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Waterborne Amitrole Affects the Predator–Prey Relationship Between Common Frog Tadpoles (*Rana temporaria*) and Larval Spotted Salamander (*Salamandra salamandra*)

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Abstract. Within their aquatic habitats, larval amphibians are often subjected to multiple natural and anthropic stressors. Among these, predation and waterborne pollution represent two types of stressing factor that frequently co-occur. In this connection, the present laboratory study was designed to investigate the effects of amitrole, a commonly used triazole herbicide, on the predator–prey relationship between common frog tadpoles (*Rana temporaria*) and larval spotted salamander (*Salamandra salamandra*). Tadpoles were exposed for 3 days to 0, 0.01, 0.1, 1, and 10 mg/L amitrole, either in the absence or in the presence of larval salamanders. Tadpole behavior (refuge use, movements) was monitored every day, and the predation efficiency was assessed at the end of the experiment by counting the number of surviving tadpoles. In the absence of the predator, amitrole-exposed tadpoles (at 0.01, 0.1, and 1 mg/L) increased their refuge use and decreased their rate of movements. In the presence of the predator, amitrole contamination did not affect tadpole behavior, except on the first day, where tadpoles exposed to 10 mg/L were found to be significantly more active than unexposed control tadpoles. Throughout the experiment, control tadpoles were the only group to show significant reductions of activity and visibility in response to the predator's presence. In contrast, tadpoles exposed to 0.01 and 0.1 mg/L amitrole increased their refuge use in response to the predator, whereas their rate of movements remained unaffected. Furthermore, exposures of tadpoles to the two highest amitrole concentrations (1 and 10 mg/L) resulted in the loss of both behavioral responses to the predator's presence. Interestingly, the lack of antipredator behavior in amitrole-exposed tadpoles did not enhance their vulnerability to predation by the larval salamander. Moreover, tadpoles exposed to the two highest herbicide concentrations showed a better survival than unexposed controls, indicating that amitrole contamination also had detrimental effects on the predatory behavior of the larval salamander. These findings emphasize the need to consider the effects of contaminants on both predator and prey before drawing conclusions about the possible consequences of prey behavioral modifications on the predation risk.

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In aquatic environments, organisms can be confronted with a high diversity of environmental stressors, of both natural and anthropogenic origins. In many cases, these factors do not operate independently, but rather interact to induce combined impacts on organisms and communities. Surprisingly, research in aquatic ecology and ecotoxicology has long been focused on the effects of single factors, and it is only in the recent years that the importance of considering the combined effects of multiple stressors has been recognized (Hatch and Blaustein 2000; Boone and James 2003; Bridges and Boone 2003; Chen *et al*. 2004; Rohr *et al*. 2004; Metts *et al*. 2005; Relyea 2006).

Among natural and anthropogenic stressors, predators and toxicants represent two major threats that frequently co-occur in aquatic ecosystems. In this regard, the potential interactions between these two environmental factors has been investigated in some aquatic animals, including invertebrates (Dodson *et al*. 1995; Ham *et al*. 1995; Clements 1999; Preston *et al*. 1999; Lefcort *et al*. 2000; Schultz and Dabrowski 2001; Wolf and Moore 2002; Maul *et al*. 2006), fishes (Weis and Khan 1991; Weis and Weis 1995a, 1995b; Gregg *et al*. 1997; Scherer *et al*. 1997; Smith and Weis 1997; Carlson *et al*. 1998; Scholtz *et al*. 2000) and amphibians (Lefcort *et al*. 1998; Raimondo *et al*. 1998; Bridges 1999a; Verrell 2000; Boone and Semlitsch 2001; Relyea and Mills 2001; Broomhall 2002; Ingerman *et al*. 2002; Relyea 2003; Broomhall 2004; Mills and Semlitsch 2004; Relyea 2004a, 2005). According to these studies, exposure to waterborne contaminants (mainly pesticides and heavy metals) and predators may affect prey organisms in two ways. First, exposure to a chemical contaminant can increase the subsequent vulnerability to predation (Dodson *et al*. 1995; Weis and Weis 1995a; Smith and Weis 1997; Carlson *et al*. 1998; Raimondo *et al*. 1998; Clements

1999; Verrell 2000; Broomhall 2002; Broomhall 2004) and alter the antipredator behaviors (Lefcort *et al.* 1998, Bridges 1999a; Preston *et al.* 1999; Scholtz *et al.* 2000; Wolf and Moore 2002). Second, exposure to a predation stress can enhance the toxicity of the contaminants (Relyea and Mills 2001; Relyea 2003, 2004a, 2005; Maul *et al.* 2006). For example, Relyea (2003) found that waterborne carbaryl (carbamate insecticide) became more deadly to green frog (*Rana clamitans*) and bullfrog (*Rana catesbeiana*) tadpoles when combined with predator cues. On the other hand, contaminants may have a more negative effect on predators than on preys, resulting in a release from predation (Weis and Khan 1991; Weis and Weis 1995b; Gregg *et al.* 1997; Scherer *et al.* 1997; Smith and Weis 1997).

In amphibians, very few studies have investigated the impacts of chemical contaminants on both preys and predators (Bridges 1999b; Ingerman *et al.* 2002; Boone and Semlitsch 2003; Mills and Semlitsch 2004). This recent research clearly demonstrates that the outcome of the predator–prey relationship depends on the relative sensitivity of each of the protagonists to the pollutant in question. When contaminants modify the prey's defensive mechanisms but not the predator's feeding behavior, this can enhance the vulnerability of the prey (Ingerman *et al.* 2002). Conversely, contaminants can decrease the predation risk due to the greater sensitivity of the predator (Boone and Semlitsch 2003; Mills and Semlitsch 2004). Such differences in the relative toxicity of contaminants to preys and predators may have serious consequences at the community level (Boone and Semlitsch 2001, 2002).

The overall objective of the present study was to determine whether a short-term exposure to sublethal concentrations of a widely used herbicide affects the predator–prey relationship between larval spotted salamanders, *Salamandra salamandra*, and tadpoles of the common frog, *Rana temporaria*. These amphibian larvae are highly suitable models to carry out this type of study. First, both species carry out their early development in small temporary forest ponds, where they commonly represent the main predator–prey complex visible on site. As a result of their relatively low complexity, these aquatic habitats can be easily reproduced under controlled conditions. Second, as with many other amphibian species, common frog tadpoles can exhibit an important behavioral, morphological, and life-history plasticity in response to the predation risk (Laurila and Kujasalo 1999; Van Buskirk and Schmidt 2000; Laurila *et al.* 2001; Relyea 2001, 2002; Lafiandra and Babbitt 2004; Relyea 2004b; Kraft *et al.* 2005; Schmidt and Van Buskirk 2005). Third, with their permeable skins and their close dependence on the aquatic environment, larval amphibians appear particularly vulnerable to the presence of waterborne contaminants. In this context, the extensive use of pesticides in both aquatic and terrestrial environments is receiving increased attention as a potential cause of amphibian declines (Davidson *et al.* 2001; Sparling *et al.* 2001; Davidson *et al.* 2002; Mandrillon and Saglio 2005). The pesticide used in our study was amitrole, a non-selective triazole herbicide that inhibits chlorophyll formation and regrowth from buds (Tomlin 1997). This highly soluble herbicide can contaminate freshwater habitats either directly when applied for the control of aquatic weeds, or indirectly through runoff from adjacent treated fields (World Health Organisation 1994). Despite its widespread occurrence, little attention has been paid to the sublethal toxicity of waterborne amitrole in amphibians (Johnson 1976), with no data concerning its possible behavioral toxicity in aquatic organisms. In our study, common frog tadpoles were kept either unexposed or exposed for 3 days to four sublethal amitrole concentrations, either in the absence or in the presence of larval spotted salamander. Effects of herbicide exposure were assessed on both antipredator behavior of tadpoles and predatory ability of larval salamander, through the monitoring of tadpole behavior and survival rate every day and at the end of the experiment, respectively.

Materials and Methods

Experimental Animals

Four egg masses of common frog were collected from forest temporary ponds at the National Institute for Agronomic Research (INRA) station near Rennes, France on January 27, 2005. Then, each clutch was split into four equal masses that were housed in four identical glass aquaria (50 × 30 × 20 cm) filled with 30 L of aged tap water, with macerated dead leaves and an airstone on the bottom. Hatching occurred on January 31, 2005 (stage 20, Gosner 1960).

Twenty-five larval spotted salamanders (total length = 3.72 cm ± 0.27 cm, weight = 411.73 mg ± 61.8 mg) were collected from the same site as *R. temporaria* eggs on January 28, 2005. After collection, larvae were housed in the laboratory and kept in two glass aquaria (50 × 25 × 30 cm), each filled with 25 L of aged tap water, with macerated dead leaves and an airstone on the bottom. The larvae were fed *ad libitum* on common frog tadpoles derived from supplementary clutches collected at the same site as the other experimental animals.

The laboratory was maintained on a cycle of 11 hours light:13 hours dark (dawn at 08:00; dusk at 19:00), and the physical and chemical characteristics of the water used throughout the experimental period were as follows: temperature 10–12°C; pH 7–7.2; NO_3^- 20–24 mg/L; $NO_2^- < 0.01$ mg/L; $NH_4^+ < 0.01$ mg/L; $PO_4^{3-} < 0.01$ mg/L.

Experimental Design

We experimentally manipulated two factors in a fully crossed design with five replicates: predator treatment (absence or presence of one larval salamander) and herbicide treatment (0, 0.01, 0.1, 1, and 10 mg/L amitrole). Groups of 15 free-swimming tadpoles (stage 25, Gosner 1960, total length = 1.99 cm ± 0.068 cm, weight = 92.55 mg ± 12.08 mg) were introduced (at 16:00) into a set of 50 plastic test aquaria (25 × 16 × 15 cm), each filled with 4 L of aged tap water, with macerated dead leaves on the bottom. All aquaria were lined with black plastic sheeting on the outer parts of the bottom and glass walls. Larval salamanders were introduced into the appropriate aquaria just before the tadpoles. Contamination with amitrole was performed a few minutes before the introduction of organisms into the 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, an organic solvent was not used. The two lowest amitrole concentrations tested in this study (0.01 and 0.1 mg/L) have been commonly reported in surface waters adjacent to treated fields, whereas a concentration of 1 mg/L can be detected after direct spraying to control aquatic weeds (World Health Organisation 1994). The other amitrole concentration tested (10 mg/L) exceeded

Table 1. Summary of repeated-measure analyses of variance for the effects and interactions of predator, amitrole, and time on the behavior of *Rana temporaria* tadpoles

Source of variation	df	Visible tadpoles			Moving tadpoles		
		Sum of squares	*F*	*p*	Sum of squares	*F*	*p*
Between groups							
Predator	1	1.9506	291.0934	<0.001	3.1600	525.3857	<0.001
Amitrole	4	0.3433	12.8076	<0.001	0.7975	33.1483	<0.001
Predator × Amitrole	4	0.2440	9.1045	<0.001	0.2672	11.1070	<0.001
Error	40	0.2680			0.2406		
Within groups							
Time	2	0.3275	32.6752	<0.001	0.1300	22.9110	<0.001
Time × predator	2	0.0305	3.0406	0.0534	0.0511	8.9978	<0.001
Time × amitrole	8	0.0361	0.9016	0.5194	0.0406	1.7872	0.0918
Time × predator × amitrole	8	0.0646	1.6123	0.1344	0.0665	2.9276	0.006
Error	80	0.4009			0.2270		

environmentally realistic levels, albeit far below the 96 hour-LC_{50} documented in tadpoles of another anuran, the tusked frog, *Adelotus brevis* (3 g/L, Johnson 1976). To our knowledge, there are no data concerning the lethal toxicity of amitrole to larval salamander or other caudate amphibians. Amitrole concentrations were determined in the aquaria immediately after contamination with 1 mg/L amitrole, and then at the end of the 3-day exposure (Ecole National de la Santé Publique, Rennes, France). These analyses show that more than 95% of the amitrole initially present in the aquaria remained at the end of the experiment.

Response Variables

During the 3 days after their introduction into the test aquaria, tadpole behavior was monitored by both authors twice a day (at 10:00 and 16:00). Each observation was carried out for a 3-minute period during which the numbers of visible (*i.e.*, unconcealed within the litter) and moving tadpoles were recorded every 30 s. The morning and afternoon recordings were averaged because the data collected each day in each aquarium did not differ significantly between these two periods (visibility: $F_{1,\ 40} = 2.23$, $p = 0.143$; activity: $F_{1,\ 40} = 3.20$, $p = 0.080$). After 72 hours, dead leaves and salamanders were removed from the experimental aquaria and the numbers of surviving tadpoles were counted.

Data Analysis

The mean numbers of visible, moving and surviving tadpoles were converted into percentages to obtain mean proportions. These proportions were then arcsine square root-transformed to stabilize the variance and provide a closer approximation to a normal distribution (Sokal and Rohlf 1981). Behavioral data were processed with repeated-measure analyses of variance (ANOVAs) to determine the main effects and interactions of predator and amitrole all along the 3-day experiment. Because preliminary analyses indicated that the proportion of surviving tadpoles in the tanks did not affect the behavioral endpoints considered, we did not perform a repeated-measures analysis of covariance using tadpole survival as a covariate. Tukey's multiple comparison tests were then used to determine the treatments that were different from one another. Survival data were processed using a two-way ANOVA to determine the effects of predator, amitrole, and their interaction. The intertreatment differences were then assessed using Tukey's multiple comparison tests.

Results

Behavioral Data

Visibility. The proportion of tadpoles unconcealed within the litter is significantly affected by time, predator, amitrole, and the predator × amitrole interaction. By contrast, this endpoint does not appear to be significantly influenced by the other two-way and three-way interactions (Table 1).

Irrespective of the treatment, the proportion of visible tadpoles is significantly higher 1 day after their introduction into the test aquaria than 2 or 3 days later. This proportion also decreases significantly between days 2 and 3.

In the absence of the larval salamander, tadpoles exposed to 0.01, 0.1, and 1 mg/L amitrole are significantly less visible than control tadpoles. These differences do not appear subsequently in the presence of the predator. In the presence of the larval salamander, control tadpoles and tadpoles exposed to 0.01 and 0.1 mg/L amitrole are the only groups showing a significant reduction of visibility compared to the treatment without predator. In contrast, tadpoles exposed to the two highest amitrole concentrations (1 and 10 mg/L) show similar visibility levels regardless of the presence/absence of the larval salamander (Figure 1).

Movements. The proportion of moving tadpoles is significantly affected by time, predator, and amitrole, as well as the interactions time × predator, predator × amitrole and time × predator × amitrole. In contrast, the time × amitrole interaction does not significantly influence this endpoint (Table 1).

Irrespective of the treatment tested, the proportion of moving tadpoles is significantly higher 1 day after their introduction into the aquaria than 2 or 3 days later. This proportion does not differ significantly between days 2 and 3.

In the absence of the larval salamander, and during the 3 days of exposure, we find the proportion of moving tadpoles to be significantly decreased in response to 0.01 mg/L amitrole (Figure 2 a–c). Additional amitrole effects are observed on day 2, with tadpoles exposed to 0.1 and 1 mg/L amitrole showing a

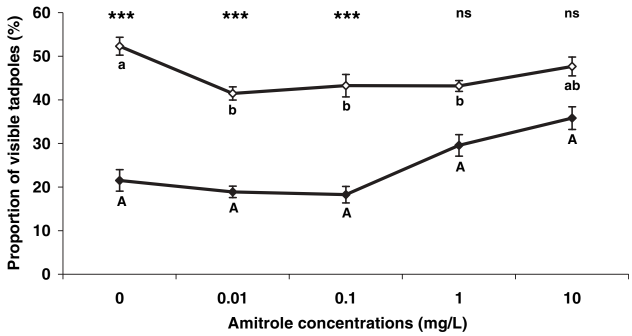

Fig. 1. Proportion of visible *Rana temporaria* tadpoles in the absence (white diamonds) or presence (black diamonds) of larval *Salamandra salamandra* across all amitrole treatments. Because the visibility pattern was the same at all times (as indicated by the nonsignificant "time × predator," "time × amitrole," and "time × predator × amitrole" interactions, Table 1), the data from each day were averaged and combined into one single graph. Treatments labeled with different letters denote significant differences at $p < 0.05$, based on Tukey's multiple comparison tests. Intertreatment differences in the absence and presence of the predator are represented by small and capital letters, respectively. For each amitrole concentration, differences between treatments without and with predator were indicated as following: *ns* nonsignificant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

significant reduction of movements compared to the controls (Figure 2b). There is no herbicide effect in the presence of the predator, except on the first day, when tadpoles exposed to 10 mg/L amitrole appear significantly more active than control tadpoles (Figure 2a). In the presence of the predator, and throughout the experiment, control tadpoles are the only group showing a significant decrease of activity compared to the treatment without predator (Figure 2a–c). A similar reduction of movements in the presence of a predator is only recorded in the group exposed to 10 mg/L, and this occurs only on the last day of the experiment (Figure 2c). Otherwise, tadpoles exposed to the four amitrole concentrations exhibit similar rates of activity irrespective of the presence/absence of the larval salamander.

Survival Data

The two-way ANOVA indicates that the survival rate of tadpoles is significantly affected by predator and amitrole. The significant effect of the herbicide can be mainly attributed to its impact in the predator's presence. Indeed, as indicated by the significant predator × amitrole interaction, amitrole effects on tadpole survival are highly dependent on the presence of the larval salamander (Table 2).

In the absence of the larval salamander, the survival rates do not differ between the different groups of tadpoles: all tadpoles survive regardless of the level of amitrole contamination. In the presence of the predator, the survival rates are significantly decreased compared with the treatments without predator. Tadpoles exposed to the two lowest amitrole concentrations (0.01 and 0.1 mg/L) show similar survival rates compared with unexposed control tadpoles. On the other hand, tadpoles exposed to 1 and 10 mg/L amitrole survive significantly better

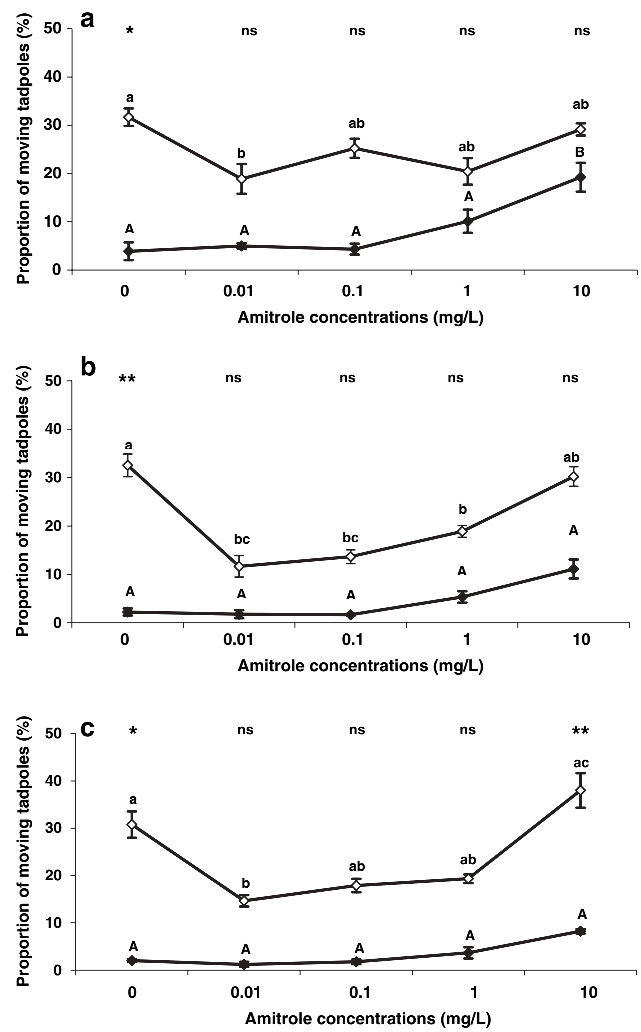

Fig. 2. Proportion of moving *Rana temporaria* tadpoles in the absence (white diamonds) or presence (black diamonds) of larval *Salamandra salamandra* across all amitrole treatments, on (**a**) day 1, (**b**) day 2, and (**c**) day 3 of the experiment. Treatments labeled with different letters denote significant differences at $p < 0.05$, based on Tukey's multiple comparison tests. Intertreatment differences in the absence and presence of the predator are represented by small and capital letters, respectively. For each amitrole concentration, differences between treatments without and with predator were indicated as following: *ns* nonsignificant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 2. Summary of the two-way analyses of variance for the effects of predator, amitrole, and their interaction on the survival rate of *Rana temporaria* tadpoles

Source of variation	df	Sum of squares	F	p
Predator	1	4.58848	2361.70	<0.001
Amitrole	4	0.17648	22.71	<0.001
Predator × amitrole	4	0.17648	22.71	<0.001
Error	40	0.07771		

in the presence of the larval salamander compared with the unexposed controls. Furthermore, we find a concentration-dependent increase in survival: tadpoles exposed to 10 mg/L

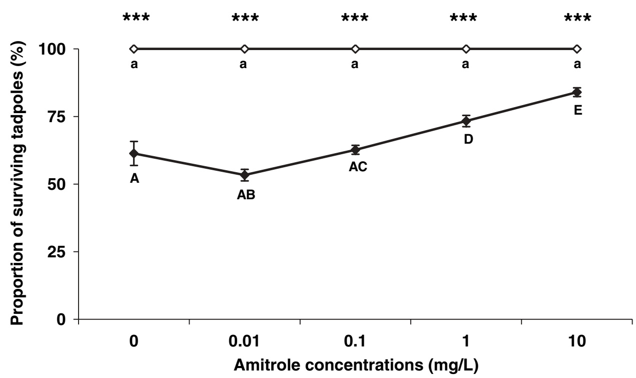

Fig. 3. Proportion of surviving *Rana temporaria* tadpoles, in the absence (white diamonds) or presence (black diamonds) of larval *Salamandra salamandra* across all amitrole treatments, at the end of 3 days of exposure. Treatments labeled with different letters denote significant differences at $p < 0.05$, based on Tukey's multiple comparison tests. Intertreatment differences in the absence and presence of the predator were represented by small and capital letters, respectively. For each amitrole concentration, differences between treatments without and with predator were indicated as following: *ns* nonsignificant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

amitrole show a significantly better survival rate than those exposed to 1, 0.1, or 0.01 mg/L. Tadpoles exposed to 1 mg/L survive significantly better than those exposed to 0.1 and 0.01 mg/L. Finally, tadpoles exposed to 0.1 mg/L survive significantly better than those exposed to 0.01 mg/L (Figure 3). All larval salamanders survive to amitrole exposures.

Discussion

The results of this laboratory experiment clearly show that short-term exposures to sublethal amitrole concentrations can alter the predator–prey relationship between common frog tadpoles and larval spotted salamanders, by affecting both prey behavior and predation rate.

In the absence of the predator, amitrole-exposed tadpoles show a significant tendency to spend more time concealed within the litter and be less active compared with the unexposed control tadpoles. These behavioral changes appear after 1 day of exposure to the lowest concentration tested (0.01 mg/L). Thus, environmentally realistic levels of exposure to this herbicide can prevent tadpoles from taking advantage of an absence of predation risk to maximize their foraging activity. Such alterations might have detrimental effects on common frog tadpoles that rely on temporary ponds for their development. In particular, less active tadpoles might incur the risk of not acquiring the energetic resources necessary to achieve metamorphosis before the pond dries up. Further experiments are thus needed to assess whether amitrole-mediated behavioral alterations can affect larval development and growth. In the gray treefrog (*Hyla versicolor*), Bridges (1999a) similarly found that, compared with unexposed controls, tadpoles subjected to a short exposure (24 hours) to sublethal concentrations of carbaryl spent more time in refuge when no predator (red-spotted newt, *Notophthalmus viridescens*) was present. Moreover, this author showed that, in comparison with controls, carbaryl-exposed tadpoles spent less time under cover in the newt's presence. In contrast, the present results indicate that the refuging behavior of *R. temporaria* tadpoles in the presence of larval *S. salamandra* is unaffected by short-term exposures (1 to 3 days) to sublethal amitrole concentrations. These contrasting results could be related to specific differences in the behavioral ecology of the observed organisms, as well as methodological differences in both the contaminants tested and the modes of exposure. Bridges (1999a) used carbaryl, a carbamate insecticide known to alter swimming behavior through its inhibitory effect on acetylcholinesterase activity (Zinkl *et al*. 1991; Bridges 1997). In addition, our behavioral observations were made on pesticide-exposed tadpoles in the presence of a free-foraging predator, whereas Bridges exposed tadpoles to the pesticide before introducing them into an uncontaminated testing chamber with a caged predator. In the present study, it is also noteworthy that, on the first day of the experiment, tadpoles exposed to the highest amitrole concentration (10 mg/L) showed higher rates of movement in the salamander's presence than the controls. Although this high concentration is unlikely to occur under natural conditions, such a behavioral change might represent a risk for tadpoles because it could increase the probability of being detected by a predator. At environmentally more realistic concentrations, amitrole does not change the behavior of tadpoles in the presence of a larval salamander. However, a growing body of evidence suggests that exposure to multiple stressors can have more serious consequences on amphibian larvae than single stressing factors (Zaga *et al*. 1998; Hatch and Blaustein 2000; Relyea and Mills 2001; Relyea 2003; Chen *et al*. 2004; Edginton *et al*. 2004; Relyea 2004a; Sih *et al*. 2004; Metts *et al*. 2005; Relyea 2005). For example, Relyea and Mills (2001) and Relyea (2003, 2004a, 2005) found that several species of anuran larvae simultaneously exposed to pesticides and predatory stress suffered from a massive increase in mortality compared to their counterparts exposed to pesticides alone. In our study, we did not enhance the effects of the herbicide by combining predation risk with amitrole contamination. On the contrary, tests in the presence of a predator failed to detect any increase of refuge use or decrease in activity produced by amitrole contamination. Furthermore, survival data show that the consumption of tadpoles by the salamander larvae decreases with increasing amitrole concentration. Further experiments are required associating predation risk and chemical stressors in amphibians to assess the relative influences of diverse factors related to the predator (species, origin, diet, etc.) and contaminant (chemistry, concentration, and duration of exposure, etc.), testing their joint effects on prey organisms.

Control tadpoles reacted to the presence of the larval spotted salamander by decreasing their rate of movements and by taking refuge within the litter. However, the results also showed that this antipredator behavioral strategy can be affected by waterborne amitrole. At the four concentrations tested, amitrole-exposed tadpoles do not significantly reduce their activity level in the presence of this predator. Moreover, tadpoles exposed to the two highest amitrole concentrations do not increase their refuge use when confronted with the larval salamander. Recent studies in aquatic invertebrates (Lefcort *et al*. 2000; Wolf and Moore 2002), fishes (Saglio and Trijasse 1998; Scholz *et al*. 2000), and amphibians (Lefcort *et al*. 1998;

Mandrillon and Saglio in press) indicate that negative impacts of contaminants on antipredator behaviors can result from alterations of the chemosensory systems involved in predator detection. In this connection, the presence of waterborne contaminants has been shown to affect the behavioral response to chemical cues from the predator and conspecific substances indicating a predation risk. In the Columbia spotted frog (*Rana luteiventris*), Lefcort *et al*. (1998) found that heavy metals reduced the antipredator response of tadpoles to chemical cues from the rainbow trout (*Oncorhynchus mykiss*). In addition, the alarm reactions resulting from the chemical detection of injured conspecifics can be modified in the presence of aquatic contaminants (Saglio and Trijasse 1998, Lefcort *et al*. 2000, Scholz *et al*. 2000, Wolf and Moore 2002). Moreover, we recently demonstrated that aquatic contamination using an environmentally realistic amitrole concentration (0.1 mg/L) can impair the Pavlovian mechanism allowing common toad tadpoles (*Bufo bufo*) to learn to recognize chemical cues from a non-native predator, the Turkish crayfish (*Astacus leptodactylus*) (Mandrillon and Saglio 2007). Such toxicant effects, which disrupt antipredator responses probably through negative impacts on the olfactory system, are likely to increase the vulnerability of exposed preys to predation.

The present study shows that the alteration of behavioral defenses in amitrole-exposed tadpoles did not result in an increased predation by the larval salamander. Instead, salamander larvae show a concentration-dependent decrease in predation efficiency. In the presence of the two highest amitrole concentrations (1 and 10 mg/L), the predation rate is significantly reduced compared to control treatment, indicating that amitrole contamination affects the predator to a greater extent than the prey. Such an adverse effect of chemical contaminants on predators has been previously documented in fishes (Weis and Khan 1991; Weis and Weis 1995b; Scherer *et al*. 1997). Weis and Khan (1991) and Weis and Weis (1995) showed that mummichogs (*Fundulus heteroclitus*) from a metal-polluted site or derived from exposure to methylmercury did not capture preys as effectively as the control fishes. Similarly, Scherer *et al*. (1997) reported a significant concentration-dependent decrease of foraging rates in lake trout (*Salvelinus namaycush*) exposed to cadmium. In amphibians, Boone and Semlitsch (2003) and Mills and Semlitsch (2004) found similar results in tadpoles of the bullfrog (*Rana catesbeiana*) and southern leopard frog (*Rana sphenocephala*) exposed to waterborne carbaryl, respectively. They demonstrated that this insecticide released tadpoles from predation, by killing predators. In the case of the present study, we still need to elucidate the mechanisms underlying the detrimental effects of amitrole on the predatory behavior of larval salamander. This herbicide could either interfere with the salamander's ability to detect preys or impair its capacity to attack and capture them. Recent studies in aquatic and terrestrial salamanders have shown that the olfactory sense (including the primary olfactory system and the vomeronasal organ) can play a major role in prey detection (Placyk and Graves 2002; Mathis 2003; Eisthen and Park 2005). As has been shown for some other pesticides (Scholz *et al*. 2000; Wolf and Moore 2002), our recent observations on common toad tadpoles indicate that waterborne amitrole can impair the chemical detection of predation cues (Mandrillon and Saglio in press). Thus, we propose that amitrole could also affect the chemical recognition of prey species. Consequently, we require further research to assess whether the alteration of the predatory ability observed here results from the negative impact of amitrole on the chemical sense of larval salamander. To specify the effects of amitrole on this predator, further experiments need to be carried out focusing on the consequences of exposure on feeding motivation, latency to attack, and capture rates. On the other hand, waterborne contaminants are able to alter predation efficiency through indirect effects on prey activity. In this connection, Relyea and Hoverman (2006) have argued that pesticides can have positive effects on prey survival by modifying their activity in the presence of predators. Based on the well-established assumption that predation risk is positively correlated with activity level (Lawler 1989; Skelly 1994), a pesticide should make prey less vulnerable to predation if it induces a reduction of activity. However, the results of our study show that the reduction of predation efficiency cannot be attributed to a behavioral change in the prey, because movement rates in unexposed and amitrole-exposed tadpoles do not differ in the presence of the salamander.

All tadpoles survived in the absence of the predator irrespective of the amitrole concentration used in our study, implying that the levels of contamination used are not lethal for larval common frog. This result is not surprising because the concentrations tested here are much lower than the amitrole 96 hour-LC_{50} (3 g/L) documented in tadpoles of another anuran, the tusked frog (*Adelotus brevis*, Johnson 1976). This latter value is 300 times the highest concentration tested here (10 mg/L).

To conclude, our study demonstrates that short-term exposures to environmentally relevant amitrole concentrations can disrupt the predator–prey relationship between *R. temporaria* tadpoles and *S. salamandra* larvae. The herbicide alters both the antipredator behavior of the tadpoles and the predation rate of the salamander. At high concentrations (1 and 10 mg/L), the outcome of the predator–prey relationship appears biased in favor of the tadpoles, which were released from the predation risk in spite of their altered behavior. Further studies are now required in these species and other amphibians to investigate the nature of the mechanisms responsible for the adverse impacts of amitrole on antipredator behaviors and predation efficiency. Additional experiments are also needed to investigate the effects of longer periods of exposure to this herbicide and to confirm these results under ecologically more realistic conditions.

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