Chemosphere 81 (2010) 1233–1238

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

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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ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form 22 August 2010
Accepted 14 September 2010

Keywords:
Tadpoles
Marbled swamp eel
Lateral line
Predation rate
Butyrylcholinesterase
Acetylcholinesterase

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.

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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doi:10.1016/j.chemosphere.2010.09.035

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 ($CoCl_2{\cdot}6H_2O$) (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. 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. catesbiana)* 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.