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Toxicity of the fungicide trifloxystrobin on tadpoles and its effect on fish–tadpole interaction

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ABSTRACT

Contamination of aquatic systems is a major environmental stress that can interfere with predator–prey interactions, altering prey or predator behavior differentially. We determined toxicity parameters of the fungicide trifloxystrobin (TFS) and examined its effects on predation rate, using a fish predator (*Synbranchus marmoratus*) and four anuran tadpole species as prey (*Rhinella arenarum, Physalaemus santafecinus*, *Leptodactylus latrans*, and *Elachistocleis bicolor*). TFS was not equally toxic to the four tadpole species, *E. bicolor* being the most sensitive species, followed by *P. santafecinus*, *R. arenarum*, and *L. latrans*. Predation rates were evaluated using different treatments that combined predator and prey exposed or not to this fungicide. TFS would alter the outcome of eel–tadpole interaction by reducing prey movements; thus, prey detection would decrease and therefore tadpole survival would increase. In addition, eels preyed selectively upon non-exposed tadpoles avoiding the exposed ones almost all throughout the period evaluated. Predation rate differed among prey species; such differences were not due to TFS exposure, but to interspecific differences in behavior. The mechanism that would explain TFS-induced reduction in predation rates remains unclear; however, what is clear is that sublethal TFS concentrations have the potential to alter prey behavior, thereby indirectly altering predator–prey interactions. In addition, we consider that predator–prey relationships are measurable responses of toxicant exposure and provide ecological insight into how contaminants modify predator–prey interactions.

1. Introduction

Predatory fish are known to have dramatic effects on amphibian populations and several studies have demonstrated direct negative effects on anuran larvae (Hecnar and M'Closkey, 1997; Babbitt, 2001; Hartel et al., 2007), often leading to the reduction of some tadpole species (Heyer et al., 1975). In addition, the presence of xenobiotics may alter the intensity of these predator–prey interactions (Broomhall, 2002, 2004; Reeves et al., 2011). Sublethal concentrations of environmental toxicants have the potential to alter predator–prey interactions, affecting prey or predator behavior differentially, and consequently modifying the composition of the ecological community (Boone and Semlitsch, 2001, 2002; Reeves et al., 2010; Relyea and Edwards, 2010). Some investigations that considered amphibian as prey showed increased vulnerability of prey exposed to methoxychlor due to modifications in their defensive mechanisms (Ingermann et al., 2002). Recently, Reeves et al. (2011) demonstrated that a chemical contaminant (Copper) combined with a chemical cue from an odonate predator (*Aeshna sitchensis*) reduced the activity of *Rana sylvatica* tadpoles and altered microhabitat use. By contrast, it has been indicated that contaminants may reduce predation risk when the predator is more sensitive than the prey, with consequent changes in predator feeding behavior (Boone and Semlitsch, 2003; Mills and Semlitsch, 2004). Such disparite findings indicate the need to evaluate how sublethal concentrations of xenobiotics influence interactions between amphibian prey species and potential predators.

In recent years, fungicides have gained popularity around the world in the control of the pathogenic fungus *Phakopsora pachyrhizi*, responsible for Asian soybean rust, and in the prevention of plant disease with the aim of increasing soybean crop yields (Sconyers et al., 2006; Battaglin et al., 2010). This increased fungicide application might lead to greater environmental load over the next few years, which poses a risk on the environment (Debjani et al., 2009; Ochoa-Acuña et al., 2009). In Argentina, the soybean production (91%) is concentrated in the Humid Pampa (Viglizzo et al., 2009). This area includes South West of Córdoba, Centre

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and South of Santa Fe, South East of Entre Rios and North of Buenos Aires provinces, in which *P. pachyrhizi* is present (Ivancovich, 2005). Triazole fungicides (cyproconazole, difenoconazole, epoxyconazole, and tebuconazole) and strobilurin fungicides (azoxystrobin, pyraclostrobin, and trifloxystrobin) are the most used to control soybean rust in this area (Sillon et al., 2005). Flint® is the first fungicide of the strobilurin group in the Bayer Crop Science product portfolio. This formulation contains Trifloxystrobin (TFS) (CAS Registry Number 141517-21-7) as active ingredient (Gisi et al., 2000). TFS is considered nontoxic to birds, mammals, bees, other beneficial insects and earthworms (CASAFE, 2007); however, it has been classified as highly toxic to non-target aquatic organisms. For example, toxic effects of TFS on *Bufo cognatus* tadpoles were observed at 40 μg L^{-1} (Belden et al., 2010), whereas the median lethal concentration (96-h LC_{50}) for *Oncorhynchus mykiss* trout ranged between 15 and 78 μg L^{-1}, and for the marine crustacean *Mysidopsis bahia* the median effective concentration (EC_{50}) ranged from 9 to 34 μg L^{-1} (APVMA, 2000). TFS is infrequently detected in aquatic habitats (Battaglin et al., 2010), because it degrades rapidly in water and soil, with an environmental half-life of 16.8–31.2 h (Banerjee et al., 2006). However, its primary metabolite [(E,E)-trifloxystrobin acid] is soluble in water; hence, aquatic organisms may be at risk of exposure to these products through spray drift, direct overspray, atmospheric transport, runoff, and movement of animals through fields during application (Belden et al., 2010).

The purpose of our study was to experimentally determine the toxicity of TFS on four common species of anuran tadpoles, and examine the effects of sublethal exposure to TFS on predation rates of tadpoles using eels (*Synbranchus marmoratus*) as fish predator. We also investigated whether eels preyed differentially on tadpoles exposed or not to TFS, and whether predation differed among anuran species.

2. Materials and methods

2.1. Fungicide

The 50 WG (Wettable Granular) formulation (commercial grade; 50% a.i.) of trifloxystrobin (Flint®, Bayer CropScience A.G., Argentina), chemical name: (E,E) methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl] phenyl}-acetic acid methyl ester (IUPAC) was used in all experiments. The fungicide was tested using formulation instead of pure active ingredient because some studies demonstrated that other inert ingredients contained in formulations may contribute to amphibian pesticide toxicity (e.g., Jones and Relyea, 2009; Lajmanovich et al., 2010). A stock solution was prepared at a concentration of 10 mg L^{-1} immediately before the start of the experiment. The solutions at various nominal concentrations were prepared by appropriate dilution of the stock solution.

2.2. Predator eel

S. marmoratus (Bloch 1795), commonly known as eel, is a teleost fish that belongs to the order Synbranchiformes (Kullander, 2003). This species is widely distributed from Mexico to central Argentina, mainly due to its ability to breathe air, tolerance to salinity, and capacity to undergo sex reversal (Lo Nostro and Guerrero, 1996; Ravaglia and Maggese, 2002). Eels are "sit-and-wait" predators (Scarabotti et al., 2011) and use tactile and visual stimuli to locate prey during the day, and the lateral line to detect prey at night, and rely on the movement of their prey to find and catch them (Junges et al., 2010). As many gape-limited predators (Urban, 2007), eels typically suck in and swallow their prey whole (Mittelbach and Osenberg, 1994). Probably the most common tactic for overcoming gape limitation is nibbling (Helfman et al., 2009). This means eels can spin rapidly around their long body axis while holding on to food and thus tear chunks from the larger mass of a prey item. Besides, eels frequently use macrophyte stands to ambush their preys. Because tadpoles and eels are natural inhabitants of the same aquatic systems (Ringuelet, 1975; Scarabotti et al., 2011), eels are considered potential predators of anuran tadpoles (Junges et al., 2010). Indeed, Maffei et al. (2011) found that *S. marmoratus* is the only predator fish that coexists with anuran larvaes in a pond in the municipality of Borebi, middle-western region of the São Paulo state, Southeastern Brazil.

Eel juveniles ($n = 48$) used in this experiment were collected from an unpolluted temporary pond in the floodplain of Paraná River (Santa Fe Province, Argentina; 31°42′34″S; 60°34′16″W). During 1 week before the start of the trials, similar-sized test eels (mean length ± S.D. = 23.04 ± 1.94 cm, mean weight ± S.D. = 13.47 ± 2.82 g) were acclimated to experimental conditions and fed on non-experimental tadpoles daily. To standardize hunger levels, all eels were starved for 24 h before each trial.

2.3. Tadpole prey species

To examine patterns of vulnerability to predation among species (Jones et al., 2009; Lajmanovich et al., 2010), as prey organisms we selected four native species of anuran tadpoles that co-occur in wetlands in the floodplain of Paraná River: *Rhinella arenarum* (Bufonidae), *Physalaemus santafecinus* (Leiuperidae), *Leptodactylus latrans* (Leptodactylidae), and *Elachistocleis bicolor* (Microhylidae). These anurans have extensive neotropical distributions (IUCN, 2010) and are frequently found in forests, wetlands, agricultural lands, and urban regions (Peltzer et al., 2006; Peltzer and Lajmanovich, 2007). These species generally breed in agricultural ponds during the soybean cultivation period (Attademo et al., 2005; Peltzer et al., 2006; Lajmanovich et al., 2010).

Anuran tadpoles were collected from a semipermanent pond at the University Ecological Reserve in Santa Fe city (Santa Fe Province, Argentina, 31°38′26″S, 60°40′22″W). In the laboratory, tadpoles of each species were placed in separate aquaria containing dechlorinated tap water (pH 7.4 ± 0.05; conductivity, 165 ± 12.5 μmhos cm^{-1}; dissolved oxygen concentration, 6.5 ± 1.5 mg L^{-1}; hardness, 50.6 mg L^{-1} of $CaCO_3$ at 22 ± 2 °C) and fed on lettuce at the beginning of the experiment. Prometamorphic stages (35–38, Gosner, 1960) of tadpoles of *R. arenarum* (mean snout-to-vent length [SVL; cm] ± SD = 0.91 ± 0.12), *P. santafecinus* (mean SVL ± SD = 0.81 ± 0.13), *E. bicolor* (mean SVL ± SD = 0.86 ± 0.13), and *L. latrans* (mean SVL ± SD = 0.93 ± 0.09) were used in the experiments. All the tadpoles were matched to be similar in size (one-way ANOVA: $F_{3,46} = 2.53$, $p = 0.06$).

2.4. Experimental design

The experiment consisted in a toxicity phase to elucidate the TFS toxicity on four anuran species followed by an exposure phase of tadpoles and eels, and then a testing phase. In the exposure phase, tadpoles and eels were exposed either to a sublethal concentration of TFS or to water 6-h before the testing phase to generate groups of individuals with differential risk associated with the fungicide (exposed to water or to TFS). The testing phase included predation experiments in which tadpoles from both groups were exposed to eels previously treated or not with TFS.

2.4.1. Acute toxicity tests

Because of the lack of information in the literature about the effects of TFS exposure on amphibians, particularly on native species, the first step was to elucidate the direct toxicity of the fungicide on four anuran species. Range-finding toxicity tests consisted

in exposing larvae of each species to TFS solutions to estimate the lethal concentration 50% (LC_{50}), the lowest-observed-effect concentration (LOEC), and the no-observed-effect concentration (NOEC). Static toxicity tests were performed in 1.5-L glass containers (12.5 cm in diameter and 13.5 cm in height) with 1 L of test solution at 25 ± 1 °C and 12 h light:12 h dark for a 48-h period. Each toxicity test was carried out in triplicate with eight different concentrations plus a negative control, and seven tadpoles per container (1.28 g L^{-1}). The nominal concentrations ranged from 0.077 to 0.35 mg a.i. L^{-1}. Larval mortality was monitored once every 24 h, and dead larvae were removed every 24 h. Animals were not fed during toxicity trials.

2.4.2. Exposure phase

6 h before the start of the testing phase, a subsample of eels ($n = 24$) and tadpoles ($n = 120$ of each species) were randomly assigned to the 'TFS exposure' treatment, whereas the other subsample ($n = 24$ eels and $n = 120$ tadpoles of each species) was assigned to the 'water exposure' treatment. In the 'TFS exposure' treatment, the LOEC previously calculated in toxicity tests for each tadpole species was used as sublethal concentration of exposure of tadpoles and eels. Therefore, each tadpole species was exposed to their LOEC, respectively (see concentrations of exposure in Table 1) while the eels were exposed to the same LOEC as prey species with which they were tested. On the other hand, in the 'water exposure' treatment, tadpoles and eels were kept in dechlorinated tap water. During exposure, neither eels nor tadpoles were fed. Following exposure, individuals were transferred to an aquarium containing pesticide-free water, and then placed in plastic test aquaria for the testing phase.

2.4.3. Testing phase: predator–prey experiments

We estimated the predation rate of eels (E) on tadpoles (T) exposed to TFS (+) and not exposed TFS (−) using four treatments: (1) neither eels nor tadpoles were exposed (E−, T−), (2) both eels and tadpoles were exposed (E+, T+), and either tadpoles (3) or eels (4) were exposed (E−, T+ and E+, T−, respectively). At the end of the exposure period, one eel predator (exposed or not to TFS, depending on the treatment) and groups of 20 tadpoles (exposed or not) were introduced into the plastic test aquaria (40 cm in length, 26 cm in width, and 12 cm in height), each containing 6 L of dechlorinated tap water and three aquatic ferns *Salvinia herzogii* to provide structural complexity. The assay began at the end of the exposure phase, with the introduction of eels into the aquaria, and lasted 24 h. In addition, to evaluate natural tadpole mortality a treatment involving each tadpole species was performed without the presence of eels. The experiments were conducted in a temperature-controlled room, with light/dark cycles that reflected natural day length, and in triplicate. Because of differences in breeding times among anurans, predation rate experiments were conducted separately for each prey species.

Table 1
Summary of median lethal concentrations (LC50), lowest-observed-effect concentrations (LOEC), and no-observed-effect concentrations (NOEC) (mg L^{-1}) of TFS on anuran tadpoles after 24-h exposure.

Species	LC_{50}	NOEC	LOEC
Rhinella arenarum	0.22 (0.19–0.25)^{ac}	0.096	0.125
Physalaemus santafecinus	0.14 (0.12–0.16)^{ab}	0.096	0.125
Elachistocleis bicolor	0.10 (0.09–0.11)^{b}	0.077	0.096
Leptodactylus latrans	0.26 (0.23–0.28)^{c}	0.180	0.230

Toxicity endpoints were calculated based on nominal concentrations. Values in parenthesis correspond to the 95% confidence interval of each estimate. Different letters (a, b, c) indicate significant differences in LC50 among species (Kruskall-Wallis ANOVA with post-hoc Dunnett's test; $p < 0.05$).

2.5. Response variables

During the 24 h of the testing phase, predation rate of the four tadpole species was determined at 1, 6, 18 and 24 h, and was calculated as the instantaneous mortality rate of prey using the following equation taken from Bergström and Englund (2002): $z = -\ln(n_t/n_0)\ t^{-1}$, where n_0 and n_t are the densities of prey at the start and the end of the experiment and t is the duration of the experiment in hours.

2.6. Statistical analyses

Median lethal concentration (LC_{50}) for each species and the respective confidence intervals (95%) were calculated using the Trimmed Spearman Karber method (Hamilton et al., 1977). In all experiments, replicates were tested for differences using ANOVA (Hurlbert, 1984). No significant differences were found among replicates ($p > 0.05$); thus, no tank effect was identified and replicates were pooled. The LC_{50} estimates were subjected to non-parametric Kruskall-Wallis ANOVA followed by the Dunnett's test for post-hoc comparison of means to determine the LOEC and the NOEC. Data from the predation experiment were analyzed using two-way ANOVA for each time tested (at 1, 6, 18 and 24 h). Treatments (four levels: E−, T−; E+, T−; E−, T+; E+, T+) and tadpole species (four levels: *R. arenarum, P. santafecinus, L. latrans, E. bicolor*) were used to test the null hypothesis that predation rates (response variable) of tadpoles would be the same. Dunnett's and Tukey's HSD tests were used as post-hoc multiple comparison tests. We also performed a Student's *t*-test to compare the means of exposed and not exposed tadpoles of all species consumed by eels, as well as to compare the means of tadpoles of all species eaten by eels exposed and not exposed to TFS. Assumptions of normality and homoscedasticity were confirmed with Kolmogrov-Smirnov and Levene tests. Statistical analyses were performed with SPSS 17.0 software at 95% significance level.

3. Results

3.1. Acute toxicity tests

In toxicity tests, mortality of tadpoles occurred within the first 24 h of exposure. LC_{50} values at 24 h ranged from 0.1 to 0.26 mg L^{-1}, and analysis of variance on LC_{50} values of TFS tadpoles showed significant variations among species (Table 1).

3.2. Exposure phase

No mortality occurred in tadpoles or eels during 6-h exposure to LOEC of TFS. No signs of reduced swimming performance or altered behavior were observed in tadpoles or eels after 6-h exposure.

3.3. Predator–prey experiments

Predation rate differed among treatments after 1 h ($F_{3,32} = 19.78$, $p < 0.0001$), 6 h ($F_{3,32} = 6.76$, $p < 0.05$), 18 h ($F_{3,31} = 20.78$, $p < 0.0001$), and 24 h ($F_{3,32} = 10.79$, $p < 0.0001$) of the start of the assay. At each of these times, predation rates were highest in the control treatment (E−, T−) and lowest in the treatment in which tadpoles and eels were simultaneously exposed to TFS (E+, T+). Fig. 1 shows the effect, pooled on all species, of sublethal TFS exposure on predation rates. Dunnett's test showed significant differences in predation rates between control (E−, T−) and the TFS-exposed groups: E + T+, E + T−, and E−T+ (Fig. 1) at 1 h, 18 h, and 24 h, whereas at 6 h, differences in predation rates were found between control (E−, T−) and two of the fungicide-exposed groups: E + T + and E−T+ (Fig. 1).

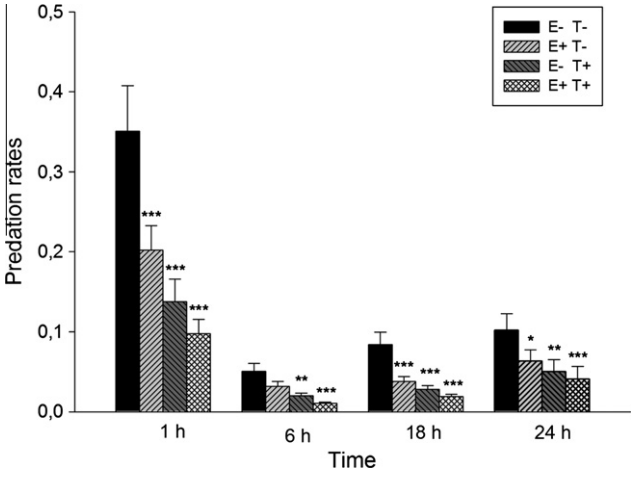

Fig. 1. Effects-pooled on all species-of sublethal TFS exposure on predation rates. Data are expressed as mean ± SE. Significant differences from control (E−T−) are indicated as: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$ based on Dunnett's post-hoc test.

Multiple comparison tests (Tukey HDS test) of all treatment means did not show significant differences between treatments with tadpoles exposed to TFS (E−T + and E + T+); however, significant differences were found between treatments with eels exposed (E + T− and E + T+), only at 1 h ($p = 0.028$). In addition, predation rates were statistically significant among tadpole species at 1 h ($F_{3,32} = 15.30$, $p < 0.0001$), 18 h ($F_{3,31} = 8.86$, $p < 0.01$), and 24 h ($F_{3,28} = 49.16$, $p < 0.0001$), but not at 6 h ($F_{3,32} = 0.95$, $p = 0.42$). Fig. 2 shows the effects, pooled of all treatments, on predation rates of each tadpole species. Comparing all four species in all treatments, *L. latrans* was less consumed than *P. santafecinus* (at 1 and 6 h) and *R. arenarum* (at 18 h), whereas at 24 h, *E. bicolor* was the least consumed species and *P. santafecinus* was the most consumed (Fig. 2). However, the interaction between treatments and tadpole species was not significant at 1 h ($F_{9,32} = 1.56$, $p = 0.16$), 6 h ($F_{9,32} = 1.52$, $p = 0.99$) and 24 h ($F_{9,28} = 0.33$, $p = 0.95$), but this interaction was significant at 18 h ($F_{9,31} = 2.45$, $p < 0.05$).

Non-exposed tadpoles (T−) of all species were captured at a higher rate than exposed ones (T+) at 1, 6 and 18 h ($t = 4.09$, degrees of freedom [df] = 46, $p = 0.0002$; $t = 4.11$, df = 46, $p = 0.0002$; $t = 3.85$, df = 45, $p = 0.0004$, respectively; Fig. 3), whereas at 24 h no differences in predation rates were found between T + and T− ($t = 1.80$, df = 42, $p = 0.078$). Similarly, the same trend was observed for eels exposed (E+) and not exposed (E−), where E− consumed more tadpoles of all species than E + at 1, 6 and 18 h ($t = 2.18$, degrees of freedom [df] = 46, $p = 0.034$; $t = 2.01$, df = 46, $p = 0.05$; $t = 2.60$, df = 46, $p = 0.012$, respectively; Fig. 4), whereas at 24 h no differences in predation rates were found between E + and E− ($t = 0.84$, df = 42, $p = 0.401$).

4. Discussion

To understand the effects of TFS fungicide on amphibians and their influence on predator–prey relationship, previous knowledge of the direct toxicity of fungicide on amphibians is necessary. Data of toxicity presented here suggest that TFS is not equally toxic to the four species of tadpoles studied, *E. bicolor* being the most sensitive species, followed by *P. santafecinus*, *R. arenarum*, and *L. latrans*. Indeed, LC_{50} values of the most sensitive species were at least twice as high as those of the least sensitive species (*E. bicolor* = 0.1 mg L^{-1} and *L. latrans* = 0.26 mg L^{-1}), indicating that larval species had differential sensitivity to TFS. This variability in toxicity of pesticides was also observed across several species of amphibians by Jones and Relyea (2009) and Jones et al. (2009), suggesting that amphibian sensitivity might have a phylogenetic basis. Furthermore, Lajmanovich et al. (2010) reported that different sensitivity to pesticides among species is related to variations in enzymatic levels (B-esterases, cholinesterases and carboxylesterases), since such enzymes play significant roles in the metabolism and subsequent detoxification of many agrochemicals. Understanding which tadpole species are sensitive to TFS will help us anticipate indirect effects that may cascade up and down the food web (Boone et al., 2007). However, a sublethal behavioral response instead of a mortality one in original acute toxicity tests could be interesting to introduce in future research using eels as predator and other native tadpole species as prey.

In natural systems, tadpoles respond to the presence of fish predator by reducing activity levels (Azevedo-Ramos et al., 1992). In environments where both predator and prey are exposed to contaminants, the outcome of the eel–tadpole interaction can be determined by the interplay between predator hunting mode and prey antipredator behavior plus the effect of toxicant exposure. In our experiments, predation rates were lower when predator and prey were exposed simultaneously to

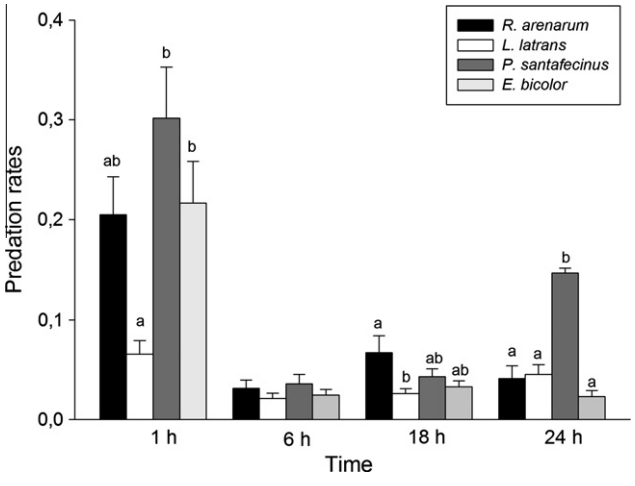

Fig. 2. Predation rates (mean ± SE) on each larval anuran species over the 24 h assay. All treatments were pooled. Different letters (*a*, *b*, *c*) denote significant differences in predation rates among species (Tukey's HSD post-hoc test; $p < 0.05$).

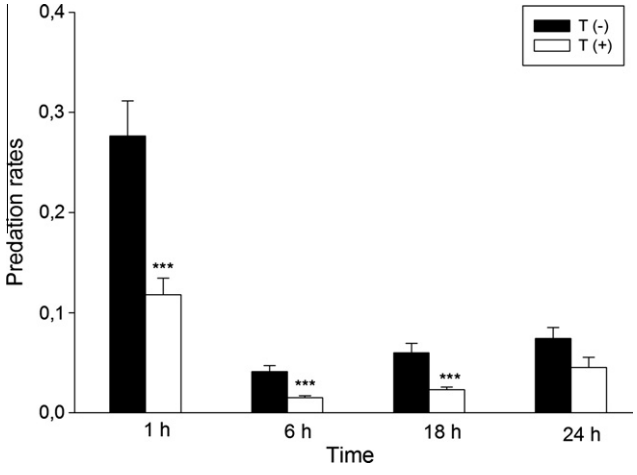

Fig. 3. Predation rates (mean ± SE) of all species on tadpoles exposed (T+) and not exposed (T−) to TFS over the 24-h assay. For (T+), E + T + and E−T + treatments were pooled, and for (T−), E−T− and E + T− treatments were pooled. Asterisks show significant differences between groups ($^{***}p < 0.001$; $^{**}p < 0.01$; Student's *t*-test).

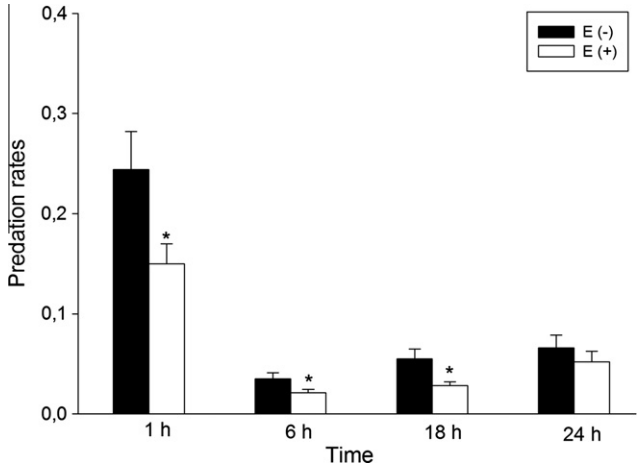

Fig. 4. Predation rates (mean ± SE) of eels exposed (E+) and not exposed (E−) to TFS of all tadpole species over the 24-h assay. For (E+), E + T + and E + T-treatments were pooled, and for (E−), E−T− and E−T + treatments were pooled. Asterisks show significant differences between groups (*$p < 0.05$; Student's *t*-test).

fungicide (E+, T+) and when only prey were exposed (E−, T+), than in the remaining treatments. Conversely, when neither prey nor predator was exposed, predation rates increased. Hence, TFS would alter the outcome of eel–tadpole interaction by reducing prey movements; thus, prey detection would decrease and therefore tadpole survival would increase, probably because the movement generated by the great activity of non-exposed tadpoles attracts the attention of predators (Werner and Anholt, 1993; Teplitsky et al., 2003). These assumptions are consistent with prior studies that have shown similar reductions in predation rate between tadpoles of *H. pulchellus* (prey) and eels exposed to an ecologically relevant fenitrothion dose (2.5 mg L^{-1}) (Junges et al., 2010). In addition, Relyea and Edwards (2010) demonstrated that a short-term exposure to sublethal concentrations of carbaryl and malathion affect prey behavior by reducing the activity of three tadpole species (*Hyla versicolor*, *Rana Clamitans*, and *R. catesbeiana*), thereby reducing predation rates. Broomhall (2002, 2004) also documented reduced per-capita predation rates at two endosulfan concentrations (0.03 and 1.3 mg L^{-1}) in tadpoles. Likewise, in aquatic communities exposed to malathion, Relyea and Hoverman (2008) found reduced predation rates on two species of tadpoles with increasing malathion concentration across a range of sublethal concentrations.

We also found that exposed and non-exposed tadpoles were differentially preyed upon by eels, which tended to avoid the exposed tadpoles almost all throughout the period evaluated. This could be indirectly inferred through the observed increase in predation rates in the different treatments, mainly those in which neither prey nor predator was exposed (E−, T−) and when only predator was exposed (E+, T−). In addition, we expected that the chance of tadpoles to escape from eel attack could be affected by TFS exposure. However, at 24 h no significant changes in predation rates were found for exposed and non-exposed tadpoles, probably because at the end of the assay tadpoles became more active (TFS environmental half-life is 16.8–31.2 h), which increased risk of predation. Overall, our data support the hypothesis that sublethal exposure to TFS, as to other pesticides, might confer an advantage to exposed tadpoles, allowing amphibian larvae to reduce potential encounters with predators (Abrams, 1984), and therefore to reduce the risk of mortality due to predation.

The lack of significant differences in the interaction between treatments and species may indicate that the differential predation rate among tadpole species is not due to the effect of TFS exposure, but to interspecific differences. Therefore, it is not surprising that predation rates on each of the four prey species were different and that were influenced by the activity of tadpoles because the predator did not chase the prey but usually stayed immobile at the bottom of the aquarium waiting for the prey. Tadpoles of *L. latrans* were the prey least captured by eel predator, followed by *E. bicolor, R. arenarum* and *P. santafecinus*. Low predation cannot be explained by greater prey size, since all tadpole species were chosen to be similar in size. The length duration of our experiments (24 h), the use of starved eels, and the "no-choice" design used, which did not allow for alternative prey items, likely played a role in the differential predation rates observed among species.

Gregariousness of *L. latrans* species (Vaz-Ferreira and Gehrau, 1975) may have served as an antipredatory mechanism to reduce the risk of predation by eels, because predators are more likely to make mistakes (confusion effect) when trying to capture prey in a large group, which reduces predation rates (Spieler, 2003; Whitfield, 2003; Abrahams et al., 2009). On the other hand, both tadpoles of *P. santafecinus* and *E. bicolor* are benthic, and suspension feeders (Perotti and Céspedez, 1999; Vera Candioti, 2006). However, *P. santafecinus* is highly active, whereas *E. bicolor* usually stays motionless in the presence of a predator and thus rarely offers a visual stimulus to a visual predator such as *S. marmoratus*. Therefore, the immobility of *E. bicolor* tadpoles may help them avoid detection by visually oriented predators. In addition, bufonid tadpoles are generally unpalatable to many vertebrate predators (Wassersug, 1971; Lawler and Hero, 1997; Alstyne, 2001; Jara and Perotti, 2006). Unpalatable tadpoles commonly present black coloration, which is generally associated with aposematism (Heyer et al., 1975; Crossland and Azevedo-Ramos, 1999; Hero et al., 2001). Additionally, it is well known that unpalatable tadpoles do not show strong reductions in foraging activity upon perceiving predation risk (D'Heursel and Haddad, 1999; Jara and Perotti, 2009, 2010). Although *R. arenarum* tadpoles are known to be unpalatable at some developmental stages (Kehr and Schnack, 1991), they are conspicuous and in constant activity, which would make them more easily detectable by eel and would therefore increase the predation rate, as suggested by Skelly (1994) and Relyea (2001). Perhaps this response in the predation rate would probably be due to the fact that the tadpole developmental stage range used in our study was more palatable to eels.

Overall, the mechanism underlying the TFS-induced reduction in predation rates remains unclear. What is clear is that sublethal concentrations of TFS have the potential to alter prey behavior and thereby indirectly alter predator–prey interactions. Further studies are needed to investigate the nature of the mechanisms responsible for the effects of pesticides on interspecific interactions such as predation on tadpoles by other native invertebrate and vertebrate predators.

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