Learned recognition of introduced predators determines survival of tadpole prey

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Summary

1.   Alien predators are one of the major causes for rapid decline and extinction of native species, because they often create novel ecological contexts in which the antipredatory responses of native organisms are no longer fit.

2.   Although larval amphibians are often capable of innately responding to chemical cues from local predators through changes in morphology and behaviour, naïve tadpoles generally cannot recognise introduced predators with which they have not shared an evolutionary past. However, in a few documented cases, aquatic organisms have been observed to alter morphology or behaviour in response to alien predators. Such a response may have evolved as adaptive recognition, increasing their repertoire of innate responses to include the novel predator, or may have evolved as the prey's ability to learn new threats by association with conspecific alarm cues.

3.   The red swamp crayfish, *Procambarus clarkii*, is a harmful invasive species in aquatic systems worldwide, causing great ecological impact on native amphibian populations during the last decades through intense predation of eggs and tadpoles. We demonstrate that naïve tadpoles of the western spadefoot toad, *Pelobates cultripes*, are not capable of innately recognising water-borne predator cues from the red swamp crayfish. Nevertheless, we demonstrate that *P. cultripes* tadpoles can learn to recognise the cues of the invasive predatory crayfish as a threat when they are exposed to predator cues combined with conspecific alarm cues. Finally, we show that tadpoles conditioned by joint exposure to crayfish and alarm cues enjoy higher survival during predation trials with invasive crayfish.

4.   Learning to recognise a newly introduced predator through association with conspecific alarm cues may allow successful generalisation of antipredatory responses by tadpoles. This cognitive ability of tadpoles may contribute to reduce their vulnerability to alien predators and soothe the impact of invasions in natural populations.

Key-words: alien predators, chemical cues, learning, phenotypic plasticity, predator recognition

Introduction

The introduction of species outside their natural range is one of the greatest threats to global biodiversity (Wilcove *et al*. 1998; Gurevitch & Padilla 2004). In particular, alien predators are considered to be one of the major causes of decline and extinction of prey species, since they can create novel ecological contexts in which the antipredatory responses of native prey may no longer be adaptive (Vitousek *et al*. 1997; Callaway & Aschehoug 2000; Shea & Chesson 2002). Because adaptive responses of animals to predation risk have evolved in specific habitats in which prey have coexisted with predators for a long evolutionary time, prey are innately equipped with morphological and behavioural traits that reduce the risk of predation by local predators and favour survival and reproductive success in such environments (Williams & Nichols 1984). However, native prey lack evolutionary history with newly introduced predators and may lack specific antipredatory strategies to cope with them (Schlaepfer *et al*. 2005). For this reason, alien predators often result more dangerous to prey populations than native predators (Salo *et al*. 2007).

Amphibians are the most vulnerable group of vertebrates with ~41% of the species endangered (Hoffmann *et al*. 2010). Amphibians are especially vulnerable to the introduction of new predators because they often present complex life cycles with aquatic eggs and larvae (Gomez-Mestre, Pyron & Wiens 2012), which are consumed in large amounts by aquatic alien predators (Gamradt & Kats 1996; Gillespie 2001; Kats & Ferrer 2003). Naïve tadpoles of many amphibian species typically respond to the presence of chemical cues from local predators by reducing their activity levels as an adaptive antipredatory strategy (Stauffer & Semlitsch 1993; Wilson & Lefcort 1993; Holomutzki

1995; Kiesecker, Chivers & Blaustein 1996; reviewed in Kats & Dill 1998; Chivers & Smith 1998). In contrast, tadpoles are often incapable of recognising water-borne cues from introduced predators with which they lack joint evolutionary history (Kiesecker & Blaustein 1997; Marquis, Saglio & Neveu 2004; Polo-Cavia *et al*. 2010; Gomez-Mestre & Diaz-Paniagua 2011). A number of studies have pointed to this common lack of adaptive responses to alien predators as a major cause of global amphibian declines (Gamradt, Kats & Anzalone 1997; Gillespie 2001; Kats & Ferrer 2003; Cruz, Rebelo & Crespo 2006; Cruz *et al*. 2008). Nonetheless, tadpoles in some amphibian populations have been reported to recognise alien predators and respond to the new threats by altering their morphology or behaviour (Chivers & Smith 1995; Kiesecker & Blaustein 1997; Chivers *et al*. 2001; Pearl *et al*. 2003; Moore *et al*. 2004). These studies suggest that given enough time, native amphibians may evolve the ability to innately detect and avoid introduced predators. Learning by association with conspecific alarm cues may contribute to reduce the impact of introduced predators on tadpoles until innate recognition evolves.

The North-American red swamp crayfish, *Procambarus clarkii*, is one of the most harmful invasive predators in aquatic systems worldwide, causing great ecological impacts on native amphibian populations through intense predation of eggs and tadpoles (Gamradt & Kats 1996; Cruz & Rebelo 2005; Gherardi & Acquistapace 2007; Ficetola *et al*. 2011, 2012). Native to southeastern USA, *P. clarkii* was introduced in 1973 into the Iberian Peninsula for commercial aquaculture purposes. In Doñana National Park (southwestern Spain), the species has expanded its populations unevenly, colonising a vast area within the Guadalquivir marshes and even reaching temporary ponds where native amphibians commonly breed (Geiger *et al*. 2005). Adaptive responses to introduced crayfish in morphology, behaviour, and life cycles of tadpoles have been reported in other amphibian populations (Pearl *et al*. 2003), especially if crayfish were fed conspecific tadpoles (Cruz & Rebelo 2005; Nunes *et al*. 2013). In contrast, common frog (*Pelophylax perezi*)

populations from Doñana National Park showed lack of antipredatory responses to the same invasive crayfish (Gomez-Mestre & Díaz-Paniagua 2011). This apparent inconsistency across studies suggests that the simple presence of predator cues (i.e. kairomones) may often not be sufficient to elicit antipredatory responses in tadpoles against introduced predators. Instead, antipredatory defences may be triggered by invasive predators mostly or only if combined with cues released from injured conspecifics (i.e. alarm cues, Summey & Mathis 1998; Marquis, Saglio & Neveu 2004; Ferrari, Wisenden & Chivers 2010).

Alarm cues are chemical compounds released from damaged prey epidermis during a predatory attack, which reliably advertise a high risk of predation. In consequence, a large number of species, including amphibians, have been shown to strongly respond to conspecific alarm cues (Chivers & Smith 1998; Chivers *et al*. 2001; Marquis, Saglio & Neveu 2004; reviewed in Ferrari, Wisenden & Chivers 2010). Alarm cues may be also present in the postdigestion cues released by predators that have eaten conspecific prey (Schoeppner & Relyea 2005), and thus, in most case studies it is not possible to distinguish whether prey responses are triggered by predator kairomones, by conspecific alarm cues, or both. On the other hand, through association with alarm cues, naïve prey can learn to recognise the cue of a novel predator as threatening (Mirza *et al*. 2006; Gonzalo, López & Martín 2007; Ferrari, Messier & Chivers 2008a,b). Apart from innate recognition of risk, learning via association with alarm cues allows prey to refine detection and avoidance of predatory threats in dynamic environments. We hypothesised that this ability of prey to learn to recognise cues from novel predators may be critical for amphibian populations in the new ecological contexts posed by alien predators. Therefore, behavioural plasticity might enable tadpoles to survive introduced predators.

Learned predator recognition has often been claimed to confer adaptive value to prey, but only a few studies to date have demonstrated increased survival of predator-

experienced prey during predatory attacks (Mirza & Chivers 2000; Gazdewich & Chivers 2002; Lonnstedt *et al*. 2012). Here we present data from three experiments aimed at studying the potential for learned predator recognition in reducing the impact of invasive predators. First we examined the capacity of tadpoles of the western spadefoot toad (*Pelobates cultripes*) to innately recognise and respond to water-borne chemical cues from either an invasive predator (*P. clarkii*) or a native one (dragonfly nymphs of *Anax imperator*). Predators in this experiment were either unfed or fed *P. cultripes* tadpoles to experimentally distinguish the effect of predator cues from that of alarm cues produced by attacked conspecific tadpoles. We then tested whether *P. cultripes* tadpoles were capable of learning predator recognition of the invasive crayfish through conditioning with conspecific alarm cues. Finally, we tested whether learned predator recognition increased tadpole survival during staged encounters with invasive crayfish.

Materials and methods

STUDY ANIMALS

We collected four egg clutches of western spadefoot toad, *Pelobates cultripes*, at Doñana National Park, southwest Spain, from sites where invasive crayfish were absent. All eggs were in early stages of development (<10 Gosner; Gosner 1960). We transported the eggs to Doñana Biological Station in Seville and housed them in a walk-in climatic chamber to guarantee naivety of experimental tadpoles to predator cues. Upon hatching, tadpoles were raised individually in 4 L plastic buckets with carbon-filtered dechlorinated tap water at 20 ºC and under a natural photoperiod (12:12 L:D). We renewed water twice weekly, and subsequently fed tadpoles with ground rabbit chow.

We also dip-netted ten dragonfly nymphs (*Anax imperator*), and used fyke-nets to capture adult red swamp crayfish (*P. clarkii*) at various ponds within the Park, to be used as native and invasive predator cue donors, respectively. Dragonflies and crayfish were

also housed individually in a climatic chamber separated from that of tadpoles, to avoid chemical or visual contact with the predators prior to the experiments. Dragonflies were kept in 1 L plastic buckets whereas crayfish, being larger, were kept in 4 L ones. Temperature and photoperiod were the same as those of tadpoles. All surviving tadpoles were kept until metamorphosis and released as juveniles at their ponds of origin after standard prophylaxis procedures, whereas no dragonflies survived and crayfish were euthanized after the experiment.

PREPARATION OF CHEMICAL STIMULI

To prepare predator chemical cues, we filled each donor dragonfly aquarium with 0·5 L and each donor crayfish aquarium with 1·5 L of dechlorinated tap water, to be pervaded with predator cues. We used different volumes of water for native and invasive predators to roughly account for size differences among predator types, since dragonflies were substantially smaller than crayfish, although the concentration of chemical cues in each case was unknown. To provide necessary perches to the dragonfly nymphs, we placed plastic plants in all aquaria, thoroughly rinsed to eliminate any strange cues. Dragonflies and crayfish were divided in two sets and were cyclically fed *P. cultripes* tadpoles and deprived of food to provide appropriate cues for the 'unfed predator' and the 'fed predator' treatments (Gomez-Mestre & Diaz-Paniagua 2011). Dragonflies and crayfish in the 'unfed predator' treatment starved for two consecutive days while dragonflies and crayfish in the 'fed predator' treatment were fed *P. cultripes* tadpoles from a stock tank, once per day, during the same two consecutive days. We then extracted and mixed the water within treatment (i.e. 'unfed dragonfly', 'fed dragonfly', 'unfed crayfish' and 'fed crayfish') and froze it in 10 ml aliquots until use. Since predator cues last approximately 2-4 days in water (Peacor et al. 2006), we ensured that water-borne cues were collected within 48 h after the first tadpole was supplied to the predators. After collecting the water containing

the cues, predators in the 'fed predator' treatments were kept for two days without feeding to ensure that no tadpole matter remained in the digestive tract and then, we switched the two set of predators between the 'unfed' and the 'fed' treatments. Control water was prepared following the same procedure but without placing dragonflies or crayfish in the aquaria (Woody & Mathis 1998; Gonzalo, López & Martín 2007; Polo-Cavia *et al*. 2010).

Tadpole alarm cues were prepared from three conspecific donor tadpoles. Tadpoles were euthanized by immersion in a highly concentrated solution of MS-222 and immediately snap-frozen in liquid nitrogen. We then homogenised these preserved specimens in 600 ml of carbon-filtered, dechlorinated tap water with a bench top homogeniser (Miccra D-1, Müllheim, Germany). We then filtrated the homogenate with filter paper to remove solid particles. The water containing the alarm cues was immediately frozen in 10 mL portions until use (Woody & Mathis 1998).

INNATE PREDATOR RECOGNITION

In a first experiment, we examined the innate responses of *P. cultripes* tadpoles to water-borne cues from native and invasive predators. We measured basal activity of 15 individual tadpoles across five different treatments each ('clean water' *vs.* 'unfed dragonfly cues' *vs.* 'fed dragonfly cues' *vs.* 'unfed crayfish cues' *vs.* 'fed crayfish cues') in a random sequence. The experiment was conducted over five consecutive days, so that each individual tadpole was tested only once a day and given 24 h to rest in between assays. The 'clean water' treatment was included to compare tadpole responses in a predator-free environment with responses to different predator cues. Tadpoles were tested individually in grey, U-shaped, gutters (101 x 11·4 x 6·4 cm) sealed at both ends with plastic caps. We traced four markings inside the gutter to visually divide each gutter in five equal parts. Each gutter was filled with 3 L of carbon-filtered dechlorinated tap water and we added 10 mL test solutions of clean water or predator cues to each end of each gutter (two frozen

aliquots per gutter) to favour cue diffusion. We waited 5 min for the aliquots to thaw entirely and then we placed a single tadpole in the middle of each gutter, waiting another 5 min before the trials began to allow tadpoles to acclimate. We then monitored each tadpole for 30 min, using the instantaneous scan sampling method, and recording every 1 min the quadrant that each tadpole occupied in the gutter (30 scans per tadpole in total). We calculated swimming activity of tadpoles from the number of lines crossed by each tadpole in each trial (Rohr & Madison 2001; Gonzalo, López & Martín 2007; Polo-Cavia *et al*. 2010). All behavioural observations in the study were carried out blindly so that the observer was unaware of the type of cue placed in each gutter in each trial.

CONDITIONING WITH CHEMICAL ALARM CUES

In a second experiment, we analysed the capacity of *P. cultripes* tadpoles to learn to recognise the cues from invasive crayfish through conditioning with conspecific alarm cues. Twenty-seven new tadpoles were randomly assigned to the 'conditioned' treatment and other twenty-seven to the 'non-conditioned' treatment. Tadpoles in the 'conditioned' treatment were exposed to predator cues from crayfish in association with conspecific alarm cues to induce conditioning, whereas tadpoles in the 'non-conditioned' treatment were exposed to predator cues alone. We added 10 mL of test solution containing unfed crayfish cues and 10 mL of test solution containing tadpole alarm cues in each housing bucket of tadpoles in the 'conditioned' treatment. Likewise, we added 10 mL of water containing unfed crayfish cues plus 10 mL of clean water in each housing bucket of tadpoles in the 'non-conditioned' treatment. We then left individual tadpoles undisturbed in their buckets overnight. The next day we tested basal activity levels of all tadpoles ($n = 54$) in clean water and in water with predator cues from the invasive crayfish, in random order. For the trials, we added two frozen aliquots of clean water or water containing crayfish cues to each gutter, which had previously been filled with 3 L of dechlorinated tap

water and followed the same experimental procedure described in *Experiment 1* to calculate tadpole swimming activity. To avoid interference between predator and diet cues on responses of tadpoles (i.e. interference between kairomones and postdigestion alarm cues; Ferrari, Wisenden & Chivers 2010), we used 'unfed crayfish cue' and not 'fed crayfish cue' stimuli for conditioning and trials in this experiment.

EFFECT OF CONDITIONING ON SURVIVORSHIP

In a third experiment we tested whether learning by conditioning with alarm cues increased tadpole survival by comparing survival rates of conditioned *vs*. non-conditioned tadpoles from the conditioning experiment in real predation trials with invasive crayfish. Each tadpole from the 'conditioned' treatment was randomly paired up with a tadpole from the 'non-conditioned' treatment, and each mixed pair ($n = 27$) was introduced in a 10 L bucket with one crayfish. From that moment on we recorded the number of surviving tadpoles from each treatment every 10 min. Trials lasted for 3 h and we applied the instantaneous scan sampling method (18 scans per pair in total). Surviving tadpoles in each trial were unequivocally identified through specific anatomical and coloration features such as pigmentation patterns, tail shape and/or position of tail veins, previously recorded for each tadpole pair.

DATA ANALYSIS

To analyze differences between behavioural responses of naïve tadpoles in clean water and in water with predator cues (predator recognition experiment) we used a one-way repeated measures analysis of variance with treatment (five levels: 'clean water', 'unfed dragonfly cues', 'fed dragonfly cues', 'unfed crayfish cues', or 'fed crayfish cues') as a within-subject factor. To test for differences between conditioned and non-conditioned tadpoles in responses to water-borne cues from the invasive crayfish (conditioning experiment), we

used a two-way repeated measures ANOVA with treatment ('conditioned' *vs.* 'non-conditioned') as a between-subject factor and the experimental chemical stimulus ('clean water' *vs.* 'crayfish cue') as a within-subject factor. In both experiments we used activity level (i.e. the number of lines crossed by tadpoles over the total observation time) as dependent variable. We verified that our data met parametric assumptions checking for normality of the data with Kolmogorov-Smirnov's test ($P \geq 0.2$ for all variables) and homogeneity of variances with Levene's test ($P \geq 0.6$ for all variables). We conducted *post hoc* comparisons among treatments with protected Fisher's LSD tests (Sokal & Rohlf 1995).

To analyze the effect of learned predator recognition on tadpole survival in direct encounters with crayfish (predation trials), we conducted a survival analysis using Cox regression with treatment ('conditioned' *vs.* 'non-conditioned') as independent factor, experimental container as random factor and the timing of each predation event as dependent variable. Analyses were performed using Statistica 8.0 and R 2.15.0 software.

Results

INNATE PREDATOR RECOGNITION

We found significant differences in overall basal activity of naïve tadpoles between predator cue treatments (One-way repeated measures ANOVA; $F_{4,56} = 11.04$, $P < 0.0001$; Fig. 1). Tadpoles reduced their swimming activity by 23 % in the presence of dragonfly cues, compared to their activity in clean water (Fisher's LSD; $P = 0.043$). Tadpoles reduced activity even further (57 %) when exposed to cues from dragonflies that had fed on conspecific tadpoles ($P < 0.0001$). However, tadpoles did not reduce their activity level in the presence of chemical cues from invasive crayfish, regardless of whether crayfish were fed or unfed conspecific tadpoles ($P > 0.78$ for both fed and unfed crayfish treatments). Consequently, swimming activity of tadpoles was significantly lower when exposed to

dragonfly cues (either fed or unfed) than when exposed to crayfish cues (all $P \leq 0.02$).

CONDITIONING WITH CHEMICAL ALARM CUES

Overall swimming activity of tadpoles was significantly lower in water with chemical cues from unfed crayfish than in clean water (Two-way repeated measures ANOVA; cue treatment: $F_{1,52} = 11.3$, $P = 0.002$) (Fig. 2). Activity levels of conditioned and non-conditioned tadpoles did not significantly differ overall (conditioning: $F_{1,52} = 0.46$, $P = 0.49$), but there was a significant interaction between cue treatment and conditioning ($F_{1,52} = 9.55$, $P = 0.003$; Fig. 2). Tadpoles conditioned with conspecific alarm cues added in combination with crayfish cues significantly reduced swimming activity in the presence of crayfish cues compared to clean water (Fisher's LSD; $P < 0.0001$), whereas activity of non-conditioned tadpoles did not vary in the presence of crayfish cues compared to clean water ($P = 0.85$). In addition, swimming activity of conditioned and non-conditioned tadpoles did not differ significantly in clean water ($P = 0.39$), but conditioned tadpoles were significantly less active than non-conditioned tadpoles in the presence of crayfish cues ($P = 0.04$).

EFFECT OF CONDITIONING ON SURVIVORSHIP

Conditioned tadpoles had significantly higher overall survival against crayfish than non-conditioned tadpoles: 67 % *vs*. 44 % survival, respectively (Cox regression; $\chi^2 = 5.79$, $P = 0.016$; Fig. 3). The effect of experimental container was non-significant ($\chi^2 = 20.18$, $P = 0.78$). Conditioned tadpoles also survived for a longer time (mean ± SE: 137·4 ± 13·4 min *vs*. 109·6 ± 13·4 min). We observed predatory events in 19 out of the 27 tadpole pairs over the observational period. Non-conditioned tadpoles were often consumed before conditioned tadpoles (13 *vs*. 6 cases). In total, 24 out of 54 tadpoles were eaten after 3 h, 15 of them non-conditioned and 9 conditioned.

Discussion

Spadefoot toad tadpoles were capable of innately recognising water-borne cues from native dragonflies and adaptively reduced their activity level, as found in other amphibian species (reviewed in Kats & Dill 1998; Chivers & Smith 1998; Fig. 1). The behavioural response of tadpoles was even greater when dragonflies where fed conspecific tadpoles, indicating a synergistic effect of the combination of dragonfly kairomones and postdigestion cues. In contrast, *P. cultripes* tadpoles failed to discriminate chemical cues from invasive crayfish, regardless of whether crayfish were fed conspecific tadpoles or not (Fig. 1). Tadpoles of another anuran species in Doñana National Park also fail to recognise invasive *P. clarkii* (Gomez-Mestre & Diaz-Paniagua 2011). This suggests that the lack of innate recognition of invasive crayfish might be common to many amphibian species in the area. Alien crayfish were introduced in Doñana ca. 35 years ago, which may not be a long evolutionary time for adaptation to occur given the generation times of temperate amphibians (1-2 years). Nevertheless, some amphibian species have been able to adapt to novel thermal regimes via behavioural plasticity within a 30-year timeframe (Skellly & Freidenburg 2000; Freidenburg & Skelly 2004).

Dietary cues have been demonstrated to elicit antipredatory behaviour in response to novel predators in aquatic prey (Mathis & Smith 1993; Chivers & Mirza 2001; Nunes *et al*. 2013), but our results suggest that the presence of postdigestion cues in the water may not be sufficient to trigger recognition of alien predators by naïve larval anurans. In fact, Ferrari et al. (2007) suggest that generalisation of predator recognition is more based on chemical similarities among phylogenetically related predators than on predator's diet, assuming that related predators produce similar chemical cues (i.e. similar kairomones). Our results are consistent with this hypothesis, since the invasive red swamp crayfish is not related to natural predators of *P. cultripes* tadpoles at Doñana National Park; the only

native crayfish in the Iberian Peninsula, the white-clawed crayfish *Austropotamobius italicus*, has never been present in the Doñana marshes (Alonso, Temiño & Diéguez-Uribeondo 2000). Also, *A. italicus* mostly inhabits streams, whereas *P. cultripes* mainly breeds in temporary ponds (García-París, Montori and Herrero2004).

Despite a lack of innate recognition of novel predators, learning may be an effective way for native prey to assess environmental risk (Mirza *et al*. 2006; Gonzalo, López & Martín 2007; Ferrari, Messier & Chivers 2008a,b). Hence, learned predator recognition might reduce the impact of invasive predators. Here we show that *P. cultripes* tadpoles can learn to recognise chemical cues from invasive *P. clarkii* as a threat through their association with conspecific alarm cues, and modify their behaviour adaptively based on experience (Fig. 2). Associative learning through alarm cues has been reported in different species (see Ferrari, Wisenden & Chivers 2010 for a review), including flatworms (Wisenden & Millard 2001), molluscs (Rochette *et al*. 1998; Dalesman *et al*. 2006), insects (Ferrari, Messier & Chivers 2008a), crustaceans (Hazlett & Schoolmaster 1998), fishes (Brown & Smith 1998; Larson & McCormick 2005) and amphibians (Mirza *et al*. 2006; Gonzalo, López & Martín 2007; Ferrari, Messier & Chivers 2008b). Despite most of these studies having been conducted in the laboratory, learning through alarm cues is likely to happen in the wild too, since prey are continuously exposed to predator's kairomones jointly with alarm cues released by attacked conspecifics (Ferrari, Wisenden & Chivers 2010). This may be particularly so in aquatic lentic environments, although the degree of spatial and temporal variation in cue availability is currently unknown. By cognitive association of these cues, prey may dynamically adjust risk assessment, expanding their ability to detect and avoid predators by responding plastically to new threats through changes in morphology and/or behaviour. Furthermore, prey populations might then rely on a critical mechanism to cope with newly introduced predators and buy time for innate recognition to evolve.

Previous experience with predators has often been predicted to influence prey fitness and survival, but only a few studies to date have demonstrated adaptive value of learned predator recognition in direct encounters with predators (Mirza & Chivers 2000; Gazdewich & Chivers 2002; Lonnstedt *et al*. 2012). Our results show for the first time a quantifiable effect of learned predator recognition on survival rates of amphibian larvae during staged encounters with an exotic, globally introduced predator. We found that *P. cultripes* tadpoles previously exposed to chemical cues from the red swamp crayfish together with conspecific alarm cues gained a survival benefit of more than 50 % in such encounters, in comparison with tadpoles that were not conditioned. Enhanced survival is likely to occur also in natural conditions, since experienced tadpoles might rapidly identify predatory crayfish as threatening and exhibit avoidance behaviour or increase refuge use (Kiesecker & Blaustein 1997; Chivers *et al*. 2001; Pearl *et al*. 2003). We thus found that learned recognition of introduced predators successfully triggers antipredatory behaviour increasing survival against novel predators. This finding suggests that invasive crayfish may be positively selecting for learning ability of larval amphibians (Trussell & Smith 2000; Langerhans *et al*. 2004; Arendt & Reznick 2005).

Cognitive abilities of tadpoles might therefore be critical for amphibian populations to persist in the presence of newly introduced predators, tempering the immediate impact of invasions through behavioural plasticity and persist long enough for genetic variants to appear and respond to selection (Losos, Schoener & Spiller 2004; Strauss, Lau & Carroll 2006). Moreover, facultative changes in morphology and behaviour induced by novel predators in experienced tadpoles may allow them to effectively cope with the new selective challenge without necessarily or immediately leading to genetic shifts in populations (Carroll & Corneli 1999; Trussell & Smith 2000; Huey, Hertz & Sinervo 2003). In that sense, learning-mediated behavioural plasticity may lead the way towards adaptation to a novel predator, whereas the innate responses might evolve at a later time

(West-Eberhard 2003). Evolution of behavioural plasticity hence seems to be decisive for native amphibians to adapt to selective impacts from introduced predators (Strauss, Lau & Carroll 2006).

In conclusion, our results show that despite lacking innate recognition of invasive red swamp crayfish, native tadpoles can learn to recognise this alien predator when jointly perceiving crayfish cues and alarm cues from attacked conspecifics. This cognitive ability of tadpoles increases their odds of survival in direct encounters with crayfish, which might be critical for native amphibian populations to endure predator invasions until adaptation in the cue recognition system evolves.

Acknowledgements

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Figure legends:

Fig. 1. Activity levels (mean ± SE number of lines crossed during 30 min) of *Pelobates cultripes* tadpoles, in trials with clean water, water with chemical cues from a native predator (*Anax imperator*), and cues from an invasive predator (*Procambarus clarkii*). Predators were either unfed or fed conspecific tadpoles. Bars labelled with different letters differed significantly from one another.

Fig. 2. Activity levels (mean ± SE number of lines crossed during 30 min) of *Pelobates cultripes* tadpoles, either in clean water (open bars) or in the presence of chemical cues from invasive crayfish (dotted bars). Tadpoles had either been previously exposed to a combination of crayfish cues with alarm cues from conspecific tadpoles (conditioned) or to crayfish cues alone (non-conditioned). Bars labelled with different letters differed significantly from one another.

Fig. 3. Survival of non-conditioned (open circles) and conditioned with conspecific alarm cues (filled circles) *Pelobates cultripes* tadpoles, in real predation trials with the invasive crayfish *Procambarus clarkii*.
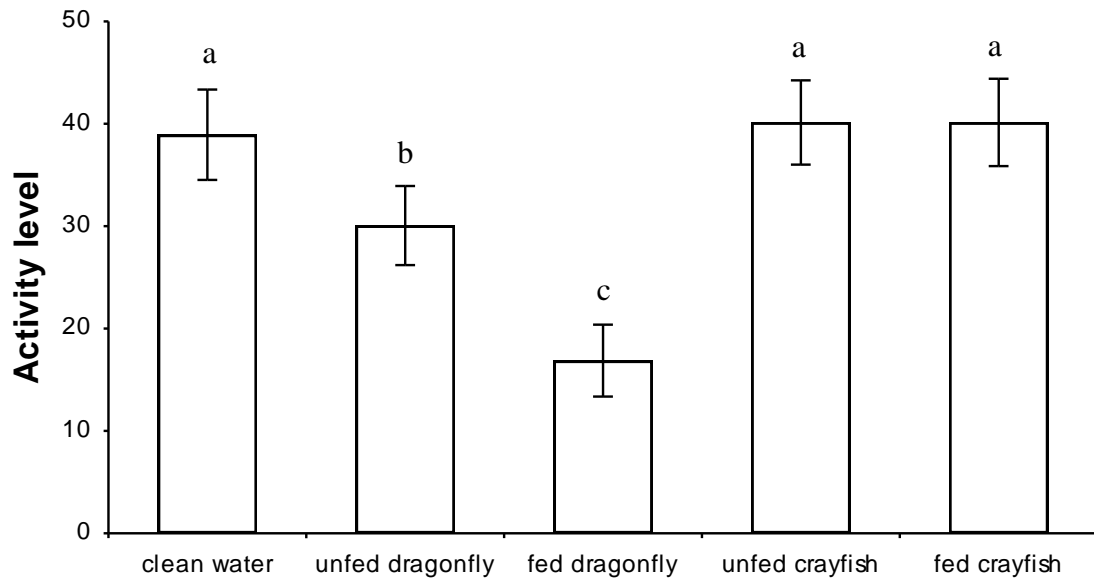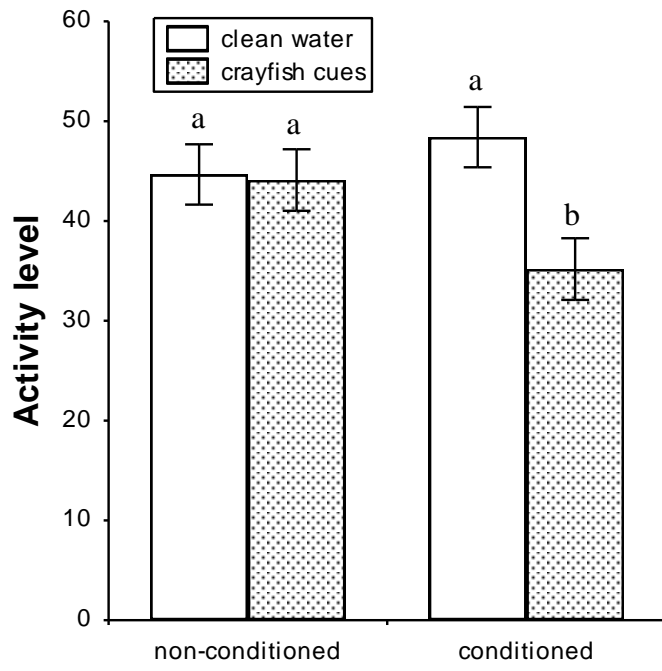
Fig. 1

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