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Health status of tadpoles and metamorphs of *Rhinella arenarum* (Anura, Bufonidae) that inhabit agroecosystems and its implications for land use

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ABSTRACT

Perturbations of water bodies near agricultural and livestock systems can affect embryonic and larval stages of anurans and negatively impact adult populations and structure of amphibian communities. This study is focused on early development of *Rhinella arenarum*, for which body growth, abnormalities in the oral disc and genetic damage on erythrocytes were analyzed to establish the impact of agroecosystems on local populations of amphibians. Tadpoles and metamorphs of *R. arenarum* were collected in three agroecosystems (namely, C1, C2, and C3) and in a site without agricultural and livestock activities (SM) from central Argentina. Egg masses of C1 were extracted for breeding tadpoles under laboratory conditions (Lab). Tadpoles were in small size and lighter in weight in C1 and C2. Metamorphs were shorter and lighter in weight in C1 and C3. In SM and Lab samples, no tadpoles with abnormal LTRF (labial tooth row formula) or without labial teeth were observed. In C1, the highest frequency of abnormal LTRF was recorded and was the only site in which tadpoles without labial teeth were found. In C1 and C2 the tadpoles had highest micronucleus frequencies and nuclear abnormalities. C1 can be considered as the site with the highest anthropogenic perturbation and with less healthy tadpoles. Livestock practices such as alternating cattle between parcel and keeping a buffer between crops and water bodies, would allow a better development of the first aquatic stages that are essential for the conservation of the anuran populations.

1. Introduction

Agriculture is the human activity that occupies most of earth's terrestrial surface (Devine and Furlong, 2007) and its expansion is the main cause of habitat fragmentation and perturbation. At the present, agriculture affects 89% of the anuran species (GAA, 2004; Young et al., 2004). Farming and livestock alter the geomorphology of the aquatic and terrestrial environment and modify the hydroperiod, water quality, vegetation cover, productivity of the environment and food webs (Knutson et al., 2004; Nori et al., 2013). Likewise, the chemical products used in farming activities can contaminate water bodies through leaching, causing damages to the aquatic environments (Knutson et al., 2004). High concentrations of elements such as phosphorous and nitrogen have been detected in agricultural areas (Hamer et al., 2004). These elements cause the eutrophication of the aquatic systems with a drastic decrease of dissolved oxygen and the further death of aerobic organisms (Mitsch and Gosselink, 2000).

Deteriorated pools in agricultural and livestock systems affect to the tadpoles, reducing its survival and affecting parameters such as growth and development and increasing the frequency of morphological malformations, variations of oral disc and cellular abnormalities (Marco and Blaustein, 1999; Peltzer et al., 2008; Carezzano and Cabrera, 2011). Impacts on the early stages of development can negatively affect the recruitment of metamorphs (Schmutzer et al., 2008; Burton et al., 2009) and the regulation of populations size (Heyer, 1974; Wilbur, 1980), so the populations

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and structure of the amphibian communities are affected (Gray et al., 2004).

The central region of Argentina has been greatly affected by agricultural expansion (INTA, 2003; Rossi, 2006). Agriculture is intensive and it requires the application of pesticides and fertilizers in great amounts (CASAFE, 1999; Lajmanovich et al., 2005a). Many of the aquatic habitats in this area have been altered and the richness and abundance of amphibian communities, as well as the diet of the larval stages were affected (Bionda et al., 2011a, 2012a, 2013a, 2013b). In addition, genotoxic damage (Caraffa et al., 2014) and morphological abnormalities in adults (Peltzer et al., 2011; Bionda et al., 2012b) have been recorded. Due to the fundamental role of tadpoles in the regulation of adult phase and the total dependency of tadpoles on the aquatic systems, is necessary to know the impact of agroecosystems on early stages of development of local anuran populations. This study focuses on tadpoles and metamorphic individuals of *Rhinella arenarum*. We use field and lab observations and we analyzed different aspects of body growth, abnormal oral disc and genetic damage in erythrocytes.

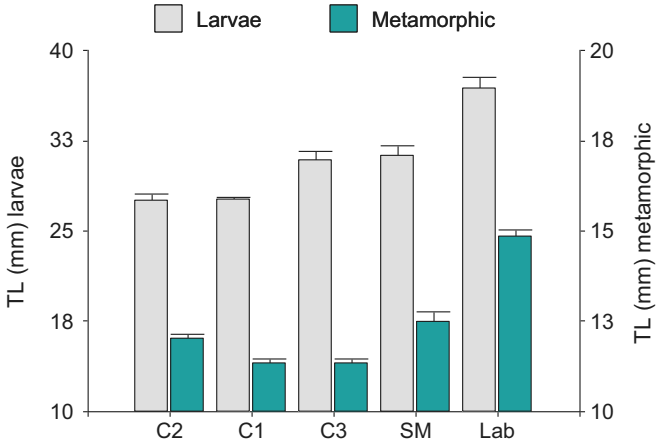

Fig. 1. Mean total length (TL) and standard error in millimeters (mm) for tadpoles (in stage 41) and metamorphic individuals (in stage 45) of each site: C1, C2 and C3, agroecosystems; SM, semi-modified site; Lab, containers in the lab.

2. Material and method

2.1. Study sites and bioindicator specie

Sampling was conducted at sites near Río Cuarto (33°10′S – 64°20′O; 420 m.a.s.l), Córdoba province, Argentina. The main socio-economic activities of this area are farming and livestock. Because of its size and population is important in the central region of the country. This area has a temperate climate characterized by dry winters and warm summers. Annual average rainfall ranges between 700 and 800 mm and the maximum rainfall occurs between October and March (Bridarolli and di Tada, 1996; PEGRC, 2011).

Four sampling sites with different degrees of human disturbance were selected: **C1** (coordinates 33°05′51″S, 64°26′02″O; 471 m.a.s.l.; 9 ha): rural landscape with a pond near to the crop (about 50 m) used by cattle to drink water; **C2** (coordinates 33°06′09″S, 64°25′32″O, 467 m.a.s.l.; 5 ha): rural landscape with pond close to the crop (about 60 m) and not used by cattle; **C3** (coordinates 33°05′39″S, 64°25′58″O, 468 m.a.s.l.; 5 ha): rural environment with pond away from the crop (about 900 m) and used

Table 1
Values of environmental variables per sites. Mean ± standard deviation (maximum and minimum values).

	C1	C2	C3	SM	Lab
Air temperature (°C)	21.3 ± 4.9 (13–28) A	21.8 ± 5.4 (12–31.2) A	21.5 ± 4.42 (14.5–28) A	23.8 ± 3.3 (19–28.5) A	25.1 ± 1.3 (24.2–26) A
Water temperature (°C)	21.8 ± 4.5 (13–32.8) A	22.5 ± 5.5 (12.2–33.4) A	24.1 ± 6.1 (15.4-37.8) A	25.2 ± 3.9 (18–30.5) A	25.3 ± 1.7 (24.1–26.5) A
Conductivity (μS)	1176 ± 404 (299–1973) A	171 ± 43 (97–264) B	525 ± 393 (154–1585) B	338 ± 187 (190–854) B	492 ± 122 (405–578) B
Salinity (mg L^{-1})	542 ± 214 (1.02–840) A	139 ± 210 (8.56–928) B	264 ± 191 (74.9–791) B	163 ± 91 (92.8–418) B	238 ± 60 (195–280) B
TDS (mg L^{-1})	597 ± 358 (1–1000) A	121 ± 31 (72.2–187) B	337 ± 235 (1.1–975) B	241 ± 133 (139–606) B	349 ± 87 (287–410) B
pH	8.8 ± 0.5 (7.9–9.6) A	7.7 ± 0.7 (6.6–9.1) B	9.3 ± 0.7 (7.3–10.4) A	8.3 ± 0.6 (7.7–9.8) B	8.2 ± 0.1 (8.1–8.2) B
Nitrate (mg L^{-1})	0.5 ± 0.5 (0–1)	0.5 ± 0.5 (0–1)	0.0 ± 0.0 (0–0)	0.0 ± 0.0 (0–0)	0.0 ± 0.0 (0–0)
Phosphorus (mg L^{-1})	5.6 ± 3.4 (2.2–9)	5.82 ± 3.18 (2.64–9)	6.45 ± 2.55 (3.9–9)	3.08 ± 2.62 (0.46–5.7)	2.32 ± 2.18 (0.14–4.5)

DGC test: means of a row with the same letter are not significantly different ($p > 0.05$). C1, C2 and C3: agroecosystems; SM: semi-modified site; Lab: containers in the lab.