufo bufo	0.784 ± 0.109	0.787 ± 0.094	0.003
Hyla meridionalis	0.751 ± 0.114	0.819 ± 0.087	0.068
Pelophylax perezi	0.671 ± 0.163	0.738 ± 0.120	0.067

could displace or reduce the abundance of native species in these breeding habitats and thus modify the organization of the community. Our results suggest that native amphibian larvae communities could change from the pre-invasion structured co-occurrence pattern to a more random pattern after the arrival of the non-native species. This shift may be produced because the species that overlap most frequently with the invasive species showed the greatest increase in niche overlap indices in the invaded area. Both *B. calamita* and *P. punctatus* show a displacement in the breeding habitat from ephemeral pools, previously free of competitors and with relatively few resources, to other richer environments but with superior competitors (see "Results" section and Table 3).

These results should be treated with caution, as they were obtained without considering pre-invasion data for the invaded area. Factors other than the presence of *Discoglossus pictus* could explain differences in the community structure between regions [e.g. quantity and quality of breeding habitats and quality of terrestrial matrix (Richter-Boix et al. 2007a)]. Our findings demonstrate one mechanism by which the non-native species *D. pictus* may have altered community structure. In spite of the lower larval survival of *D. pictus* in the presence of both intraspecific and interspecific competition, the other sympatric species in ephemeral ponds showed greater competition effects (especially *B. calamita*). This interspecific asymmetric competition could make it harder for *B. calamita* to reach metamorphosis, especially in water-stress scenarios. Another possible effect could be the earlier breeding of *B. calamita* in ephemeral ponds, as observed in some newly created ponds near the study area (unpublished data). Moreover, there are other potential competitors of anurans, although in the terrestrial phase, such as newts, which can also influence the community structure; however, our study only focused on the larval phase.

In order to document changes in community structure in the future, community organization should be compared before and after invasion in areas at the present limit of the distribution range of *D. pictus*. A previous study of the Argentine ant (*Linepithema humile*) in North America structured over several consecutive years demonstrated that the invasive species caused rapid and drastic changes in ant community structure (Sanders et al. 2003). Other studies show that both the extinction (Hecnar and

M'Closkey 1997) and introduction (Pearl et al. 2004) of bullfrogs (*Lithobates catesbeiana*) have an effect on the structure of amphibian communities. Similarly, a 14-year study documents the strong effects of competition from the invading fish species vendace (*Coregonus albula*) on the native European whitefish (*C. lavaretus*) whose population density reducing by more than 90 % (Bøhn et al. 2008).

As the invasion of *Discoglossus pictus* is a dynamic process and the amphibian community structure in the area is well documented, we have a valuable opportunity to test hypotheses and make predictions. In a few years, we will be in a position to verify the effects of *D. pictus* on the native amphibian community into which it is currently expanding, and especially on the sympatric species in ephemeral ponds.

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