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Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics

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Many antipredator adaptations are induced by the prey's ability to recognize chemical cues from predators and to act according to the threat level posed by that predator. However, predator recognition often requires learning by prey individuals. We tested the ability of Iberian green frog, *Rana perezi*, tadpoles to assess the magnitude of predation risk and adjust their behaviour by using perceived cues from a predatory snake, when this stimulus was found alone or associated with chemical alarm cues from conspecific tadpoles. Tadpoles exposed to alarm cues and the predatory snake scent together reduced their movement rates to a greater extent than when the snake scent was found alone, and reduced movement even more in the subsequent exposure to the predator snake scent alone. We also tested whether tadpoles were able to associate novel chemical cues (i.e. from an exotic nonpredatory fish) with predation risk after a simultaneous exposure with conspecific alarm cues. Tadpoles exposed to nonpredatory fish cues and alarm cues together reduced their activity levels, and reduced activity in the subsequent exposure to the fish cue alone, in a similar way as they reduced movement in the presence of predatory snake cues. Therefore, tadpoles learnt to perceive the fish cues as risky when these were previously associated with alarm cues. Predator recognition learning ability may be particularly advantageous for organisms whose environment may have a wide range of types of predators, even new exotic introduced species of predators that can affect the survival of prey.

Keywords: alarm cue; chemical cue; iberian green frog; learning; predation risk; predator recognition; *Rana perezi*

An important component of antipredator behaviour is the ability to detect and recognize predators (Lima & Dill 1990). Many antipredator adaptations are induced or mediated by the prey's ability to recognize chemical cues from predators (Kats & Dill 1998). Many aquatic animals, including some invertebrates, fish and amphibians, use chemical cues to assess predation risk (e.g. Von Frisch 1938; Petranka et al. 1987; Dodson et al. 1994; Kiesecker et al. 1996; Chivers & Smith 1998). Chemical cues may arise from the predators, but often they may be released by prey animals when they are captured by a predator (i.e. alarm cues; Chivers & Smith 1998; Kats & Dill 1998), which serve as a reliable and imminent indicator of risk for conspecifics (Chivers & Smith 1998).

Prey animals can reduce the probability of being captured by a predator by altering their behaviour after detecting cues that indicate increased predation risk (reviewed in Kats & Dill 1998). Thus, a higher responsiveness to predator or alarm cues may increase prey survival probabilities (Downes 2002). However, according to the threat-sensitive hypothesis (Helfman 1989), natural selection should favour individuals that take action appropriate to the magnitude of threat, which would require an accurate discrimination of the current level of risk that each predator poses. Thus, the response of prey to predators may be context dependent (Maerz et al. 2001). For example, many prey species only respond to chemical cues of a predator when the predator is fed a diet that contains conspecifics (e.g. Mathis & Smith 1993; Wilson & Lefcort 1993; Chivers et al. 1996).

In some cases, predator recognition requires learning by prey individuals (e.g. fish: Mathis & Smith 1993; damselfly

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larvae: Chivers et al. 1996; crayfish: Hazlett 2003). Individuals must experience simultaneously a predator cue and a danger cue, such as an alarm chemical cue released by a crushed conspecific, before the predator cue is considered as a danger signal. The ability to acquire recognition of predation risk is of obvious fitness benefit. Chemical alarm signals are important in facilitating learned recognition of predation risk by prey animals such as fish (Göz 1941; Magurran 1989; Mathis & Smith 1993; Chivers & Smith 1994, 1998; Larson & McCormick 2005) or adult newts (Woody & Mathis 1998). Releaser-induced recognition learning involves the simultaneous exposure to aversive stimulus and a neutral stimulus causing learned aversion to the neutral stimuli (Yunker et al. 1999). The result of this learning mechanism is acquired predator recognition in which predator naïve individuals show appropriate antipredator behaviour to the cue of a potential predator even though they have had no direct exposure to the predator. Several authors showed such acquired predator recognition in fish by pairing alarm cues with the visual or chemical cues of a predator (e.g. Chivers & Smith 1994, 1995; Larson & McCormick 2005). Therefore, the ability of prey to learn to recognize a novel predator should minimize the prey's risk of capture.

Predator recognition abilities of prey animals may have implications for current conservation issues. For example, although the effects of introduced predators on native species are complex (Kiesecker & Blaustein 1997, 1998), many amphibian populations have declined after the introduction of exotic predator species (Kupferberg 1997; Kiesecker & Blaustein 1998). Tadpoles have evolved a number of behavioural and morphological adaptations to survive and coexist with their natural predators, but those antipredator mechanisms that exist for native predators may not be sufficient to allow coexistence with introduced predator species (Gamradt & Kats 1996). Thus, several studies have found that amphibian populations were affected by new introduced predators (Kiesecker & Blaustein 1997; Adams 1999; Goodsell & Kats 1999; Knapp & Matthews 2000; Murray et al. 2004), but not all amphibian species were negatively affected (Hecnar & M'Closkey 1997). Recently, Bosch et al. (2006) have found that *Rana iberica* tadpoles could detect chemical cues from both native and exotic trout species and reacted by decreasing their activity, although the response towards native predators was stronger than the response towards exotic trout. The authors suggested that these antipredator behavioural responses were inefficient against the introduced trout, but did not reveal the origin of this antipredator behaviour in response to exotic predators. We hypothesized that tadpoles' responses might be elicited because exotic trout released chemicals cues similar to those released by native trout, but it also remains possible that *R. iberica* tadpoles were able to learn recognition of new potential predators.

In this study, we examined whether tadpoles of the Iberian green frogs, *R. perezi*, can use chemical cues of predators and/or alarm substances released from conspecifics to adjust their behaviour in response to the perceived predation risk. Furthermore, we aimed to determine whether tadpoles can learn to associate chemical cues to which tadpoles cannot be genetically predisposed (e.g. those from nonpredatory exotic fish species) with predation risk through their association with the simultaneous presence of alarm cues of conspecifics.

METHODS

Study Animals

We collected 63 Iberian green frog tadpoles (SVL, $\overline{X} \pm \mathrm{SE} = 1.3 \pm 1.2$ cm, Gosner's stage: 24; see Gosner 1960) by netting during July 2005 at several small ponds in Collado Mediano (Madrid, central Spain). Tadpoles were housed individually at 'El Ventorrillo' Field Station, 10 km from the capture area, in plastic aquaria (18 × 25 cm and 10 cm high) with water at ambient temperature and under a natural photoperiod. They were fed every 2 days with commercial fish flakes.

We also captured in a larger pond at the same locality two viperine snakes, *Natrix maura*, to be used as native predator scent donors. This snake is predominantly aquatic and mainly feeds on amphibians, both larvae and adults, and fish (Haley & Davies 1986; Braña 1998). Snakes were housed individually in plastic cages (36 × 25 cm and 13 cm high) containing sawdust and tree bark for cover and a pond with water (10 cm diameter). The snakes' cages were placed in a different room than the tadpoles' aquaria to avoid contact with the scent and visual stimuli before they were tested. To avoid potential confounding effects of the diet on the results, all snakes were fed small pieces of commercial freshwater fish, obtained from a fish market, for 3 weeks before collecting their chemical stimuli.

We obtained from a commercial dealer nonpredatory zebra danio fish, *Brachydanio rerio*, to be used as source of neutral scent. Before and after the experiment, fish were maintained in a large filtered aquarium and regularly fed with commercial fish flakes.

All the animals were healthy during the trials, all maintained or increased their original body mass, and all tadpoles metamorphosed into subadult frogs. These frogs and the snakes were returned to their exact capture site. The experiments were carried out under licence from the 'Consejería de Medioambiente de la Comunidad de Madrid' (the Environmental Agency of the local Government of Madrid). Procedures are conformed to recommended guidelines for use of live Amphibians in laboratory research (ASIH 2004).

Preparation of Chemical Stimuli

Alarm cues of tadpoles were prepared from three tadpoles (SVL, $\overline{X} \pm \mathrm{SE} = 1.2 \pm 0.1$ cm). They were cold anaesthetized by placing at 4°C for 20 min, inducing them deep hypothermia, and, then, euthanized with a quick blow to the head to avoid suffering (ASIH 2004). We did not use a chemical anaesthetic, because these chemicals may interfere with natural tadpoles' chemical cues in subsequent trials. The extract was then prepared

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by putting these dead tadpoles in a clean disposable plastic dish, and macerating them in 600 ml of distilled water. The stimulus water was then filtered through absorbent paper to remove solid particles, and immediately frozen in 10 ml portions until used (Woody & Mathis 1998).

The snake scent was prepared by placing the snakes individually in cages (36 × 25 cm and 13 cm high) containing 500 ml of clean water and left overnight. Then, we extracted and mixed the water, and frozen it in 10 ml portions until use. Clean water was collected from a nearby high mountain spring that did not house frogs, fish or snakes.

The neutral stimulus was prepared by placing zebra danio fish in groups of three into a 3-litre aquarium with clean water for 3 days. These aquaria were aerated but not filtered. Fish were not fed during this short period to avoid contaminating water with food odour. Thereafter, water was drawn from the aquaria and frozen in 10 ml portions until its use in experiments. Fish were returned and fed in their home large aquaria. We prepared control water in an identical manner but without placing fish or snakes in the aquaria (Woody & Mathis 1998).

Experimental Design

We planned an experiment with sequential determined trials to condition the tadpoles, and tried to determine whether frog tadpoles were able to assess predation risk and to learn recognition of novel predators. Between trials we allowed the tadpoles to rest for 1 day to avoid stress. We randomly distributed the tadpoles in four different treatments of 15 tadpoles each, and conducted the experiment in two different series. In each trial single individual tadpoles were tested separately. Thus, different trials were considered as replicates of each treatment. In the first series (Series 1), on Day 1 ('response to control clean water alone') individual tadpoles ($N = 30$) from two treatments ('control' and 'experimental') were tested with clean water. The objective of this trial was to determine the basal activity levels of tadpoles in a predator-free environment, and to use this number as a control for the effect of predator and alarm chemical cues in further trials. On Day 3 ('initial response to chemical cues') individual tadpoles from the 'control' treatment were exposed to the snake predator chemical cues alone mixed with clean water. Individual tadpoles from the 'experimental' treatment were exposed to both the scent of the predatory snake and conspecific chemical alarm cues, thus, simulating the cues from a predatory snake that was eating a conspecific tadpole. This allowed us to measure the effect of the alarm cues on tadpole behaviour. The objective of this trial was to determine whether frog tadpoles would react to the paired presentation of conspecific alarm cues and predator chemical cues. On Day 5 ('conditioned response to the predator stimulus') individual tadpoles from the two treatments were exposed to the predatory snake chemical cues alone mixed with clean water. The objective of this trial was to determine whether tadpoles were able to adjust their behaviour accordingly to the predation risk perceived in the previous trial. We predicted that tadpoles from the experimental treatment would have a greater fright reaction than tadpoles from the control treatment because the previous simultaneous presentation of predator and alarm cues would indicate that the predator was more dangerous than when the predator cues were presented alone.

In the second series (Series 2), we used different individual tadpoles ($N = 30$), to avoid previous experience. We followed the same procedure as in previous series: Day 1 ('response to control clean water alone'), Day 3 ('initial response to chemical cues'), and Day 5 ('conditioned response to the nonpredatory cues'). However, we used nonpredatory fish scent instead of predatory snake scent. The objective of this experiment was to determine whether tadpoles would react to the paired presentation of conspecific alarm cues and neutral chemical cues from nonpredatory fish (i.e. cues not previously associated with danger). Such paired stimuli might be experienced by a predator naïve prey exposed simultaneously to conspecific alarm cues and the scent of a predator, or to chemical cues from a predator that was eating a conspecific. We aimed to determine whether tadpoles were able to learn to recognize and associate the neutral cue with danger after the previous exposure. We predicted that only tadpoles of the experimental treatment (i.e. conditioned with conspecific alarm cue and the neutral cue) would respond with a fright reaction to the neutral cue alone. The two series were carried out in parallel, and observations were carried out blind.

Tadpoles were tested individually in grey, U-shaped gutters (101 × 11.4 cm and 6.4 cm high) sealed at both ends with plastic caps. We marked the internal part of the gutters with four crossing lines that created five subdivisions of equal surface. We made rectangular release cages (21 × 7.6 cm and 6.4 cm high) by sewing together clear, perforated plastic normally used for needlepoint (2 mm square holes), which were placed in the middle of the central subdivisions. We filled each gutter with 3 litre of clean water from a mountain spring at 20°C, and placed clear plastic over each trough on either side of the cage to isolate the system from air movements in the testing room (see Rohr & Madison 2001).

We made different test solutions, 20 ml each (2 ice aliquots), using combinations of clean water or water with alarm cues, and water with snake or fish scent, and a control treatment of clean water alone. We assigned test solutions to one end of each trough (right or left) by stratified randomization, and assigned 20 ml of clean water (2 ice aliquots) to the opposite end.

We placed a single tadpole covered with a release cage in each gutter, and waited 5 min for habituation. Then we deposited the test solution ices and we began trials by slowly lifting the cages above each tadpole 5 min after we deposited the test solution ices aliquots (i.e. after the ices aliquots had entirely thawed). We subsequently stood as motionless as possible recording from a hidden point the quadrant that each tadpole occupied at 1 min intervals for 30 min. We calculated levels of activity from the number of lines crossed by each tadpole during the observation period (Rohr & Madison 2001). Diffusion of chemicals in still water may be a slow process. However, all individual tadpoles used in the experiment were observed at least once in all of the subdivisions of the gutter, so we

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were confident that all tadpoles were really exposed to the chemical stimuli. Moreover, tadpoles often showed episodes of fast swimming which should contribute to diffuse chemicals in water.

Levels of activity (number of lines crossed) were log transformed and then tested by general linear modelling (GLM; Grafen & Hails 2002). We used day of the 'trial' as a within variable, and 'predator' (i.e. snake versus fish) and 'alarm' (i.e. control treatment with the absence of alarm cues versus experimental treatment with the presence of alarm cues) as categorical between variables. We included the interactions between variables in the model to test for the effects of the different treatments (with or without alarm cues) depending on the type of predator cues and the day of the trial. Subsequent post hoc multiple comparisons were made using Tukey's pairwise comparisons (Sokal & Rohlf 1995).

RESULTS

On average, tadpoles reduced their activity more with predatory snake chemical cues (Series 1) than with nonpredatory fish chemical cues (Series 2; 'predator' effect; Table 1, Fig. 1). Also, control tadpoles were more active than experimental tadpoles conditioned with conspecific alarm cues ('alarm' effect), and there were significant differences between the 3 days of the experiments ('trial' effect); all tadpoles being more active on the first day than the rest of the days (Table 1). However, all the two-way interactions between factors were significant (Table 1).

In Series 1 (Fig. 1a), activity levels on Day 1 (water alone) did not differ between control and experimental tadpoles (Tukey test: $P = 0.60$). On Day 3 (predatory snake chemical cues with or without alarm cues) tadpoles from the two treatments decreased their activity in relation to the previous day ($P = 0.001$ in both cases), but experimental tadpoles exposed to snake cues combined with alarm cues decreased significantly their activity than control tadpoles exposed to snake cues alone ($P < 0.0001$). Finally, on Day 5 (snake cues alone) the activity of control tadpoles did not change with respect to their activity on Day 3 ($P = 0.90$), but activity increased significantly in experimental tadpoles ($P = 0.0001$), although it did not reach the activity levels of the control treatment on this Day 5 ($P = 0.0001$).

Table 1. Results of a GLM testing the effects of 'predator' type (snake versus fish) and the presence or absence of 'alarm' chemical cues (between effects) on the activity levels of Iberian green frog tadpoles in the different days of the 'trials' (within effect; see Methods)

Effect	Sum of squares	*df*	*F*	*P*
Intercept	311.47	1	5986.14	<0.0001
Predator	0.53	1	10.14	0.002
Alarm	8.72	1	167.57	<0.0001
Predator×alarm	0.28	1	5.31	0.02
Error	2.91	56		
Trial	10.66	2	173.64	<0.0001
Trial×predator	0.51	2	8.34	0.0004
Trial×alarm	4.29	2	69.85	<0.0001
Trial×predator×alarm	0.01	2	0.10	0.90
Error	3.44	112		

In Series 2 (Fig. 1b), activity levels on Day 1 (water alone) did not differ between the two treatments of tadpoles (Tukey test: $P = 0.99$). On Day 3 (fish chemical cues with or without alarm cues), activity decreased significantly only in experimental tadpoles exposed to fish cues combined with alarm cues ($P = 0.0001$), but it did not change significantly in control tadpoles exposed to fish chemical cues alone ($P = 0.60$). On Day 5 (fish cues alone), activity of control tadpoles did not change with respect to the previous Days 1 and 3 ($P = 0.90$), whereas activity of experimental tadpoles increased significantly with respect to the previous Day 3 ($P = 0.0001$), but without reaching the activity levels of control tadpoles ($P = 0.009$).

In addition, there was a no significant three-way interaction between the factors and the trials, which indicated that tadpoles reacted in a similar way in the two series of the experiment, and showed that the alarm cue effect was more important than the effect of the type of predator (snake versus fish; Table 1). Thus, comparing the two series there were no significant differences between the four treatments of tadpoles in the level of activity on Day 1 (water alone: Tukey test: $P > 0.60$, in all cases). On Day 3, there were no significant differences between tadpoles from the two experimental treatments, which decreased their activity level in a similar way in the presence of alarm chemical cues, regardless of whether these were chemical cues from a predatory snake or from a nonpredatory fish. Also on Day 3, there were no significant differences between tadpoles from the two control treatments ($P = 0.90$). This showed that, although tadpoles did recognize the snake cues alone as a predator (because they decreased activity with respect to the water alone condition; see above) and did not recognize the fish cues as a predator (because there were not differences with respect to the water alone, see above), the effect of the predator chemical cues alone was lower than the effect of the predator cues combined with conspecific alarm cues. Finally, on Day 5, there were no significant differences between tadpoles from the two control treatments ($P = 0.71$), but there were significant differences between tadpoles from the two experimental treatments ($P = 0.04$). This showed that, although conditioned tadpoles were able to recognize the fish as a potential predator, tadpoles reacted more strongly to the snake chemical cues alone than to the fish cues alone (see above).

DISCUSSION

Our results first show that *R. perezi* tadpoles display antipredator behaviours (i.e. a reduction in activity) in response to chemical cues released from conspecifics. This is a typical antipredatory response to the presence of alarm signals, commonly reported in tadpoles of other frog species, but that was, however, generally assumed absent in Ranid frogs (e.g. Gohner & Pfeiffer 1996), such as our study species. Furthermore, our results indicated that *R. perezi* tadpoles were able to modify their antipredatory

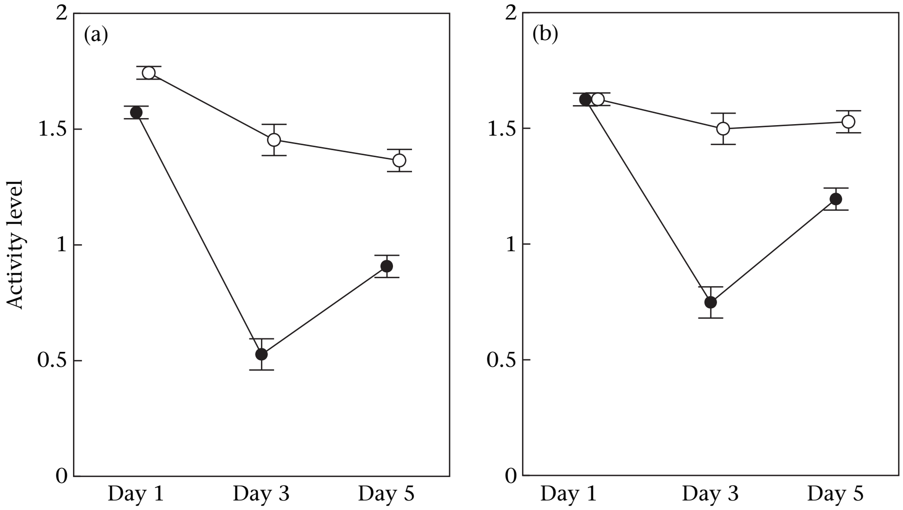

Figure 1. Activity levels of tadpoles ($\overline{X} \pm$ SE log transformed number of lines crossed by tadpoles during 30 min) in successive trials with clean water alone (Day 1), and water with chemical cues from (a) predatory snake or (b) nonpredatory fish (Days 3 and 5), with (●: alarm) or without (○: control) conspecific alarm cues added on Day 3 only.

behaviour according to their previous experience with chemical cues of the predator. At the end of the Series 1, conditioned experimental tadpoles decreased their activity more than control tadpoles when exposed to snake chemical cues alone, after the previous presentation of snake chemical cues in conjunction with alarm cues. This solution mix could simulate the chemical cues released by a predator that was eating a conspecific tadpole, and, thus, a tadpole can attribute to that predator a higher risk than to a potential predator but that was not actually attacking tadpoles. Thus, tadpoles seemed to assess the magnitude of predation risk and adjusted their behaviour by using perceived cues that vary according to the simulated predator diet.

The results suggested that the response of frog tadpoles represented a form of threat-sensitivity (Helfman 1989). According to the threat-sensitivity hypothesis, prey species should behave flexibly towards a varying degree of predator threat and, consequently, leave more time for other activities when the threat is low (Helfman 1989). The behavioural response elicited by the predatory snake in control tadpoles was weaker than that elicited by the same snake in experimental tadpoles. This suggested that snakes were not perceived to be very dangerous predators unless the tadpoles had been previously exposed to snakes that 'had eaten conspecifics' (i.e. mix of alarm cues plus snake chemical cues). Thus, alarm cues marked snakes, and allowed tadpoles to recognize snakes as dangerous predator in future encounters regardless of the snake's recent diet. In our case, this snake species preys mainly on adult (or metamorphosed) frogs, although it can also preys on tadpoles in lower proportions (Braña 1998). Thus, it is possible that tadpoles perceived this snake species not to be a very dangerous predator, unless they assessed that this snake was actually preying on tadpoles.

Furthermore, tadpoles were able to become conditioned to recognize a novel and nondangerous chemical cue as dangerous through associations of this cue with conspecific chemical alarm cues. Thus, experimental tadpoles were conditioned to recognize *B. rerio* (a nonpredatory fish) as a predator after the simultaneous exposure to conspecific alarm cues. As in the previous experiment, when we simulated a predator eating tadpoles, the alarm cues marked that 'novel predator' as dangerous, and fish were recognized as predators in subsequent exposures.

Fish are well known for their ability to acquire recognition of novel stimuli as dangerous. A single, simultaneous exposure to conspecific alarm cues and a novel stimulus transfers risk to the novel stimulus whether or not it is a novel chemical cue (e.g. Göz 1941; Suboski et al. 1990; Chivers & Smith 1995; reviewed in Smith 1992, 1997). Fish learned to recognize and avoid predator chemical cues after a single simultaneous encounter with predator and conspecific alarm cues. The ability to acquire recognition of novel predators has also been found in platyhelminthes (Wisenden & Millard 2001), crayfish (Hazlett 2003) and damselflies (Chivers et al. 1996). In amphibians we are only aware of one paper which found this ability in adults newts *Notophthalmus viridiscens* (Woody & Mathis 1998). These authors found that newts that spent all of their adult life in water were able to associate chemical alarm cues with chemicals from an unfamiliar predator. Our results showed that green frog tadpoles were able to learn a dangerous chemical cue from a nonpredator fish. Therefore, this learning mechanism may be especially important for the survivorship of prey species that are likely to find a high variety of predators while they are in the aquatic phase. To learn to recognize novel predators also may be of particular importance to prey species that live in habitats where the predator species vary across seasons or where new species of predators could appear (such as introduced predators). Prey species that live and breed in different kinds of aquatic habitats (such as green frogs) may experience a wide range of types of predators. Having the capacity of learning about the actual danger of new species could be very advantageous for their tadpoles, even more than having a genetically determined capacity to recognize diverse types of predators.

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Some studies have documented the decline of native frogs following the introduction of exotic predator species (e.g. Gamradt & Kats 1996; Goodsell & Kats 1999; Gillespie 2001), but other studies reported a lack of effects (Kiesecker & Blaustein 1997). According to Chivers et al. (2001) this may be in part due to the success of these frogs in recognizing and avoiding introduced predators. In the experiment of Chivers et al. (2001), juvenile treefrogs were able to recognize chemicals from an introduced predatory species (bullfrog), but it was not determined whether treefrogs learnt that bullfrogs were a threat or whether the recognition was genetically determined, because treefrogs came from a population syntopic with bullfrogs. Thus, most studies about new predator-species recognition have ignored the mechanisms which allow the recognition of a novel chemical stimulus as a potential predator. In our study, we found that this mechanism may be the association between the conspecific alarm cues with an unknown stimulus. Therefore, we could expect that this mechanism was also used in other species which use alarm cues.

Our results suggest that learning to recognize novel predators could be a possible mechanism to face up exotic introduced predators. The effects of introduced predators on native species are complex (Kiesecker & Blaustein 1997, 1998). The ability of prey to recognize an introduced predator should minimize the prey's risk of capture (Kiesecker & Blaustein 1997; Chivers et al. 2001). However, recognition of the predator does not by itself imply that there will not be significant predator effects, or the response elicited could be inefficient against the introduced species (Bosch et al. 2006). Our results showed that, although conditioned tadpoles were able to recognize the fish as a potential predator, tadpoles reacted more strongly to the snake chemical cues than to the fish cues. This result may imply that tadpoles were more sensitive to native predators than to novel introduced ones. Recognition of snakes may be, thus, genetically determined, but also it is likely that tadpoles may require more frequent exposures to the novel fish chemical cue combined with alarm cues to elicit a fright response similar to that elicited from native predatory snakes (reinforcement). Nevertheless, since we only tested one native and one novel predator species, the different responses to snake and fish might be predator-class or -species specific, and, for example, the response to a novel snake might be as great as for the native snake predator. Further experiments that replicate predator types (i.e. multiple novel and native predators) are needed to explain these differences.

Our knowledge of amphibian capacity for learning recognition of new predators is poor. But, it seems an important mechanism to avoid new or introduced predators. The results of Woody & Mathis (1998) and our own data suggest that amphibian species with a higher capacity of learning recognition of new aquatic predators may be those that spend a lot of time in the water, such as aquatic newts or anuran species whose tadpoles have long periods of growth before metamorphosis (e.g. Iberian green frogs). Further experiments are needed to ascertain which species are able to learn about new predators, and which species cannot, as these would be the species most at risk from introduced predator species.

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