

# Effects of the invasive crayfish *Procambarus clarkii* on growth and development of *Pelophylax perezi* tadpoles in field conditions

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## Abstract

*Effects of the invasive crayfish Procambarus clarkii on growth and development of Pelophylax perezi tadpoles in field conditions.* Introduced predatory aquatic invertebrates may contribute to the global decline of amphibians as their larval are extremely vulnerable to predators. The objective of this study was to examine the effects of the predatory invasive crayfish *Procambarus clarkii* on the growth and development of native Iberian green frog tadpoles, *Pelophylax perezi*, in field conditions. We hypothesized that *P. clarkii* might affect *P. perezi* development by (a) inducing a delay in its metamorphosis and (b) reducing survival and mass of metamorphs. The experiment was developed in two ponds (with and without *P. clarkii*'s presence) in the Natural Park of Aiguamolls de l'Empordà (NE of the Iberian Peninsula). For each pond, groups of 10 tadpoles were randomly assigned to 15 cylindrical field enclosures. These enclosures avoided direct contact (i.e. predation) between both species. Our results suggest that, in field conditions, the presence of *P. clarkii* accelerates metamorphosis of *P. perezi* tadpoles. The higher growth rate of *P. perezi* through shorter larval periods could be the result of behavioural plasticity in response to the strong pressure imposed by *P. clarkii*. This conclusion would be in accordance with the hypothesis that phenotypic plasticity plays an important role in the conservation of *P. perezi* in front of biological invasions.

Key words: Tadpole, Predator, Invasion, Non–native, Larval development, Fitness

## Resumen

*Efectos del cangrejo exótico, Procambarus clarkii, en el crecimiento y el desarrollo de los renacuajos de Pelophylax perezi en condiciones de campo.* Los invertebrados acuáticos depredadores introducidos pueden contribuir a la disminución general de los anfibios, cuyas larvas son extremadamente vulnerables a los depredadores. El objetivo de este estudio fue examinar los efectos del cangrejo exótico, *Procambarus clarkii*, en el crecimiento y el desarrollo de los renacuajos de la rana verde ibérica (*Pelophylax perezi*) en condiciones de campo. Concretamente, planteamos la hipótesis de que *P. clarkii* podría afectar al desarrollo de *P. perezi* de dos formas: (a) induciendo un retraso en su metamorfosis y (b) reduciendo la supervivencia y la cantidad de renacuajos en fase de metamorfosis. El experimento se realizó en dos estanques (con y sin presencia de *P. clarkii*), en el Parque Natural Aiguamolls de l'Empordà (NE de la península ibérica). En cada estanque se introdujeron aleatoriamente grupos de 10 renacuajos en 15 cilindros cerrados de malla. Estos cilindros evitaron el contacto directo (es decir, la depredación) entre ambas especies. Nuestros resultados sugieren que, en condiciones de campo, la presencia de *P. clarkii* podría acelerar la metamorfosis de los renacuajos de *P. perezi*. La plasticidad en el comportamiento de *P. perezi* como respuesta a la fuerte presión ejercida por *P. clarkii* permite acelerar la tasa de crecimiento reduciendo los periodos larvales. Además, este estudio avalaría la hipótesis de que la plasticidad fenotípica juega un papel importante en la conservación de *P. perezi* frente a las invasiones biológicas.

Palabras clave: Renacuajo, Depredador, Invasión, Alóctono, Desarrollo larvario, Eficacia biológica

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## Introduction

Global change, including biological invasions, habitat fragmentation and destruction, acidity, pollution (such as insecticides and herbicides) and emerging infectious diseases, is known to be causing amphibian declines worldwide (Houlahan et al., 2000; Saura–Mas et al., 2002; Collins and Storfer, 2003; Kats and Ferrer, 2003; Stuart et al., 2004; Hayes et al., 2010). It is difficult to determine the exact causes of the decline in amphibian populations as their demography is characterized by fluctuations in the number of individuals due to their biological dependence on environmental conditions and their meta–population traits. However, the joint action of natural fluctuations in populations' and anthropogenic might result in local extinction more easily than either alone (Pechmann et al., 1991). Biological invasions are a major factors in global change due to their effects on the natural ecosystems (Vitousek et al., 1996; Garcia–Berthou, 2010; Simberloff et al., 2013). Humans have deliberately introduced animals outside their natural range for a variety of motives (commercial and non–commercial). As a result, allochthonous species often cause declines and even extinctions of native amphibian populations around the world (Carey et al., 2003; Lever, 2003).

Invasive species can affect amphibians in aspects such as predation, competition, parasitism, and habitat disruption. The bullfrog (*Lithobates catesbeiana*) is a native of North–America and considered to be one of the most harmful invasive species worldwide since it negatively affects native amphibians through competition and predation (Kats and Ferrer, 2003; Beebee and Griffiths, 2005; GISD, 2018). Other invasive species such as introduced predatory aquatic invertebrates and fishes are a concern for amphibian populations and may contribute to global amphibian decline because larval amphibians are extremely vulnerable to vertebrate and invertebrate predators (Alford and Richards, 1999). In recent years, one of the most important pests in Iberian Peninsula wetlands, streams and ponds has been the crayfish *Procambarus clarkii*, which has affected the ecosystem dynamics and caused large socio–economic damage, mainly to rice fields in these areas (Beja, 1996; Gutiérrez–Yurrita, 1997; Twardochleb et al., 2013; Carreira et al., 2014). *P. clarkii* is a native of North–America and it has become invasive in many continental aquatic Mediterranean ecosystems (Taylor et al., 1996; Gonçalves et al., 2015). Several studies show that anuran tadpoles can detect chemical cues coming from predators and injured prey during predation events (Petranka et al., 1987; Schoeppner and Relyea, 2005; Fraker et al., 2009), Sublethal effects can thus be produced in amphibians by altering habitat conditions, behaviour, and development and growth (Anholt and Werner, 1995; Relyea, 2001, 2002; Rodríguez et al., 2005; Tejedo et al., 2010).

The eradication of *P. clarkii* from most Mediterranean wetlands is nearly impossible due to its reproductive and invasive traits. It has been suggested that the species plays an important role in the decline of local populations of amphibians in such ecosystems because

of its predatory role, and particularly its direct or indirect effects on tadpoles (Renai and Gherardi, 2004; Rodríguez et al., 2005; Cruz et al., 2006). Many authors have described changes in behaviour, morphology and growth during metamorphosis processes of native tadpoles in interaction with *P. clarkii* (Almeida et al., 2011; Gonçalves et al., 2011). Nunes et al. (2014a) observed that eight of nine species of tadpoles changed their morphology or life history when reared with the fed dragonfly, but only four made such changes when reared with the fed crayfish, suggesting among–species variation in the ability to respond to a novel predator.

Nowadays, one of the most important challenges in animal ecology is to know more about phenotypic plasticity of species in front of environmental changes. Many studies have been conducted to obtain more information on phenotypic plasticity of prey induced by predators. Some studies have focused in anuran species report a clear lack of response to invasive predators (Smith et al., 2007; Gomez–Mestre and Diaz–Paniagua, 2011; Vázquez et al., 2017), while other studies report different types of behavioural and morphological responses after a relatively short period of coexistence with the invasive predator (Kiesecker and Blaustein, 1997; Pearl et al., 2003; Almeida et al., 2011; Gonçalves et al., 2011; Pujol–Buxó et al., 2013; Nunes et al., 2014a). The phenotypic plasticity of prey in front of invasive predator species is also an important factor to take into account. Heritable phenotypic plasticity to native species might thus be a key step to understanding the effects of global environmental changes, such as biological invasions.

The main objective of this study was to examine the effects of the presence of the predatory invasive crayfish *P. clarkii* (with non–lethal effects) on the growth and development of the Iberian green frog *Pelophylax perezi* in field conditions in the Natural Park of Aiguamolls de l'Empordà (NE of the Iberian Peninsula). Most previous studies examining the effects of invasive predators to anuran development have been developed under lab or mesocosm conditions. An innovative aspect of our study is that the experiment was developed under field conditions. We aimed to answer the following questions: first, does the presence of *P. clarkii* hasten metamorphosis of *P. perezi*? We hypothesized that the time to metamorphosis would be delayed because of diminished activity and consequent lower energy intake (Tejedo et al., 2010; Touchon et al., 2015); and second, does the presence of *P. clarkii* affect survival, growth rate and mass of metamorphs? We hypothesize that detection of the predator *P. clarkii* could induce differences in feeding activity, resulting in smaller sizes at metamorphosis (Orizaola et al., 2012; Richter–Boix et al., 2004).

## Material and methods

### Study species

*P. perezi* is the most common and widespread frog species in the Iberian Peninsula (Bosch et al., 2009; Masó and Pijoan, 2011). Adults are essentially aquatic,

although they have a certain terrestrial dispersion capacity (Egea–Serrano, 2009). *P. perezi* is present in many types of Mediterranean and Eurosiberian habitats, such as wetlands, ponds, lakes, rice fields, and rivers. In the study area, it shares habitats with natural predators such as autochthon fish and dragonflies, as well as with invasive predators such as *P. clarkii*. The Natural Park of Aiguamolls de l'Empordà consists of wetlands that can be considered a long-term invaded area because *P. clarkii* has been present since the 1980s (Moreno–Amich and Vila–Gispert, 2000), and by the 1990s it had become abundant throughout the Park (Moreno–Amich and Vila–Gispert, 2000). The red swamp crayfish (*P. clarkii*) has a cylindrical body with a clearly marked abdomen and differentiated and segmented thoracic limbs. It is considered an opportunistic omnivore (Gutiérrez–Yurrita et al., 1998) and it tends to inhabit swamp areas with abundant vegetation (especially macrophytes) (Gherardi et al., 2002). It exhibits characteristics of an r-selected species, including early maturity at small body size (10 g), rapid growth rates (50 g in 3–5 months), large numbers of offspring at a given parental size (a female of an average size producing 400 pleopodal eggs), and relatively short life spans (Gherardi, 2006).

#### Experimental design and study site

Field work was conducted at the Natural Park of Aiguamolls de l'Empordà (North–East of the Iberian Peninsula). Two ponds were created for the experiment in the natural park wetlands area at the beginning of autumn 2003 (UTM: 507473, 4674382). The area of each pond was 4 x 4 m and 1 m in depth, and they were separated from each other by 5 m. After they were made, the ponds were surrounded by a plastic fence of 1 m in height. This fence was sunk 30 cm into the ground to avoid amphibians and crayfishes entering the ponds. The bottom of one of the ponds, considered a control pond (without *P. clarkii*), was lined with screen-mesh under the soil to avoid the entrance of individuals of *P. clarkii*. During autumn 2003 and spring 2004, the ponds were naturally colonized by aquatic vegetation with species such as *Typha latifolia* and *Chara vulgaris*. As the ponds were alongside each other, they were under the same environmental conditions with the same forest canopy and similar to sunlight. We therefore assume that the two ponds had similar physical–chemical water features with around 600  $\mu\text{S}/\text{cm}$  conductivity, 8.24 pH, and near 100 % exposure of oxygen saturation (these variables were measured twice during the experiment, in June, and in August). Water temperature was measured 10 times during the experiment and was similar between ponds, ranging from the 29°C in July and August to a minimum of 21°C in October.

In 27<sup>th</sup> April of 2004 two egg masses of *P. perezi* were collected in the Natural Park of Aiguamolls de l'Empordà. Eggs hatched in the laboratory at 23–25 °C and were held until tadpoles were free-swimming (Gosner stage 25; Gosner, 1960). We mixed tadpoles from the different clutches before use in the experiment to homogenize genetic variation. For each pond, groups of 10 tadpoles were randomly assigned to 15 cylin-

dric field enclosures made of plastic screen mesh (2.0 mm mesh; 19 cm diameter, 46 cm height; depth of enclosure submergence was approximately 40 cm [11-liter volume]) and were divided among three spatial blocks. There was thus a total of 30 replicates, 15 for each treatment (i.e. for each pond), and 300 tadpoles. One of the ponds was a control, without *P. clarkii* (hereafter referred to as 'control pond'). The pond with the invasive predator (hereafter referred to as 'invaded pond') had a density of individuals of *P. clarkii* similar to that of natural conditions (6 crayfish/m<sup>2</sup>) (Gherardi and Acquistapace, 2007). *P. clarkii* individuals were collected with crayfish traps in the Natural Park of Aiguamolls de l'Empordà and we added predators, regardless of sex, to the experimental pond on the same day they were collected.

We searched ponds daily for metamorphs, defined by the emergence of at least one forelimb (stage 42; Gosner, 1960). When metamorphs were detected, they were removed immediately from the enclosure and total weight was measured to the nearest 0.0001 g. On day 156 (10th of October 2004), we ended the experiment because most surviving animals had reached metamorphosis.

#### Data analyses

Mass at metamorphosis (weight of metamorph), larval period (number of days until metamorphosis, day of Gosner stage 42) and survival at metamorphosis (Sm, metamorphosed) were used to measure the response of tadpoles to the invasive predator *P. clarkii*. Total survival (Ts, number of metamorphosed individuals and tadpoles that survived at the end of the experiment) was also considered since some individuals did not metamorphose at the end of the experiment, but they were alive. Survival data was binomial: alive or death.

A linear regression was performed to analyze mass at metamorphose and larval period linear dependence. This analysis was done at an individual level because working with the means rules out intravariability within the species and we could have lost information concerning the relationship between these two variables. Treatment effects (control pond or invaded pond) on mass of metamorph, larval period and growth rate were analysed using the SPSS program, and the univariate model, considering individuals as experimental units. The enclosure was nested within a particular pond; this term was considered random in an overall mixed GLM (Df are in table 1). Larval period, mass of metamorph, and growth rate were log transformed to achieve normality distribution. Survival at metamorphosis and total survival were analysed using a generalized linear model with a binomial distribution and a logit function. All statistical analyses were performed using SPSS 15 (SPSS Inc., Chicago, IL, USA, 1989–2006).

#### Results

Overall, our results showed that tadpoles reared under the presence of *P. clarkii* had a shorter larval period (pond with *P. clarkii*: 67.30 days (SE 1.62);

Table 1. General linear model results for effects of treatments to mass at metamorphosis and larval period and growth rate (log transformed to assume normality).

Tabla 1. Resultados obtenidos con el modelo lineal generalizado de los efectos de los tratamientos en la masa en la metamorfosis, el período larvario y la tasa de crecimiento (log transformado para suponer normalidad).

		Df	Mean square	Error	F	P-value
Larval period	Pond	1	0.72	0.15	104.48	< 0.0001
	Pond * enclosure	17	0.01	0.27	2.80	0.001
Mass at metamorphosis	Pond	1	0.04	0.71	1.60	0.22
	Pond * enclosure	17	0.02	1.28	1.36	0.18
Growth rate	Pond	1	1.11	0.57	43.6	< 0.0001
	Pond * enclosure	17	0.03	1.06	2.61	0.002

pond without *P. clarkii*: 105.9 days (SE. 4.89); fig. 1, table 1). The metamorph mass and larval period were linearly correlated for both treatment groups (fig. 2; pond without *P. clarkii*,  $R^2 = 0.348$  ( $p = 0.001$ ) and pond with *P. clarkii*,  $R^2 = 0.314$ ,  $p = 0.001$ ). Therefore, the final weight of metamorph increased with the increase of the larval period, and growth rate was significantly higher in the pond with *P. clarkii* (table 1, pond with *P. clarkii*, 0.006 g/d, SE 0.0003; pond without *P. clarkii*: 0.004 g/d, SE 0.0002). No significant differences were found between treatments for the metamorph mass (table 1; pond with *P. clarkii*, 0.43 g, S.E. 0.19; pond without *P. clarkii*, 0.40 g, SE 0.0). Figure 2 shows the segregation of the two groups during the larval period, but no segregation occurred between groups along the metamorph mass axis, indicating treatment had no effect on this variable. The presence of *P. clarkii* did not promote significant differences in total survival or in survival to metamorphosis.

## Discussion

Our results suggest that the presence of the invasive species *P. clarkii* influences tadpole development since tadpoles reared in the presence of *P. clarkii* had a shorter larval period, reaching metamorphosis earlier than tadpoles reared in an environment without *P. clarkii*. We hypothesized that the time to metamorphosis would probably be longer because of diminishing activity and consequently less energy intake (Tejedo et al., 2010, Touchon et al., 2015). However, discussion on this issue continues and our results indicate other patterns could operate in this predatory–prey relation. Denver (1995, 1997a) showed that tadpoles in drying or stressed conditions initiated metamorphosis early due to activation of the corticotrophin–releasing hormone, known to be responsible to advance metamorphosis for tadpoles (Denver, 1997b). *P. clarkii* might act as a stressor to *P. perezi*, as it reportedly preys on egg masses, tadpoles and even adult amphibians (Gherardi et al., 2001). Therefore, in our study, *P. clarkii* might

have accelerated metamorphosis of tadpoles through such activation of corticotrophin–releasing hormone. Orizaola et al. (2012) and Richter–Boix et al. (2004) suggested that the presence of predators will result in smaller sizes at metamorphosis. Nevertheless, although mass at metamorphosis was not significantly different between treatments in our study, our results show that growth rate was significantly higher for individuals reared under *P. clarkii* presence. This agrees with Nunes et al. (2014b), who showed that *P. perezi* tadpoles tended to grow faster in the presence of crayfish than in non–predatory environments. A larger prey size might provide an advantage from predation, so that increasing growth rate could also be a direct and adaptive response to predation (Urban, 2007). Importantly, however, the growth/predation risk trade–off is a common constraint documented for many organisms, with higher growth rates coming at the expense of increased vulnerability to predators (Lima and Dill, 1990; McPeck, 2004).

Nunes et al. (2014b) studied *P. perezi* and *P. clarkii* relations considering frog populations differing in historical exposure to the invasive predator. Tadpoles from non–invaded populations responded to the presence of *P. clarkii* with behavioural plasticity (they reduced behavioural activity), whereas long–term invaded populations showed canalized antipredator behavior (they presented a constant low activity level). Their results suggest that, while native *P. perezi* populations responded behaviourally to *P. clarkii*, the strong predation pressure imposed by the crayfish has induced the evolution of qualitatively different antipredator defences in populations with longer coexistence time. The Natural Park of Aiguamolls de l'Empordà consists of wetlands that can be considered a long–term invaded area (*P. clarkii* has been present for more than 20 years). In our experiment, therefore, tadpoles (from both treatments) might show a constant low activity level. As a result, tadpoles reared under the presence of the crayfish, might not present changes in behavioural activity levels but higher growth rates, while tadpoles without the presence of crayfishes might not accelerate growth

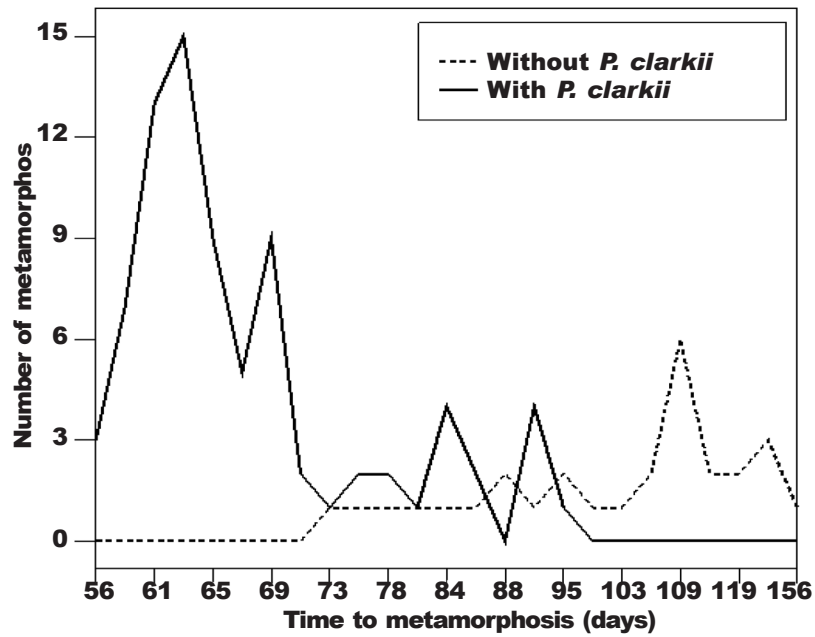


Fig. 1. Relation between number of metamorphoses and time to metamorphosis for the two treatments.

Fig. 1. Relación entre el número de renacuajos en fase de metamorfosis y la duración de la metamorfosis para los dos tratamientos.

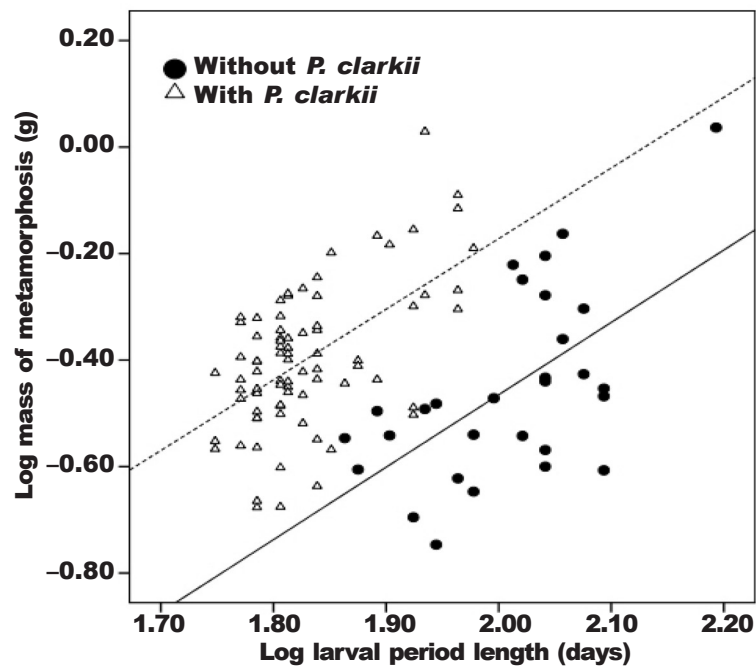


Fig. 2. Tadpole mass at metamorphosis linearly dependent on the tadpole larval period for each treatment: pond without *P. clarkii* ( $R^2 = 0.348$ ,  $p = 0.001$ ) and pond with *P. clarkii* ( $R^2 = 0.314$ ,  $p = 0.001$ ).

Fig. 2. Relación lineal entre el número de renacuajos en fase de metamorfosis y el período larval para cada tratamiento: estanques in *P. clarkii* ( $R^2 = 0,348$ ,  $p = 0,001$ ) y estanques con *P. clarkii* ( $R^2 = 0,314$ ,  $p = 0,001$ ).

since they did not detect cues and danger. Nunes et al. (2014b) stated that a negative correlation between growth and behaviour agrees well with the low activity levels found in long coexistence populations.

The role of antipredator phenotypic plasticity might be crucial in population dynamics during biological invasions (Miner et al., 2005). Relyea (2005) first examined the heritability of predator-induced defences, and observed that predator-induced traits can frequently be heritable, although the magnitude of heritability can be wide ranging across environments. Beyond the direct predation impact, *P. clarkii* is known for its capacity to destroy macrophytes and to increase water turbidity by digging tunnels, all actions that contribute to increase algal growth in invaded ponds and lakes (Rodríguez et al., 2005). As a result, tadpoles would feed much better in a more eutrophic and algal dominated pond than in an oligotrophic pond dominated by macrophyte. This could explain the higher growth rate among tadpoles in the pond with crayfish, but we cannot make this conclusion as we did not measure changes in the algae community or in turbidity. As some studies indicate how location (field or laboratory, for example) can affect survival (Mitchell, 1990; Saura–Mas et al., 2002), we hypothesized that presence or absence of *P. clarkii* could also affect survival rates by increasing stress variables related to growth. Nevertheless, we did not detect a significant impact on survival rates, probably because there was no direct contact between prey and predator. Our results suggest that stress promoted by the presence of *P. clarkii* might promote shorter life cycles but not changes in survival if there is no contact between prey and predator.

A potential weakness in our study is that our statistical analysis did not consider the possibility that native predators could also induce defences in tadpoles. While tadpoles in this study have similar habitats and experience similar native predator regimes, we cannot exclude the possibility that these differences may result from adaptation to other local habitat features such as competitors or food availability (Relyea, 2002; Richter–Boix et al., 2010).

Finally, we conclude that, in field conditions, the invasive species *P. clarkii* might accelerate a metamorphosis of *P. perezi* tadpoles in Mediterranean wetlands ecosystems. Here we show that in addition to direct predation, this invasive predator may also alter *P. perezi* populations by increasing their growth rate by decreasing the larval period and, as a result, maybe also decreasing the length of the life cycle. These *P. perezi* life cycle changes might not be synchronised with the food web in Mediterranean wetlands, causing effects at a community level. These results represent a preliminary approach to the study of changes that this invasive species can drive in *P. perezi* populations. Further studies with higher statistical power are needed to confirm our trends. Our findings, however, coincide with observations from other studies indicating that phenotypic plasticity in *P. perezi* may play an important role in population dynamics in the face of global changes such as those involving invasive predators.

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