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Effect of exposure to contaminated pond sediments on survival, development, and enzyme and blood biomarkers in veined treefrog (*Trachycephalus typhonius*) tadpoles

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ABSTRACT

Sediments are important elements of aquatic ecosystems and in general sediments accumulate diverse toxic substances. Amphibians potentially have a greater risk of exposure to contaminants in sediments, and the test of sediments provides first lines of evidences. Sediment outdoor microcosm experiments were conducted to analyze biological endpoints (survival, development, growth, and morphological and organ malformation), enzyme activity (butyrylcholinesterase, BChE; glutathione-S-transferase, GST; and catalase, CAT) and blood biomarkers in veined treefrog *Trachycephalus typhonius* tadpoles, a widespread neotropical species. Hatching (stage 23) of *T. thyphonius* was exposed until they reached metamorphosis (stage 46). Sediment tests were performed and four different treatments were used: three ponds (LTPA, ISP, and SSP) influenced by industrial and agricultural activities and a reference treatment from a forest (RFS). Physical and chemical variables and concentration of nutrients, pesticide residues, and metals were determined. One treatment was metal-rich (LPTA) and two were nutrient-rich (ISP and SSP). Sediment treatments had no significant effect on survival; in contrast they had significant sublethal effects on *T. typhonius* larval development and growth rates, and affected overall size and shape at stage 38. Principally, in LPTA animals were significantly larger than in RFS, exhibiting swollen bodies, tail muscles and tail fin. In addition, metamorphs from LPTA, ISP, and SSP were smaller and showed signs of emaciation by the end of the experiment. Statistical comparisons showed that the proportions of each type of morphological abnormalities (swollen bodies and diamond shape, gut uncoiling, diverted gut, stiff tails, polydactyly, and visceral and hindlimb hemorrhaging) were significantly greater in metal- and nutrient-rich sediment treatments. Moreover, activities of BChE, GST and CAT, as well as and presence of micronuclei, immature, mitotic, anucleated erythrocytes varied significantly among treatments. Our biological effects-based sediment study highlights the use of different biological endpoints and biomarkers on anuran larvae at sites where pond sediment is risky and sediment management should be considered. Finally, the information of those biological endpoints and biomarkers would be useful as a management tool to decide if there are sufficient exposures of tadpoles to suspected pollutants on sediment.

1. Introduction

Sediments represent important elements of aquatic ecosystems, playing a variety of roles in maintaining their structure and function. Despite of sediment importance, worldwide available information on their quality conditions indicates that sediments are contaminated by diverse toxic and bioaccumulative substances (Millennium Ecosystem Assessment, 2005). Although certain chemicals are highly sorbed to sediment, these compounds may still be available to the biota (Chapman et al., 2002). In this sense, sediment toxicity test can be considered as first lines of evidences in ecological risk assessments (ERA) and it is used to explore whether chemical contaminants in sediment are risky to aquatic organisms (US-EPA, 1996a).

Outdoor experiments are one method of directly assessing sediment toxicity that can be replicated and manipulated (US-EPA, 1996a),

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yielding similar results to large farm ponds and where environment conditions approximate to natural systems (Boone et al., 2007). In addition, sediment quality triad approach consisting in combine biological, chemical, and toxicological data has become more usually used in sediment quality assessment (Chapman et al., 2002).

Many key contaminants, such as nutrients, metals, and agrochemicals presented in aquatic systems have lethal toxic effects (mortality) and a number of sublethal effects (malformation, behavioral shifts, timing of development and metamorphosis) on amphibians (Rowe et al., 2002; McDaniel et al., 2004; Metts et al., 2012). Thus, studies that focus on how contaminants present in water bodies and associated sediments affect health of amphibian larvae provide crucial information to their conservation (Ficken and Byrne, 2012).

Moreover, since these contaminants are frequently present in complex mixtures, efforts of ecotoxicologists have been focused toward identification on effects or specific biomarker of exposure on sentinel species (Monferrán et al., 2011; Ossana et al., 2013). Recently, the use of non-lethal biological techniques such as the analyses of enzyme and blood biomarkers have been gained attention due to their unquestionable value as early signals of the adverse effects of exposure to chemical contamination on fishes, reptiles, birds (for example, Barata et al. 2010; Bassó et al., 2012; Costa et al., 2011) and amphibians (Attademo et al., 2011; Ossana et al., 2013). Hence, modifications in biological endpoints and enzymatic and blood biomarkers as integrated responses of multiple changes occurred in the test organisms turn into a consistent warning sign of the level of modification of a given environment, and has been considered a priority in characterization environmental risk for amphibian larvae (Lajmanovich et al., 2010; Da Rocha, 2011).

The goal of this study was to determine and characterize the toxicity of pond sediment in anthropogenic areas using the veined frog (*Trachycephalus typhonius*) tadpole as bioindicator species in the mid-western Entre Ríos province in Argentina. Biological responses were determined based on the evaluation of survival, development endpoints and a multiple biomarker approach (butyrylcholinesterase, glutathione-S-transferase and catalase activities, useful indicators of exposition to pesticides and metals and alteration of erythrocytes, recognizing for it use as cytotoxicity and genotoxicity, Costa et al., 2011). Due to the spread of contaminants mainly by agricultural, urban, and industrial discharges and runoff from uplands on aquatic environments of the mid-eastern region of Argentina (SADSN, 2013), examining sediment toxicity based on the biological effects-based assessment provide useful information to estimate potential ecological risks for wildlife (den Besten et al., 2003), and would be helpful for allocating scarce resources for environmental assessments. In this region few studies demonstrated biological negative effects on invertebrates (Pavé and Marchese, 2005) and vertebrates (Peltzer et al., 2008) by different nutrients, agrochemicals and metals presented on water and sediment of several aquatic systems.

2. Materials and methods

2.1. Study area and sediment sampling sites

Sediment toxicity of different ponds from the mid-western Entre Ríos province of Argentina was investigated. The climate in this region is temperate, with a mean annual temperature of 18.5 °C and mean annual precipitation of 995 mm. The landscape is highly exploited by humans, and is dominated by the presence of greater urban areas, agriculture and industries. In the last decades, forests have been mainly modified by large-scale production of genetically modified (GM) Roundup Ready (RR) soybeans and, to a lesser extent, corn, rice and sorghum (Aizen et al., 2009). Moreover, industrial activities in the region include petroleum refinery, iron and steel manufacturing, paper pulp mills, foodstuff, car batteries, leather and textile productions, affecting the quality of bottom sediments of lentic and lotic aquatic environments (Fiorenza, 2009). In this region is demonstrated that amphibian population's dependent on ponds imbedded within or around anthropogenic areas for their survival, development and reproduction (Peltzer et al., 2003).

Four ponds were selected: three ponds that differed in runoff origin were categorized as contaminated ponds, and one pond was considered the reference site (Fig. 1). (1) Reference site (RFS, 31°44′35.04″S; 60°20′5.15″W) is situated in "Parque Urquiza", on the ravines of Paraná River and it is far from industrial and agricultural areas (more than 10 km). (2) Inside soybean field pond (ISP, 31°42′44.83′′S; 60°32′50.25″W): A temporal pond located in a depression inside a soybean field. (3) Surrounded soybean pond (SSP, 31°43′50.81″S; 60°28′2.42″W): A pond surrounded by a soybean field and a two-lane highway (Acceso Norte). (4) Las Tunas pond (LTPA 31°44′30.37″S; 60°25′39.47″W): a pond close to the industrial zone and soybean fields. The sites named as contaminated ponds represented potential habitats for the selected species for the present study *T. typhonius* that is, sites in which concentrations of contaminants were recorded in previous analysis (Pavé and Marchese, 2005; Peltzer inedited data) and native anuran species (*Rhinella fernandezae, Scinax nasicus* and *Hypsiboas pulchellus*) use these ponds to reproduce and survive (Peltzer et al., 2003). In these sites, alterations on developments and growths of amphibian tadpoles, decrease of their health status, and mortalities, and malformations in adults were also observed (Peltzer et al., 2008, 2011).

Sediments (approximately 4–5 kg) were collected from each site from the top 10-cm stratum with a pointed shovel and stored in a portable refrigerator until the start of the experiment. Sediments from all the sites were sampled in summer (27 January 2011), the season with greatest precipitation and greatest amount of nutrients and chemical contaminants present in water and sediment runoff from uplands converging in ponds.

2.2. Experimental design

The effect of sediment treatment on biological responses was examined in outdoor microcosms during January–April 2011, and they were located at Facultad de Bioquímica y Ciencias Biológicas (FBCB) of Universidad Nacional del Litoral (UNL). Microcosms consisted of plastic 30-l enclosures (37 cm long × 20 cm wide × 25 cm deep) covered with iron frames fitted with 2 × 2-mm mesh to avoid predation or oviposition by insects or other anuran species colonists.

2.2.1. Species selected

Six egg surface films of veined treefrog *T. typhonius* (Anura, Hylidae) were collected from a temporal, fishless pond located in the Paraná River floodplain (31°44′36″S; 60°19′40″W) on 27 January 2011. This anuran is distributed from southern Mexico to the north of Argentina, and it is frequently found in a number of habitat types comprising vegetated areas, wetlands, agricultural land, and urban areas (Lavilla et al., 2000). The eggs were mixed and held them and larvae at environmental temperature (24–26 °C), and light > 200 lx for 48 h, after absorption of their yolk sac.

2.2.2. Sediment test

Sediment tests followed the sediment exposure protocols for amphibians (US-EPA, 1996a; NAVFAC, 2004; ASTM, 2007). Before using them, sediment samples were ground and wet-sieved to remove extraneous organic matter (e.g. grass, roots), and fine mixture of silt, loam and clay particles for maximum particulate suspension were obtained.

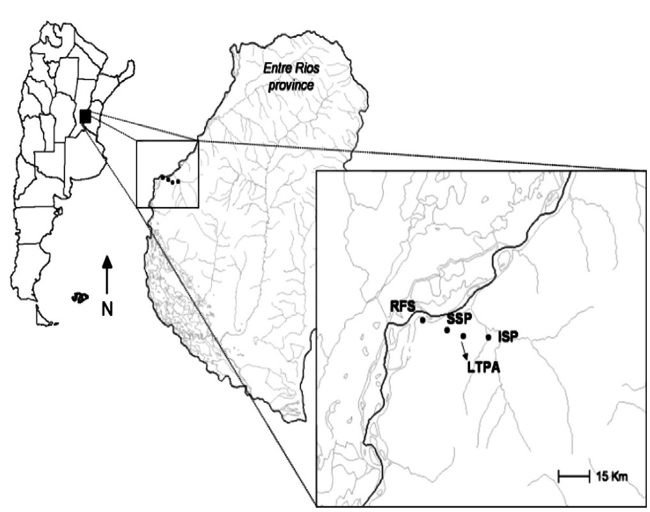

Fig. 1. Location of sampled sites from the mid-western Entre Ríos province, Argentina.