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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.

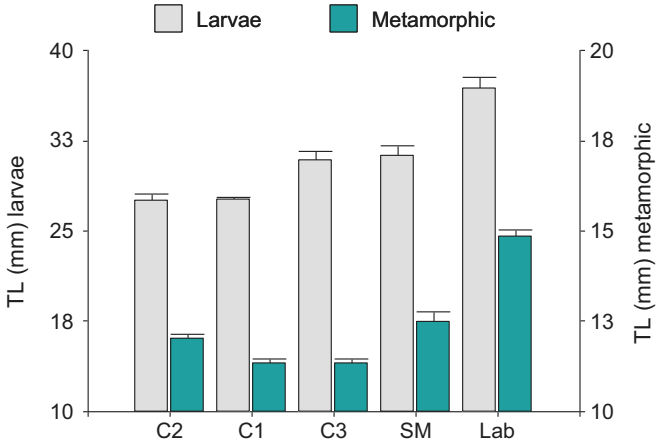

Fig. 1. Mean total length (TL) and standard error in millimeters (mm) for tadpoles (in stage 41) and metamorphic individuals (in stage 45) of each site: C1, C2 and C3, agroecosystems; SM, semi-modified site; Lab, containers in the lab.

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.

by cattle discontinuously (every three days); **SM** (coordinates 33°06′42″S, 64°18′12″O, 428 m.a.s.l.; 8 ha): semi-modified landscape; pond not affected by crops or livestock, near to a protected natural area, the native forest "El Espinal", located within the National University of Río Cuarto, with grasslands and forest formations of native and non-native trees (Doffo, 1989). Ponds of all study sites are temporary and have no fishes. Additionally, tadpoles were bred in the lab -Lab- at controlled temperature using dechlorinated water.

Amphibians are good bioindicators because possess several characteristics that may make them more sensitive to environmental disturbances than other wildlife (Rowe et al. 2003). We select the native specie *R. arenarum* (Hensel, 1867), because provides a suitable and useful experimental model for biomonitoring aquatic ecosystems (Vera Candioti et al., 2010). Sensitivity of *R. arenarum* was proven in several studies (Howe et al., 1998; Venturino et al., 2003; Bosch et al., 2011; Lajmanovich et al., 2014). This anuran species has a wide Neotropical distribution (is present in Argentina, Bolivia, Brazil, Uruguay and very likely in Paraguay) (Frost, 2014). It is commonly found in forests, wetlands, riverside areas, urban and agricultural lands. Besides, this species is easy to handle and acclimate to laboratory conditions (Kwet et al., 2004). Studies about the biology of its larvae stages are rare (Kehr, 1994; Lajmanovich and Fernández, 1995; Bionda et al., 2012a). Tadpoles of *R. arenarum* are black, the labial tooth row formula (LTRF) is 2 (2)/3 or 2(2)/(1)3 and possess benthic habits (Cei 1980; Echeverria et al., 1987; Gallardo, 1987).

2.2. Field work

Field work was carried out from October 2012 to February 2013. The environmental variables that were surveyed at each site per week were: water and air temperature (at 100 cm from the ground), pH, conductivity, total dissolved solids (TDS) and salinity of water bodies using a digital multiparameter 35-series 35425-10 test (Oakton Instruments 625 E Bunker Court Vernon Hills, IL 60061, USA). Two water samples from each pond were taken to determine the levels of nitrate and phosphorus through the colorimetric and visible spectrophotometric methods of analysis, respectively. These analyses were performed by the area of Hydrology, Department of Geology, National University of Río Cuarto. The Visual Encounter Survey (VES) methodology was used for sampling of individuals. VES consists in walks around the borders of the pond to observe tadpoles and metamorphs. Individuals captured were anesthetized with a solution at 0.5% of MS 222 or Methanesulfonate Salt (3-Aminobenzoic Acid Ethyl Ester Sigma-Aldrich™) and preserved in Phosphate Buffer for further analysis.

2.3. Lab analysis

A 10% of the total mass of two ovipositions from C1 site was extracted for the husbandry of tadpoles in the lab. The eggs were placed in two transparent plastic containers (42 cm long, 32 cm wide and 20 cm high) with dechlorinated water. Each container contained 5% of each oviposition and was conserved near to a natural light source. In order to generate movement on the water, a submersible water pump was placed inside each container. To avoid the suction of the eggs or tadpoles, the water pump was covered with a micronet. An aerator was also placed inside each container. The feeding of tadpoles was *ad limitum* with *Taraxacum* sp. boiled for 20 min.

The following measures were recorded for tadpoles: development stage (following Gosner, 1960); mass, using a Mettler balance (P11N 0-1000g); total length (TL; length from the snout to the end of the tail); maximum width (MW; maximum width of the body, dorsal view); length of left leg (LL); body length (BL; snout-vent length) and tail muscle height (TMH; tail muscle height bundle the vent tube, lateral view). In addition, the oral discs were examined to record possible abnormalities on labial tooth row formula. Morphometric measurements and observations of oral discs were performed with a Zeiss West Germany binocular magnifying glass.

The following parameters were measured on metamorphs: mass (with Mettler balance, P11N 0-1000g); total length (TL; length from the snout to the end of the tail); maximum width (MW) and length of left leg (LL). We considered the individuals to be metamorphs since stage 42 (tadpoles that have lost its larval characteristics and acquire adult structures, i.e. emergence of at least one forelimb) until their complete metamorphosis (stage 46) following Peltzer et al. (2013). Morphometric measurements were performed with a Zeiss West Germany binocular magnifying glass.

A micronucleus test with tadpoles from stages 38 and 41 of Gosner (Gosner, 1960) was carried out to analyze genetic damage. Tadpoles were anesthetized for three minutes in a solution of chloroethylene. The blood was obtained from each tadpole by

Table 2

A. Analysis of the covariance for tadpoles (stages 33–41) and DGC post-hoc test DGC.
Tadpoles: stages 33–41
ANCOVA

Variable	*F*-value	*p*-Value	*p*-Value Cov: stage	Regression coefficients	Post-hoc test
TL	$F_{5,\ 85}$: 56.6	< 0.0001*	< 0.0001*	0.75	C1–C2≠C3–SM≠LAB
BL	$F_{5,\ 85}$: 35.3	< 0.0001*	< 0.0001*	0.36	C1–C2≠C3–SM ≠LAB
LL	$F_{5,\ 85}$: 60.9	< 0.0001*	< 0.0001*	1.24	C1–C2≠SM–C3–LAB
MW	$F_{5,\ 85}$: 13.9	< 0.0001*	< 0.0001*	0.32	C1–C2–C3–SM≠LAB
TMH	$F_{5,\ 38}$: 6.98	0.0001*	0.1847	0.02	C2≠C3–SM–C1≠LAB
Weight	$F_{5,\ 17}$: 15.6	< 0.0001*	< 0.0001*	0.03	C2–C1≠C3–SM–LAB

B. Analysis of the variance for metamorphosed (stage 45) and DGC post-hoc test.
Metamorphosed: stage 45
ANOVA

Variable	*F*-value	*p*-Value	Post-hoc test
TL	$F_{4,\ 61}$: 91.38	< 0.0001*	C1–C3≠C2≠SM≠LAB
MW	$F_{4,\ 61}$: 40.81	< 0.0001*	C3≠C1–C2≠ SM≠ LAB
LL	$F_{4,\ 61}$: 34.65	< 0.0001*	C1–C3≠C2–SM≠LAB
Weight	$F_{4,\ 61}$: 92.66	< 0.0001*	C1–C3≠C2–SM≠LAB

C1, C2 and C3: agroecosystems; SM: semi-modified site; Lab: containers in the lab.

* Significant *p*-value; α: 0.05

cardiac puncture (Lajmanovich et al., 2005b) and blood smears were prepared on clean slides, fixed, and stained by means of the May-Grünwald/Giemsa method (Dacie and Lewis, 1995; Barni et al., 2007). Coded and randomized slides were scored by a single observer using a Carl Zeiss trinocular Primo Star microscope (Pack 5) with 100 × objective lens with immersion oil (Feng et al., 2004). It is important to note that red blood cells in amphibians are nucleated and undergo cell division in the circulation, particularly during the developmental stages (Duellman and Trueb, 1986). The criteria for distinguishing a micronucleus (Mn) are: (a) the intensity of a stained Mn should be similar to that of the principal nucleus but with an inferior diameter, (b) it should be round with a nuclear membrane and not connected to the principal nucleus, (c) it should not overlap with the principal nucleus and has to be located within the cytoplasm (Schmid, 1975; Ferrier et al., 1998; Meintières et al., 2001).

Presence of other nuclear abnormalities (NA) were assessed according to the procedures of Guilherme et al. (2008) in mature erythrocytes, by determining the frequency of the following nuclear lesions: lobed nuclei, binucleate or segmented nuclei, kidney-shaped nuclei, notched nuclei, and picnotic nuclei (Pollo et al., 2012; Lajmanovich et al., 2014). Mn and NA frequencies were determined in 2000 erythrocytes from each tadpole and the results were expressed per 1000 cells (‰).

2.4. Statistical analysis

For each statistical analysis, data distributions for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene test) were assessed, and different software packages according to

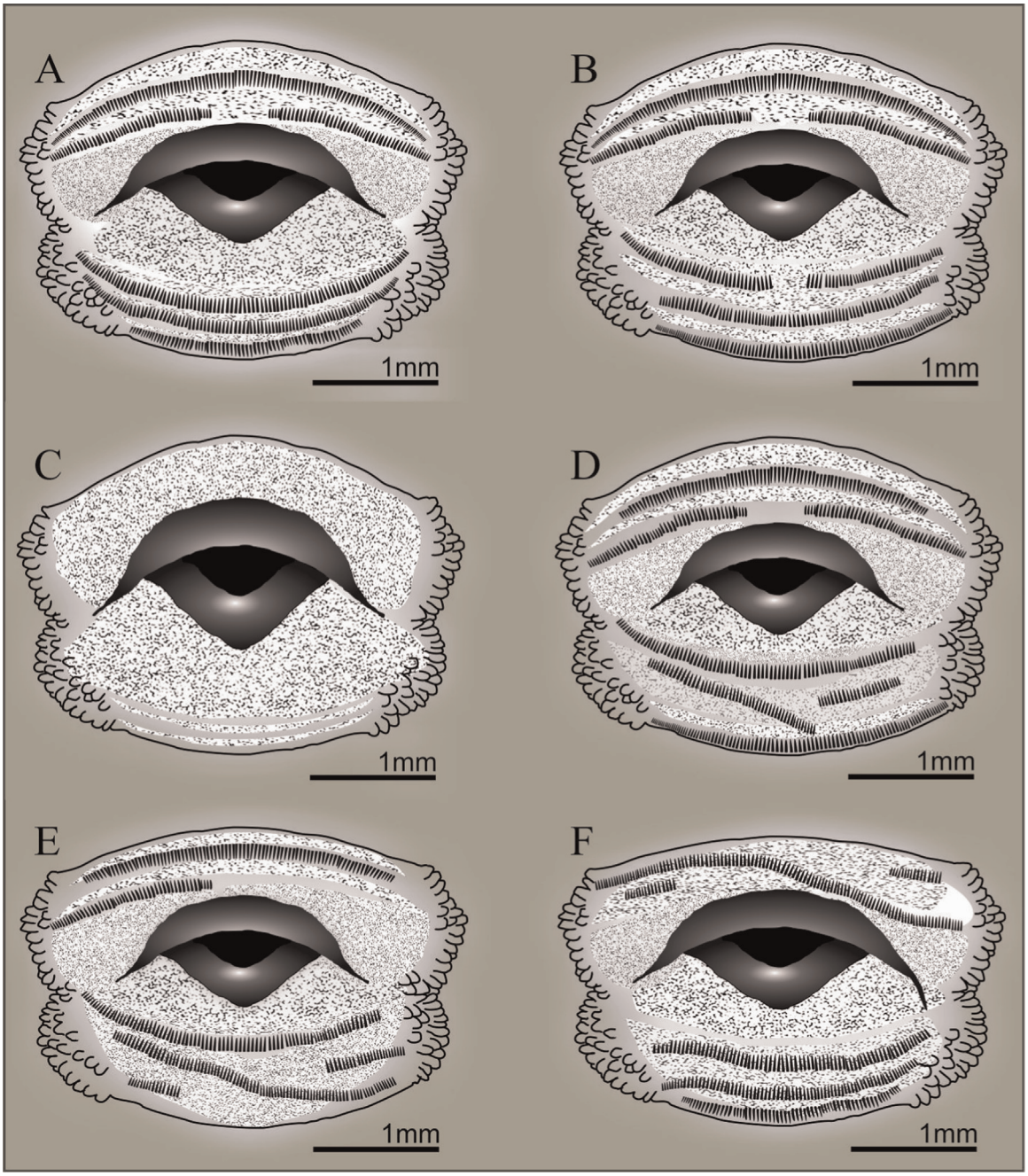

Fig. 2. Record of oral discs classified according to the labial tooth row formula: (A) 2(2)/3; (B) 2(2)/3(1); (C) without labial teeth (0/0); (D) abnormal LTRF (abnormality in 2nd lower labial tooth row); (E) abnormal LTRF (abnormality in 2nd upper and lower labial tooth row); and (F) abnormal LTRF (abnormality in 1st upper labial tooth row).

the analysis were used, such as InfoStat/P version 1.1 (Di Rienzo et al., 2012) and Statistica (StatSoft, 2001). A descriptive analysis and ANOVA of the environmental variables was performed. Morphometric measures of the tadpoles were analyzed by analysis of covariance (ANCOVA). Larval stage is the covariate due to the fact that the stage has an influence on the body size of tadpoles linearly. The statistic values of Chi-square of Pearson and Chi-square MV-G^2 were calculated for the analysis of the labial tooth row formula. Chi-square and Chi-square MV-G^2 are asymptotically equivalent (Balzarini et al., 2008). Frequency of micronucleus and nuclear abnormalities were examined with analysis of variance (ANOVA). The DCG post-hoc test (Test Di Rienzo, Guzmán and Casanoves) was used. This test for mean comparison (Di Rienzo, et al., 2002) utilizes the multivariate technique of cluster analysis (average chain or UPGMA) on a distance matrix obtained from the sample means (Balzarini et al., 2008).

3. Results

Values of environmental variables are shown in Table 1. Air temperature ($F_{4, 79}$: 0.84; p-value: 0.5015) and water temperature ($F_{4, 89}$: 1.24; p-value: 0.2978) varied similarly in all sites, recording the lowest minimum temperatures in the three agroecosystems. The highest conductivity ($F_{4, 85}$: 38.36; p-value < 0.0001), salinity ($F_{4, 85}$: 17.09; p-value < 0.0001) and TDS ($F_{4, 85}$: 13.7; p-value < 0.0001) values were registered in C1. C1 and C3 showed a wide range of conductivity and TDS. In the three agroecosystems, a wide range of salinity was registered. In C2 pH values lower than 7 were recorded ($F_{4, 86}$: 20.7; p-value < 0.0001). Nitrate was detected only in water samples of C1 and C2. In the three agroecosystems, the highest concentrations of phosphorous were recorded.

A total of 100 tadpoles and 75 metamorphs was analyzed. The mean total length to each site reached by tadpoles in the final stage (stage 41) and by metamorphic individuals (stage 45) is plotted on Fig. 1. On sites SM and Lab, the biggest tadpoles and metamorphs were recorded.

Analysis of covariance (Table 2A) showed a significant positive linear relation (p-value < 0.05; regression coefficients > 0.0) between the larval stage and the morphometric variables (except for TMH; p-value: 0.1847). The analysis showed significant differences among the sites for all the morphometric variables (p-value < 0.05). The lowest means of TL, BL, LL and weight were in C1 and C2 (DCG test; Table 2A). The highest values of maximum width were recorded in the Lab tadpoles.

In relation to the metamorphs (Table 2), ANOVAs showed differences for all variables. In C1 and C3 sites, the individuals with lower total length and weight, and shorter hind limbs were recorded. In C3, the metamorphic individuals had the lowest maximum width. The highest media values of all variables were registered in Lab.

The recorded oral discs were classified in the following labial tooth row formula (LTRF): 2(2)/3; 2(2)/3(1); abnormal and without labial teeth (0/0) (Fig. 2). The Chi-square tests were significant (Chi-square Pearson: 29.06; df: 12; p-value: 0.0039. Chi-square MV-G2: 29.12; df: 12; p-value: 0.0038). In the SM and Lab samples no tadpoles with abnormal LTRF or without labial teeth were recorded. On C1 site the highest frequency of abnormal LTRF was recorded (relative frequency: C1: 0.13; C2: 0.09; C3: 0.08; SM: 0.00; and Lab: 0.00) and the only site in which tadpoles without labial teeth were found (relative frequency: C1: 0.13; C2: 0.00; C3: 0.00; SM: 0.00; and Lab: 0.00) (Fig. 3).

Micronucleus test was performed with 25 tadpoles (five per site). ANOVAs showed a significant difference in the frequency of micronucleus ($F_{4, 20}$: 5.66; p-value: 0.0033) and nuclear abnormalities ($F_{4, 20}$: 9.89; p-value: 0.0001) between sites (Table 3). The DGC test differentiated C1 and C2 from the remanent sites, with higher micronucleus frequencies and nuclear abnormalities than those recorded in the tadpoles of the other sites.

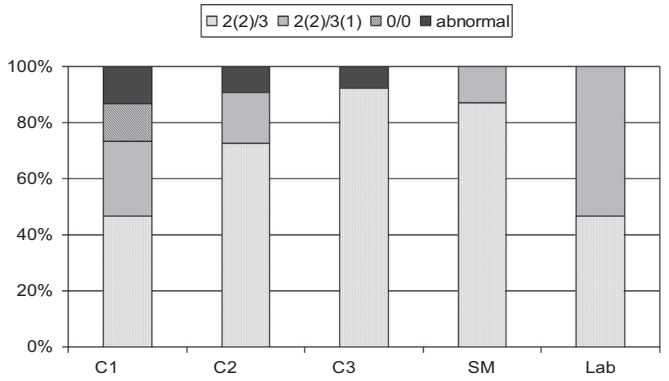

Fig. 3. Percentage of the labial tooth row formula recorded per site: C1, C2 and C3, agroecosystems; SM, semi-modified site; Lab, containers in the lab.

4. Discussion

Negative relations between the agricultural activity and the amphibian populations had been reported since more than two decades (Berger, 1989). In our study is reported that tadpoles and metamorphs inhabiting agricultural and livestock lands showed differences in their traits. The bioindicator anuran species that was utilized has a wide distribution in South America (Frost, 2014), thus the results could be applied to other region's agroecosystems.

On C1 site, high conductivity, salinity and total dissolved solids values were recorded, which indicates mineralization processes that could be caused by the anthropogenic activity developed in this site (Gatica et al., 2012). On C2, acidic pH values (6.6) were recorded, and on C3 the pH values were the most alkaline (10.4). The pH values for a normal development of amphibians range between 6.3 and 7.7 (García and Fontúrbel, 2003). The stress, either acid or alkaline in these environments, could be causing genetic disorders in early stages (Pough and Wilson, 1977).

Nitrate and phosphorous are the main components of the agrochemicals utilized on crops and are good indicators of the existence of disturbance by agricultural and livestock activity (Blanco et al., 2004; Camargo and Alonso, 2008; Giuliano Albo and Blarasin, 2013. In organisms, the toxic action of nitrate is because respiratory pigments are converted in unable forms to transport and release oxygen. Low concentrations of nitrate are detrimental to tadpoles. Camargo and Alonso (2006) reported in *Rana temporaria* that lowest observed effect concentration (LOEC) is 5.0 mg/L nitrate. After precipitation or fertilizer applications, perhaps nitrate concentrations in C1 and C2 (1 mg/L) are higher in ponds and are harmful concentrations for tadpoles.

In the three agroecosystems, the levels of phosphorous were superior to what was recorded in the other sites. The association of phosphorus sediment in shallow ponds, such as C1, C2 and C3 sites and a higher level 0.1 mg L^{-1}, generate eutrophication, producing a drastic diminution of dissolved oxygen (DOF, 1989; Macleod and Haygarth, 2003). In the agroecosystems the levels of phosphorous could mean that this nutrient could be damaging the amphibians and all aerobic aquatic organisms of these communities (Ongley, 1997; Mitsch and Gosselink, 2000). Likewise, in those sites, the temperature ranges were wider, which could mean another stressor for the larval phases because it has an influence on the concentration of dissolved oxygen (Palma Acuña, 2005) and on the availability and toxic effect of some substances used in agriculture (Hoffman et al., 2003).

Table 3
Mean relative frequency and standard deviation of micronucleus (Mn) and nuclear abnormalities (NA) recorded in each site.

	C1	C2	C3	SM	Lab
Mn	1.50 ± 1.17	1.19 ± 0.48	0.64 ± 0.46	0.00 ± 0	0.20 ± 0.27
	A	A	B	B	B
NA	11.36 ± 3.79	16.14 ± 10.16	3.35 ± 1.61	2.10 ± 0.64	1.90 ± 1.29
	A	A	B	B	B

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

In C1 and C2, small size (TL, BL, and LL) tadpoles were recorded and in this site the tadpoles also showed a lighter weight. Metamorphic individuals were shorter and lighter in C1 and C3. Smaller sizes may be due to trophic alterations in these ecosystems because of eutrophication processes that reduce the food quality (Smol, 2002). Food quantity and quality below the optimum value can have a significant influence on the metamorphosis time and size of tadpoles (Carey and Bryant, 1995; Taylor et al., 1995). Studies on diet and growth of anuran larvae of *Rhinella arenarum* y *Physalaemus biligonigerus* performed by Bionda et al. (2012a, 2013b), showed that the tadpoles that inhabit these same agroecosystems consumed less food and had lower body condition, besides having a lower survival rate (Bionda et al., 2013a). On the other hand, the small size of tadpoles that inhabit agroecosystems may be also due to the exposition to certain agrochemicals that can alter the growing and development of these organisms. Lab studies have demonstrated that the exposition to certain chemical substances normally used in crops, generates smaller tadpoles when the metamorphosis occur (Boone et al., 2001; Greulich and Pflugmacher, 2003).

Likewise, it was found that the majority of the abnormal labial tooth row formula (LTRF) and tadpoles without labial teeth were found in C1 site while in SM and Lab sites, both without agricultural perturbations, no abnormalities were recorded in the keratinized oral structures. Certain agricultural perturbations have been related to abnormalities of the oral disc, which has an influence on the body size of tadpoles. Deformations in rows of labial teeth can occur naturally throughout ontogeny (Drake et al., 2007). However, a high incidence of deformations in keratinized oral structures has been recorded, which is associated to factors such as temperature (Bresler and Bragg, 1954), environmental contaminators (Rowe et al., 1996, 1998; Hopkins et al., 2000) and pathogens (Drake et al., 2007). Keratinized mouthparts of tadpoles are complex structures that play a significant role in feeding. Keratinized labial teeth function in two ways: to anchor the oral disc to a substrate and to rake material from it (Venesky et al., 2010a). The absence of labial teeth reduces the efficacy of tadpoles forage (Matthew et al., 2010) and affects negatively on feeding kinematics (Venesky et al., 2010b), by feeding more frequently than the tadpoles with normal oral discs. Then, the tadpoles in C1 site with a high frequency of abnormalities in the labial teeth should spend more energy resources in the acquisition of food and reduce resources for growth and metamorphosis, which could explain the lower body size recorded in them.

Anuran erythrocytes have a high incidence of micronucleus and nuclear abnormalities after the exposure to various contaminants (Campana et al., 2003; Lajmanovich et al., 2005b; Cabagna et al., 2006). For this, are commonly useful for knowing the relation between the organisms and their medium, and with that, the health of the environment (Hoffman et al., 2003). Analysis of the genetic damage showed a high frequency of micronucleus and nuclear abnormalities in the agroecosystems C1 and C2. In these sites, Caraffa et al. (2014) reported a high frequency of micronucleus on adult individuals of the same species.

It is important to highlight that tadpoles reared in the lab came from eggs mass collected in the C1 site. These tadpoles were bigger, without abnormalities on the oral disc and with a low micronucleus frequency and nuclear abnormalities, opposite to the tadpoles and metamorphic individuals collected in situ in the C1 site. In this way, we would discard a genetic effect, because the tadpoles of the same population had different characteristics depending on the environment in which they grew.

In summary, in SM and Lab, biggest organisms were recorded, with normal LTRF and low Mn and NA frequencies. Of rural sites, C1, with a pond near of crops and intense trampling by livestock in and around the pond, could be considered as the agroecosystem with highest anthropic perturbation and with less healthy tadpoles. Levels of conductivity, TDS and salinity showed high values. Tadpoles and metamorphic individuals were smaller and had a higher amount of abnormalities in the oral disc and cells. On the other hand, C3 could be considered as the less disturbed agroecosystem, with crops far from the pond and discontinuous presence of cattle. Tadpoles in this site were bigger and had a lower frequency of abnormalities in the oral disc and in erythrocytes than in C1 and C2, being able to be classified as healthier. These results allow us to think that the environmental degradation caused by agricultural and livestock activities in the central region of Argentina would have an impact on development of early stages of *R. arenarum*. However, livestock practices such as alternating cattle between parcel and keeping a buffer between crops and ponds, would allow a better development of first aquatic stages, essential for the conservation of anuran populations.

As a final recommendation, we consider important to perform pathogenesis studies on sites where abnormalities in the oral disc were recorded, because several studies (Lips, 1999; Drake et al., 2007) indicate that the loss of keratinized dental pieces of tadpoles is a consequence of infections caused by *Batrachochytrium dendrobatidis,* which is associated to the reduction of amphibians all around the world (Fellers et al., 2001).

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References

Balzarini, M.G., Gonzalez, L., Tablada, M., Casanoves, F., Di Rienzo, J.A., Robledo, C. W., 2008. Infostat. Manual del Usuario. Editorial Brujas, Córdoba, Argentina.

Barni, S., Boncompagni, E., Grosso, A., Bertone, V., Freitas, I., Fasola, M., Fenoglio, C., 2007. Evaluation of *Rana* snk *esculenta* blood cell response to chemical stressors in the environment during the larval and adult phases. Aquat. Toxicol. 81 (1), 45–54.
Berger, L., 1989. Disappearance of amphibian larvae in the agricultural landscape. Ecol. Int. Bull. 17, 65–73.
Bionda, C.L., di Tada, I.E., Lajmanovich, R.C., 2011a. Composition of amphibian assemblages in agroecosystems from the central region of Argentina. Russ. J. Herpetol. 18 (2), 93–98.
Bionda, C., Gari, N., Luque, E., Salas, N., Lajmanovich, R., Martino, A., 2012a. Ecología trófica en larvas de *Rhinella arenarum* (Anura: Bufonidae) en agroecosistemas y sus posibles implicaciones para la conservación. Rev. Biol. Trop. 60 (2), 771–779.
Bionda, C.L., Salas, N.E., Caraffa1, E., Baraquet, M., Martino, A.L., 2012b. On abnormalities recorded in an urban population of *Rhinella arenarum* from central Argentina. Herpetol. Notes 5, 237–241.
Bionda, C., Lajmanovich, R., Salas, N., Martino, A., di Tada, I.E., 2013a. Demografía poblacional de *Rhinella arenarum* (Anura: Bufonidae) y *Physalaemus biligonigerus* (Anura: Leiuperidae) en agroecosistemas de la provincia de Córdoba, Argentina. Rev. Biol. Trop. 61, 1389–1400.
Bionda, C.L., Luque, E., Gari, N., Salas, N.E., Lajmanovich, R.C., Martino, A.L., 2013b. Diet of tadpoles of *Physalaemus biligonigerus* (Leiuperidae) from agricultural ponds in the central region of Argentina. Acta Herpetol. 8, 2.
Blanco, S., Ector, L., Becares, E., 2004. Epiphytic Diatoms as water quality indicators in Spanish shallow lakes. Vie et Milieu 54, 71–79.
Bosch, B., Mañas, F., Gorla, N., Aiassa, D., 2011. Micronucleus test in post metamorphic *Odontophrynus cordobae* and *Rhinella arenarum* (Amphibia: Anura) for environmental monitoring. J. Toxicol. Environ. Health Sci. 3 (6), 154–163.
Boone, M.D., Bridges, C.M., Rothermel, B.B., 2001. Growth and development of larval green frogs (*Rana clamitans*) exposed to multiple doses of an insecticide. Oecologia 129, 518–524.
Bresler, J., Bragg, A.N., 1954. Variations in the rows of labial teeth in tadpoles. Copeia 1954, 255–257.
Bridarolli, M.E., di Tada, I.E., 1996. Algunos aspectos de la geografía física de la provincia de Córdoba. In: di Tada, I.E., Bucher, E.H. (Eds.), Biodiversidad de la provincia de Córdoba. Fauna, Volumen I. Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina, pp. 15–38, 373.
Burton, E.C., Gray, M.J., Schmutzer, A.C., Miller, D.L., 2009. Differential responses of postmetamorphic amphibians to cattle grazing in wetlands. J. Wildl. Manag. 73, 269–277.
Cabagna, M., Lajmanovich, R.C., Peltzer, P.M., Attademo, M., Ale, E., 2006. Induction of micronucleus in tadpoles of *Odontophrynus americanus* (Amphibia: Leptodactylidae) by the pyrethroid insecticide cypermethrin. Environ. Toxicol. Chem. 88, 729–737.
Camargo, J.A., Alonso, A., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ. Int. 32, 831–849.
Camargo, J.A., Alonso., A., 2008. Contaminación por nitrógeno inorgánico en los ecosistemas acuáticos: problemas medioambientales, criterios de calidad del agua, e implicaciones del cambio climático. Ecosistemas 16, 98–110.
Campana, M.A., Panzeri, A.M., Moreno, V.J., Dulout, F.N., 2003. Micronuclei induction in *Rana catesbeiana* tadpoles by the pyrethroid insecticide lambda-cyhalothrin. Genet. Mol. Biol. 26, 99–103.
Caraffa, E., Bionda, C., Pollo, F., Salas, N., Martino, A., 2014. Determinación de la frecuencia de micronúcleos en eritrocitos de *Bufo arenarum* que habitan ambientes urbanizados. Acta Toxicol. Argent. 21, 2.
Carey, C., Bryant, C.J., 1995. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. Environ. Health Perspect. 103, 13–17.
Carezzano, F.J., Cabrera, M.R., 2011. Variación en la fórmula dentaria larval de *Physalaemus biligonigerus* (Amphibia, Leptodactylidae) de humedales en agroecosistemas del sur de Córdoba, Argentina. Bol. Soc. Zool. Urug. 20, 28–33.
CASAFE (Cámara de S anidad Agropecuaria y Fertilizantes), 1999. Guía de Productos Fitosanitarios para la República Argentina, 6ta Ed. Argentina, pp. 1600.
Cei, J.M., 1980. Amphibians of Argentina. Monitore Zoologico Italiano 2: Nuova Serie. Firenze, pp. 609.
Dacie, J.V., Lewis, S.M., 1995. Practical Haematology, 8ed. Churchill Livingstone, Edinburgh, pp. 84–90.
Devine, G.J., Furlong, M.J., 2007. Insecticide use: contexts and ecological consequences. Agric. Hum. Values 24, 281–306.
Di Rienzo, J.A., Guzmán, A.W., Casanoves, F., 2002. A multiple comparisons method based on the distribution of the root node distance of a binary tree. J. Agric. Biol. Environ. Stat. 7 (2), 1–14.
Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C. W., 2012. Grupo InfoStat Professional, FCA. Universidad Nacional de Córdoba, Argentina 〈http://www.infostat.com.ar〉.
DOF (Diario Oficial de la Federación), 1989. Criterios ecológicos de calidad del agua. CECCA001/89. 13 de diciembre. México, pp. 7–23.
Doffo, N., 1989. Geomorfología del área urbana de la ciudad de Río Cuarto y de la cuenca del arroyo El Bañado, algunas consideraciones aplicadas al manejo del medio natural Trabajo Final. Universidad Nacional de Río Cuarto.
Drake, D.L., Altig, R., Grace, J.B., Walls, S.C., 2007. Occurrence of oral deformities in larval anurans. Copeia 2007, 449–458.
Duellman, W.E., Trueb, L., 1986. Biology of Amphibians. McGraw-Hill Book Company, San Francisco, New York, p. 670.
Echeverria, D.D., Fiorito de Lopez, L.E., Vaccaro, O.M., Filipello, A.M., 1987. Consideraciones acerca de las formulas dentarias de las larvas de *Bufo arenarum* Hensel (Anura: Bufonidae). Cuad. Herpetol. 3, 3.
Fellers, G.M., Green, D.E., Longcore, J.E., 2001. Oral chytridiomycosis in the mountain yellow-legged frog (*Rana muscosa*). Copeia 2001, 945–953.
Feng, S., Kong, Z., Wang, X., Zhao, L., Peng, P., 2004. Acute toxicity of two novel pesticides on amphibian *Rana N. Hallowel*. Chemosphere 56, 457–463.
Ferrier, V., Gauthier, L., Zoll-Moreux, C.L., Haridon, J., 1998. Genotoxicity tests in amphibians: a review. In: Wells, et al. (Eds.), Microscale Testing in Aquatic Toxicology: Advances, Techniques and Practice. CRC Press LLC, Boca Raton, London, New York, Washington, DC, pp. 507–519.
Frost, D.R., 2014. Amphibian Species of the World: An Online Reference. Version 6.0 (Date of access), Electronic Database accessible at: 〈http://research.amnh.org/herpetology/amphibia/index.html〉. American Museum of Natural History, New York, USA. (accessed 12.05.14.).
GAA (Global Amphibian Assessment), 2004. Summary of key findings. On line: 〈http://www.globalamphibians.org〉 (accessed 12.05.14.).
Gallardo, J.M., 1987. Anfibi os Argentinos, guía para su identificación. Biblioteca Mosaico, Buenos Aires, Argentina, pp. 98.
García, G.C., Fontúrbel, F.R., (Eds.), 2003. Propuestas para un desarrollo sostenible: Lago Titikaka por estrategas K. Editorial Publicaciones Integrales. La Paz, pp. 105.
Gatica, E.A., Almeida, C.A., Mallea, M.A., Corigliano, C.M., González, P., 2012. Water quality assessment, by statistical analysis, on rural and urban areas of Chocancharava River (Río Cuarto), Córdoba, Argentina. Environ. Monit. Assess. 184 (12), 7257–7274.
Giuliano Albo, M.J., Blarasin, M.T., 2013. Hidrogeoquímica y estimación del fondo natural de nitratos del agua subterranea en un agroecosistema del pedemonte de la sierra de Comechingones. Córdoba (Argentina). Rev. Asoc. Geol. Argent. 71, 3.
Gosner, K.L., 1960. A simplified table for stading anuran embryos and larvae with notes on identification. Herpetologica 16, 183–190.
Gray, M.J., Smith, L.M., Leyva, R.I., 2004. Influence of agricultural landscape structure on a Southern High Plains, USA, amphibian assemblage. Landsc. Ecol. 19, 719–729.
Greulich, K., Pflugmacher, S., 2003. Differences in susceptibility of various life stages of amphibians to pesticide exposure. Aquat. Toxicol. 65, 329–336.
Guilherme, S., Válega, M., Pereira, M.E., Santos, M.A., Pacheco, M., 2008. Erythrocyticnuclear abnormalities in wild and caged fish (*Liza aurata*) along an environ-mental mercury contamination gradient. Ecotoxicol. Environ. Saf. 70, 411–421.
Hamer, A.J., Makings, J.A., Lane, S.J., Mahony, M.J., 2004. Amphibian decline and fertilizers used on agricultural land in south-eastern Australia. Agric. Ecosyst. Environ. 102, 299–305.
Hensel, R., 1867. Beiträge zur Kenntnis der Wirbelthiere Südbrasilens. Archiv für Naturgeschichte, Berlín, vol. 33, pp. 120–162.
Heyer, W.R., 1974. Niche measurements of frog larvae from a seasonal tropical location in Thailand. Ecol. Tempe 55, 651–656.
Hoffman, D.J., Rattner, B.A., Burton Jr., G.A., Cairns Jr., J., 2003. Handbook of Ecotoxicology, 2nd ed. FL: Lewis Publishers, Boca Raton, p. 1290.
Hopkins, W.A., Congdon, J.D., Ray, J.F., 2000. Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal ash burning power plant. Environ. Toxicol. Chem. 19, 862–868.
Howe, G.E., Gillis, R., Mowbray, R.C., 1998. Effect of chemical synergy and larval stage on the toxicity of atrazine and alachlor to amphibian larvae. Environ. Toxicol. Chem.
INTA. EEA MANFREDI, 2003. Proyecto Regional de Agricultura Sustentable, On line: 〈http://www.inta.gov.ar/manfredi/info/boletines〉.
Kehr, A.I., 1994. Patrones de dispersión espacio-temporales y su influencia en la biología larval de *Bufo arenarum* (Amphibia: Anura). Neotropica 36 (96), 67–81.
Knutson, M.G., Richardson, W.B., Reineke, D.M., Gray, B.R., Parmelee, J.R., Weick, S. E., 2004. Agricultural ponds support amphibian populations. Ecol. Appl. 14, 669–684.
Kwet, A., Reichle, S., Silvano, D., Úbeda, C., Baldo, D., Di Tada, I., 2004. *Rhinella arenarum*. The IUCN Red List of Threatened Species. Version 2014.3. 〈www.iucnredlist.org〉 (downloaded on 14.04.15.).
Lajmanovich, R.C., Fernández, V.C., 1995. Alimentación de larvas de *Bufo arenarum* Hensel (Amphibia: Bufonidae) en ambientes del río Paraná, Argentina. Boletín del Museo de Historia Natural de Chile 45, 7–18.
Lajmanovich, R., de La Sierra, P., Marino, F., Peltzer, P., Lenardón, A., Lorenzatti, E., 2005a. Determinación de residuos de organoclorados en vertebrados silvestres del Litoral Fluvial de Argentina, INSUGEO. Miscelánea 14, 255–262.
Lajmanovich, R.C., Cabagna, M., Peltzer, P.M., Stringhini, G.A., Attademo, A.M., 2005b. Micronucleus induction in erythrocytes of the *Hyla pulchella* tadpoles (Amphibia: Hylidae) exposed to insecticide endosulfan. Mutat. Res. 587, 67–72.
Lajmanovich, R.C., Cabagna-Zenklusen, M.C., Attademo, A.M., Junges, C.M., Peltzer, P.M., Bassó, A., Lorenzatti, E., 2014. Induction of micronuclei and nuclear abnormalities in tadpoles of the common toad (*Rhinella arenarum*) treated with the herbicides Liberty (®) and glufosinate-ammonium. Mutat. Res.—Genet. Toxicol. Environ. Mutagen. 769, 7–12.
Lips, K.R., 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conserv. Biol. 13 (1), 117–125.
Macleod, C., Haygarth, P., 2003. A review of the significance of non-point source agricultural phosphorus to surface water. Scope Newslett. 51, 1–10.
Marco, A., Blaustein, A.R., 1999. The effects of nitrite on behavior and metamorphosis in cascades frogs (*Rana cascadae*). Environ. Toxicol. Chem. 18, 946–949.
Matthew, D.V., Wassersug, R.J., Parris, M.J., 2010. The impact of variation in labial tooth number on the feeding kinematics of tadpoles of southern leopard frog

(*Lithobates sphenocephalus*). Copeia 2010 (3), 481–486.
Meintières, S., Biola, A., Pallardy, M., Marzin, D., 2001. Apoptosis can be a confusing factor in in vitro clastogenic assays. Mutagenesis 16 (3), 243–250.
Mitsch, W.J., Gosselink, J.G., 2000. Wetlands, 3rd ed. Wiley, New York.
Nori, J., Lescano, J.N., Illoldi-Rangel, P., Frutos, N., Cabrera, M.R., Leynaud, G.C., 2013. The conflict between agricultural expansion and priority conservation areas: making the right decisions before it is too late. Biol. Conserv. 159, 507–513.
Ongley, E.D., 1997. Lucha contra la contaminación agrícola de los recursos hídricos. Cuadernos de Riego y Drenaje N 55. FAO. Roma. 1997.On line: ⟨http://www.fao.org/docrep/W2598S/W2598S00.htm⟩.
Palma Acuña, P.S., 2005. Ensayo de micronúcleos en eritrocitos de *Oncorhynchus mykiss* como herramienta para evaluar la exposición a pesticidas potencialmente genotóxicos en el Río Traiguén (Tesis de Grado). Universidad Católica de Temuco, Chile.
PEGRC (Plan Estratégico Gran Río Cuarto), 2011 Estudio: 1.EG. Ministerio de economía y finanzas Públicas-Argentina, Préstamo BID 1896-AR, Programa Multisectorial de Preinversión II. Proyecto: "Plan estratégico y código de planeamiento urbano para el aglomerado del Gran Río Cuarto". Documento de Trabajo N° 2: Diagnóstico Eje Político y Documento de Trabajo N° 3: Diagnóstico Eje Urbano. Coordinador Gustavo Busso.
Peltzer, P.M., Lajmanovich, R.C., Sánchez Hernández, J.C., Cabagna, M., Attademo, A. M., Bassó, A., 2008. Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. Ecotoxicol. Environ. Saf. 70, 185–197.
Peltzer, P.M., Lajmanovich, R.C., Sanchez, L.C., Attademo, A.M., Junges, C.M., Bionda, C.L., Martino, A.L., Bassó, A., 2011. Morphological abnormalities in amphibian populations from the mid-eastern region of Argentina. Herpetol. Conserv. Biol. 6 (3), 432–442.
Peltzer, P.M., Lajmanovich, R.C., Attademo, A.M., Junges, C.M., Cabagna-Zenklusen, M.C., Repetti, M.R., Sigrist, M.E., Beldoménico, H., 2013. Effect of exposure to contaminated pond sediments on survival, development, and enzyme and blood biomarkers in veined treefrog (*Trachycephalus typhonius*) tadpoles. Ecotoxicol. Environ. Saf. 98, 142–151.
Pollo, F.E., Salas, N.E., Mancini, M.A., Martino, A.L., 2012. Estudio comparativo de la frecuencia de micronúcleos y anormalidades nucleares en eritrocitos de tres especies ícticas. Acta Toxicol. Argent. 20 (2), 62–67.
Pough, F.H., Wilson, R.E., 1977. Acid precipitation and reproductive success of *Ambystoma salamanders*. Water, Air Soil Pollut. 7, 307–316.
Rossi, R.L., 2006. Impactos recientes de la soja en la Argentina. Panorama productivo del cultivo. Agromercado, Cuadernillo Clásico vol. 129, pp. 4–7.
Rowe, C.L., Kinney, O.M., Fiori, A.P., Congdon, J.D., 1996. Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: effects on grazing ability and growth. Freshw. Biol. 36, 723–730.
Rowe, C.L., Kinney, O.M., Congdon, J.D., 1998. Oral deformities in tadpoles of the Bullfrog (*Rana catesbeiana*) caused by conditions in a polluted habitat. Copeia 1998, 244–246.
Rowe, C.L., Hopkins, W.A., Bridges, C., 2003. Physiological ecology of amphibians in relation to susceptibility to natural and anthropogenic factors. In: Linder, G., Krest, S., Sparling, D. (Eds.), Amphibian Decline: An Integrated Analysis of Multiple Stressor Effects. Society of Environmental Toxicology and Chemistry Press, Pensacola, pp. 9–58.
Schmid, W., 1975. The micronucleus test. Mutat. Res. 31, 9–15.
Schmutzer, A.C., Gray, M.J., Burton, E.C., Miller, D.L., 2008. Impacts of cattle on amphibian larvae and the aquatic environment. Freshw. Biol. 53, 2613–2625.
Smol, J.P., 2002. Pollution of Lakes and Rivers: A Paleoenvironmental Perspective. Arnold Publishers, London; Oxford University Press, New York, pp. 280.
StatSoft, 2001. Statistica for Windows, Release 6.0, Computer Program Manual, StatSoft, Inc.. Tulsa, OK, USA.
Taylor, C.L., Altig, R., Boyle, A., 1995. Can anuran tadpoles choose among foods that vary in quality? Alytes 13, 81–86.
Venesky, M.D., Wassersug, R.J., Parris, M.J., 2010a. The impact of variation in labial tooth number on the feeding kinematics of tadpoles of southern leopard frog (*Lithobates sphenocephalus*). Copeia 3, 481–486.
Venesky, M.D., Wassersug, R.J., Parris, M.J., 2010b. How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenocephalus*)? Biol. Bull. 218 (2), 160–168.
Venturino, A., Rosenbaum, E., Caballero De Castro, A., Anguiano, O.L., Gauna, L., Fonovich De Schroeder, T., Pechen De D'Angelo, A.M., 2003. Biomarkers of effect in toads and frogs. Biomarkers 8 (3–4), 167–186.
Vera Candioti, J.V., Natale, G.S., Soloneski, S., Ronco, A.E., Larramendy, M.L., 2010. Sublethal and lethal effects on *Rhinella arenarum* (Anura, Bufonidae) tadpoles exerted by the pirimicarb-containing technical formulation insecticide Aficida®. Chemosphere 78, 249–255.
Wilbur, H.M., 1980. Complex life cycle. Annu. Rev. Ecol. Syst. 11, 67–93.
Young, B.E., Stuart, S.N., Chanson, J.S., Cox, N.A., Boucher, T.M., 2004. Joyas que están desapareciendo: El estado de los anfibios en el nuevo mundo. Nature Serve, Arlington, Virginia, USA, p. 53.