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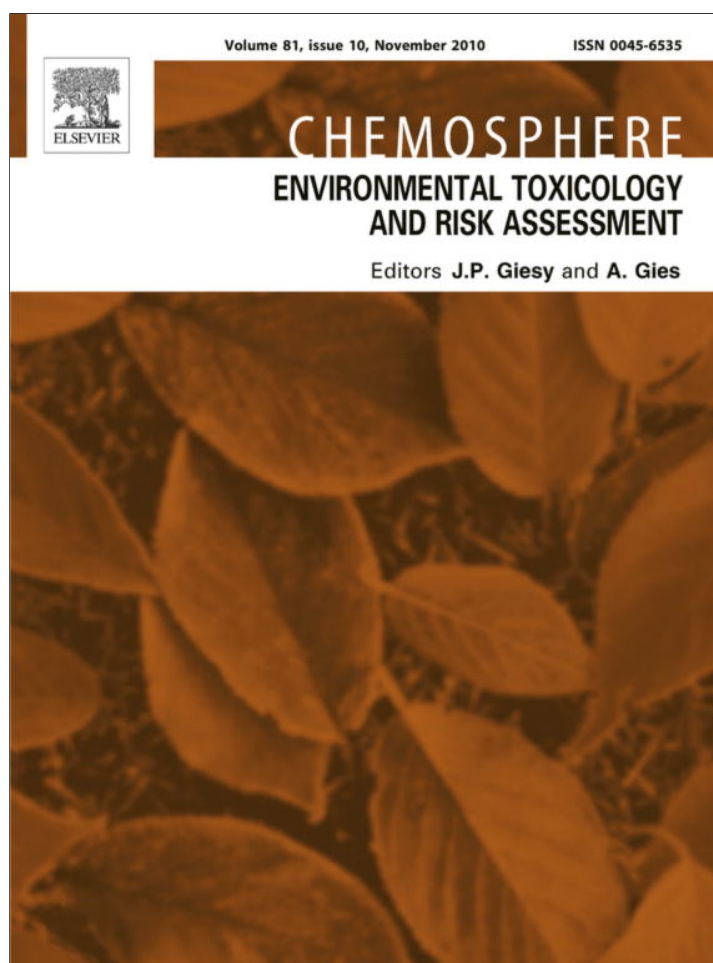


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Predator–prey interactions between *Synbranchus marmoratus* (Teleostei: Synbranchidae) and *Hypsiboas pulchellus* tadpoles (Amphibia: Hylidae): Importance of lateral line in nocturnal predation and effects of fenitrothion exposure

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ABSTRACT

Environmental contaminants can disrupt interactions between aquatic species by altering community structure. We explored predator–prey interactions between marbled swamp juvenile eels (*Synbranchus marmoratus*; predator) and anuran tadpoles (*Hypsiboas pulchellus*; prey) in relation to two aspects: the importance of lateral line in the predator and whether the absence of light modifies predation rates; and the effect of a sub-lethal concentration of fenitrothion on both predator and prey. Eels were tested under two sensory conditions (lateral line intact and lateral line blocked by cobalt chloride) in dark conditions. Predation rates were evaluated using different treatments that combined predator and prey exposed or not to insecticide. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were also measured in muscle samples of eels and tadpoles to explore whether fenitrothion affects predator and prey differentially. Marbled swamp eels were more efficient in feeding on tadpoles during the night than during the day, showing that lateral line makes an important contribution to prey detection and capture. Regarding pesticide effects, short-term (6 h) exposure to an ecologically relevant fenitrothion dose of 2.5 mg L⁻¹ altered the predator–prey relationship by changing prey behaviour, reducing prey detection and therefore increasing tadpole survival. At this concentration, the outcome of the predator–prey relationship appears biased in favor of the exposed tadpoles, which were released from predation risk, despite their altered behaviour and the higher inhibition percentages of tail BChE (70%) and AChE (51%) than in control individuals. Our study involving these model species and agrochemicals demonstrates that fenitrothion affected the outcome of a predator–prey relationship. Further studies are needed, in these species and other native amphibians, to investigate the nature of the mechanisms responsible for the adverse effects of pesticides on antipredator behaviour and predation efficiency.

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1. Introduction

Sub-lethal concentrations of agrochemicals, both synthetic fertilizers and pesticides, can disrupt interactions between species, and the ecological functioning of communities may be compromised before the effects can be detected at species level (Mann et al., 2009). To understand the effects of agrochemicals on amphibians and their influence on interactions such as predator–prey

relationships in aquatic systems, the direct toxicity of an agrochemical on predator and prey should be studied simultaneously, mimicking natural conditions (Rohr and Crumrine, 2005; Relyea, 2009). In this context, Boone et al. (2007) investigated the role of multiple stressors on amphibian decline and found that the predator (bluegill fish) and the presence of a carbamate (carbaryl) and ammonium nitrate fertilizer in mesocosms affect survival and abundance of anuran tadpoles. A recent review (Kerby et al., 2010) argues that amphibians might not be described as “canaries in a coal mine”, based on the fact that amphibians are not always the first indicators of an environmental impact, pointing out that the primary impacts of contaminants on the decline of these vertebrates are from indirect effects (Relyea et al., 2005; Relyea and Diecks, 2008). Indeed, little is

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known about the indirect effects of organophosphate (OP) pesticides on predator (fish)–prey (amphibians) relationships.

In the present work, we selected two model organisms for the analysis of predator–prey interactions (the prey anuran tadpoles: *Hypsiboas pulchellus*, and the predator marbled swamp juvenile eel: *Synbranchus marmoratus*). Because they are natural inhabitants of the same aquatic systems (Ringuelet, 1975), marbled swamp eel is considered a potential tadpole predator. This species uses the lateral lines (a mechanosensory system distributed on the skin surface and in fluid-filled dermal canals on the head and body of all fish species) to detect and capture prey (Liao and Chang, 2003; Pohlmann et al., 2004) in absence of light (at night, in turbid water, or in highly vegetated habitats). Because little is known about strategies and senses that aquatic predators use to detect and locate their prey in temperate aquatic systems where vision is often limited, we checked the importance of lateral line in nocturnal predation by eels. In addition, we determined predation rates in anuran tadpoles of *H. pulchellus* by juveniles of marbled swamp eels exposed to a sub-lethal concentration of fenitrothion. Because predation rate may differ over a 24-h period according to the feeding behaviour of the predator, predation trials were performed during day and night. Fenitrothion is an insecticide widely used in agriculture (IPCS, 1992), but whose sub-lethal toxicity has been poorly studied in amphibians. This OP inhibits the activity of cholinesterases (ChEs), specific biomarkers for assessing exposure of wildlife to OP and carbamate pesticides (Sánchez-Hernández, 2001; Lajmanovich et al., 2004, 2009). We also examined whether a single dose of fenitrothion affected predator and prey differentially by measuring acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in predator and prey.

2. Materials and methods

2.1. Species selection

H. pulchellus (Duméril and Bibron, 1841) is a common arboreal frog categorized as stable (Kwet et al., 2004) with an extensive distribution in the Neotropical region (IUCN, 2005). It is frequently found in both natural and altered lentic water bodies of agricultural and urban areas, with marginal vegetation composed of small shrubs and riparian trees, interspersed with assemblages of Poaceae, Polygonaceae, and Cyperaceae (Peltzer and Lajmanovich, 2004; Peltzer et al., 2006).

S. marmoratus (Bloch, 1795) is a fish widely distributed from Mexico to northern 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). It is frequently found in mud caves of rivers, ponds, swamps, marshy areas, drains, rice fields, and waters poor in oxygen (Graham, 1981).

The common aquatic fern *Salvinia herzogii* (incorporated in all the experiments to provide refuge, mimicking natural conditions) and eight egg clutches of *H. pulchellus* were collected from a semi-permanent pond at the University Ecological Reserve in Santa Fe city (Santa Fe Province, Argentina, 31°38'26"S, 60°40'22"W). Clutches were mixed before use to homogenize genetic variation. Marbled swamp eel juveniles ($n = 38$; total length = 21.99 ± 0.078 cm, weight = 16.42 ± 0.07 g) were collected from an unpolluted temporary pond in the floodplain of the Paraná River (Santa Fe Province, Argentina; 31°42'34"S; 60°34'16"W).

Eels and eggs were transported to laboratory and maintained in separate 10-L glass aquaria filled with dechlorinated water under natural photoperiod 12L:12D (Light:Dark) at 25 ± 1 °C. The eggs were allowed to develop until tadpoles reached Gosner stage 32 (Gosner, 1960). Tadpoles ($n = 456$; total length = 2.69 ± 0.06 cm, weight = 0.023 ± 0.008 g) were fed boiled lettuce until the start of

the experiment; eels were fed non-experimental *H. pulchellus* tadpoles.

2.2. Experimental design

2.2.1. Importance of mechanoreception during nocturnal predation

Heavy metals, such as Co^{2+} , have been shown to block the mechano-sensitivity of the lateral line in fish (Baumann and Roth, 1986; Karlsen and Sand, 1987). This effect of Co^{2+} is completely reversible and is also antagonized by Ca^{2+} . In our experiments, the lateral line system of eels was chemically blocked by exposure to cobalt hexachloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) (CAS No. 6551) obtained from Anedra S.A.[®], Argentina, using a procedure adapted by Karlsen and Sand (1987). Marbled swamp eels ($n = 3$) were immersed in a 0.1 mM, calcium-free CoCl_2 solution (adjusted with 0.5 N NaOH to produce a final pH of 7.2) for 6 h. Control marbled swamp eels ($n = 3$) without CoCl_2 were also treated under the same test conditions. Test solutions were prepared immediately before each experiment. After 6 h of exposure to CoCl_2 , eels were transferred to rectangular plastic containers containing 6 L clean water to determine nocturnal predation rates. In each tank, we added a marbled swamp eel individual ($n = 1$), tadpoles ($n = 20$), and aquatic ferns ($n = 3$). The assays were carried out in triplicate. To differentiate the function of mechanoreceptors for nocturnal feeding, we compared the nocturnal predation rate of lateral line-blocked eels on tadpoles with that of untreated eels. After 12-h of darkness, we removed the marbled swamp eel and counted the number of surviving tadpoles.

2.2.2. Effect of fenitrothion on marbled swamp eel–tadpole interactions and analysis of enzymatic activity

Exposure to fenitrothion [O,O-Dimethyl O-(3-methyl-4 nitrophenyl) phosphorothioate] was purchased from Agroparque S.R.L. (Argentina). It is a commercial product, trade name “Hormigal-L” (CAS No. 122-14-5 commercial grade) containing 10% (w/v). There was no need to use organic solvent because fenitrothion is readily soluble in water. Hashimoto and Nishiuchi (1981) demonstrated that fenitrothion concentration (LC_{50}) was lethal to anuran tadpoles (*Bufo bufo japonicus*, *Rana brevipoda*, and *R. catesbeiana*) at a dose between 1.2 and 15 mg L^{-1} . Although the concentration used in our experiment (2.5 mg L^{-1}) falls within the lethal LC_{50} values reported by Hashimoto and Nishiuchi (1981), we considered that this value reflects the concentration commonly observed in nature, particularly in surface waters adjacent to treated fields (Ernst et al., 1991). Indeed, in preliminary experiments (data not published) we determined the sub-lethal effects of 2.5 mg L^{-1} of fenitrothion on *H. pulchellus* tadpoles. Likewise, Berrill et al. (1994) observed abnormal swimming behaviour, paralysis, reduced growth, and retarded development in larval amphibians (*Rana pipiens*, *R. clamitans*, *R. catesbeiana*) exposed to 0.2–5.5 mg L^{-1} fenitrothion under laboratory conditions. To our knowledge, there are no data about fenitrothion lethal toxicity on marbled swamp eel.

Both eels and tadpoles were exposed to either fenitrothion or a clean water solution. For fenitrothion exposure, a sub-sample of marbled swamp eel juveniles ($n = 16$) and *H. pulchellus* tadpoles ($n = 168$) was taken from the stock animals and exposed to 2.5 mg L^{-1} of fenitrothion for 6 h. At the same time, another sub-sample ($n = 16$ eels and $n = 168$ tadpoles) was kept in dechlorinated water as control. Neither fish nor tadpoles were fed during exposure. After 6 h of exposure, eels and tadpoles were transferred to aquaria containing pesticide-free water. Then, 16 eels ($n = 8$ exposed and $n = 8$ control) and 16 tadpoles ($n = 8$ exposed and $n = 8$ control) were randomly selected and euthanized (ASIH, 2004; Nickum and Bart, 2004). Muscle samples were immediately excised, weighed, and frozen until analysis of AChE and BChE activity.

The remaining eels and tadpoles were retrieved from their aquaria and placed in plastic containers (40 cm in length, 26 cm in width, and 12 cm in height) to determine predation rates.

Predation rate experiments. We estimated the predation rate of eels (E) on tadpoles (T) exposed to fenitrothion (+) and not exposed to fenitrothion (–) using four treatments: (1) neither eels nor tadpoles were exposed (E–,T–), (2) both eels and tadpoles were exposed (E+,T+), and (3, 4) either eels or tadpoles were exposed (E–,T+ and E+,T–, respectively). The experiments were carried out during the day (7:00–19:00, at light hours) and at night (19:00–7:00, dark hours). Groups of 20 tadpoles (exposed or not to fenitrothion, depending on the treatment) were introduced into a set of 16 plastic test aquaria, each containing 6 L of dechlorinated water. After that the corresponding (exposed or not) juveniles of marbled swamp eel were incorporated into the aquaria. Three aquatic ferns were placed in each aquarium to provide animals with refuge, simulating natural conditions. Four replicates of each treatment were performed, and tadpoles and marbled swamp eels were used only once. The number of surviving tadpoles was recorded at 12 and 24 h after the beginning of the experiments.

Enzymatic activity. After thawing, muscle samples were homogenized in phosphate buffer (0.1 M, pH 8.0) using a polytron over ice and the extracts were centrifuged at 9000 rpm for 10 min. Enzymatic analyses were carried out at room temperature (25 °C) and assayed on the supernatant according to Ellman's method (Ellman et al., 1961). The reaction medium consisted of 25 μ L of muscle homogenate, 915 μ L 25 mM Tris-HCl, 1 mM CaCl_2 (pH = 7.6), 50 μ L 2 mM DTNB (3×10^{-4} M, final concentration), and 10 μ L butyrylthiocholine iodide (BuSCh) and acetylthiocholine iodide (AcSCh) (2×10^{-3} M, final concentration). The substrates, acetylthiocholine (ASCh) and butyrylthiocholine iodide (BSCh), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), Tris-HCl, and CaCl_2 were purchased from Sigma–Aldrich (Germany). Changes in absorbance were recorded at a wavelength of 412 nm for 1 min using a JENWAY UV-VIS spectrophotometer model 6405. Activities were standardized against total protein, which was measured according to the Biuret method described by Kingsley (1942) using albumin as a standard, and expressed as $\text{nmol min}^{-1} \text{mg}^{-1}$ protein. All assays were performed in triplicate on each homogenate and mean values and standard error (SE) were calculated.

2.3. Data analysis

Predation rate was estimated as the number of tadpoles consumed by a marbled swamp eel individual/number of tadpoles available. Predation data were processed with a repeated measures analysis of variance (ANOVA) to determine the main effects of night and day cycles and fenitrothion over the 24-h experiment. Data were previously tested for normality (Shapiro–Wilk normality test) and homogeneity of variance (Levene's test) (Zar, 1996). Dunnett's post-hoc multiple comparison tests were then used to determine differences between treatments. Student's *t*-test was performed to compare differences between mean AChE and BChE activities of pesticide-exposed individuals and controls, and to determine differences in predation rates between eels exposed to CoCl_2 and control groups. Statistical analyses were performed with SPSS 17.0 software package. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Importance of mechanoreception during nocturnal predation

During the period of exposure (6 h) to CoCl_2 , no signs of general behavioural disorders (production of mucus, altered swimming, increased activity, or death) were observed in exposed marbled

swamp eels compared with control ones. The lowest predation rate was recorded in the treatment involving lateral line-blocked eels; the value was significantly different from that of untreated eels ($t = 12.01$; $p = 0.0003$; $df = 4$) (Fig. 1).

3.2. Effect of fenitrothion on marbled swamp eel–tadpole interactions and analysis of enzymatic activity

Exposure to fenitrothion. Survival of tadpoles and marbled swamp eels was 100% in the control treatments and after 6-h exposure to a sub-lethal fenitrothion concentration (2.5 mg L^{-1}). However, tadpoles showed reduced swimming performance, while the exposed marbled swamp eels arched their bodies and produced a notable amount of mucus compared to control eels. These effects disappeared when both species were transferred to a container filled with clean water.

Predation rate experiments. Significant differences among treatments were found after 24 h of predation ($F = 4.17$; $p = 0.031$). The Dunnett's post-hoc multiple comparisons test showed significant differences in predation rates between control (E–,T–) and fenitrothion-exposed (E+,T+) groups ($p = 0.018$) (Fig. 2). The maximum predation rate was recorded in the control treatment (E–,T–), whereas the lowest value was detected when eels and tadpoles were simultaneously exposed to fenitrothion (E+,T+) (Fig. 2).

Predation rate was statistically significant between nocturnal and diurnal cycles ($F = 9.372$; $p = 0.005$). While 19 ± 6 tadpoles (\pm SD) were preyed upon at night, only 7 ± 3 tadpoles were consumed during the day. During night hours there were significant differences between treatments ($F = 5.221$; $p = 0.015$). (Fig. 3) The Dunnett's post-hoc multiple comparisons test showed significant differences in predation rates between control (E–,T–) and fenitrothion-exposed (E+,T+) groups ($p = 0.011$). However, differences between treatments were not statistically significant during the day ($F = 1.615$; $p = 0.238$) (Fig. 3).

Enzymatic activity. Sub-lethal exposure of tadpoles to 2.5 mg L^{-1} of fenitrothion for 6 h inhibited BChE and AChE activities (Table 1). In tadpoles, we observed a 70% and 51% decline in tail BChE ($t = 3.515$, $p = 0.0043$, $df = 12$) and AChE ($t = 3.538$; $p = 0.0041$; $df = 12$) activity, respectively, compared with controls. However, no significant differences in muscle BChE ($t = 1.515$; $p = 0.0820$;

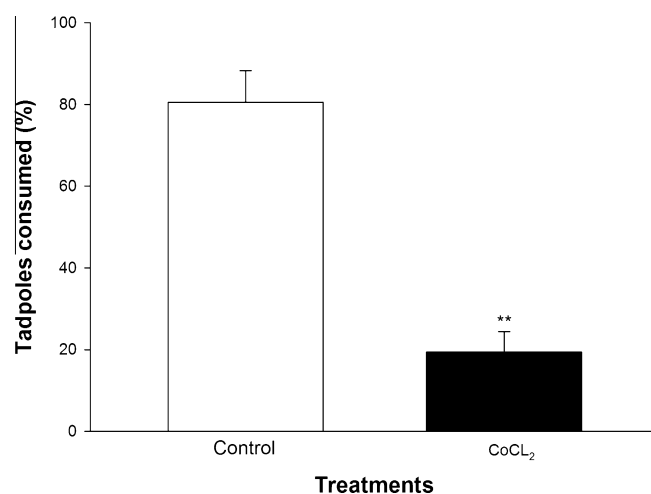


Fig. 1. Percentage of *H. pulchellus* tadpoles consumed (number of tadpoles consumed/number of tadpoles available) by an eel individual with lateral line blocked and control individuals. Vertical bars represent ± 1 standard error. **Asterisks show significant differences (Student's *t*-test; $p < 0.01$).

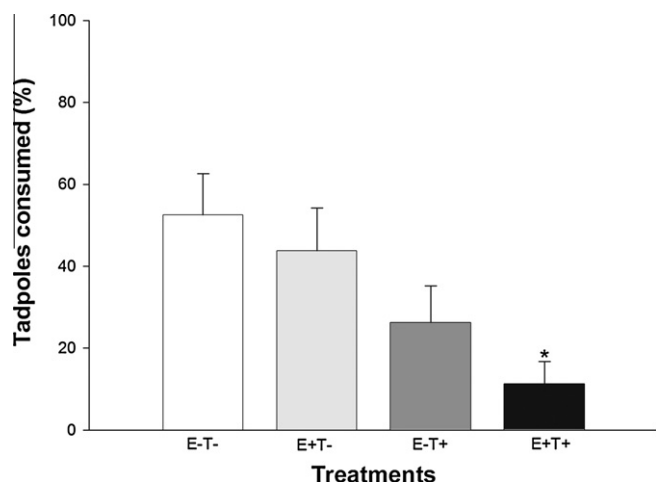


Fig. 2. Total percentage of *H. pulchellus* tadpoles preyed upon by *S. marmoratus* in each treatment. (T = tadpole, E = eel; (+) = exposed to fenitrothion, (–) = not exposed to fenitrothion). Vertical bars represent ± 1 standard error. *Asterisk shows significant differences between treatments based on Dunnett's post-hoc multiple comparisons test; $p < 0.05$.

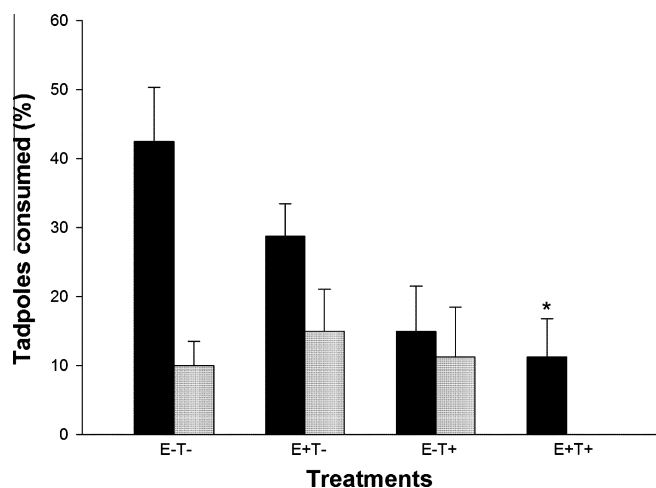


Fig. 3. Percentage of tadpoles consumed during nocturnal (■) and diurnal (□) cycles (in different treatments (T = tadpole, E = eel; (+) = exposed to fenitrothion, (–) = not exposed to fenitrothion). Vertical bars represent ± 1 standard error. *Asterisk shows significant differences between treatments based on Dunnett's post-hoc multiple comparisons test; $p < 0.05$.

Table 1
BChE and AChE activities of *Synbranchus marmoratus* and *Hypsiboas pulchellus* tadpoles after fenitrothion exposure.

Species		N	BChE activity ^a Mean \pm SE	AChE activity ^a Mean \pm SE
<i>S. marmoratus</i>	Control	8	101.5 \pm 10.7	202.4 \pm 63.5
	Exposed	8	70.7 \pm 13.5 ^{NS}	100.4 \pm 24.6 ^{NS}
<i>H. pulchellus</i>	Control	8	4.09 \pm 0.58	41.2 \pm 4.2
	Exposed	8	1.22 \pm 0.20*	20.2 \pm 5.3*

N samples analysed, SE standard error.

^a Expressed as $\text{nmol min}^{-1} \text{mg}^{-1}$ protein of muscle.

* Activities significantly different between exposed and control values were identified by Student's *t*-test (* $p < 0.05$, NS not significant).

df = 12) or AChE ($t = 1.217$; $p = 0.2491$; df = 12) activity were detected between eels exposed to the pesticide and controls.

4. Discussion

The effects of environmental contaminants on aquatic communities are a matter of increasing concern due to their potential influence on predator–prey relationships and community structure (Boone and James, 2003; Relyea, 2005; Relyea and Hoverman, 2008). Pesticides may have positive (Cooke, 1971; Raimondo et al., 1998; Broomhall, 2004), negative (Boone and Semlitsch, 2003; Mills and Semlitsch, 2004), or no impact (Bridges, 1999) on predator–prey interactions, depending on the sensitivity of species to contaminant exposure. The results of our laboratory experiments suggest that the outcome of the predator–prey relationship appears biased in favor of the exposed tadpoles. Fenitrothion induced changes in prey behaviour, decreasing prey–predator encounters and therefore reducing predation rate between tadpoles of *H. pulchellus* (prey) and eels (*S. marmoratus*). Relyea and Hoverman (2006) found that pesticides can have positive effects on prey survival by modifying their activity in the presence of predators. The decrease in predation rate we recorded in tadpoles exposed to fenitrothion, indicates that this chemical made tadpoles less active and, therefore, less conspicuous to predators. Although we did not quantify level of activity immediately after the 6-h exposure to pesticide, our results indicate that predation rates were lower when predator and prey were exposed simultaneously and when only prey were exposed, than in the remaining treatments. Conversely, when neither prey nor predator was exposed, predation rates increased, both during the day and night, probably because the movement generated by the great activity of unexposed tadpoles attracts the attention of predators (Werner and Anholt, 1993; Skelly, 1994; Teplitsky et al., 2003). Fenitrothion contributed to a decrease in predatory encounters of *H. pulchellus* tadpoles, thus reducing predation rate.

Predation rate of marbled swamp eel juveniles was higher at night than during the day. This result suggests that tadpoles are vulnerable to nocturnal predation, especially in the presence of predators that are able to detect prey by their movement, such as eels. Previous studies have shown that in complete darkness fish use their lateral line system for detecting prey (Montgomery et al., 1995; Kanter and Coombs, 2003), but no information is available on lateral line-mediated prey detection in eels. In our study, eels with a blocked lateral line system could not catch prey as they usually do, although olfaction was left intact. Moreover, inactivation of the lateral line eliminates the feeding response to live prey (Hoekstra and Janssen, 1985; Janssen, 2000). Even though in nature there is little chance that the lateral line system can be blocked, the chance for a fish experiencing darkness or great turbidity levels is high. Therefore, when the marbled swamp eel feeds at night, non-visual senses such as the lateral line system make an important contribution to prey detection and capture (Liao and Chang, 2003), because predators detect swimming vibrations of prey using mechanoreceptors (Pastorok, 1981). Under daylight conditions, the vision of eels plays the main role in prey detection, because they detect tadpoles through movement. However, with low tadpole activity and a complex habitat structure, prey visibility may decrease (Babbitt and Jordan, 1996; Babbitt and Tanner, 1997; Denton and Beebe, 1997; Baber and Babbitt, 2004). Additional studies on synergistic effects between agrochemicals and potential blocking compounds commonly found in nature are necessary for a better understanding of predator–prey interaction.

As noted above, sub-lethal levels of pesticides can change an individual's behaviour, altering predator–prey dynamics. Also, Clements et al. (1989) showed that the effects of toxicants on predation are more likely to be detected if the behaviour of one species is differentially affected. For example, Ingermann et al. (2002) demonstrated that when contaminants modify prey defences but not predator feeding behaviour, they can enhance prey vulnerability.

In our experiment, the *H. pulchellus* tadpoles were more sensitive to fenitrothion effects than its predator, the marbled swamp eel. We hypothesize that water permeability of the skin of tadpoles and their smaller body size (tadpoles are seven times smaller than eels) contribute to the sensitivity of this organism. While eels are able to tolerate higher fenitrothion concentrations than tadpoles, exposed eels arched their bodies and had greater mucus production compared to control eels. Although we did not quantify these responses, other studies have reported muscle twitches but not mucus production in the European eel (*Anguilla anguilla*) exposed to sub-lethal fenitrothion doses (Sancho et al., 1998). Arora and Kulshrestha (1984), however, described copious mucus secretion in fish (*Channa punctatus*) exposed to carbaryl and phenthoate. Those authors postulated that mucus secretion is regarded as a defence response in protecting vital organs, like gills against pesticide toxicity. Similar observations have been made in studies on fish exposure to pesticides (Jiraungkoorskul et al., 2003; Ortiz et al., 2003). The mucus secreted by the marbled swamp eel would therefore play an important role as a defence mechanism against exposure to fenitrothion insecticide.

Traditional ecotoxicological investigations have focused on measuring impacts of anticholinesterase pesticides by using single-species toxicity tests (i.e., mortality rates). Few studies determined the impacts of pesticides on enzymatic activity in predator and prey. Our results demonstrate that at the fenitrothion concentration tested inhibition of BChE and AChE in prey (tadpoles) was higher (70% and 51%, respectively) than that in the predator (marbled swamp eel), showing a 30% and 50% inhibition, respectively. This finding provides further evidence that the prey was more sensitive to fenitrothion exposure than its predator, and that AChE activity is important for many physiological functions of marbled swamp eels (such as prey location and orientation toward food). Indeed, when AChE activity decreases acetylcholine is not broken and accumulates within synapses, which therefore cannot function normally (Dutta and Arends, 2003).

Although the physiological role of BChE remains uncertain, it appears to have a protective function by sequestering circulating organophosphate compounds and decreasing the toxic effect of these compounds on AChE (Allon et al., 1998). Recently, Stefanidou et al. (2009) suggested that BChE is the most appropriate tool for assessing OP poisoning or simple exposure to OP, especially at sub-acute levels. Sparling et al. (1997) reported that amphibian plasma BChE appears more sensitive to pesticide exposure than brain AChE. The present results not only reinforce this assumption in tadpoles but also demonstrate that both cholinesterases showed similar trends in the muscle.

To conclude, the results of our study clearly show that short-term (6 h) exposure to an ecologically relevant fenitrothion dose can alter the predator–prey relationship between marbled swamp eel juveniles and *H. pulchellus* tadpoles, by affecting both prey behaviour and predation rate. At this concentration the outcome of the predator–prey relationship appears biased in favor of the exposed tadpoles, which were released from predation risk despite their altered behaviour. Further studies in these species and other native amphibians are needed to investigate the nature of the mechanisms responsible for the adverse effects of pesticides, such as fenitrothion, on antipredator behaviour and predation efficiency.

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