

## Herbicide exposure affects the chemical recognition of a non native predator in common toad tadpoles (*Bufo bufo*)

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**Summary.** In amphibians and fishes, evidence is increasing that chemical cues from injured conspecifics can play a role in the chemical labelling and learned recognition of unfamiliar predators. In this laboratory study, we tested the prediction that prior chemical exposure to a non-native predator feeding on conspecific tadpoles will subsequently allow tadpoles of the common toad (*Bufo bufo*) to recognize the chemical cues specifically released by this starved predator. Furthermore, we investigated the vulnerability of this chemically-mediated process to herbicide contamination. With these aims in view, groups of tadpoles were kept either unexposed or exposed for ten days to chemical cues from Turkish crayfish (*Astacus leptodactylus*) previously fed on tadpoles, both in uncontaminated water and in the presence of four sublethal concentrations of amitrole (0.01, 0.1, 1 and 10 mg.l<sup>-1</sup>). We then assessed the effects of the six conditioning treatments on general activity and behavioural response to chemical cues from starved crayfish. Larval treatments did not affect the general activity of the tadpoles. By contrast, the treatments had significant effects on the behavioural response to the test solution prepared from starved crayfish. The only tadpoles to show an antipredator behavioural response to the chemical stimulation from starved crayfish belonged to the groups derived from chemical exposure to tadpole-fed crayfish in uncontaminated water and in contaminated water with the lowest concentration of amitrole (0.01 mg.l<sup>-1</sup>). Conversely, this chemical stimulation produced no behavioural change in the control group or in the groups derived from exposure to tadpole-fed crayfish in contaminated water containing 0.1, 1 and 10 mg.l<sup>-1</sup> of amitrole. This study demonstrates that chemical cues released during the predator's feeding activity can subsequently be used by common toad tadpoles in the recognition of an unfamiliar predator. In addition, our results show that the presence of sublethal amitrole concentrations can impair this recognition process. Such a pesticide effect might be especially detrimental for amphibian populations threatened by invasive predators.

**Key words.** *Bufo bufo* tadpoles – acquired predator recognition – chemical cues – *Astacus leptodactylus* – amitrole

### Introduction

In human-dominated ecosystems, organisms can be confronted with multiple environmental stressors, of both natural and anthropogenic origins. In this regard, considerable research efforts have focused on the stress factors contributing to the global decline of amphibian populations across the globe. Potential causes include habitat destruction or fragmentation, climate change, introduced predators or competitors, disease, and the presence of chemical contaminants (Berger 1989; Alford & Richards 1999; Davidson *et al.* 2001; Sparling *et al.* 2001; Blaustein & Kiesecker 2002; IUCN 2004; Rachowicz *et al.* 2006). As these factors often occur concomitantly, it is of a particular importance to consider the possible interactions of multiple stressors. Although the relative importance of interaction types may vary among sites, the presence of non-native predators and agricultural contaminants are of frequent occurrence in amphibian habitats (Relyea 2003).

In this context, some recent studies on amphibians have shown a possible interaction between the effects of pesticides and predators. For example, the toxicity of pesticides can become increased in the presence of chemical cues indicating a predation risk (Relyea & Mills 2001; Relyea 2003, 2004, 2005). Some data also indicate that the predator-prey relationship can be modified in the presence of pesticides (Verrell 2000; Ingermann *et al.* 2002; Broomhall 2004). In particular, waterborne pesticides might affect chemically-mediated behaviours through their detrimental effects on the olfactory system (Delaleu & Sicard 1994; Park *et al.* 2001; Park & Propper 2002). Such effects might result in population loss since in larval amphibians chemoreception plays a key role in the identification of the risk associated with the presence of predaceous species. In this regard, data indicate that inexperienced larvae may change their behaviour, morphology and life history in response to the chemical presence of their natural predators (Kiesecker & Blaustein 1997; Griffiths *et al.* 1998; Gallie *et al.* 2001). On the other hand, larvae often fail to respond to chemical cues from unfamiliar predators (Kiesecker & Blaustein 1997; Griffiths *et al.* 1998; Marquis *et al.* 2004; Mandrillon & Saglio 2005). When confronted with novel predators, survival of amphibian larvae thus appears to be conditioned by their ability to quickly evolve appropriate antipredator adaptations. In this connection, a growing body of evidence suggests that

aquatic invertebrates and vertebrates have the ability to learn to recognize the chemical cues released by unfamiliar predators. Such a chemical conditioning appears to be based on an association between the odour *per se* of the predator and the presence of alarm substances from preyed conspecifics (Mathis & Smith 1993; Chivers & Smith 1994a, b; Wisenden *et al.* 1997; Brown & Smith 1998; Woody & Mathis 1998; Wisenden 2000; Korpi & Wisenden 2001; Wisenden & Millard 2001; Wisenden *et al.* 2004; Darwish *et al.* 2005; Ferrari *et al.* 2005; Larson & McCormick 2005; Mandrillon & Saglio 2005). In the common toad (*Bufo bufo*), we recently found that tadpoles were able to establish a conditioning association between conspecific and predator chemicals. Following an exposure to a combination of chemical cues from a non-native predator, the Turkish crayfish (*Astacus leptodactylus*) and chemical cues from crushed conspecifics, toad tadpoles subsequently showed a behavioural antipredator reaction (decrease of activity) to crayfish cues alone (Mandrillon & Saglio 2005).

In Western Europe, common toad breeds in permanent ponds where *A. leptodactylus* and other recently introduced and invasive predators of amphibian larvae can co-occur. Among those predators are various crayfish (spiny crayfish *Orconectes limosus*, signal crayfish *Pascifastacus leniusculus*, red swamp crayfish *Procambarus clarkii*), fish (brown bullhead *Ictalurus nebulosus*, mosquitofish *Gambusia spp.*), and the bullfrog *Rana catesbeiana* (Gamradt & Kats 1996; Kiesecker & Blaustein 1998; Goodsell & Kats 1999; Kats & Ferrer 2003). Introduced into France in the 1970's (Arrignon 1997) and now integrated in the French fauna, the Turkish crayfish is of growing occurrence in the western part of France (Changeux 2006). Although common toad tadpoles are unpalatable to most fish, they can be preyed upon by diverse invertebrate predators, and mostly, by aquatic insects (Semlitsch & Gavasso 1992; Manteifel & Reshetnikov 2002; Nöllert & Nöllert 2003). Alien crayfish might also represent a serious threat for common toad tadpoles (Nyström & Abjörnsson 2000; Gherardi *et al.* 2001), and our recent laboratory experiments indicated that *A. leptodactylus* can readily capture and consume *B. bufo* tadpoles (Marquis *et al.* 2004; Mandrillon & Saglio 2005). Thus, the ability to learn to recognize the chemical stimuli from this predator might be of significant survival value for common toad tadpoles. To date, studies which considered this learning process in amphibians documented recognition of predator odour through its pairing with chemical cues from conspecifics experimentally crushed by the authors (Woody & Mathis 1998; Mandrillon & Saglio 2005). However, no attention has been paid to the role played by chemical cues related to the predator diet which yet provide tadpoles the opportunity to associate the conspecifics chemical cues with the predator odour in a more realistic way.

This study was designed with two objectives in mind. The first was to test whether pre-exposure to a non-native predator, the Turkish crayfish, fed with conspecifics can enable the common toad tadpole to make a learning association between the predator's odour and chemical cues globally resulting from the predator's feeding activity. The second was to assess the effects of a pesticide on this chemically-mediated process. The pesticide used in our study was amitrole, a widely used non-selective herbicide that can

contaminate water bodies directly when applied for the control of aquatic weeds, or indirectly through runoff waters from treated fields (World Health Organisation 1994). Despite its widespread occurrence, little attention has been given to the possible effects of sublethal concentrations of amitrole on aquatic organisms.

## Materials and Methods

### Test animals

On 23 March 2005, eight egg strings of common toad (stage 17, Gosner 1960) were collected from an experimental permanent pond devoid of crayfish and brought back to the laboratory at the Institut National de la Recherche Agronomique (INRA, Rennes, France). Then, each egg string was split into four equal masses that were kept in four identical glass aquaria (50 × 30 × 20 cm) filled with 30 l of aged tap water. Hatching occurred on 25 March 2005 (stage 18, Gosner 1960).

Juvenile Turkish crayfish ( $n = 60$ , mean weight = 5.84 g ± 0.77 g, mean cephalothorax length = 2.78 cm ± 0.13 cm) were taken from a population reared in outdoor concrete tanks (5 × 3 × 0.5 m) devoid of anuran larvae. Crayfish were housed in the laboratory and kept in a plastic tank (2 × 0.5 m, water depth = 10 cm) receiving a continuous flow (100 ml.min<sup>-1</sup>) of dechlorinated and aerated tap water, with a litter of macerated dead leaves. They were fed a mixed diet of periphyton covering dead leaves and fish food flakes (Tetramin, Germany).

The laboratory was maintained on a cycle of 12 hrs light/12 hrs dark (dawn at 08:00; dusk at 20:00), and the physical and chemical characteristics of the water used throughout the experimental period were as follows: temperature 13–18°C; pH 7–7.2; NH<sub>4</sub><sup>+</sup> < 0.01 mg/l, NO<sub>2</sub><sup>-</sup> < 0.01 mg/l, NO<sub>3</sub><sup>-</sup> 22–26 mg/l, PO<sub>4</sub><sup>3-</sup> < 0.01 mg/l.

### Conditioning treatments

Conditioning treatments started ten days after hatching, and were maintained for ten days. To mimic natural densities, 200 tadpoles (mean weight = 34.43 mg ± 1.07 mg, mean length = 14.47 mm ± 0.24 mm, stage 25, Gosner 1960) were introduced into twelve identical glass aquaria (80 × 20 × 20 cm, water volume = 30 l). Each tank contained a litter of macerated dead leaves and an airstone to ensure aeration. Tadpoles were fed thawed lettuce *ad libitum* grown from organic farming.

The groups of tadpoles were subjected to the following conditioning treatments:

- (1) Unex: tadpoles were kept unexposed.
- (2) TFA: tadpoles were exposed to chemical cues from tadpole-fed *Astacus*.
- (3) TFA + A(0.01): tadpoles were exposed to chemical cues from tadpole-fed *Astacus*, in water contaminated with 0.01 mg.l<sup>-1</sup> amitrole.
- (4) TFA + A(0.1): tadpoles were exposed to chemical cues from tadpole-fed *Astacus*, in water contaminated with 0.1 mg.l<sup>-1</sup> amitrole.
- (5) TFA + A(1): tadpoles were exposed to chemical cues from tadpole-fed *Astacus*, in water contaminated with 1 mg.l<sup>-1</sup> amitrole.
- (6) TFA + A(10): tadpoles were exposed to chemical cues from tadpole-fed *Astacus*, in water contaminated with 10 mg.l<sup>-1</sup> amitrole.

Each treatment was replicated twice.

Chemical cues from tadpole-fed *Astacus* (TFA) were obtained by holding twenty crayfish and 400 common toad tadpoles (Stage 25, Gosner 1960) for 72 hours in a glass aquarium (80 × 20 × 20 cm, water volume = 10 l), with macerated dead leaves and an airstone on the bottom. At the end of this period, we removed the

crayfish and dead leaves and checked that all tadpoles had been consumed. Then, the aquarium water was stirred and 60-ml samples were collected with a syringe and stored at  $-20^{\circ}\text{C}$ . Every day during the ten days of the conditioning period, we introduced a defrosted 60-ml water sample into each of the aquaria exposed to TFA treatments. In order to reproduce the mechanical disturbance caused by liquid introduction, 60 ml of aged tap water were also introduced each day into the control aquarium.

Additions of amitrole were carried out immediately before the introduction of tadpoles into the conditioning aquaria. Amitrole (1*H*-1, 2, 4-triazol-3-amine, 99.9 % purity) was purchased from Cluzeau Info Labo (France). Because amitrole is readily soluble in water, we did not use an organic solvent. The two lowest amitrole concentrations tested in this study (0.01 and 0.1  $\text{mg}\cdot\text{l}^{-1}$ ) have been commonly reported in surface waters, while a concentration of 1  $\text{mg}\cdot\text{l}^{-1}$  can be detected after direct spraying to control aquatic weeds (World Health Organisation 1994). Although the other tested concentration (10  $\text{mg}\cdot\text{l}^{-1}$ ) is higher than environmentally realistic levels, it is nevertheless far below the 96h-LC<sub>50</sub> (3  $\text{g}\cdot\text{l}^{-1}$ ) documented in larval anurans (Johnson 1976).

Conditioning treatments were interrupted after ten days. To avoid handling stress, groups of tadpoles were left in their respective aquaria. However, dead leaves were removed and the water was slowly drained off, leaving a minimum volume (about 0.5 l) for tadpole maintenance. Thirty litres of aged tap water were then added into each tank. This operation was repeated twice, followed by the addition of a new litter of macerated dead leaves. Amitrole concentrations were determined in the aquarium exposed to (TFA + A(1)) immediately after contamination, ten days later, and then after the final rinsing (Ecole Nationale de la Santé Publique, Rennes, France). These analyses show that 81.44 % of the amitrole initially present in the tank remained at the end of the ten-day exposure. Furthermore, rinsing of the aquarium resulted in the elimination of 96.03 % of the initial herbicide concentration.

#### Behavioural testing

Behavioural observations started immediately after the end of the exposures and lasted 11 days, being conducted in four identical glass aquaria (80 × 20 × 20 cm, water volume = 16 l). Except for the observation-side wall, the outer parts of the glass walls were lined with beige plastic sheeting. To minimise stress, each aquarium was isolated with silent blocks and black plastic curtains. Tadpole behaviour was observed through small openings (2 × 2 cm) in the curtains.

Behavioural observations were run each day on groups of six previously untested tadpoles. The observations started at 10:30, after a 90-minute period allowing tadpoles to acclimatize to the test aquaria. Behavioural monitoring was carried out for two consecutive 15-minute periods (control period, followed by test period), during which the number of tadpoles showing tail movements were recorded every 30 s. During the second period, 1 l of a control or a test solution was dripped (volume 1 l, flow rate 66.6  $\text{ml}\cdot\text{min}^{-1}$ ) into either end of the aquarium. The control solution (C<sub>sol</sub>) consisted of 1 l of aged tap water. The test solution (SA<sub>sol</sub>) was obtained from water conditioned with starved *Astacus*. To prepare the starved-*Astacus* test solution, we applied the same protocol as for TFA conditioning treatments, except that starved crayfish were used instead of tadpole-fed crayfish. On the day of testing, the obtained samples were defrosted and homogenized in 1 l of aged tap water. The control solution (C<sub>sol</sub>) was tested on control tadpoles (Unex). The test solution (SA<sub>sol</sub>) was tested on each group of tadpoles (Unex, TFA, TFA+A(0.01), TFA+A(0.1), TFA+A(1), TFA+A(10)). Control and test solutions were tested six times on the appropriate groups of tadpoles. Among these six tests, one half was performed on tadpoles from replicate 1, and the other half was performed on tadpoles from replicate 2. Olfactometric tests were conducted in a randomized order, and were coded so that observations were performed blind. All aquaria were cleaned thoroughly between behavioural measurements, and the tested tadpoles were then released in their original pond.

#### Data analysis

The mean numbers of tadpoles showing tail movements were transformed in percentages to obtain the mean proportion of moving tadpoles. To stabilize the variance and obtain a closer approximation to a normal distribution, behavioural data were arcsine square root-transformed (Sokal and Rohlf 1981). The mean proportion of moving tadpoles during the control period was used to investigate the effects of conditioning treatments on general activity, and were processed using a one-way analysis of variance (ANOVA). Differences in the mean proportion of moving tadpoles between the test and control observation periods were considered to reflect the response of the different groups of tadpoles to the tested solution. To assess that the starved-*Astacus* solution (SA<sub>sol</sub>) did not affect the behaviour of naïve tadpoles, we compared the response of unexposed tadpoles to this solution with their response to the control solution (C<sub>sol</sub>), using a Student *t*-test. The effects of the conditioning treatments on behavioural response to the starved-*Astacus* test solution were investigated using a one-way analysis of variance (ANOVA), and inter-treatment differences were then assessed using Tukey's multiple comparison tests. The absence of differences between the two sets of replicates was assessed, using Student's *t*-test. Both replicate series showed similar general activity ( $t = -1.86$ ,  $P = 0.104$ ) and responses to the test solution ( $t = -0.79$ ,  $P = 0.449$ ).

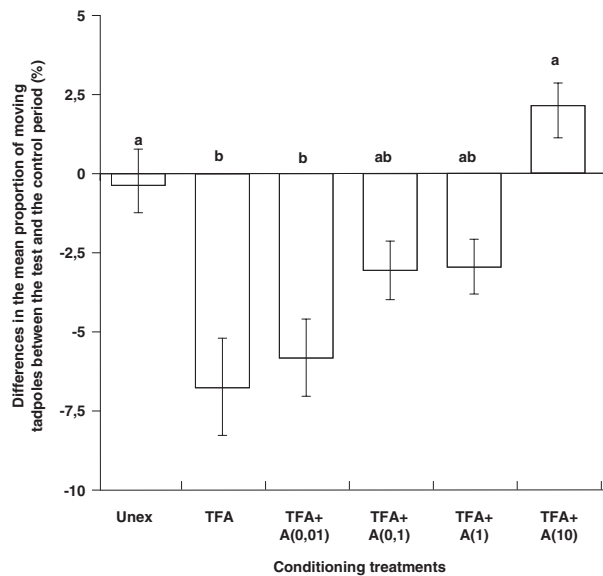
#### Results

Before the introduction of the test solution (control period), the proportion of moving tadpoles did not differ between the different groups ( $F_{5,11} = 0.95$ ,  $P = 0.51$ ), indicating that conditioning treatments did not affect the general activity of tadpoles.

The response of the unexposed tadpoles to the test solution from starved *Astacus* did not differ from their response to the control solution ( $t = 1.98$ ,  $P = 0.19$ ), suggesting that naïve tadpoles did not identify the chemical cues from starved crayfish as a predation risk. However, conditioning treatments produced significant effects on the behavioural response of tadpoles to the test solution prepared from starved *Astacus* ( $F_{5,11} = 10.61$ ,  $P = 0.006$ , Fig. 1). Pairwise comparisons with unexposed control (Unex) indicate that tadpoles derived from exposure to tadpole-fed *Astacus* (TFA,  $P = 0.0129$ ) and TFA in the presence of 0.01  $\text{mg}\cdot\text{l}^{-1}$  amitrole (TFA+A(0.01),  $P = 0.0245$ ) were the only groups showing a significant reduction of movements in response to starved *Astacus*. By contrast, this chemical stimulation from starved *Astacus* did not produce any significant behavioural change in tadpoles exposed to TFA in the presence of amitrole at 0.1  $\text{mg}\cdot\text{l}^{-1}$  (TFA+A(0.1)), 1  $\text{mg}\cdot\text{l}^{-1}$  (TFA+A(1)) or 10  $\text{mg}\cdot\text{l}^{-1}$  (TFA+A(10)). Further inter-treatment comparisons revealed that the responses to chemical cues from starved *Astacus* significantly differed between tadpoles derived from exposure to TFA+A(10) and those from TFA ( $P = 0.0108$ ) and TFA+A(0.01) ( $P = 0.0201$ ).

#### Discussion

The present olfactometric tests show that the chemical recognition of a non-native predator can be significantly influenced by the chemical experience of tadpoles. While tadpoles naïve to *Astacus* did not change their behaviour in response to a chemical stimulation from this starved



**Fig. 1** Effects of the larval conditioning treatments on the behavioural response of *Bufo bufo* tadpoles to chemical cues from starved *Astacus leptodactylus*. Conditioning treatments: Unex = unexposed, TFA = exposed to chemical substances from tadpole-fed *Astacus* in aged tap water, TFA+A(0.01) = exposed to chemical substances from tadpole-fed *Astacus* in water contaminated with 0.01 mg.l<sup>-1</sup> amitrole, TFA+A(0.1) = exposed to chemical substances from tadpole-fed *Astacus* in water contaminated with 0.1 mg.l<sup>-1</sup> amitrole, TFA+A(1) = exposed to chemical substances from tadpole-fed *Astacus* in water contaminated with 1 mg.l<sup>-1</sup> amitrole, TFA+A(10) = exposed to chemical substances from tadpole-fed *Astacus* in water contaminated with 10 mg.l<sup>-1</sup> amitrole. Control period: observation session before the introduction of the starved-*Astacus* solution (SA<sub>sol</sub>), test period: observation session during the introduction of the starved-*Astacus* solution (SA<sub>sol</sub>). Treatments labelled with different letters denote significant differences at  $P < 0.05$ , based on Tukey's multiple comparison tests

predator, the same stimulation produced a reduction of swimming movements in tadpoles having experienced a nonlethal exposure to tadpole-fed *Astacus*. These results support the hypothesis that naïve larval amphibians do not show antipredator behaviour in response to chemical cues from unfamiliar starved predators (Kiesecker & Blaustein 1997; Griffiths *et al.* 1998; Marquis *et al.* 2004; Mandrillon & Saglio 2005). In addition, the behavioural response to starved *Astacus* indicates that tadpoles can learn to recognize the crayfish's odour through a previous association between direct chemical cues specifically released by this predator and indirect chemical cues resulting from its feeding activity. The effect is observed here in tadpoles derived from exposure to conspecific-fed crayfish. A similar learning process, known as "releaser-induced recognition learning", has been well described in fish, where it is now recognized that chemicals released by injured conspecifics play a major role in the acquired chemical recognition of an unfamiliar predator (Mathis & Smith 1993; Chivers & Smith 1994a, b; Brown & Smith 1998; Wisenden 2000; Korpi & Wisenden 2001; Wisenden *et al.* 2004; Darwish *et al.* 2005;

Larson & McCormick 2005). Recent observations on common toad tadpoles (Mandrillon & Saglio 2005) and adults of the central newt *Notophthalmus viridescens* (Woody and Mathis 1998) indicate that amphibians can similarly learn to recognize an unfamiliar odour as representing a predation risk based on a prior association between this odour and the chemical cues from experimentally crushed conspecifics. However, we demonstrate here for the first time in amphibians that chemical cues naturally released through the predator's feeding activity can be used in the learned recognition of an unfamiliar predator. Further investigations are now required to assess the relative importance of conspecific alarm cues and predator excretory products in this learning process. Additional experiments are also needed to assess whether this learning mechanism can operate at earlier developmental stages. In the present work, the conditioning association leading to the learned chemical recognition of the predator resulted from exposure of free-swimming tadpoles. However, recent studies have shown that amphibian eggs and hatchlings can exhibit phenotypic plasticity in response to chemical cues indicating predation (Chivers *et al.* 2001; Laurila *et al.* 2001, 2002; Johnson *et al.* 2003; Orizaola & Braña 2004; Saglio & Mandrillon 2006). Consequently, it might be speculated that these early developmental stages have also the ability to develop the Pavlovian conditioning (Pavlov 1906) involved in the chemical recognition of novel predators. In another connection, further investigations are required to precise the timing of this memorization process. Here, the behavioural response to predator's chemical cues was tested for eleven days following the cessation of a ten-days conditioning session. Knowing that our experimental design did not allow to test for any time trend, additional experiments remain necessary to assess whether this recognition process can result from shorter conditioning periods or persist through further developmental stages.

To our knowledge, this study is also the first to show that the presence of sublethal concentrations of a pesticide can impair the process of chemical recognition of predators. Tadpoles exposed to chemical cues from conspecific-fed crayfish in water containing 10 µg.l<sup>-1</sup> amitrole subsequently reacted to the introduction of a test solution from starved crayfish by a decrease of their movements. Conversely, tadpoles derived from exposure to conspecific-fed crayfish in the presence of 100 µg.l<sup>-1</sup>, 1 mg.l<sup>-1</sup> and 10 mg.l<sup>-1</sup> amitrole did not show any behavioural change in response to the chemical stimulation from starved crayfish. These results suggest that amitrole contamination might have some detrimental consequences in amphibian populations exposed to the threat of invasive predaceous species. Only one study had previously investigated the effects of a chemical stressor on the acquisition of predator recognition. Leduc *et al.* (2004) reported that the learning ability of juvenile rainbow trout (*Oncorhynchus mykiss*) to recognize a novel predator (yellow perch, *Perca flavescens*) was impaired under weakly acid conditions. Aside from chemical stressors, data concerning the possible impacts of other types of environmental stressing factors (*e.g.* temperature, UV...) on the acquired predator recognition are lacking, and additional studies on the effects of such factors will be required to gain

a fuller understanding of this process. In the present work, the mechanism responsible for the detrimental effects of amitrole on the predator chemical recognition by common toad tadpoles remains to be precised. It might result from specific impacts of amitrole on the learning ability, as well as on the olfactory system. Further studies are thus needed to clarify this point.

To conclude, this study demonstrates that dietary chemical cues from the predator can be used in the learned recognition of a non-native predator by tadpoles of the common toad. Such a learning ability might be of high survival value for amphibian larvae when confronted with unfamiliar or invasive predators. However, our results also indicate that the chemical recognition of the predator can be inhibited by the presence of sublethal concentrations of amitrole. It is noteworthy that environmentally appropriate concentrations are sufficient to induce such an inhibition, even though they are hundreds of times lower than the documented 96h-LC<sub>50</sub> in larval anurans (Johnson 1976). Therefore, the simultaneous presence of invasive predators and pesticides represents a double hazard for amphibian larvae since individuals exposed to pesticides might not establish the chemically-mediated process necessary to evolve a behavioural response to these predators. Further studies are now required in larval amphibians to check whether sublethal exposures to other commonly used pesticides can induce such modifications, and to confirm these results under more ecologically realistic conditions.

### Acknowledgements

We thank A. Neveu and P.-M. Lucas for help in collecting the common toad eggs. We are also grateful to M. Carpenter for reviewing the english version. The study was performed with permit (006910) from the Regional Department of Veterinary Services.

### References

- Alford RA, Richards SJ (1999) Global amphibian declines: A problem in applied ecology. *Annu Rev Ecol Syst* 30: 133–165
- Arrignon JCV (1997) Status of the foreign crayfish in France. Pp 665–670 in Momot W T (eds), *Freshwater Crayfish* 11. Proc. Internat. Assoc. Astacol., Lakehead University, Thunder Bay, Ontario, Canada
- Berger L (1989) Disappearance of amphibian larvae in the agricultural landscape. *Ecol Internat Bull* 17: 65–73
- Blaustein AR, Kiesecker JM (2002) Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecol Lett* 5: 597–608
- Broomhall SD (2004) Egg temperature modifies predator avoidance and the effects of the insecticide endosulfan on tadpoles of an Australian frog. *J Appl Ecol* 41: 105–113
- Brown GE, Smith RJF (1998) Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery – reared fish to recognize chemical cues of predator. *Can J Fish Aquat Sci* 55: 611–617
- Changeux T (2006) Evolution de la répartition des écrevisses en France métropolitaine selon les enquêtes nationales menées par le Conseil supérieur de la pêche de 1977 à 2001. Conseil Supérieur de la Pêche, Circulaire du 09/06/06, 22 p
- Chivers DP, Smith RJF (1994a) Fathead minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Anim Behav* 48: 597–605
- Chivers DP, Smith RJF (1994b) The role of experience and chemical alarm signalling in predator recognition by fathead minnows, *Pimephales promelas*. *J Fish Biol* 44: 273–285
- Chivers DP, Kiesecker JM, Marco A, De Vito J, Anderson MT, Blaustein AR (2001) Predator induced life history changes in amphibians: egg predation induces hatching. *Oikos* 92: 135–142
- Darwish TL, Mirza RS, Leduc AOHC, Brown GE (2005) Acquired recognition of novel predator odour cocktails by juvenile glowlight tetras. *Anim Behav* 70: 83–89
- Davidson C, Shaffer HB, Jennings MR (2001) Declines of the California red-legged frog: Climate, UV-B, habitat, and pesticides hypotheses. *Ecol Appl* 11: 464–479
- Delaleu J-C, Sicard G (1995) Physiological and histological recovery of the olfactory mucosa of the frog after a dichlobenil injection. *Chem Senses* 20: 433–440
- Ferrari MCO, Trowell JJ, Brown GE, Chivers DP (2005) The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. *Anim Behav* 70: 777–784
- Gallie JA, Mumme RL, Wissinger SA (2001) Experience has no effect on the development of chemosensory recognition of predators by tadpoles of American toad, *Bufo americanus*. *Herpetologica* 57: 376–383
- Gamradt SC, Kats LB (1996) Effects of introduced crayfish and mosquitofish on California newts. *Conserv Biol* 10: 1155–1162
- Gherardi F, Renai B, Corti C (2001) Crayfish predation on tadpoles: a comparison between a native (*Austroptamobius pallipes*) and an alien species (*Procambarus clarkii*). *Bull Fr Pêche Piscic* 361: 659–668
- Goodsell J A, Kats LB (1999) Effect of introduced mosquitofish on Pacific treefrogs and the role of alternative prey. *Conserv Biol* 13: 921–924
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190
- Griffiths RA, Schley L, Scharp PE., Dennis JL, Roman A (1998) Behavioural responses of Mallorcan midwife toad tadpoles to natural and unnatural snake predators. *Anim Behav* 55: 207–214
- Ingermann RL, Bencic DC, Verrell P (2002) Methoxychlor alters the predatory-prey relationship between dragonfly naiads and salamander larvae. *B Environ Contam Tox* 69: 771–777
- IUCN (2004) Species Survival Commission, conservation International Center for Applied Biodiversity Science, NatureServe, IUCN Global Amphibian Assessment
- Johnson CR (1976) Herbicide toxicities in some Australian anurans and the effect of subacute dosages on temperature tolerance. *Zool J Linn Soc-Lond* 59: 79–83
- Johnson JB, Saenz D, Adams CK, Conner RN (2003) The influence of predator threat on the timing of a life-history switch point: predator-induced hatching in the southern leopard frog (*Rana sphenoccephala*). *Can J Zool* 81: 1608–1613
- Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Divers Distrib* 9: 99–110
- Kiesecker JM, Blaustein AR (1997) Population differences in response of red-legged frogs (*Rana aurora*) to introduced bullfrogs. *Ecology* 78: 1752–1760
- Kiesecker JM, Blaustein AR (1998) Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth and survival of native red-legged frogs (*Rana aurora*). *Conserv Biol* 12: 776–787
- Korpi NL, Wisenden BD (2001) Learned recognition of novel predator odour by zebra danios, *Danio rerio*, following time-shifted presentation of alarm cue and predator odour. *Environ Biol Fish* 61: 205–211
- Larson JK, McCormick MI (2005) The role of chemical alarm signals in facilitating learned recognition of novel chemical cues in a coral reef fish. *Anim Behav* 69: 51–57
- Laurila A, Crochet P-A, Merila J (2001) Predation-induced effects on hatchling morphology in the common frog (*Rana temporaria*). *Can J Zool* 79: 926–930

- Laurila A, Pakkasmaa S, Crochet P-A, Merila J (2002) Predator – induced plasticity in early life history and morphology in two anuran amphibians. *Oecologia* 132: 524–530
- Leduc AOHC, Ferrari MCO, Kelly JM, Brown GE (2004) Learning to recognize novel predators under weakly acidic conditions: the effect of reduced pH on acquired predator recognition by juvenile rainbow trout. *Chemoecology* 14: 107–112
- Mandrillon A-L, Saglio P (2005) Prior exposure to conspecific chemical cues affects predator recognition in larval common toad (*Bufo bufo*). *Arch Hydrobiol* 124: 1–12
- Manteifel YB, Reshetnikov AN (2002) Avoidance of noxious tadpole prey by fish and invertebrate predators: adaptivity of a chemical defence may depend on predator feeding habits. *Arch Hydrobiol* 153:657–668
- Marquis O, Saglio P, Neveu A (2004) Effects of predators and conspecific chemical cues on the swimming activity of *Rana temporaria* and *Bufo bufo* tadpoles. *Arch Hydrobiol* 160: 153–170
- Mathis A, Smith RJF (1993) Fathead minnows, *Pimephales promelas*, learn to recognize Northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. *Anim Behav* 46: 645–656
- Nöllert A, Nöllert C (2003) Guide des amphibiens d'Europe. Pp 286–292 in Delachaux et Nieslé (eds)
- Nyström P, Abjörnsson K (2000) Effects of fish chemical cues on the interactions between tadpoles and crayfish. *Oikos* 88: 181–190
- Orizaola G, Braña F (2004) Hatching responses of four newt species to predatory fish chemical cues. *Ann Zool Fennici* 41: 635–645
- Park D, Hempleman SC, Proppper CR (2001) Endosulfan exposure disrupts pheromonal systems in the red-spotted newt: a mechanism for subtle effects of environmental chemicals. *Environ Health Persp* 109: 669–673
- Park D, Proppper CR (2002) Endosulfan affects pheromonal detection and glands in the male red-spotted newt, *Notophthalmus viridescens*. *B Environ Contam Tox* 69: 609–616
- Pavlov IP (1906) The scientific investigation of the psychical faculties or processes in the higher animals. *Science* 24: 613–619
- Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, Briggs CJ (2006) Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87: 1671–1683
- Relyea RA, Mills N (2001) Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *PNAS* 98: 2491–2496
- Relyea RA (2003) Predator cues and pesticides: a double dose of danger for amphibians. *Ecol Appl* 13: 1515–1521
- Relyea RA (2004) Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environ Toxicol Chem* 23: 1080–1084
- Relyea RA (2005) The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Arch Environ Con Tox* 48: 351–357
- Saglio P, Mandrillon A-L (2006) Embryonic experience to predation risk affects tadpoles of the common frog (*Rana temporaria*). *Arch. Hydrobiol.* 166: 505–523
- Semlitsch RD, Gavasso S (1992) Behavioural response of *Bufo bufo* and *Bufo calamita* tadpoles to chemical cues of vertebrate and invertebrate predators. *Ethol Ecol Evolut* 4: 165–173
- Sokal RR, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Company, San Francisco
- Sparling DW, Fellers GM, McConnell LS (2001) Pesticides and amphibian population declines in California, USA. *Env Toxicol Chem* 20: 1591–1595
- Verrell P (2000) Methoxyflor increases susceptibility to predation in the salamander *Ambystoma macrodactylum*. *B Environ Contam Tox* 64: 85–92
- Wisenden BD, Chivers DP, Smith RJF (1997) Learned recognition of predation risk by *Enallagma* damselfly larvae (Odonata, Zygoptera) on the basis of chemical cues. *J Chem Ecol* 23: 137–151
- Wisenden BD (2000) Olfactory assessment of predation risk in the aquatic environment. *Philos T Roy Soc B* 355: 1205–1208
- Wisenden BD, Millard MC (2001) Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk and to associate risk with novel cues. *Anim Behav* 62: 761–766
- Wisenden BD, Klitzke J, Nelson R, Friedl D, Jacobson PC (2004) Predator-recognition training of hatchery-reared walleyed (*Stizostedion vitreum*) and a field test of a training method using yellow perch (*Perca flavescens*). *Can J Fish Aquat Sci* 61: 2144–2150
- Woody DR, Mathis A (1998) Acquired recognition of chemical stimuli from an unfamiliar predator: associative learning by adult newts, *Notophthalmus viridescens*. *Copeia* 4: 1027–1031
- World Health Organisation (1994) Amitrole Health and Safety Guide. International Program on Chemical Safety. Health and Safety Guide 85

Received 21 June 2006; accepted 23 September 2006.  
Published Online First 25 November 2006.