

Research Article

Differential trophic traits between invasive and native anuran tadpoles

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Abstract

How trophic resources are managed is a key factor in our understanding of the success of invasive species. In amphibians that usually occupy ephemeral ponds, the capacity to acquire resources and food selection are especially important because as a pond dries, the larval density increases and food resources are limited. Abundant and high-quality food can increase the final size and reduce the duration of development of amphibians. The aim of this work was to assess the trophic traits of tadpoles of the invasive (originally North African) anuran *Discoglossus pictus* compared to those of native European *Epidalea calamita* tadpoles under laboratory conditions. Food of two different levels of quality was supplied, and the feeding activity and food preference of the two species were analysed alone and in co-occurrence. *D. pictus* was capable of modifying its behaviour and food preferences; while *E. calamita* displayed much milder differences between treatments. Both alone and in co-occurrence with the native species, the invasive tadpoles obtained higher feeding activity values and showed a stronger preference for high-quality food. Additionally, when high densities of the two species shared food resources, the feeding activity results indicated potential displacement of the native tadpoles to low-quality resources. *D. pictus* thus presents trophic traits that are favourable for invasion and could limit the fitness of *E. calamita* when resources are limited or there is a risk of pond desiccation.

Key words: diet quality, feeding activity, food preference, *Discoglossus pictus*, invasiveness, anuran tadpoles

Introduction

Invasion by alien species is one of the most important threats to the balance of ecosystems and community structure, and the second most significant cause of biodiversity loss on the global scale (Levine et al. 2003). Approximately ten new species of living organisms become established in Europe each year (Hulme et al. 2009); however, only a small fraction of introductions (20%–30% worldwide) result in invasion (Pimentel et al. 2001). The success of a species in a novel environment is likely to depend on several factors, including the abiotic physical environment, biological interactions and the traits of the introduced

species (Shea and Chesson 2002; Duncan et al. 2003; Blackburn et al. 2009). The availability of resources and the capacity of an invasive species to exploit them are critical factors for its survival and reproduction (Thébaud et al. 1996; Tilman 2004).

When an alien amphibian species is introduced, its efficiency at exploiting the available resources during larval stages can be a key factor for the successful establishment of the species and may therefore define its invasiveness, because of the vulnerability during this phase and the crucial effect of food on the development of tadpoles and the subsequent development of toadlets (Werner 1986; Scott 1994; Tejedo et al. 2000). The larval period is considered the most vulnerable phase for amphibians since it is when

the highest mortality rate is registered, especially for species that occupy temporary ponds (Calef 1973; Wilbur 1980; Newman 1987; Denver 1998). On the other hand, the effects of diet on the breeding success and metamorphic fitness of amphibians have been repeatedly documented (Kupferberg 1997; Babbitt et al 2000; Álvarez and Nicieza 2002; Jefferson 2014). Both food quantity and food quality are crucial for their development: tadpoles cannot fully develop under desiccated conditions when food is scarce (Enriquez-Urzelai et al. 2013), or low in protein content (Nathan and James 1972; Steinwascher and Travis 1983; McCallum and Trauth 2002; Richter-Boix et al. 2007). Moreover, when food is abundant or highly proteic, tadpoles are capable of accelerating their growth and development (Gotthard and Nylin 1995; Morey and Reznick 2000; Lind and Johansson 2007). Therefore, optimal exploitation of available resources by tadpoles ensures fast growth and high performance, thus favouring the establishment and spread of introduced amphibian species.

Discoglossus pictus auritus (Otth, 1837) is an anuran species introduced from Algeria to Banylus-sur-Mer (France) around 100 years ago (Wintrebert 1908). Its population is currently increasing and its range expanding annually to areas of north-eastern Spain and south-eastern France (Martens and Veith 1987; Montori et al. 2007; Franch et al. 2007). It mostly occupies ephemeral wetlands that are characterized by their low species richness and the low occurrence of potential predators. At the local level, the use of such ponds can be an advantage for the success of this species in the invaded area because in this way it avoids competition and predation risks. However, at the community level, ephemeral pond use leads to spatial limitations on larval development derived from inevitable pond drying (Babbitt et al. 2000; Newman 1987). Traits that favour short developmental periods and high growth rates improve success in colonising this type of ponds.

Often *D. pictus* shares breeding ponds with the native species *Epidalea calamita* Laurenti, 1768. Ephemeral freshwater environments are closed systems with limited resources that intensify interactions between organisms (Wilbur 1980). Competitive superiority in direct interactions or better exploitation of available resources can increase the survival or fitness of one species over that of another when the two share such environments. The greater capacity of the introduced species to take up and use nutrient resources in ephemeral ponds, is a positive biological trait for

its establishment and spread, despite the shared habitat. Both *D. pictus* and *E. calamita* exhibit explosive breeding characterized by a high number of eggs per clutch, which leads to high densities of tadpoles in ponds and this intensifies their interactions. Also, *D. pictus* and *E. calamita* present high similarities in the exploitation of their trophic niche, both in larval morphology and from the analysis of digesta (Díaz-Paniagua 1985; Escoriza and Boix 2012; Richter-Boix et al. 2012; San Sebastián et al. 2015).

The aim of this study is to evaluate the capacity of the invasive species (*D. pictus*) to exploit resources, and compare this with the native species. The capacity to exploit resources is evaluated via two measurements: the percentage of tadpoles feeding (feeding activity) and rate of selection of high-quality food (food preference). In order to obtain these measurements, we designed two experiments. The first evaluated whether the invasive species adopts a better strategy to exploit nutrient resources than the native species when they do not co-occur. The second analysed the capacity of the two species to exploit resources in aquariums shared at low and high densities. We expect greater success in the wild of the amphibian species that better exploits high quality resources and/or has a higher activity rate.

Methods

Animals and rearing conditions

Two experiments were conducted in different years (2011 and 2013) but with the same experimental laboratory conditions of temperature (19°C–20°C) and photoperiod (12D:12L). The tadpoles used in each experiment were collected as eggs on the 24th and 25th March 2011 (*D. pictus* and *E. calamita*, respectively) and on the 8th April 2013, from 3–4 clutches for each species and year, near to Girona (northeast Spain). The same procedure was followed in each experiment. Clutches of each species were transported to the laboratory in separate containers of dechlorinated water. Eight days after hatching, the tadpoles were mixed together to avoid genetic biases and were then held in plastic containers. The tadpoles were reared and fed with rabbit food until the start of the experiment. The experiments started on 5th April 2011 and 10th April 2013 (day 0) when the tadpoles reached Gosner stage 25 (Gosner 1960). All the individuals were released to their original location after the experiments finished.

Experimental design

Experimental units consisted of 30 L tanks containing dechlorinated water ($1.2 \times 0.45 \times 0.40$ m). The tadpoles were randomly allocated to the experimental treatments. The water was changed every two days to prevent eutrophication and the accumulation of lethal levels of metabolic products. We used two food types in the feeding trials that differed in composition: (LP) a commercial rabbit food, which has a low-protein and high-carbohydrate content (16% protein, 3% lipids, 17% carbohydrates, 10% ash); and (HP) the commercial fish food Sera O-nip, which is rich in protein (46% protein, 22% lipids, 2% carbohydrates, 9% ash). Food was supplied *ad libitum* during the experiments. In all the treatments and experiments, the food was available in two small Petri dishes placed randomly in the corners of the containers to avoid learning effects and ensure selection by tadpoles according to diet quality.

The first experiment on the differential selection of diet quality by *D. pictus* and *E. calamita* consisted of three treatments for each species, each replicated six times. All the containers had the same density of tadpoles (10 larvae per container). To evaluate the dietary selection of each species, there were two treatments with one food type (LP or HP) and one treatment with both food types (HLP) for each species: *D. pictus* with HP food (DHP), LP food (DLP), and HP + LP food (DHLP); and *E. calamita* with HP food (BHP), LP food (BLP), and HP + LP food (BHLP).

The second experiment involved two treatments, both containing HP and LP food, and tadpoles at either low density (LD) or high density (HD). The LD treatment consisted of a total of 10 tadpoles, at the same density as in the first experiment, but with five larvae of *D. pictus* and five larvae of *E. calamita*. The HD treatment was designed to increase potential interactions between the species in a more realistic scenario, with 20 larvae of *D. pictus* and 20 larvae of *E. calamita*.

Response variables

Throughout the experiments, we checked each tank daily and we fed the tadpoles with variable frequency to ensure *ad libitum* feeding conditions. For each tank, we checked tadpole behaviour once 5 minutes after food administration, to avoid potential disturbances after dish manipulation. The average number of registers was 16 per aquarium in the first experiment and 23 in the

second experiment (more details in Appendix 1). We stopped collecting activity data when 30% of the larvae in the aquarium underwent metamorphosis, to avoid recording behavioural changes as a consequence of reduced tadpole density.

The resource exploitation capacity of *D. pictus* and *E. calamita* were analysed via two variables: feeding activity and food preference. Feeding activity is considered to be a measure of the capacity a species has for resource exploitation, and thereby of competitive capacity (Smith et al. 2004); while food preference helped us to determine whether the tadpoles of each species followed indiscriminate or random foraging, or had a preference for one of the food types.

Feeding activity was calculated as the number of tadpoles feeding (in dishes with HP food, dishes with LP food or two types of food HLP) divided by the total number of tadpoles in each aquarium and then expressed as a percentage, as observed at first glance (Peacor and Werner 1997; Relyea 2002; Pujol-Buxó et al. 2013). Food preference was estimated as the proportion of tadpoles consuming each kind of food divided by the total number of tadpoles in feeding attitude in treatments with both types of food: HP and LP. While feeding activity was calculated including zero activity records, to estimate food selection, we only used counts with tadpole activity higher than zero.

Statistical analysis

Firstly, we compared feeding activity between the species and treatments, and tested their interaction. Then feeding activity in each treatment and food type was explored between species and within the same species. Secondly, the food preference was analysed for each species only in treatments with two types of food (HLP in the first experiment, and LD and HD in the second experiment). Given the presence of a certain amount of zero values and that most assumptions needed to perform classic parametric or non-parametric analyses of variance were not met (McElduff et al. 2010), differences in feeding activity between treatments, species and food types were explored via non-parametric randomization ANOVA (NP-R-ANOVA). We used percentages as the dependent variable and tested the differences applying the function *aovp* from the package *lmPerm* (Wheeler 2010). Potential random variations between aquariums were considered including aquarium as an error in the model. The function *aovp()* assumes a model of the form: $Y = Xb + Zg + e$, where X is the incidence matrix for fixed effects,

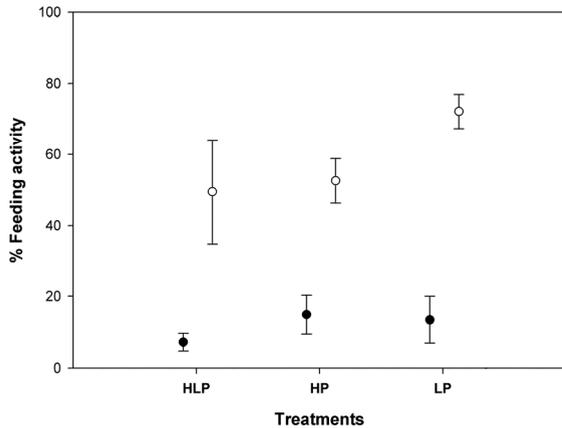


Figure 1. Mean (\pm SE) feeding activity of *D. pictus* (white circle) and *E. calamita* (black circle) in each treatment.

and Z is the incidence matrix for random effects, with columns representing the different error strata. The algorithm projects Y into strata creating a single error term in each case while X is also projected so that the model in this stratum becomes $P(Y)=P(X)bi+ei$. This way the model accounts for the lack of independence of the data within each experimental unit.

Our p and F values were calculated after 1,000 permutations, and the level of significance was set at values of p lower than 0.05.

For the food preference analysis, we excluded records with no activity and used only the percentage of feeding larvae in the HP treatment (real distribution). In this case, we generated a new distribution (simulated distribution) that represented our null hypothesis of non-preference for both types of food. This simulated data was generated using a binomial distribution with a "success" probability of 0.5 (that is, an average of 50% tadpoles in each side as null hypothesis: given no preference, half of the active larvae should be eating HP food and the other half LP food) with the standard deviation of the real distribution for each species. This probability was applied on a simulated number of active tadpoles (=events) which followed a Poisson distribution with the same parameters of the real distribution. We simulated the same number of null hypothesis data as the number of real data, and then differences between these two distributions were tested using the same procedures as applied to feeding activity. All the analysis were conducted in R (Development R Core Team 2013).

Results

Experiment 1: Differential food selection by D. pictus and E. calamita

Feeding activity: species, treatments and diet quality

We found differences in feeding activity between the species ($F_{1,29}=337.92$ $p<0.001$), treatments ($F_{2,29}=7.95$ $p=0.001$) and an interaction between the two ($F_{2,29}=6.87$ $p<0.005$). *D. pictus* showed higher feeding activity than *E. calamita* in treatments with one ($F_{DHP-BHP\ 1,176}=124.46$ $p<0.001$; $F_{DLP-BLP\ 1,203}=319.03$ $p<0.001$) and two types of food ($F_{DHLP-BHLP\ 1,151}=41.76$ $p<0.001$). The feeding activity of *D. pictus* ranged from 25% to 72%, while that of *E. calamita* did not exceed 20% in any treatment.

Within species, feeding activity levels varied between treatments. *D. pictus* showed significantly more activity in tanks with LP food (DLP) than in DHP ($F_{1,166}=38.31$ $p<0.001$) or DHLP treatments ($F_{1,161}=14.43$ $p<0.005$), with no significant difference between DHP and DHLP ($F_{1,151}=0.33$ $p=0.5$) (Figure 1). When we compared the feeding activity on each type of food between treatments, we obtained similar results. There were no significant differences in the feeding activity on HP food between the DHP and DHLP treatments ($F_{1,151}=5.18$ $p<0.05$). However, the feeding activity of tadpoles on the LP food was lower in the DHLP treatment than in the DLP treatment ($F_{1,161}=697.50$ $p<0.001$). Although *D. pictus* showed higher activity in the DLP treatment ($72\% \pm 4.8\%$), when it had two types of diet available (DHLP) it showed $41\% \pm 12\%$ feeding activity on HP food and $9\% \pm 3\%$ on LP ($F_{1,166}=38.30$ $p<0.001$).

The feeding activity of *E. calamita* was low in all treatments (Figure 1). Levels of activity were similar in the BHP and BLP treatments ($F_{1,213}=0.14$ $p=0.7$), and in the BHLP and BLP treatments ($F_{1,193}=5.80$ $p=0.05$). However, we observed higher feeding activity in the BHP treatment than in the BHLP treatment ($F_{1,176}=9.68$ $p=0.01$) (Figure 1). When we compared feeding activity on each type of diet, we detected differences between feeding activity on HP food between the BHLP and BHP treatments ($F_{1,176}=19.17$ $p=0.001$), and on LP food between the BHLP and BLP treatments ($F_{1,193}=14.64$ $p<0.005$). The percentage of *E. calamita* tadpoles eating HP food in the BHLP treatment was lower than in BHP, being $5\% \pm 1\%$ and $15\% \pm 5\%$ respectively. Also, the BHLP treatment registered lower values of feeding activity on LP food than the BLP treatment. The

BHLP treatment showed $3\% \pm 2\%$ of tadpoles eating LP food while BLP registered $13\% \pm 6\%$. In the BHLP treatment, the native species showed similar feeding activity on the different diets ($F_{1,213}=0.14$ $p=0.7$).

Food preference

D. pictus showed preferential selection in treatments with two types of food ($F_{1,133}=47.37$ $p<0.001$). On average, 81% of tadpoles were observed feeding on HP food, and only 19% on LP food. However, *E. calamita* displayed no food preference in this treatment ($F_{1,58}=1.31$ $p=0.5$), with 60% feeding on HP food and 40% on LP (Figure 2).

Experiment 2: Competitive or opportunistic strategy by *D. pictus* co-occurring with *E. calamita* (LD and HD)

Feeding activity: species, treatments and diet quality

When the species co-occurred in the same environment, *D. pictus* showed higher feeding activity in both the LD and HD treatments ($F_{LD\ 1,255}=32.04$ $p<0.001$; $F_{HD\ 1,303}=97.13$ $p<0.001$) (Figure 3). Moreover, in both treatments, the feeding activity of *D. pictus* was higher on the HP food ($F_{LD\ 1,255}=33.60$ $p<0.001$; $F_{HD\ 1,303}=206.2$ $p<0.001$). Although feeding activity on the LP food was similar for the two species at LD, ($F_{1,255}=0.75$ $p=0.4$), higher *E. calamita* feeding activity was recorded on this food at HD ($F_{1,303}=5.36$, $p=0.02$) (Figure 3).

Density had a significant effect on the feeding activity of the two species (*D. pictus*: $F_{1,274}=12.16$, $p=0.005$; *E. calamita*: $F_{1,274}=27.76$, $p<0.001$), which was higher under HD conditions than LD (Figure 3). Analysis of the type of food and species revealed that increased density provoked an activity gain of 8% on HP food ($F_{1,274}=8.04$ $p=0.01$) and 3% on LP food ($F_{1,274}=20.03$ $p=0.001$) for *D. pictus*; and a gain of only 1% on HP food and 6% on LP food for *E. calamita*. The proportion of *E. calamita* larvae feeding on the HP food did not vary between density treatments ($F_{1,274}=0.41$ $p=0.5$); whereas for the LP food there was an increase in feeding activity in HD conditions ($F_{1,274}=72.819$ $p<0.001$).

Differences in feeding activity between types of diet were evident for *D. pictus* ($F_{LD\ 1,255}=67.76$ $p<0.001$, $F_{HD\ 1,303}=253.34$ $p<0.001$). At all densities the invasive species showed higher feeding activity on HP food than on LP (LD: 18% vs. 2%; HD: 26% vs. 5%). However, the native *E. calamita* only showed higher feeding activity on HP food compared to LP food under LD conditions ($F_{1,255}=17.14$ $p<0.001$; 6% vs. 1%). In the HD

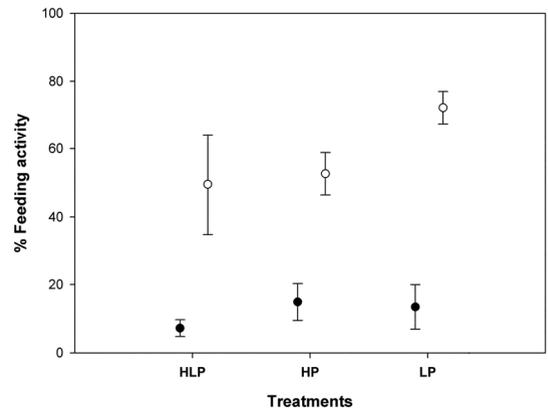


Figure 2. Mean (\pm SE) food preference for both species in treatments with two types of food. *D. pictus* (white circle) and *E. calamita* (black circle).

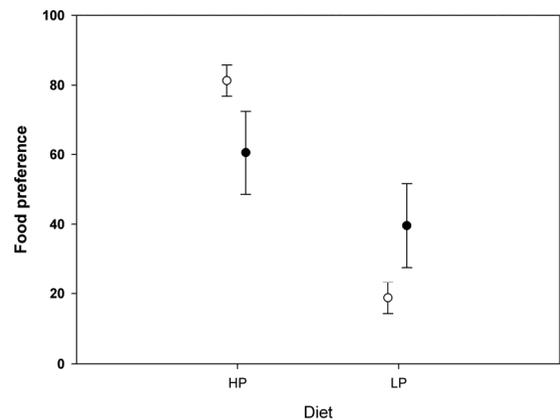


Figure 3. Mean (\pm SE) feeding activity of *D. pictus* (white circle) and *E. calamita* (black circle) at each density (LD or HD) and quality of diet (LP or HP).

conditions, the feeding activity of *E. calamita* on the two diets was similar ($F_{1,303}=0.65$ $p=0.42$; 8% LP food vs. 7% HP food).

Food preference

In this experiment we detected a preference for type of food by *D. pictus*. More active *D. pictus* chose HP food than LP food in the LD ($F_{1,135}=42.95$ $p<0.001$) and HD treatment ($F_{1,277}=224.62$ $p>0.001$). A total of 88% and 85% of active larvae of *D. pictus* selected HP food over LP, in the LD and HD treatments respectively. Despite the level of activity recorded for *E. calamita*, it showed no dietary selection in terms of quality of diet. The preference for HP or LP food was similar under LD ($F_{1,67}=1.75$ $p=0.3$) and HD ($F_{1,243}=0.65$ $p=0.7$) conditions. Sixty-one per

cent of active *E. calamita* larvae were observed feeding on HP food under LD and 51% under HD conditions.

Discussion

The present study suggests that trophic traits could favour the success of *D. pictus* in the invaded area. *D. pictus* presented higher feeding activity and selection of high-quality food resources, contrasting with the lower feeding activity and apparent non-food selection of the native species either when co-occurring or when alone. As found in other species (Kupferberg 1997; Niecieza and Álvarez 2002) the capacity of amphibian species to exploit resources could predict fast development, high reproductive success and the increased fitness of its metamorphs; especially advantageous for invasive species and with potential negative effects for less competitive species.

Resource exploitation capacity of the invasive and the native species

It is widely documented, in both the field and the laboratory, that food quality is of primary importance in mediating interspecific differences in amphibian reproductive success and performance (for a review see Kupferberg 1997). These effects have been confirmed in *E. calamita* and in *Discoglossus galganoi* (Capula, Nascetti, Lanza, Bullini and Crespo 1985) (in Griffiths et al. 1993; Niecieza and Álvarez 2002; Martins et al. 2013), among other species (Schiesari 2006; Pandian and Marian 1985; Richter-Boix et al. 2007). Those studies show that high-protein food increases the final size and reduces the time required for development in amphibians. These features are directly associated with post-metamorphosis survival rates, a reduction of mortality risk in drying ponds, juvenile performance, enhanced fecundity and presumably juvenile dispersion capacity (Howard 1980; Smith 1987; Babbitt et al. 2000; Tejedo et al. 2000; Martins et al. 2013). Accordingly, high-quality food selection could favour and ensure short-term reproductive success and indirectly enhance the invasive capacity of introduced amphibian species. The results of the present study indicate differential selection of food quality by the invasive species (*D. pictus*) while the native species (*E. calamita*) seems to follow a random feeding pattern, with no clear dietary selection when in isolation.

Although some species seem to show indiscriminate feeding behaviour (Jenssen 1967; Diaz-Paniagua 1985; Diaz-Paniagua 1989; Hoff et al. 1999), tadpole diets can vary under different

environmental conditions (Kupferberg 1996; Taylor et al. 1995; Babbitt et al. 2000; de Sousa et al. 2014). *Pelodytes punctatus*, for example, shows no preference in the absence of stress factors but becomes selective under stress factors such as competition, predation, and pond desiccation risk (Richter-Boix et al. 2007). This species is another native anuran of invaded areas with high co-occurrence with *D. pictus* and *E. calamita* in ephemeral ponds (Montori et al. 2007; Escoriza and Boix 2012; Richter-Boix et al. 2012). While the native species (*E. calamita* and *P. punctatus*) apparently show no dietary quality selection in conditions without unfavourable factors, either in laboratory experiments or in nature (Diaz-Paniagua 1985; Diaz-Paniagua 1989), invasive *D. pictus* always shows a preference for high-quality food. This preference could be due to the intrinsic behaviour of the species or to particular stimuli or stress linked to its introduction and process of expansion. It is possible that invasive populations have already undergone an adaptive selection favouring some traits (Phillips et al. 2006). In fact, it has been documented that some invasive species present traits linked to their expansion, with higher competitive capacity (Blossey and Notzold 1995; Müller-Schärer et al. 2004), higher growth rates (Davis 2005; Mason et al. 2008) and changes in food preference or diet that ensure rapid development and improve reproductive success (Lach et al. 2000; Bøhn et al. 2004).

Additionally, *D. pictus* seems to be capable of modifying its behaviour to achieve more favourable outcomes. It selected HP food when two types of food were available; but when only low-quality (LP) food was available, the species increased its feeding activity with respect to the DHP and DHLP treatments. As the quantity of food has similar effects on larval development as the quality of the food (Morey and Reznick 2000; Lind et al. 2008; Enriquez-Urzelai et al. 2013), the observed increase in activity in the low-quality treatment suggests active compensation by the species for the low quality of the food available via a higher intake. Therefore, it seems that even in an environment free of threats and under *ad libitum* feeding conditions, the invasive tadpoles maximize resources by consuming either higher-quality food or more. Additional comparative studies between the native and invaded ranges could establish whether the invasive species has these specific trophic traits (favouring its success in recipient systems) in their home ranges also, or whether this behaviour is a consequence of its introduction (Moloney et al. 2009).

Resource exploitation capacity of the invasive and the native species under co-occurrence

D. pictus presented similar trophic traits when it was in co-occurrence with the native species and when it was alone. As found in other invasive species (Petren and Case 1996; Holway 1999; Byers 2000), *D. pictus* showed a greater capacity to exploit available resources than did the native residents. *D. pictus* registered higher feeding activity than *E. calamita* and a preference for high-protein food under both LD and HD conditions. Moreover, the increase in the activity of the two species under HD conditions and some changes in feeding activity of the native species suggested an increase in interactions and indeed competitive processes between the two species.

Feeding activity is a trait associated with competitive capacity and capacity for resource exploitation. The introduction of more competitive species often has negative consequences on native species with less competitive capacity. More active individuals are often more competitive and can expropriate resources from less active individuals (Woodward 1982; Werner 1992; Laurila 2000; Dayton and Fitzgerald 2001). *D. pictus* showed higher feeding activity than *E. calamita* in all the treatments. Our results are also consistent with those of Richter-Boix et al. (2012), who reported a higher competitive capacity of *D. pictus* when it co-occurred with *E. calamita*. That study showed that the survival, body mass, and activity of the native species decreased and the time to metamorphosis suffered an increase, in the presence of the invasive species. In summary, it seems that *D. pictus* not only shows good resource exploitation, but it could also have negative consequences on *E. calamita* when the two co-occur.

The results obtained for food preference seem to corroborate the hypothesis that *D. pictus* has a higher competitive capacity. When the invasive and native species co-occurred (under both LD and HD conditions), the invasive species showed a clear preference and selection for high-protein food, whereas the native species showed no preference. In all habitat types, including those with no other kind of threat, competitive interaction is one of the factors that leads to differential dietary selection in amphibians (Griffiths et al. 1993). Although the native species showed no food preference, its feeding activity experienced some changes. When the two species co-occurred at LD, the feeding activity of *E. calamita* was slightly higher on high-protein food than on

low-protein food, which could indicate a mild response to a stress factor such as the presence of another species. At HD, the native species significantly increased its total feeding activity only on low-protein food; while its level of feeding activity on high-protein food was maintained. In view of the preference for HP food shown by *D. pictus*, the increase in feeding activity on LP food by *E. calamita* could suggest a reclusion of the native species to the lower-quality food. *E. calamita* could be exploiting those resources, since they are less used by the alien species. Recently, these laboratory results have been corroborated in nature (San Sebastián et al. 2015). Patterns of displacement and trophic niche partitioning have also been detected in ephemeral ponds occupied by *D. pictus* and *E. calamita*. Despite the similarity in diet of the two species (Díaz-Paniagua 1985; Escoriza and Boix 2012), the hierarchy of the communities seems to be repeated in both experimental and field experiments.

As with other invasive species (Fausch and White 1981; Holway 1999), *D. pictus* seems to present a higher competitive capacity than the native species with which it frequently shares its habitat, thereby favouring invasion in the recipient area.

Conclusion

Our conclusions are consistent with other studies conducted on this invasive species: *D. pictus* presents favourable traits that provide it with a high invasive capacity (see Enriquez-Urzelai et al. 2013; Pujol-Buxó et al. 2013; San Sebastián et al. 2015). In this work, this invasive species was found to show a preference for high-quality food and to modify its levels of activity according to the quality of diet available, which could ensure rapid larval development and good performance of metamorphs. It also displayed good resource exploitation capacity when sharing nutrients with its most common competitor in nature, *E. calamita*, suggesting high success rates in ephemeral ponds. This invasive species even seems to exhibit a higher competitive capacity than the native one, which could trigger a reduction in the metamorphic fitness and even in the recruitment success of the native species due to the presence of *D. pictus* under stress factors such as pond desiccation. Studies that explain the coexistence of the two species from different points of view such as scale (population and individual behaviour) or skills (physiological capacity, types of food) should be conducted.

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References

- Babbitt KJ, Meshaka WE, Ross ST (2000) Benefits of eating conspecifics: effects of background diet on survival and metamorphosis in the cuban treefrog (*Osteopilus septentrionalis*). *Copeia* 2: 469–474, [http://dx.doi.org/10.1643/0045-8511\(2000\)000\[0469:BOECEO\]2.0.CO;2](http://dx.doi.org/10.1643/0045-8511(2000)000[0469:BOECEO]2.0.CO;2)
- Blackburn TM, Lockwood JL, Cassey P (2009) Avian invasions: the ecology and evolution of exotic birds. Oxford University Press, Oxford
- Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *The Journal of Ecology* 83: 887–889, <http://dx.doi.org/10.2307/2261425>
- Böhm T, Sandlund OT, Amundsen P, Primicerio R (2004) Rapidly changing life history during invasion. *Oikos* 106: 138–150, <http://dx.doi.org/10.1111/j.0030-1299.2004.13022.x>
- Byers J (2000) Competition between two estuarine snails: implications for invasions of exotic species. *Ecology* 81: 1225–1239, [http://dx.doi.org/10.1890/0012-9658\(2000\)081\[1225:CBTESI\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2000)081[1225:CBTESI]2.0.CO;2)
- Calef GW (1973) Natural mortality of tadpoles in a population of *Rana aurora*. *Ecology* 54: 741–758, <http://dx.doi.org/10.2307/1935670>
- Davis HG (2005) r-Selected traits in an invasive population. *Evolutionary Ecology* 19: 255–274, <http://dx.doi.org/10.1007/s10682-005-0912-5>
- Dayton GH, Fitzgerald LA (2001) Competition, predation, and the distributions of four desert anurans. *Oecologia* 129: 430–435, <http://dx.doi.org/10.1007/s004420100727>
- De Sousa VTT, Nomura F, Venesky MD, Rossa-Feres DC, Pezzuti TL, Andrade GV, Wassersug RJ (2014) Flexible feeding kinematics of a tropical carnivorous anuran tadpole. *Journal of Zoology* 293: 204–210, <http://dx.doi.org/10.1111/jzo.12135>
- Denver RJ, Mirhadi N, Phillips M (1998) Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondi* tadpoles to habitat desiccation. *Ecology* 79: 1859–1872, <http://dx.doi.org/10.2307/176694>
- Diaz-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, <http://dx.doi.org/10.1163/156853885X00317>
- Diaz-Paniagua C (1989) Larval diets of two anuran species, *Pelodytes punctatus* and *Bufo bufo*, in SW Spain. *Amphibia-Reptilia* 10: 71–75, <http://dx.doi.org/10.1163/156853889X00304>
- Duncan RP, Blackburn TM, Sol D (2003) The ecology of avian introductions. *Annual Review of Ecology, Evolution, and Systematics* 34: 71–98, <http://dx.doi.org/10.1146/annurev.ecolsys.34.011802.132353>
- Enriquez-Urzelai U, San Sebastián O, Garriga N, Llorente GA (2013) Food availability determines the response to pond desiccation in anuran tadpoles. *Oecologia* 173: 117–127, <http://dx.doi.org/10.1007/s00442-013-2596-9>
- Escoriza D, Boix D (2012) Assessing the potential impact of an invasive species on a Mediterranean amphibian assemblage: a morphological and ecological approach. *Hydrobiologia* 680: 233–245, <http://dx.doi.org/10.1007/s10750-011-0936-5>
- Fausch KD, White RJ (1981) Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for positions in a Michigan stream. *Canadian Journal of Fisheries and Aquatic Sciences* 38: 1220–1227, <http://dx.doi.org/10.1139/f81-164>
- Franch M, G. Llorente A, Montori A, Richter-Boix A, Carranza S (2007) *Discoglossus pictus* beyond its known distributional range. *Herpetological Review* 38: 356–358
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190
- Griffiths ARA, Denton J, Wong AL (1993) The effect of food level on competition in tadpoles: interference mediated by protothecan algae? *Journal of Animal Ecology* 62: 274–279, <http://dx.doi.org/10.2307/5358>
- Hoff K, Blaunstein AR, McDiarmid RW, Altig R (1999) Behavior: Interactions and their consequences. In: McDiarmid RW, Altig R (eds) *Tadpoles: the Biology of Anuran Larvae*. Chicago: The University of Chicago press, pp 215–239
- Holway DA (1999) Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology* 80: 238–251, [http://dx.doi.org/10.1890/0012-9658\(1999\)080\[0238:CMUTDO\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(1999)080[0238:CMUTDO]2.0.CO;2)
- Hulme PE, Pysvek P, Nentwig W, Vilà M (2009) Will threat of biological invasions unite the European Union? *Science* 324: 40–41, <http://dx.doi.org/10.1126/science.1171111>
- Howard RD (1980) Mating behaviour and mating success in woodfrogs, *Rana sylvatica*. *Animal Behaviour* 28: 705–716, [http://dx.doi.org/10.1016/S0003-3472\(80\)80130-8](http://dx.doi.org/10.1016/S0003-3472(80)80130-8)
- Jefferson DM, Hobson KA, Demuth BS, Ferrari MCO, Chivers DP (2014) Frugal cannibals: how consuming conspecific tissues can provide conditional benefits to wood frog tadpoles (*Lithobates sylvaticus*). *Naturwissenschaften* 101: 291–303, <http://dx.doi.org/10.1007/s00114-014-1156-4>
- Jenssen TA (1967) Food habits of the green frog, *Rana clamitans*, before and during metamorphosis. *Copeia* 1967: 214–218, <http://dx.doi.org/10.2307/1442196>
- Kupferberg S (1997) The role of larval diet in anuran metamorphosis. *American Zoologist* 159: 146–159, <http://dx.doi.org/10.1023/icb.37.2.146>
- Kupferberg SJ (1996) The ecology of native tadpoles (*Rana boylei* and *Hyla regilla*) and the impacts of invading bullfrogs (*Rana catesbeiana*) in a northern California river. University of California, Berkeley
- Lach L, Britton DK, Rundell RJ, Cowie RH (2000) Food preference and reproductive plasticity in an invasive freshwater snail. *Biological Invasions* 2: 279–288, <http://dx.doi.org/10.1023/A:1011461029986>
- Laurila A (2000) Competitive ability and the coexistence of anuran larvae in freshwater rock-pools. *Freshwater Biology* 43: 161–174, <http://dx.doi.org/10.1046/j.1365-2427.2000.00533.x>
- Levine JM, Vilà M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society, Series B* 270: 775–81, <http://dx.doi.org/10.1098/rspb.2003.2327>
- Lind MI, Johansson F (2007) The degree of adaptive phenotypic plasticity is correlated with the spatial environmental heterogeneity experienced by island populations of *Rana temporaria*. *Proceedings of the Royal Society of London Series B* 20: 1288–1297, <http://dx.doi.org/10.1111/j.1420-9101.2007.01353.x>
- Lind MI, Persbo F, Johansson F (2008) Pool desiccation and developmental thresholds in the common frog, *Rana temporaria*. *Proceedings of the Royal Society Series B* 275: 1073–80, <http://dx.doi.org/10.1098/rspb.2007.1737>
- Mason RAB, Cooke J, Moles AT, Leishman MR (2008) Reproductive output of invasive versus native plants. *Global Ecology and Biogeography* 17: 633–640, <http://dx.doi.org/10.1111/j.1466-8238.2008.00402.x>

- Martens H, Veith M (1987) Considerations on origin and chorology of *Discoglossus pictus* Otth, 1837 in the eastern Pyrenees. In: Van Gelder JJ, Strijbosch H, Bergers PJM (eds), Proceedings of the 4th Ordinary General Meeting Societas Europaea Herpetologica in Nijmegen 1987, Faculty of Science, Nijmegen, pp 267–269
- Martins FMS, Oom MDM, Rebelo R, Rosa GM (2013) Differential effects of dietary protein on early life-history and morphological traits in natterjack toad (*Epidalea calamita*) tadpoles reared in captivity. *Zoo Biology* 32: 457–462, <http://dx.doi.org/10.1002/zoo.21067>
- McCallum ML, Trauth SE (2002) Performance of Wood frog tadpoles (*Rana sylvatica*) on three soybean meal–corn meal rations. *Podarcis* 3: 78–85
- McElduff F, Cortina-Borja M, Chan SK, Wade A (2010) When t-tests or Wilcoxon-Mann-Whitney tests won't do. *Advances in Physiology Education* 34: 128–133, <http://dx.doi.org/10.1152/advan.00017.2010>
- Moloney KA, Holzapfel C, Tielbörger K, Jeltsch F, Schurr FM (2009) Rethinking the common garden in invasion research. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 311–320, <http://dx.doi.org/10.1016/j.ppees.2009.05.002>
- Montori A, Llorente GA, Richter-Boix A, Villero D, Franch M, Garriga N (2007) Colonización y efectos potenciales de la especie invasora *Discoglossus pictus* sobre las especies nativas. *Munibe* 25: 14–27
- Morey S, Reznick D (2000) A comparative analysis of plasticity in larval development in three species of spadefoot toads. *Ecology* 81: 1736–1749, [http://dx.doi.org/10.1890/0012-9658\(2000\)0081\[1736:ACAOPJ\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2000)0081[1736:ACAOPJ]2.0.CO;2)
- Müller-Schärer H, Schaffner U, Steinger T (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology and Evolution* 19: 417–422, <http://dx.doi.org/10.1016/j.tree.2004.05.010>
- Nathan AJM, James VG (1972) The role of protozoa in the nutrition of tadpoles. *Copeia* 1972: 669–679, <http://dx.doi.org/10.2307/1442727>
- Newman RA (1987) Effects of density and predation on *Scaphiopus couchii* tadpoles in desert ponds. *Oecologia* 71: 301–307, <http://dx.doi.org/10.1007/BF00377299>
- Nicieza A, Álvarez D (2002) Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. *Oecologia* 131: 186–195, <http://dx.doi.org/10.1007/s00442-002-0876-x>
- Pandian TJ, Marian MP (1985) Predicting anuran metamorphosis and energetics. *Physiological Zoology* 58: 538–552
- Peacor SD, Werner EE (1997) Trait-mediated indirect interactions in a simple aquatic food web. *Ecology* 78: 1146–1156, [http://dx.doi.org/10.1890/0012-9658\(1997\)078\[1146:TMIIA\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(1997)078[1146:TMIIA]2.0.CO;2)
- Petren K, Case TJ (1996) An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology* 77: 118–132, <http://dx.doi.org/10.2307/2265661>
- Phillips BL, Brown GP, Webb JK, Shine R (2006) Invasion and the evolution of speed in toads. *Nature* 439: 803, <http://dx.doi.org/10.1038/439803a>
- Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment* 84: 1–20, [http://dx.doi.org/10.1016/S0167-8809\(00\)00178-X](http://dx.doi.org/10.1016/S0167-8809(00)00178-X)
- Pujol-Buxó E, San Sebastián O, Garriga N, Llorente GA (2013) How does the invasive/native nature of species influence tadpoles' plastic responses to predators? *Oikos* 122: 19–29, <http://dx.doi.org/10.1111/j.1600-0706.2012.20617.x>
- R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>
- Relyea RA (2002) Costs of phenotypic plasticity. *The American Naturalist* 159: 272–282, <http://dx.doi.org/10.1086/338540>
- Richter-Boix A, Garriga N, Montori A, Franch M, San Sebastián O, Villero D, Llorente GA (2012) Effects of the non-native amphibian species *Discoglossus pictus* on the recipient amphibian community: niche overlap, competition and community organization. *Biological Invasions* 15: 799–815, <http://dx.doi.org/10.1007/s10530-012-0328-4>
- Richter-Boix A, Llorente GA, Montori A, Garcia J (2007) Tadpole diet selection varies with the ecological context in predictable ways. *Basic and Applied Ecology* 8: 464–474, <http://dx.doi.org/10.1016/j.baae.2006.09.009>
- San Sebastián O, Navarro J, Llorente GA, Richter-Boix A (2015) Trophic strategies of a non-native and a native amphibian species in shared ponds. *PLoS ONE* 10(6): e0130549, <http://dx.doi.org/10.1371/journal.pone.0130549>
- Schiesari L (2006) Pond canopy cover: a resource gradient for anuran larvae. *Freshwater Biology* 51: 412–423, <http://dx.doi.org/10.1111/j.1365-2427.2005.01497.x>
- Scott DE (1994) The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology* 75: 1383–1396, <http://dx.doi.org/10.2307/1937462>
- Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology and Evolution* 17: 170–176, [http://dx.doi.org/10.1016/S0169-5347\(02\)02495-3](http://dx.doi.org/10.1016/S0169-5347(02)02495-3)
- Smith DC (1987) Adult recruitment in chorus frogs: effects of size and date at metamorphosis. *Ecology* 68: 344–350, <http://dx.doi.org/10.2307/1939265>
- Smith GR, Dingfelder HA, Vaala DA (2004) Asymmetric competition between *Rana clamitans* and *Hyla versicolor* tadpoles. *Oikos* 105: 626–632, <http://dx.doi.org/10.1111/j.0030-1299.2004.12841.x>
- Steinwascher K, Travis J (1983) Influence of food quality and quantity on early growth of two anurans. *Copeia* 1983: 238–242, <http://dx.doi.org/10.2307/1444720>
- Taylor CL, Altig R, Boyle CR (1995) Can anuran tadpoles choose among foods that vary in quality? *Alytes* 13: 81–86
- Tejedo M, Semlitsch RD, Hotz H (2000) Covariation of morphology and jumping performance in newly metamorphosed water frogs: effects of larval growth history. *Copeia* 2: 448–458, [http://dx.doi.org/10.1643/0045-8511\(2000\)000\[0448:COMAJP\]2.0.CO;2](http://dx.doi.org/10.1643/0045-8511(2000)000[0448:COMAJP]2.0.CO;2)
- Thébaud CA, Finzi C, Affre L, Debusseche M, Escarre J. 1996. Assessing why two introduced *Conyza* differ in their ability to invade Mediterranean old fields. *Ecology* 77: 791–804, <http://dx.doi.org/10.2307/2265502>
- Tilman D (2004) A stochastic theory of resource competition, community assembly and invasions. *Proceedings of the National Academy of Sciences of the United States of America* 101: 10854–10861, <http://dx.doi.org/10.1073/pnas.0403458101>
- Werner EE (1992) Competitive interactions between wood frog and northern leopard frog larvae: the influence of size and activity. *Copeia* 1992: 26–35, <http://dx.doi.org/10.2307/1446532>
- Wheeler RE (2010) lmerPerm: Permutation Tests for Linear Models. R package version 1.1–2. 1–36
- Wilbur HM (1980) Complex life cycles. *Annual Review of Ecology and Systematics* 11: 67–93, <http://dx.doi.org/10.1146/annurev.es.11.110180.000435>
- Wintrebert P (1908) Présence à Banyuls-sur-Mer (Pyrenées Orientales) du *Discoglossus pictus* Otth. *Bulletin de la Société Zoologique de France* 33:54
- Woodward BD (1982) Tadpole competition in a desert anuran community. *Oecologia* 54: 96–100, <http://dx.doi.org/10.1007/BF00541115>

Appendix 1. Number of registers for each aquarium and treatment.

Experiment	Treatment	Species	Registers	Average*	Minimum	Maximum
I	CHLP	<i>E. calamita</i>	83	17	15	19
	CHP	<i>E. calamita</i>	104	17	15	21
	CLP	<i>E. calamita</i>	121	20	14	23
	CHLP	<i>D. pictus</i>	79	13	8	15
	CHP	<i>D. pictus</i>	84	14	14	14
	CLP	<i>D. pictus</i>	94	16	15	17
II	LD	<i>E. calamita</i> - <i>D. pictus</i>	131	22	20	24
	HD	<i>E. calamita</i> - <i>D. pictus</i>	155	26	25	26

*Average per aquarium