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Adaptive Forgetting in Iberian Green Frog Tadpoles (*Pelophylax perezi*): Learned Irrelevance and Latent Inhibition May Avoid Predator Misidentification

Adega Gonzalo, Pilar López, and José Martín
Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain

Predator recognition often requires learning by prey individuals. Iberian green frog tadpoles (*Pelophylax perezi*) have the ability to learn to recognize new potential predators when their chemical cues are found paired with conspecific alarm cues. However, a random pairing of alarm cues and chemical stimuli of a nonpredator might later induce costly antipredator responses to nondangerous species. Here, we studied the potential existence in this frog species of two phenomena (learned irrelevance and latent inhibition) that could help tadpoles to avoid these nonadaptive responses to chemical cues of nonpredator species. Our results showed that, when tadpoles experienced a random pattern of presence of alarm cues alone or predator cues alone over the 4 days before or after the simultaneous detection of these two cues paired, no learned association was formed. These results showed the existence of an effect of learned irrelevance on learning in Iberian green frog tadpoles. Also, tadpoles clearly inhibited the formation of a learning association between predator and alarm cues after a 4-day period during which they had been exposed to the predator cues alone. This result showed the existence of an effect of latent inhibition on learning about cues related to increased predation risk. Thus, both learned irrelevance and latent inhibition, rather than being considered to be failed predator recognition, can rather be seen as adaptive ways for dealing with conflicting information and as strategies to avoid learning irrelevant information and costly antipredatory responses to nonpredatory stimuli.

Keywords: predator recognition, conditioning learning, learned irrelevance, latent inhibition, frogs, tadpoles

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An important component of antipredator behavior is the prey's ability to detect and recognize predators (Lima & Dill, 1990), which in many cases depends on the 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, Kats & Sih, 1987; Dodson et al., 1994; Kiesecker, Chivers & Blaustein, 1996; Chivers & Smith, 1998; reviewed by Ferrari, Wisenden & Chivers, 2010). Chemical cues may arise from the predators, but chemical stimuli may also be often released by prey when they are captured by a predator (i.e., alarm cues) (Chivers & Smith, 1998; Kats & Dill, 1998; Ferrari, Wisenden & Chivers, 2010). Alarm cues serve as a reliable and imminent indicator of risk for conspecifics (Chivers & Smith, 1998).

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Adega Gonzalo, Pilar López, and José Martín, Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain.

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Correspondence concerning this article should be addressed to José Martín, Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, E-28006 Madrid, Spain. E-mail: jose.martin@mncn.csic.es

In some cases, chemically based predator recognition requires learning by prey individuals (e.g., fish: Mathis & Smith, 1993; damselfly larvae: Chivers, Wisenden & Smith, 1996; crayfish: Hazlett, 2003; amphibians: Mirza, Ferrari, Kiesecker & Chivers, 2006; Gonzalo, López & Martín, 2007). Prey must be exposed simultaneously to a predator cue and a danger cue, such as an alarm cue released by a crushed conspecific, before the predator cue is considered as a danger signal. However, in natural conditions, prey do not only find the predator and alarm chemical cues paired, but they also may find a mix of a wide range of cues. So, prey are often confronted with cues from multiple types of potential predators, but also with cues from nonpredator species, and a response caused by a random pairing of any unrelated cues inducing unnecessary antipredator behavior to nonpredatory stimuli could be very costly (Belden, Wildy, Hatch & Blaustein, 2000). Although numerous studies have investigated learning of predators by prey (see above), less attention has been directed to mechanisms for avoiding or forgetting the erroneous learning of nondangerous species as dangerous. Mechanisms for forgetting such erroneous associations are, however, very important because they help to prevent prey from continuing to exhibit maladaptive antipredator behaviors toward nonpredator species.

In classical conditioning, the subject learns about the relationship between two stimuli, an unconditioned stimulus, which produces a strong, consistent, overt unconditioned response, and a conditioned stimulus, which in itself produces either no overt response or a weak response usually unrelated to the response that eventually will be learned (Kamprath & Wotjak, 2004). However, the rate at which a subject acquires conditioned responses to paired

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presentations of conditioned and unconditioned stimuli can be affected by prior exposure to those individual stimuli (Allen et al., 2002). There are two phenomena that might be particularly relevant to the situations that prey probably experience in nature, one is "learned irrelevance" (Bennett, Wills, Oakeshott & Mackintosh, 2000), in which the random exposure to two cues alone altered later learning that would follow from exposure to the two cues paired. The other phenomenon is 'latent inhibition,' where the previous exposure to a stimulus (without any reinforcement) results in a reduction in the strength of a learned association that could be formed later (Ferguson, Cobey & Smith, 2001). Thus, if both of these phenomena also occur in the predator learning process, the detection of a cue from a potential predator that was not temporally linked to conspecific alarm cues indicating increased predation risk might later inhibit the formation of the association and learning that would occur normally when the two cues appear together.

However, only a few studies have demonstrated the existence of learned irrelevance or latent inhibition in the predator learning process. Hazlett (2003) showed learned irrelevance in virile crayfish (*Orconectes virilis*). When crayfish were given uncorrelated presentations of conspecific alarm cues and the cues of a novel predator, associative learning failed to occur. Acquistapace, Hazlett and Gherardi (2003) demonstrated latent inhibition in crayfish. Preexposure to cues of a novel predator for two hours during three consecutive days prevented associative learning of those cues with high risk. Latent inhibition has also been demonstrated in fathead minnows (Ferrari & Chivers, 2006a). Minnows preexposed to novel predator cues for one hour on five consecutive days failed in the posterior associative learning of the predator. Similarly, a coral reef fish, the lemon damselfish (*Pomacentrus moluccensis*), can learn to recognize a predator as nonthreatening through latent inhibition (Mitchell, McCormick, Ferrari & Chivers, 2011).

Amphibian tadpoles can learn to recognize unknown chemical cues as dangerous when the cues have been previously presented paired with conspecific alarm cues (Mirza, Ferrari, Kiesecker & Chivers, 2006; Gonzalo, López & Martín, 2007; Ferrari, Wisenden & Chivers, 2010). Cues from nonpredatory species can also been learned as dangerous if they have been previously associated with alarm cues (Gonzalo, López & Martín, 2007). However, the existence of mechanisms able to prevent learning association in amphibians has only be explored in embryos of a single frog species. In wood frogs (*Lithobates sylvaticus*), embryonic preexposure to the odor of tiger salamanders (*Ambystoma tigrinum*) without risk reinforcement inhibited the subsequent learning by tadpoles of salamanders as a potential predator (Ferrari & Chivers, 2009, 2011).

In this study, we investigated whether latent inhibition and learned irrelevance may inhibit the associative learning of predator cues in tadpoles of the Iberian green frog (*Pelophylax perezi*, formerly *Rana perezi*). This species lives and breeds in different kinds of aquatic habitats (García-París, 2000) that contain a wide range of predators and nonpredators. Iberian green frogs have long periods of growth before metamorphosis (García-París, 2000), and their predator species vary across seasons. Thus, accurate learning of true predator cues should be important for *P. perezi* tadpoles. Previous experiments showed that Iberian green frog tadpoles are able to learn potential predators after the presentation of predator chemical cues paired with alarm cues (Gonzalo, López & Martín, 2007). This learned association lasts for at least nine days (Gonzalo, López & Martín, 2009). Tadpoles also may learn to recognize predators from disturbance cues, although responses are weak when compared with their response to alarm cues, and the period of retention of the learned association was shorter (Gonzalo, López & Martín, 2010). However, under natural conditions frog tadpoles face a wide range of mixed cues (e.g., alarm cues, predator cues, nonpredator cues), and learning inhibition mechanisms could be an adaptive way to avoid learning irrelevant information. In this experiment, we exposed Iberian green frog tadpoles to a random pattern of nonpredator and alarm chemical cues, or to nonpredator cues alone for four days, before conditioning and examined whether tadpoles later recognized the nonpredatory fish cues as dangerous.

General Method

Study Animals

We collected 180 Iberian green frog tadpoles (SVL, mean $\pm$ *SE* = 5.2 $\pm$ 0.3 mm, Gosner stage: 24; see Gosner, 1960) by netting at several small ponds in Collado Mediano (Madrid, central Spain) during several days of August, 2006. We were able to find different cohorts of tadpoles of similar size on different dates for a prolonged period because of the long breeding period of this frog (García-París, 2000). This allowed us to use tadpoles of similar size and stage in all experiments. Tadpoles were housed individually at "El Ventorrillo" Field Station, 10 km from the capture area, in plastic aquaria (10 cm $\times$ 15 cm and 10 cm high) with water at ambient temperature and under the natural photoperiod of the area. Tadpoles were fed every day with commercial fish flakes.

We obtained zebra danio fish (*Brachydanio rerio*) from a commercial dealer to be used as source of the neutral scent. Fish were maintained in a large filtered aquarium and regularly fed with commercial fish flakes. This is a nonpredatory exotic fish species from southeastern Asia that does not coexist in the wild Iberian green frogs in Europe. No other closely related fish species are found in the habitat of Iberian green frogs. We used this nonpredatory, evolutionarily novel species for conditioning tadpoles to avoid possible innate responses to chemical cues that might resemble predatory fish species that cohabit with these tadpoles.

After completion of the experiments, tadpoles were maintained in captivity for other studies, and after metamorphosis were returned to their exact capture site. Procedures 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 of the captured tadpoles (SVL, mean $\pm$ *SE* = 4.2 $\pm$ 0.1 mm). They were cold anesthetized by inducing them deep hypothermia by placing at 4 °C for 20 min, and then euthanatized with a quick blow to the head (ASIH, 2004). We did not use a chemical anesthetic, because these chemicals may interfere with natural tadpoles' chemical cues in subsequent trials. The extract was then prepared by putting these dead tadpoles in a clean disposable plastic dish and macerating them in 3 L 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; Gonzalo, López & Martín, 2007).

The fish stimulus was prepared by placing 10 zebra danio fishes into a 10 L aquarium with clean water for three days. These aquaria were aerated but not filtered. Fish were not fed during this short period to avoid contaminating water with food odor. Thereafter, water was drawn from the aquaria, filtered through absorbent paper, and frozen in 10-mL portions until its use in experiments (Woody & Mathis, 1998; Gonzalo, López & Martín, 2007). Fish were returned and fed in their home large aquaria. We prepared clean control water in an identical manner but without placing fish in the aquaria and then freezing it in 10 mL portions. Clean water without chloro came from a mountain spring, which did not contain fish or tadpoles.

Preexposure Period

We randomly assigned tadpoles to six independent experiments of 30 individuals each. Each individual tadpole only participated in one experiment. In each experiment, all tadpoles were first exposed, in their own individual aquaria, over a period of five days to the same preexposure to chemical cues. In different experiment we used different sequences and combinations of exposure to a novel chemical cue (zebra danio fish), conspecific alarm cues, or both types of cues. The particular sequences and durations of exposure are described below for each experiment. In each test, we used different test solutions, 20 ml each (two ice aliquots), of water with alarm cues, water with fish scent, or both cues combined (two ice aliquots of each solution). The test solutions were gently placed in the aquaria after the ice aliquots had entirely thawed.

Testing Protocol

After the preexposure periods, tadpoles within each experiment were randomly assigned to a control (clean water, $n = 15$) or experimental treatment (zebrafish cues, $n = 15$). Tadpoles were tested individually in an observation aquaria consisting of gray, U-shaped gutters (101 cm $\times$ 11 cm and 6 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 filled each gutter with 3 L of clean water from a mountain spring, which did not contain fish or tadpoles, at 20 °C. We 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 randomly assigned test solutions (20 mL, two ice aliquots, of clean water or water with zebra danio fish cues) to one end of each trough (right or left), and assigned 20 mL of clean water (two ice aliquots) to the opposite end.

In all experiments, we placed a single tadpole covered with a release cage in each gutter and waited 5 min for acclimatization. Then we deposited the test solution ices (zebrafish for experimental tadpoles or clean water for control tadpoles), and we began trials by slowly lifting the cages above each tadpole 5 min after we deposited the test solution ice aliquots (i.e., after the ice aliquots had entirely thawed). We subsequently stood as motionless as possible in a hidden area and recorded the section 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; Gonzalo, López & Martín, 2007). 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 were confident that all tadpoles were exposed to the chemical stimuli. Moreover, tadpoles often showed episodes of fast swimming which should contribute to diffusing the chemicals in the water.

Each experiment was analyzed separately. We used one-way ANOVAs to test for differences in activity level of tadpoles on Day 6 (number of lines crossed in the observation aquaria; log transformed) between control (clean water) and experimental (zebrafish cues) tadpoles (Sokal & Rohlf, 1995).

Experiment 1: Exposure to Alarm Cues Alone (Basal Activity)

A previous experiment showed that basal activity of tadpoles before training with alarm cues was similar to the activity level showed after training and later tested with clean water (Gonzalo, López & Martín, 2007). Here, we performed a first experiment to test whether the preexposure in consecutive days to alarm cues alone altered the behavioral pattern of activity of tadpoles when they later found the fish cues alone.

Method

Experiment 1. All 30 tadpoles within this experiment were exposed to clean water alone on Days 1 and 3, and to alarm cues alone on Days 2 and 4. Aquaria water was not changed during these days. On Day 6, tadpoles were individually placed in the observation aquaria (see testing protocol above) and tested randomly with either zebrafish cues (experimental group, $n = 15$) or clean water (control group, $n = 15$).

Results

Experiment 1. There were no significant differences in activity on Day 6 between control and experimental tadpoles, $F(1, 28) = .4$, $p = .50$ (Figure 1A). Thus, the previous exposure to alarm cues alone had no effects on the activity of tadpoles when they later experienced fish cues alone.

Experiments 2 and 3: Classical Conditioned Learning

Iberian green frog tadpoles are able to learn new chemical cues as dangerous after conditioning with alarm cues (Gonzalo, López & Martín, 2007), so these two experiments were designed to confirm whether tadpoles were able to learn to associate nonpredatory fish cues with high predation risk. We also used these experiments to test whether tadpoles that had been conditioned with alarm cues to recognize a predator as dangerous reduced their activity as an antipredatory behavior when they were later exposed to the cues of the predator alone.

In Experiment 2, we exposed tadpoles on two different days to the simultaneous presence of the chemical cues of a simulated potential predator (zebrafish) and alarm cues paired, simulating that when fish cues appeared, a predation event also always occurred (as indicated by the presence of alarm cues).

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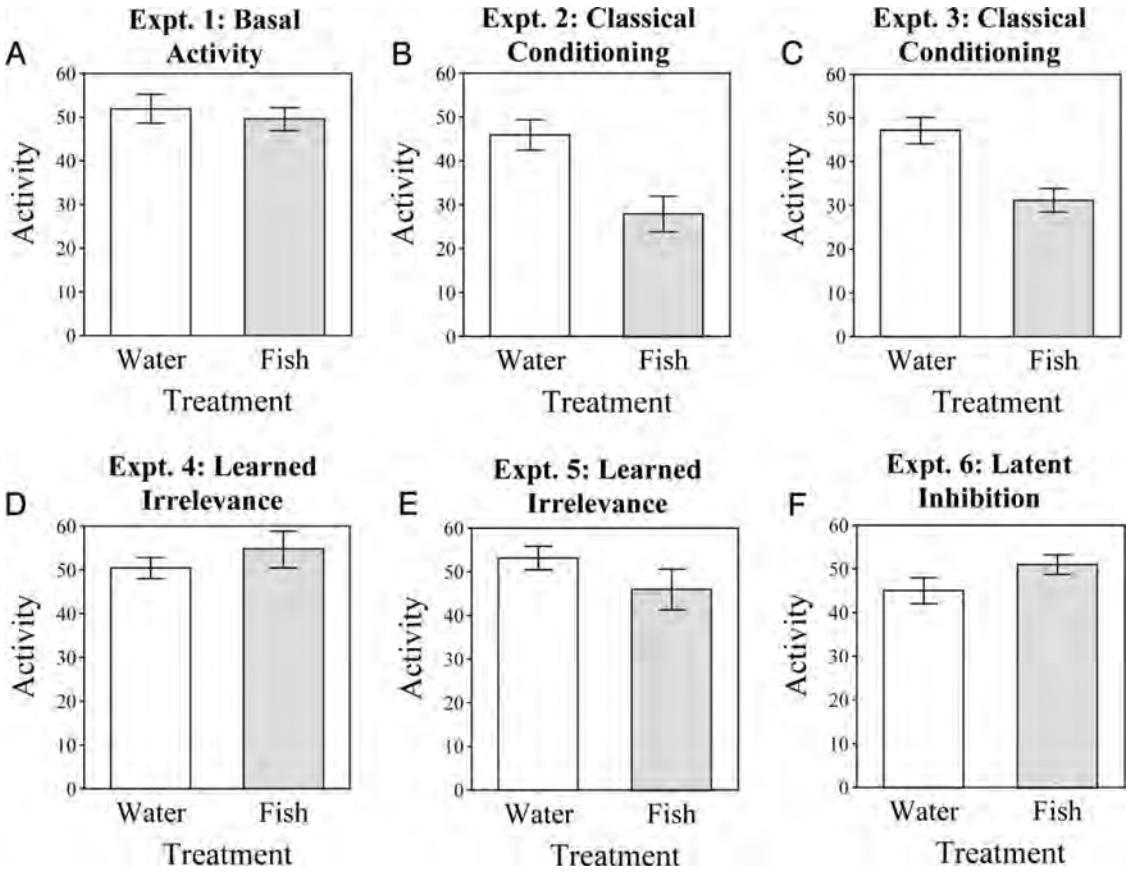


Figure 1. Mean (±*SE*) activity level (i.e., numbers of line crossing) of Iberian green frog tadpoles when exposed to zebrafish cues (experimental) or clean water (control) at the end of the different experiments (A to F) with different patterns of pre-exposure to fish and alarm cues (see Method).

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In Experiment 3, we tested the persistence of the fish cues in water as effective cues to elicit learning. We simulated a situation where tadpoles lived exposed to a simulated potential predator (zebrafish) whose chemical cues remained constantly in the water, and that tadpoles were also exposed on different days to alarm cues. Both cues did not appear simultaneously but on alternate days. However, if the fish cues persisted in water for at least a day (Hazlett, 1999, 2003; Ferrari, Messier & Chivers, 2008), they could be found paired with alarm cues by tadpoles. We then simulated a single occasion when the predator ate a conspecific tadpole, which released alarm cues that, then, appeared simultaneously mixed with predator cues.

Method

Experiment 2. All tadpoles ($n = 30$) were trained by exposing them to clean water alone on Days 1 or 3, or to zebrafish cues and alarm cues paired on Days 2 and 4. Aquaria water was not changed during these days. On Day 6, tadpoles were placed individually in the observation aquaria (see testing protocol above) and tested randomly with either zebrafish cues (experimental, $n = 15$) or clean water (control, $n = 15$).

Experiment 3. During the first four days, all 30 tadpoles were trained by exposing them to zebrafish cues alone on Days 1 and 3 and to alarm cues on Days 2 and 4. We did not change water in the aquaria between days to maximize the time that the zebrafish and alarm cues could be found together. On Day 5, all tadpoles were exposed to the simultaneous presentation of zebrafish cues and alarm cues for 2 h. On Day 6, tadpoles were individually placed in the observation aquaria (see testing protocol above) and tested randomly with either zebrafish cues (experimental, $n = 15$) or clean water (control, $n = 15$).

Results

Experiment 2. Control tadpoles showed significantly higher levels of activity on Day 6 than experimental tadpoles, $F(1, 28) = 10.0$, $p = .003$ (Figure 1B). Thus, after a classical conditioning with alarm cues, tadpoles showed evidence of the persistence of a learned association between zebra fish cues and elevated predation risk by the significant later reduction of activity in response to zebra fish cues alone.

Experiment 3. Activity levels on Day 6 were significantly higher in control than in experimental tadpoles, $F(1, 28) = 13.7$, $p < .001$ (Figure 1C). Thus, tadpoles previously exposed to independent sequential presentations of the two cues (fish and alarm), but without removal of water that allowed the maintenance of the two cues together, appeared to form a learned association

between the two cues, which resulted in a decrease of activity when the zebra fish cues were later found alone.

Experiments 4 and 5: Learned Irrelevance

These experiments were designed to test whether tadpoles showed the phenomena of learned irrelevance. We simulated a natural situation where tadpoles lived with a nonpredator (zebrafish) whose chemical cues were found independently of predation events (indicated by the presence of alarm cues). In Experiment 4, we exposed tadpoles sequentially to fish and alarm cues alone, then to both cues together. However, in nature, a series of independent exposures to one of the two types of cues alone (predator and alarm) could occur either before or after the simultaneous detection of these two cues paired. Thus, in Experiment 5, tadpoles were trained as in Experiment 4, but tadpoles were first exposed to fish and alarm cues together, then to sequential exposure to fish and alarm cues alone.

Method

Experiment 4. During the first four days, all 30 tadpoles were trained with presentation on alternate days of zebrafish cues alone followed the next day by presentation of alarm cues alone. Zebrafish or alarm cues were placed in the training aquarium for only 2 h, and thereafter we siphoned about 85% of the water from the training container (as in Hazlett, 2003) and immediately replaced it with clean water (at room temperature to avoid temperature shock). Water was removed to avoid the permanence of a high concentration of zebrafish or alarm cues in the water after the independent presentation, thus avoiding the mix of alarm and zebrafish cues in subsequent days. Exposure to fish cues alone was randomly performed on either Days 1 and 3 or on Days 2 and 4, and alarm cues alone were added on the alternate days. On Day 5, all tadpoles were exposed simultaneously to zebrafish and alarm cues paired together for 2 h. On Day 6, tadpoles were individually placed in the observation aquaria (see testing protocol above) and tested randomly with either zebrafish cues (experimental, $n = 15$) or clean water (control, $n = 15$).

Experiment 5. On Day 1, all tadpoles ($n = 30$) were first exposed simultaneously to zebrafish cues and alarm cues paired for 2 h (with draining of 85% of water after the exposure to remove the zebrafish cues) (Hazlett, 2003). All tadpoles were then exposed to zebrafish cues alone for 2 h (with draining of 85% of water after the exposure) on either Days 2 and 4, or on Days 3 and 5, and on alternate days to alarm cues alone for 2 h (with draining of 85% of water after the exposure). On Day 6, tadpoles were individually placed in the observation aquaria (see testing protocol above) and tested randomly with either zebrafish cues (experimental, $n = 15$) or clean water (control, $n = 15$).

Results

Experiments 4 and 5. There were no significant differences in activity on Day 6 between control and experimental tadpoles in either of these experiments (Experiment 4: $F(1, 28) = .4$, $p = .50$; Experiment 5: $F(1, 28) = 2.8$, $p = .10$ (Figure 1D and 1E). Thus, tadpoles that were exposed to a sequential independent presentation of the fish or alarm cues alone did not appear to form an association between the two cues when these were presented paired, and showed similar activity to control tadpoles when later finding the fish cues alone. These results suggest that tadpoles showed the phenomena of learned irrelevance, where the random exposure to fish or alarm cues alone during four days altered learning that would follow from exposure to the two paired cues. Also, this effect appeared regardless of whether the simultaneous exposure to fish and alarm cues occurred after (Experiment 4; Figure 1D) or before (Experiment 5; Figure 1E) the independent presentation of the fish and alarm cues alone.

Experiment 6: Latent Inhibition

This experiment was designed to test whether tadpoles show the phenomena of latent inhibition. We simulated a natural situation where tadpoles are constantly exposed to the chemical cues of a predator (zebrafish), but where there are no predation events until the last day of training. Thus, we made several exposures to fish cues alone, followed by a simultaneous exposure to fish and alarm cues together.

Method

Experiment 6. All tadpoles ($n = 30$) were exposed to clean water on Days 1 and 3, and to zebrafish cues alone on Days 2 and 4 (without water removal after the exposure). On Day 5, all tadpoles were simultaneously exposed to zebrafish cues and to alarm cues paired together for 2 h. On Day 6, tadpoles were tested individually (see testing protocol above) with either zebrafish cues (experimental, $n = 15$) or clean water (control, $n = 15$).

Results

Experiment 6. There were no significant differences in activity on Day 6 between control and experimental tadpoles, $F(1, 28) = 2.7$, $p = .10$ (Figure 1F). Thus, tadpoles did not show an association of zebrafish cues with predation risk when they had been exposed to zebrafish cues alone for three days before the simultaneous presentation of the zebrafish cues paired with alarm cues. This result suggests that tadpoles showed latent inhibition where the previous frequent exposure to the fish stimulus alone (without any reinforcement with alarm cues) resulted in a reduction of the strength in the learned association that could be formed later with the simultaneous presentation of fish and alarm cues.

General Discussion

The ability to learn to recognize predator chemical cues has been demonstrated in a wide variety of taxa (reviewed by Chivers & Smith, 1998; Ferrari, Wisenden & Chivers, 2010). In almost every case, the experimental protocol was classical conditioning experiment, in which a single paired presentation of a predator odor and alarm cues induces an antipredator behavior in a subsequent exposure to predator odor alone. However, animals in nature continually receive information about their environment and must filter this information to focus on those aspects most important to survival (Lima & Dill, 1990; Dukas, 2002). Our experiments clearly demonstrate that the temporal pattern of exposure to predator and alarm cues determines whether Iberian green frog tadpoles will form a learned association with inputs from a successful

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predation event, which can then be used when the predator cues are subsequently detected alone.

Predator cues can persist in the habitat for a long time (Hazlett, 2003). Fish cues persist for more than one day (Hazlett, 2003; present study), whereas the persistence of alarm cues is shorter; Ferrari, Messier and Chivers (2008) showed than alarm cues of wood frogs (*Lithobates sylvaticus*) quickly degrade under natural conditions. Hazlett (1999) tested the persistence of crayfish (*Orconectes virilis*) alarm cues in artificial conditions and concluded that alarm cues persist for at least six hours. Our study showed that tadpoles can form an association between predator cues and alarm cues even when they were presented on different days but persisted in the water and could be found together (Experiment 3). Thus, one-day-old fish cues were effective in eliciting the learning association in tadpoles.

In another laboratory experiment, classically conditioned Iberian green frog tadpoles reduced their activity in response to fish chemical cues, and this response lasted at least nine days without further reinforcement (Gonzalo, López & Martín, 2009). However, in nature, tadpoles are not exposed to the presence of a single event of paired cues, but to a mix of different types of cues. So, the ability to learn to recognize a predator could be more complex than just detecting two paired cues simultaneously. Avoiding erroneous learning could help animals to avoid nonadaptive responses to chemical cues from nondangerous species that sometimes may be randomly paired with alarm cues.

Learned irrelevance occurs when the random presentation of the two cues indicates to the prey that the two cues are not causally linked. Hazlett (2003) demonstrated the existence of this phenomenon in crayfish. Likewise, in our study, tadpoles failed to form an association between a conditioned stimulus (alarm cues) and a novel cue (fish) when they had been exposed to the two cues separately before or after the simultaneous presentation of the two cues paired (Experiments 4 and 5). These results showed that tadpoles are able to learn that the two types of cues are not causally connected if the two cues are found independently before or after they are found simultaneously. Therefore, our results demonstrate the existence of the process of learned irrelevance in Iberian green frog tadpoles.

Nevertheless, a simpler alternative explanation of these results might be that the learning process was dose-dependent such that tadpoles might be more sensitive to learn association when the time that they found together predator and alarm cues was longer, or when the dose of alarm cues in water was stronger. Thus, in Experiments 4 and 5, where alarm or predator cues were diluted after 2 h by removing most of the water, tadpoles might be less sensitized to learn associations than in Experiments 2 and 3 where alarm or predator cues were left in the water for 24 h. Further studies should consider the responses of tadpoles when different densities of predator and alarm cues are found in the water.

Latent inhibition occurs when a previous exposure to a neutral stimulus indicates to the animal that this cue is not linked with other cues with biological significance. Latent inhibition has been demonstrated in crayfish (Acquistapace, Hazlett & Gherardi, 2003), fathead minnows (Ferrari & Chivers, 2006a), a coral reef fish (Mitchell et al., 2011), and wood frogs (Ferrari & Chivers, 2009, 2011). In our experiment Iberian green frog tadpoles also failed to form the association when they were exposed to the unconditioned fish stimulus alone for four days before or after the unconditioned (fish) and the conditioned (alarm) cues were presented together. Therefore, our results demonstrate the existence of the effect of latent inhibition in Iberian green frog tadpoles.

The ability to learn potential predators may be especially important for the survival of prey species that are likely to be exposed to a wide range of predators while they are in the aquatic phase. Thus, learning may enable animals to adjust their behavior in variable environments. However, it could be very costly to always respond to a chemical cue as dangerous only because it coincidentally appeared once mixed with chemical alarm cues (Lima & Dill, 1990; Belden, Wildy, Hatch & Blaustein, 2000). Thus, both learned irrelevance and latent inhibition, rather than being seen as failed predator recognition, could rather be seen as adaptive strategies to handle conflicting information and to avoid learning irrelevant information. However, the occurrence of these phenomena in a given species and habitat should depend on the relative costs of overresponding to predation cues (and associated lost opportunities) versus underresponding (and being attacked by a predator). In many cases, given the uncertainties and cost asymmetries, it might be much more costly to not respond than to overrespond to predators. For example, these two processes could limit the ability of prey to learn new potential predators (Ferrari & Chivers, 2006a, 2011). In the case of the Iberian green frog tadpoles, these mechanisms could interfere with the learning of chemicals from those predators that usually capture other species but may opportunistically attack tadpoles too, or in the learning of introduced exotic predator species that are not recognized by naïve tadpoles (Polo-Cavia, Gonzalo, López & Martín, 2010).

Both learned irrelevance and latent inhibition seem to produce the same effect, but typically the exposure to both conditioned and unconditioned stimuli not correlated with each other retards subsequent learning to a higher degree than the exposure to one type of stimulus alone (Bennett, Maldonado & Mackintosh, 1995). Thus, learned irrelevance seems to be even more disruptive than latent inhibition (Bennett, Maldonado & Mackintosh, 1995). Ferrari and Chivers (2006b) showed that recent information plays a major role in eliciting antipredator responses with more or less intensity in fathead minnows when they are exposed to predator cues. So, if the information is always updated, it is likely that tadpoles that learned irrelevance of the fish cue, or that showed latent inhibition to the fish cue, would be able to learn later that the fish cue is a predator cue if they were reinforced repeatedly with alarm cues. Future work should examine whether both learning processes affect in a different way this possible posterior learning ability, and if learned irrelevance retards more than latent inhibition the posterior associative learning. It will be interesting to look into how long these effects last and how close or far apart multiple cue exposures can be, or how many exposures are needed, for learned irrelevance or latent inhibition to occur and persist.

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Correction to Gonzalo, López, and Martín (2012)

In the article "Adaptive Forgetting in Iberian Green Frog Tadpoles: Learned Irrelevance and Latent Inhibition May Avoid Predator Misidentification," by Adega Gonzalo, Pilar López, and José Martín (*Journal of Comparative Psychology*, Advance online publication August 6, 2012. doi:10.1037/a0029173), the title should have read "Adaptive Forgetting in Iberian Green Frog Tadpoles (*Pelophylax perezi*): Learned Irrelevance and Latent Inhibition May Avoid Predator Misidentification." All versions of this article have been corrected.

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