Available online at www.sciencedirect.com
ScienceDirect
Ecotoxicology and Environmental Safety 70 (2008) 185–197
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles☆

Paola M. Peltzer[a,b,*], Rafael C. Lajmanovich[a,b], Juan C. Sánchez-Hernandez[c], Mariana C. Cabagna[b], Andrés M. Attademo[a,b], Agustín Bassó[b]

[a]*National Council for Scientific and Technical Research (CONICET), Argentina*
[b]*Faculty of Biochemistry and Biological Sciences-ESS-FBCB-UNL, Paraje el Pozo s/n, 3000 Santa Fe, Argentina*
[c]*Laboratory of Ecotoxicology, Department of Environmental Science, University of Castilla-La Mancha, Avda. Carlos III, 54071 Toledo, Spain*

Received 1 November 2006; received in revised form 27 May 2007; accepted 2 June 2007
Available online 20 July 2007

Abstract

To test the hypothesis that eutrophication ponds modulate some aspects of the health responses and survival of anuran tadpoles, we conducted field experiments using *Scinax nasicus* as sentinel organism to evaluate the quality of two ponds filled with agricultural runoff in a dominant agricultural landscape of Mid-Western Entre Ríos Province (Argentina). The survival, growth and development rates, erythrocytes nuclei aberrations, parasite infection, and brain cholinesterase activity were monitored after seven days of exposure. Water samples from the ponds were also analyzed for physico-chemical variables and levels of pesticide residues. Residues of organochlorine pesticides and nutrients were higher in the agricultural ponds with respect to those from the control pond. We suggest that the interactions among washed-off nutrients and pesticides from agriculture and environmental factors account for deleterious effects on *S. nasicus* survival, growth and development rate, thereby compromising their health status. These effects can lead, in turn, to an increase in tadpole vulnerability to opportunistic parasites, erythrocytes nuclei aberrations or hemolysis.

Keywords: Agroecosystems; Agricultural runoff; Amphibian; Eutrophication; Tadpoles; *Scinax nasicus*

1. Introduction

Amphibian populations from around the world have apparently declined or experienced severe range reductions (Houlahan et al., 2000; Stuart et al., 2004). Habitat loss, agricultural activities, unsustainable harvesting, increasing UV radiation, climate change, introduction of exotic species, emerging diseases, or a combination of these are among the hypothesized factors that are the most plausible hypothesis for amphibian population decline (e.g. Carey et al., 1999; Kiesecker, 2002; Johnson and Chase, 2004). The need for increasing the knowledge on toxic effects of pollutants on herpetofauna stressed by some authors (Lambert, 1997) has resulted in a surge of investigations about adverse effects to these vertebrates from pesticides and fertilizers applications (Wijer et al., 2003; Relyea et al., 2005). Amphibians that breed in ponds immersed or surrounded by agricultural fields are probably exposed to high levels of agrochemicals and, according to these data,

☆This study was supported by a SEED GRANT for the Declining Amphibian Population Task Force (DAPTF)-2005. Theme: *Population and health of common toads across agricultural lands: implication in worldwide decline*; and a project CAID + D-2005 (Litoral National University-UNL)-PI No. 019-120. Theme: *Estudio ambiental sobre los efectos de los agroecosistemas en poblaciones de anfibios anuros del Litoral fluvial argentino: monitoreo y conservación*.The paper conforms to the legal requirements of our country in which it was carried out, including those relating to conservation and animal welfare, and to the Journal's policy on these matters. Indeed, the investigation was conducted according to the *Guidelines for use of live amphibians and reptiles in field research* compiled by ASIH, HL, and SSAR (2001) and the state law "*Protection and Conservation of Wild Fauna*" (Argentina National Law No. 22.421). The regulations and permits have been obtained and were provided by the Director of the Natural Reserve "Parque General San Martín" of Entre Ríos Province, Argentina.

*Corresponding author. Faculty of Biochemistry and Biological Sciences-ESS-FBCB-UNL, Paraje el Pozo s/n, 3000 Santa Fe, Argentina. Fax: + 54 0342 4750394.
E-mail address: paolapeltzer@hotmail.com (P.M. Peltzer).

0147-6513/$ - see front matter
doi:10.1016/j.ecoenv.2007.06.005

they can suffer serious consequences at the population level.

Ponds situated within or around agricultural areas can receive washed-off nutrients and pesticides from surrounding agricultural lands. In the agroecosystems, concentrations of phosphorus and nitrogen generally excess those levels detected in non-agricultural areas (Hamer et al., 2004). An excess of nitrogen and phosphorous leads to eutrophication with a drastic decrease in dissolved oxygen and subsequent mortality of aerobic aquatic organisms (Mitsch and Gosselink, 2000). Because amphibians rely on pond to reproduce and spend the greater part of their developmental stages in this aquatic environment, high concentrations of these nutrients may affect local populations and community structure (Bishop et al., 1999; Knutson et al., 2004), reducing survivorship, altering the feeding and swimming activity, and decreasing the growth and development of amphibian larvae (e.g. Baker and Waights, 1994; Xu and Oldham, 1997; Marco and Blaustein, 1999). On the other hand, the studies by Johnson et al. (2002) and Johnson and Chase (2004) suggested that nutrient inputs increase the density of planorbid snails on ponds and therefore likely increase the intensity of the parasite infection and the frequency of malformations in amphibians.

In the Mid-Eastern Argentina, traditional agriculture was replaced by a more specialized agriculture aimed at large-scale production with glyphosate-resistant (GR) soybean (*Glycine max* (L.) Merr.) as the dominant crop. This has led to the increased potential for damage by pest and disease and a gradually decrease in the capability of soil to provide nutrients. These two consequences from an intensive agriculture based on a single crop-type need the continuous application of pesticides and fertilizers (CASAFE, 1999; Lajmanovich et al., 2005a). Indeed, this modern agriculture has led to the expansion of cultivated areas thus exerting an increasing pressure on non-agriculture portions of the land and wildlife (Peltzer et al., 2005). Forest habitats in the agricultural landscapes have been substantially reduced and fragmented into numerous small plots. Many of the aquatic habitats have severely altered and the amphibian populations are dependent on ponds imbedded within or around agricultural areas for their survival, development and reproduction (Peltzer et al., 2003, 2005). In this scenario, an effective management program for amphibian conservation in agricultural landscape requires an understanding of what factors influence the anuran amphibian populations (Knutson et al., 1999; Semlitsch, 2000). Amphibians have been extensively used in standardized toxicological tests for assessing the toxic effects from nutrients and pesticides (e.g. Watt and Jarvis, 1997; Bridges, 1999; Lajmanovich et al., 1998, 2003; Feng et al., 2004). However, there are a few field experiments that have provided solid data on the impact of eutrophication on amphibian survival and health.

To test the hypothesis that agricultural pond eutrophication modulates some aspects of health status and survival of anuran tadpoles, we conducted field experiments with *Scinax nasicus* (Anura, Hylidae) tadpoles caged in agricultural ponds from the Mid-Western Entre Ríos Province. Biological endpoints include tadpole survival, development and growth rates, erythrocytes micronuclei aberrations, parasite infection, and measurements of biomarkers of pesticide exposure (brain acetylcholinesterase activity). Physico-chemical parameters and concentrations of pesticide residues of water were also determined. This work provides a basic scheme from which different but related biological parameters may be used as endpoints for monitoring the health status of anuran that live in agricultural landscape.

2. Materials and methods

2.1. Study area

The study area was located in the Mid-Western Entre Ríos Province, Argentina (31°44′S, 60°31′W) (Fig. 1), and encompassed the Espinal and Delta-Islas Eco-regions. The area belongs to the pluvial district of Argentina, with rainfall evenly distributed across the spring and summer seasons and a mean annual temperature of 18 °C. Agriculture is the predominant land use in this region, covering more than 60% of its 2500 ha. Most of the agricultural land is devoted to soy (40%), corn, and other cereal production (17%) (Peltzer et al., 2006).

2.2. Field survey

Experimental ponds were located using Landsat 7 (+ETM) images and serial aerial photographs (first order to fifth order at a scale of 1:50.000-Brigada Aérea Argentina at Paraná City of Entre Ríos Province). Three ponds were chosen for the mesocosm study: one is located within a native forest (Control site, Parque Urquiza Nuevo: PUN)—it is 25 km far from any agroecosystems, and two ponds are situated in agricultural environment (Acceso Norte: AN; La Picada: LP) which are surrounded by soybean fields. The agricultural ponds were selected according to the following features: (1) ponds had water during the period of study, (2) they were primarily permanent waterbodies, (3) ponds received pluvial runoff from agricultural lands, (4) they had similar environmental characteristics and depth (0.55 m ± 0.20), and (5) preliminary data are available, stating that anurans (e.g. *Chaunus fernandezae*, *Odontophrynus americanus*, *Hypsiboas pulchellus*, *Scinax squalirostris*) use this ponds to reproduce and survive (Peltzer et al., 2003; Peltzer and Lajmanovich, 2004). It is important to note that these two ponds with those features were only present in an area of 4000 ha of intensively agricultural activities. Moreover, cattails (*Typha latifolia*), burhead (*Echinodorus grandiflorus*), pampas grass (*Cortaderia selloana*), duckpotatoe (*Sagittaria montevidensis*), smartweed (*Polygonum punctatum*), bulrush (*Schoenoplectus californicus*), and rush (*Juncus pallescens*) were the most common emergent vegetation in three ponds, but there were no aquatic floating plants. A wide range of aquatic invertebrates, especially snails belonging to the family Planorbidae, were present in the agricultural ponds.

The survey was conducted in 2005 during the final period of soybean development corresponding to February–March. This period is coincident with the maximum pesticide applications, to protect soybean plants from harmful herbivores such as the hemipteran *Nezara viridula* (Lorenzatti et al., 2004). Nitrogen fertilizers are also applied to supply soils with enough nutrients for crop. In each pond, a water sample was obtained for physico-chemical and pesticide residue determination. Concentrations of nitrate were determined using the cadmium reduction method and soluble reactive phosphorus (orthophosphate) concentration using the phosphoantimonyl–molybdenum complex method (Eaton et al., 1995) following the APHA (1994) criteria. In addition, pH, water temperature, conductivity, turbidity, and dissolved oxygen were recorded. Pesticide residues (organochlorines, OC: α-HCH [Detection Limit, DL: 2 ng L^{-1}], β-HCH [DL: 2 ng L^{-1}], γ-HCH [DL: 2 ng L^{-1}], aldrin [DL: 2 ng L^{-1}], dieldrin [DL: 2 ng L^{-1}], heptachlor [DL: 1 ng L^{-1}], heptachlor epoxide