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Original Articles

Differential effects on life history traits and body size of two anuran species inhabiting an environment related to fluorite mine

Manuel A. Otero[a,b,*], Favio E. Pollo[a,b], Pablo R. Grenat[a,b], Nancy E. Salas[a], Adolfo L. Martino[a]

[a] Ecología, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, UNRC, ruta 36 km 601, Rio Cuarto, Córdoba, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ABSTRACT

Mining activity is an important cause of physicochemical, biological, and landscape alterations. Several studies involving disturbed environments confirmed the modification of demographic traits in anuran populations. The current study aimed to assess the effects of natural and artificial surface waters associated with a fluorite mine on the body size and life history traits of *Rhinella arenarum* and *Boana cordobae*. Sampling was done in three areas: stream running on granitic rock, with medium natural fluoride content (CN); stream running on metamorphic rock, with low natural fluoride content (LV); and artificial decantation pond (DP) with large variation in physicochemical parameters. Adult individuals of the two anuran species were measured and weighed, and their body condition (BC) was calculated. Life history traits were assessed using the skeletochronology method. Individuals from DP weighed significantly heavier than individuals from streams. The BC index in *R. arenarum* was higher at LV, whereas in *B. cordobae*, a higher index was obtained at both LV and DP. The average age differs between sites in *B. cordobae* but not in *R. arenarum*. Growth coefficient was higher for altered environment in both species (CN; DP), whereas the estimated SVLmax was slightly higher in populations from less disturbed sites (LV). The most affected species was *B. cordobae*. Therefore, for future studies, it is important to know the biology of sentinel species because not all amphibian species may respond in the same way to similar disturbances. Differences obtained in life history traits may be explained by the lack of survival of larger/older individuals or compensatory growth, required to balance fast growth with the costs on survival at more disturbed sites.

1. Introduction

Pollution of freshwater bodies generates a growing concern worldwide (Antunes et al., 2007; Marques et al., 2008). Anthropogenic activities such as urbanization, agriculture, livestock, and mining activities (Castro et al., 2003; Marques et al., 2008; Antunes et al., 2008; Bionda et al., 2011, 2013; Babini et al., 2015) contribute to such degradation. Mining activity represents one of the main sources of physicochemical, biological, and landscape alterations. Wastewater produced by this activity contains complex mixtures of pollutants (Marques et al., 2009; Zocche et al., 2013; Lanctôt et al., 2016), thereby raising possible threats to aquatic biota. Traditionally, evaluation of environmental health has been based on physicochemical measurements of water bodies, but does not necessarily provide appropriate information on exposure and response of organisms to pollution (Antunes et al., 2008; Lavoie et al., 2012).

It is well noted that anuran amphibians have large potential as bioindicators. Because they have a semipermeable skin and different life cycle stages, amphibians are susceptible to environmental alteration in both aquatic and terrestrial habitats (Alford and Richards, 1999; Simon et al., 2011; Babini et al., 2015). Amphibians may hold both the role of prey and predators, making up a fundamental element in accumulation and transfer of pollutant substances between aquatic and terrestrial environments (Marques et al., 2013). Furthermore, amphibians are good models for pollution studies because their populations generally contain elevated number of individuals and they are good representatives of aquatic environments (Burger and Snodgrass, 1998). Finally, anuran species-specific characteristics, depending on whether they belong to terrestrial, aquatic, or arboreal habitats, could exhibit greater or lesser negative effects on populations when an environmental change occurs (Keller et al., 2009).

Many studies have demonstrated the adverse effects of wastewater generated by mining activities on amphibians. The main contributions to this knowledge base have been studies under laboratory conditions related to survival, growth, larval development (Tejedo and Fieques, 2003; Muñoz-Escobar and Palacio-Baena, 2010; Lanctôt et al., 2016),

genotoxicity and cytotoxicity (Djomo et al., 2000; Marques et al., 2008, 2009, 2013). In addition, there are some fieldworks in which the species diversity (Ortiz et al., 2003; Aguilar et al., 2012), *in situ* cytogenotoxicity (Pollo et al., 2016, 2017), sex ratio, and age structure (Zhelev et al., 2014) were evaluated. However, the number of field studies that provide adequate and long-term information on exposure and response of amphibians to mining pollution is low.

The age structure may affect population growth and supply information on recent history, current state, and the future population trends (Dimmick and Pelton, 1996). Moreover, it is could be very appropriate for understanding species dynamics and for establishing reasons of population instability (Driscoll, 1999). In consequence, skeletochronology is considered a reliable technique for age determination of many vertebrates including amphibians (Sinsch et al., 2001; Marangoni et al., 2012; Otero et al., 2017a, 2017b). Several studies from disturbed agroecosystems (Spear et al., 2009; Attademo et al., 2014; Zamora-Camacho and Comas, 2017; Zhelev et al., 2017) and environments affected by coal and cooper mining activity (Zhelev et al., 2014) confirmed the modification of demographic traits and body size in anuran populations. However, to date, the effect that fluorite mining could have on life history traits of anuran species is yet unknown.

In this study, we present the first data of demographic and morphometric traits of two anuran species inhabiting an environment associated with fluorite mine. We aim to assess the potential effects of natural and artificial surface waters associated with a fluorite mine on life history traits and body size of *Rhinella arenarum* and *Boana cordobae* to test two exclusive hypothesis: H1: The alteration of an environment related to fluorite mine causes readjustments in anuran life history traits such as changes in growth rates, lower longevity, and reduced reproductive potential (RP); and H2: *R. arenarum* and *B. cordobae* show differential effects on life history traits to similar disturbances because these species have different reproductive and behavioral habits.

2. Materials and methods

2.1. Study area

The study area is located in the batholith Cerro Áspero (32° 50′ 22.85″ S; 64° 79′40.60″ W; altitude 1200 m.a.s.l), in the south-central Sierra de Comechingones, Córdoba, Argentina. In this point, rocks contain approximately 1.210 mg/kg of fluoride (Coniglio et al., 2010). This geological formation allowed installation of the fluorite mine Cerros Negros, in which fluoride extraction is carried openwork and fluoride is recovered physically by a flotation process. The mine wastes are deposited on a series of artificial earth ponds, of which two are vegetated with *Typha* sp. In these decantation ponds (DP), precipitate sediments are produced by the fluorite flotation process, and the DP are a closed system.

The landscape corresponds to a mountain environment with low level of anthropic intervention, strongly undulating, with natural vegetation typical of a xerophilous forest (Oggero and Arana, 2012). Several streams cross this area, the most important being the Cerros Negros stream, running on granitic rock (fluoride concentration = 1.90 mg/L), and Los Vallecitos stream, running on metamorphic rock (fluoride concentration = 0.3 mg/L; Pollo et al., 2017). Thus, we selected three sampling sites according to fluoride concentrations and level of disturbance produced by mining activity (Fig. 1): Site I – Vallecitos stream (LV), which runs on metamorphic rock with low natural fluoride content and low levels of mining perturbation (scrap yard); Site II – Cerros Negros stream (CN), which runs on granitic rock with medium natural fluoride content and medium levels of anthropic alteration (soil movement and mineral extraction); and Site III – Artificial DP as areas with high fluoride content and high levels of disturbance (mineral treatment, sediments produced by fluorite flotation process and heavy machinery). In all study sites, population of *R. arenarum* and *B. cordobae* were previously registered.

2.2. Species

R. arenarum has a large distribution in South America and is located in Argentina, Bolivia, Brazil, Uruguay, and Paraguay. Adult individuals generally congregate in large breeding groups at lentic and lotic water bodies. Eggs are landed in large gelatinous strings along the edges of ponds (Kehr, 1994; Bionda et al., 2011). *R. arenarum* inhabits a wide range of environments including coastal areas, subtropical or tropical forests, and rural or urban areas. It is considered as a domestic species owing to their frequent presence in the surroundings of houses. This species represent a useful experimental model for monitoring aquatic environment (Vera Candioti et al., 2010; Pollo et al., 2015, 2017), and its sensitivity has been assessed in several studies (Venturino et al., 2003; Bosch et al., 2011; Lajmanovich et al., 2014; Pollo et al., 2015).

B. cordobae has a distribution limited to San Luis and Córdoba provinces, in central Argentina. This narrow distribution and a broad altitudinal range, together with the reported IUCN status (i.e., data deficient), make this species an interesting research model. *B. cordobae* inhabits highland streams and rivers, usually associated with slight perturbation. Individuals vocalize mainly from periaquatic vegetation but can also do it partly submerged in water (Barrio, 1962). Females deposit egg masses that are stick to submerged vegetation (Verga et al., 2012).

These species present ecological features that are necessary for the selection of sentinel species to ensure the detection of local perturbations: presence in abundance within the study area, low rate of migration, and limited to a small space (Flickinger and Nichols, 1990). In addition, it is well-known that amphibians are highly philopatric; hence, the adult individuals analyzed at each site probably have gone through their larval cycle at that site (Sinsch, 1990). Both species were characterized with regard to their vertical location using Vallan's (2000) guild criteria: *R. arenarum* was found on the ground (terrestrial) and *B. cordobae* was found inhabiting herbaceous, shrubby, or arboreal habitats (arboreal).

2.3. Sampling methods and data collection

We sampled all three sites from September 2013 to April 2014, which is the period of increased reproductive activity for *R. arenarum* and *B. cordobae* and, consequently, when anurans are found around water bodies (Bionda et al., 2011). These months coincide with a season of rainfall and warmer temperatures. In each study site, metamorphosed (n = 5), juveniles (n = 3), and adult individuals of *R. arenarum* (LV = 21; CN = 28; DP = 27) and *B. cordobae* (LV = 54; CN = 14; DP = 34) were found through visual encounter surveys (Heyer et al., 1994) and captured by hand. During the night sampling, the location of the populations was based on male mating calls; consequently, the number of males captured was greater than that of females. After the capture, each individual was anesthetized for few minutes with an MS 222 (Tricaine methanesulfonate) solution. For each captured individual, we recorded snout-vent length (SVL) through a digital caliper Mahr 16 (0.01 mm), weight using a Mettler balance (P11N 0.1–1000 g), and sex according to secondary sexual features such as the presence of vocal sac and nuptial pads (Duellman and Trueb, 1994). One phalanx of each specimen was clipped off following an identification pattern for each site (according to Donnelly et al., 1994), and it was preserved in a solution of 70% alcohol. Antifungal or antibacterial and healing agents were added at the puncture site to prevent infections, and each individual was released 2 h later into their places of capture. The body condition (BC) of all individuals was calculated according to Luddecke (2002) as weight (g) * 1000/SVL^3 (mm), which relates weight and SVL and gives an estimate of the nutritional condition.

In each study site, surface water samples were collected in 1-L plastic bottles for chemical analysis. The major ions (F^-, $Na+$, Ca^{++}, Mg^{++}, $K+$, Cl^-, $SO_4^=$, and HCO_3^-) were analyzed by Department of Geology, National University of Río Cuarto, through standard methods
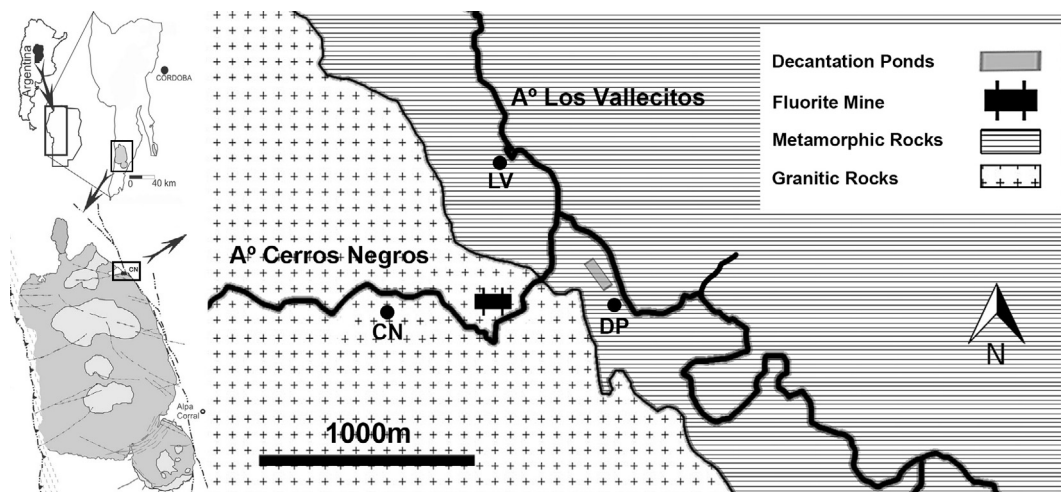
Fig. 1. Location of sampling sites in the granitic batholith Cerro Áspero in the central-south region of Sierra de Comechingones, Córdoba, Argentina. Black points indicate the sampling sites. Image modified from Coniglio et al. (2010).

(APHA-AWWA, 1999). Physicochemical parameters of water (temperature, pH, electrical conductivity, and salinity) were measured *in situ* with digital equipment Test TM35-Series Multiparameter 35425-10, and dissolved oxygen was measured by a meter HD3030.

2.4. Age determination

Standard methods of skeletochronology were performed (e.g., Martino and Sinsch, 2002; Bionda et al., 2015; Otero et al., 2017a). Each phalanx was cross-sectioned at a thickness of 8–10 μ through a rotary microtome Leica®RM2125RTS. Histological sections were stained for 3 min in Ehrlich's hematoxylin. Using a light microscope, Zeiss AxioPhot-Axio Lab (100×) equipped with digital camera Canon G10, Periosteal lines of arrested growth (LAGs) were independently recorded by two researchers. Following the suggestion of Sinsch et al. (2007), double and false lines were defined as incomplete and fainted hematoxynophilic lines. The presence of Kastschenko Line (KL; the section between the endosteal and periosteal zones; Rozenblut and Ogielska, 2005) enabled us to assess endosteal resorption. The complete resorption of LAGs was also confirmed by the diameter difference between LAGs and KL (sensu Liao and Lu, 2010; Li et al., 2013).

2.5. Demographic traits and growth models

Demographic traits such as age at maturity (ASM), longevity, and RP were calculated according to Otero et al. (2017a,b). Growth trait was estimated according to von Bertalanffy (1938) equation: $SVLt = SVLmax - (SVLmax - SVLmet) e - K(t - tmet)$, where $SVLt$ = mean SVL at age t, $SVLmax$ = maximum asymptotic SVL, $SVLmet$ = mean SVL at metamorphosis, t = number of growing season (age), tmet = proportion of the growing season until metamorphosis (fixed to 0.25 for *R. arenarum* and 0.4 for *B. cordobae*), and K = growth coefficient. The growth model was regarded as a function of age and was fitted to the average growth curve by the least square procedure.

2.6. Statistical analysis

Descriptive statistics are given as mean ± standard deviation. All parameters measured were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene test). Water temperature, pH, salinity, and conductivity were compared using one-way analysis of variance (ANOVA). We used the nonparametric Kruskal–Wallis test to explore for significant differences in dissolved oxygen and ion concentrations between sites. Comparisons of water parameters, morphometrics, and age between sites were conducted using the pairwise post

hoc Bonferroni test. To assess the relationship between SVL and age, Pearson's correlation analysis was performed. The comparisons between sites were performed by separating sexes in each species because sexual dimorphism has been proved in both *R. arenarum* (Bionda et al., 2015) and *B. cordobae* (Otero et al., 2017a). The significance level used in all tests was P < 0.05. Tests were performed using the statistical packages InfoStat (Di Rienzo et al., 2012).

3. Results

3.1. Physicochemical analysis of surface water

Average values of physicochemical variables and ion concentrations in water from each sampling site and the differences between sites are shown in Table 1. The mean fluoride concentrations varied among sites and were significantly greatest in DP. pH values showed little variation around neutrality at LV and CN, whereas this parameter varied greatly into the alkaline range at DP. Dissolved oxygen was lower in DP, whereas water temperature was significantly higher for the same site. Salinity, conductivity, and most ion concentrations showed little temporal variation between streams and significantly higher values at LV. On the other hand, these parameters in DP showed high temporal variation and were extremely high with regard to the streams.

3.2. Morphological analysis

Mean SVL, weight, and BC of *R. arenarum* and *B. cordobae* for each site are shown in Table 2. Mean SVL of *R. arenarum* were not significantly different between sites in both sexes (Males: $F_{2,37} = 1.66$, p = 0.211; Females: $F_{2,27} = 1.17$, p = 0.325). Similarly, SVL of *B. cordobae* did not show differences between sites (Males $F_{2,73} = 2.98$, p = 0.085; Females $F_{1,18} = 1.87$; p = 0.181). The results for weight of *R. arenarum* males were significantly different between sites ($F_{2,37} = 9.60$; p < 0.001), with the lowest value registered at CN and highest value at DP. With regard to females, mean weight did not differ between sites ($F_{2,27} = 1.12$; p = 0.242). In *B. cordobae* we found significant differences in the weight of males between sites ($F_{2,73} = 6.80$; p < 0.01), with DP having individuals that weigh heavier than LV and CN. By contrast, the mean weight of females was not significantly different ($F_{1,18} = 0.84$; p = 0.371). BC of *R. arenarum* was statistically significant between sites for males (H = 6.05; p < 0.05), with the index higher in LV than in CN and DP. However, in females, there was not difference in this parameter (H = 1.95; p = 0.223). In *B. cordobae*, the results for BC of males were significantly different between sites (H = 18.15; p < 0.001), with the lowest value registered at CN. With

Table 1
Chemical, physical, and ion concentration data for each sampling site.

	Sites			
	LV	CN	DP	
Temperature Water (T°W)	18.17 ± 2.7[A]	18.72 ± 2.6[A]	22.83 ± 4.1[B]	$F_{2,62} = 8.8$; $p < 0.001$
pH	7.80 ± 0.2[A]	7.55 ± 0.3[B]	8.61 ± 0.3[B]	$F_{2,62} = 21.18$; $p < 0.001$
Salinity (S), ppm	54.75 ± 10.6[A]	26.46 ± 8.9[A]	476.23 ± 382.0[B]	$F_{2,62} = 20.1$; $p < 0.001$
Conductivity (Cond), μS/cm	112.50 ± 24.5[A]	49.04 ± 23.5[A]	1495.81 ± 6[B]	$F_{2,47} = 77.25$; $p < 0.001$
Dissolved Oxygen (O),%	91.40 ± 10.6[A]	94.90 ± 12.1[A]	75.30 ± 12.3[B]	$H = 7.96$; $p = 0.018$
HCO_3^-, mg/L	70.65 ± 8.0[A]	22.50 ± 7.1[B]	375.0 ± 240.4[C]	$H = 12.74$; $p < 0.001$
Sulfates ($SO_4^=$), mg/L	25.00 ± 0.3[A]	8.25 ± 1.6[A]	102.05 ± 30.2[B]	$H = 12.16$; $p = 0.0023$
Chloride (Cl⁻), mg/L	3.60 ± 0.1[A]	2.9 ± 0.1[A]	378.6 ± 232.4[B]	$H = 11.13$; $p = 0.0025$
Sodium (Na^+), mg/L	7.30 ± 0.1[A]	5.65 ± 1.5[A]	422.65 ± 277.4[B]	$H = 11.7$; $p = 0.0029$
Potassium (K^+), mg/L	0.85 ± 0.2[A]	0.30 ± 0.1[A]	9.60 ± 2.1[B]	$H = 12.16$; $p = 0.0022$
Calcium (Ca^{++}), mg/L	12.6 ± 5.1[A]	4.40 ± 0.6[B]	18.40 ± 1.1[C]	$H = 10.34$; $p = 0.0054$
Magnesium (Mg^{++}), mg/L	3.65 ± 1.7[A]	2.20 ± 0.9[A]	6.85 ± 2.8[B]	$H = 10.07$; $p = 0.0063$
Fluoride (F⁻), mg/L	0.29 ± 0.1[A]	2.06 ± 0.5[B]	13.8 ± 3.6[C]	$H = 9.85$; $p < 0.001$

LV = Los Vallecitos stream, CN = Cerros Negros stream, DP = decantation ponds. Mean ± standard deviation.
Different letters show inter-site pairwise differences, according to post hoc Bonferroni test $p < 0.05$.

regard to females, mean BC was not different between sites (H = 0.76; p = 0.383).

3.3. Skeletochronological analysis

All individuals analyzed showed recognizable bone features that enabled us to estimate age (Fig. 2). The hematoxylin-stained cross-sections of phalanges showed a series of concentric rings (LAGs) separated by broad growth layers in the periosteal bone. Well-defined Kastschenko lines were observed. In 30 individuals of *R. arenarum* (39%) and 23 individuals of *B. cordobae* (22.5%), endosteal resorption was present, but it did not hamper age assessment because the first LAG was never fully reabsorbed. In some cases, the outer LAGs were closely adjacent, but at the insertion site of the phalanx ligament, it was feasible to discern the peripheral LAGs and to account them reliably. False and double LAGs were observed, but they did not affect LAGs counted in the sample.

Life history traits of *R. arenarum* showed little variation and did not show a defined trend between sites (Table 3). Mean age was not significantly different between sites (Males: H = 0.57; p = 0.525; Females: H = 0.47; p = 0.459). Females from DP showed that ASM was three years and was higher than ASM of all other sites. Maximum longevity was five years, and it was registered in LV females and DP

males. Moreover, individuals from these sites showed a higher RP than in all other sites.

The Kruskal–Wallis test was carried out for data on the age of *B. cordobae,* and the results showed that CN males differed significantly from DP and LV males (H = 5.30; P < 0.05) and that LV females differed significantly from DP females (H = 6.2; P < 0.05). ASM was two years at LV, whereas in other sites, it was three years. The greatest longevity recorded was six years in LV females and the lowest longevity was four years in DP females. Estimated RP was higher in LV (Males = 4 years; Females = 5 years) than in the remaining sites.

3.4. Growth patterns

SVL was significantly and positively correlated with age in *R. arenarum* for all sites (LV: r = 0.73, P < 0.01; CN: r = 0.42, P < 0.01; DP: r = 0.19, P < 0.05). Meanwhile, SVL of *B. cordobae* was correlated with age in LV (r = 0.82, P < 0.05) and DP (r = 0.42, P < 0.05) but not in CN (r = 0.24, P = 0.173).

Mean and standard deviation of recently metamorphosed SVL (SVLmet) of *R. arenarum* was slightly different between sites (LV: 9.24 ± 0.78; CN: 9.3 ± 0.57; DP: 8.33 ± 0.84), whereas the SVLmet of *B. cordobae* was not different between sites (LV: 27.95 ± 2.9; CN: 29.4 ± 3.1; DP: 29 ± 2.7). The von Bertalanffy growth model

Table 2
Morphological features of adult *R. arenarum* and *B. cordobae* for each site. Mean values ± standard deviation and range (in parentheses) are given.

	Variables	Males			Females		
		LV	CN	DP	LV	CN	DP
Rhinella arenarum	n	8	16	19	13	12	8
	SVL, mean (mm)	89.6 ± 14[A] (62.1–102.3)	88.6 ± 8.6[A] (74.4–105.3)	92.3 ± 5.8[A] (78.83–104.1)	95.4 ± 13.6[A] (60.1–117.3)	94 ± 6.2[A] (85.78–106)	100.2 ± 3.4[A] (96–109.6)
	Weight, mean (g)	87.2 ± 14.9[AB] (60.8–101.9)	74.1 ± 11.5[A] (48.5–90)	95.8 ± 16.3[B] (78–132.6)	89.9 ± 30.9[A] (42–144.5)	91.2 ± 20.6[A] (57.9–119)	101.4 ± 13.1[A] (93–120.8)
	Body Condition, mean	0.14 ± 0.41[A] (0.04–0.21)	0.11 ± 0.33[B] (0.08–0.18)	0.12 ± 0.03[B] (0.09–0.19)	0.11 ± 0.03[A] (0.06–0.17)	0.10 ± 0.02[A] (0.09–0.14)	0.09 ± 0.01[A] (0.08–0.11)
Boana cordobae	n	40	14	25	14	–	9
	SVL, mean (mm)	48.7 ± 4.4[A] (41–58.26)	51.0 ± 3.9[A] (42–55.37)	47.8 ± 3.2[A] (43.26–54)	52.8 ± 5[A] (42–60)	–	52.2 ± 2.6[A] (49–55)
	Weight, mean (g)	8.7 ± 1.8[A] (6.3–13.6)	8.9 ± 1.6[A] (6.2–12)	9.9 ± 2.2[B] (7–15.5)	11.7 ± 3.3[A] (7.5–17.3)	–	12.7 ± 2.1[A] (9.6–16.1)
	Body Condition, mean	0.08 ± 0.016[A] (0.05–0.11)	0.07 ± 0.012[B] (0.05–0.09)	0.09 ± 0.011[A] (0.06–0.11)	0.08 ± 0.017[A] (0.06–0.12)	-	0.09 ± 0.011[A] (0.07–0.10)

LV = Los Vallecitos stream, CN = Cerros Negros stream, DP = decantation ponds.
Data for females of *B. cordobae* were not obtained at CN.
Different letters show inter-site pairwise differences, according to post hoc Bonferroni test $p < 0.05$.
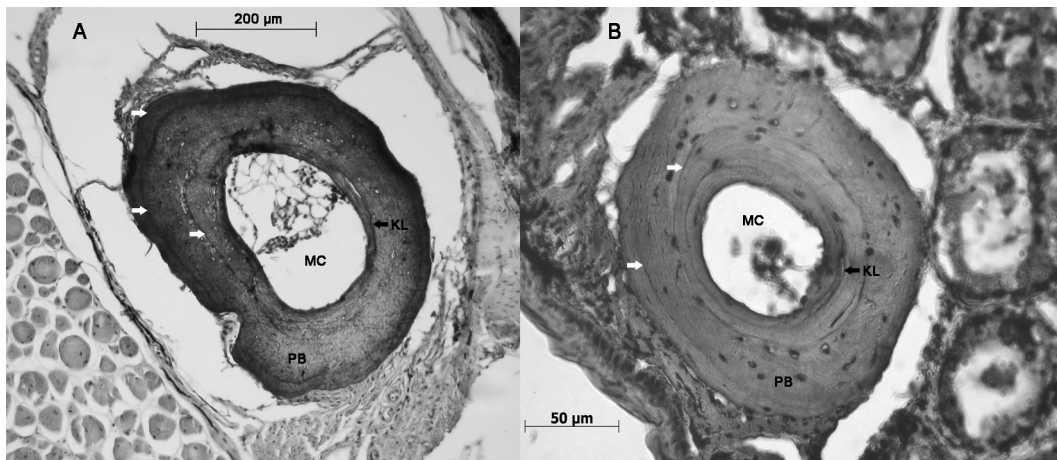
Fig. 2. Examples of phalangeal cross-sections (10-μm thick). MC = medullar cavity; PB = periosteal bone; KL = Kastschenko line; White arrows = LAGs. (A) *Rhinella arenarum* at 10×, and (B) *Boana cordobae* at 40×.

adequately fitted the age/body length data, with $r^2 > 0.842$ in *R. arenarum* and $r^2 > 0.897$ in *B. cordobae* for all sites. Growth models showed a similar shape in both species (Fig. 3).

For *R. arenarum,* the growth coefficient (K) was significantly higher in CN and DP than in LV. However, the estimated maximum asymptotic SVL (SVLmax) was higher in LV than in CN and DP (Table 3). In *B. cordobae,* a similar trend was observed in the estimated parameters by the von Bertalanffy growth model. The growth coefficient (K) of *B. cordobae* was higher in CN and DP than in LV. By contrast, SVLmax was higher in LV than in other sites.

4. Discussion

Mining activity is a cause of large alterations in environments. Many research reports have documented the impact of mining activities on hydrological and chemical properties, but there are few field studies that provide information on the response of anuran amphibians to mining pollution. Furthermore, the evaluation of pollutant effects on

naturally exposed organisms gives information on the environmental health (Hoffman et al., 2003). Simon et al. (2011) suggested that the skeletal age determination in anurans may help environmental load assessments in facilitating further monitoring to assess the effects of environmental pressures on both amphibians and their habitats. In this study, we provide the first data of life history traits and body size of two anuran species inhabiting water surfaces associated with a fluorite mine to promote the understanding of interactions between mining pollution, water quality, and the response of related anuran populations.

Fluoride is a necessary element for all animals and in appropriate doses can promote the growth and development of the skeletal system (Camargo, 2003; Zhao et al., 2013). However, it is also well-recognized worldwide as an important inorganic pollutant in water and a cause of toxicity for numerous aquatic invertebrates, fishes, and amphibians (Camargo, 2003; Goh and Neff, 2003; Chai et al., 2016). The Canadian Council of Ministers of Environment (CCME, 2002.) suggests a maximum limit of 0.12 mg/L of F-content. However, all study sites showed values above this limit. CN and DP showed higher concentrations than

Table 3
Demographic life history traits of *Rhinella arenarum* and *Boana cordobae*. For all estimates, the recently metamorphosed and juveniles of each species were included.

	Variables	Males			Females		
		LV	CN	DP	LV	CN	DP
Rhinella arenarum	n	8	16	19	13	12	8
	Age, mean	3.2 ± 0.84[A]	3.13 ± 0.72[A]	3.37 ± 0.83[A]	3.08 ± 0.98[A]	3.18 ± 0.79[A]	3.3 ± 0.55[A]
	ASM	2	2	2	2	2	3
	Longevity	4	4	5	5	4	4
	PR	3	3	4	4	3	2
	K	0.39 ± 0.06	0.7 ± 0.11	0.77 ± 0.10	0.4 ± 0.06	0.76 ± 0.11	0.62 ± 0.12
	(95% CI)	(0.27–0.5)	(0.49–0.92)	(0.55–0.97)	(0.26–0.51)	(0.53–0.96)	(0.48–0.79)
	SVL max	125.5 ± 7.95	101.6 ± 4.95	102 ± 3.42	135.5 ± 10.7	105.8 ± 4.08	120.1 ± 13.6
	(95% CI)	(104.4–145)	(91.3–113)	(94.8–109.1)	(113–156.1)	(97–114)	(95.7–140)
Boana cordobae	n	40	14	25	14	–	9
	Age, mean	3.37 ± 0.82[A]	3.73 ± 0.65[B]	3.4 ± 0.58[A]	3.76 ± 0.66[A]	–	3.33 ± 0.53[B]
	ASM	2	3	3	2	–	3
	Longevity	5	5	5	6	–	4
	PR	4	3	3	5	–	2
	K	0.3 ± 0.09	0.45 ± 0.19	0.46 ± 0.13	0.32 ± 0.12	–	0.47 ± 0.16
	(95% CI)	(0.18–0.55)	(0.09–0.88)	(0.19–0.78)	(0.08–0.61)	–	(0.04–0.86)
	SVL max	63.2 ± 4.12	57.6 ± 7.15	54.5 ± 4.72	65.8 ± 5.79	–	59.1 ± 5.66
	(95% CI)	(54.8–70.1)	(41.9–73)	(44–64.3)	(50.5–76)	–	(46–70.1)

LV = Los Vallecitos stream, CN = Cerros Negros stream, DP = decantation ponds.

Variables: *n* (sample size); Age Mean ± SE; ASM = age at sexual maturity (years); Longevity (years); PR = potential reproductive (years). K (growth coefficient) and SVL max (maximum asymptotic SVL) estimated by von Bertalanffy growth model and 95% confidence intervals (in parentheses).

Data for females of *B. cordobae* were not obtained at CN.

Different letters show inter-site pairwise differences, according to post hoc Bonferroni test $p < 0.05$.
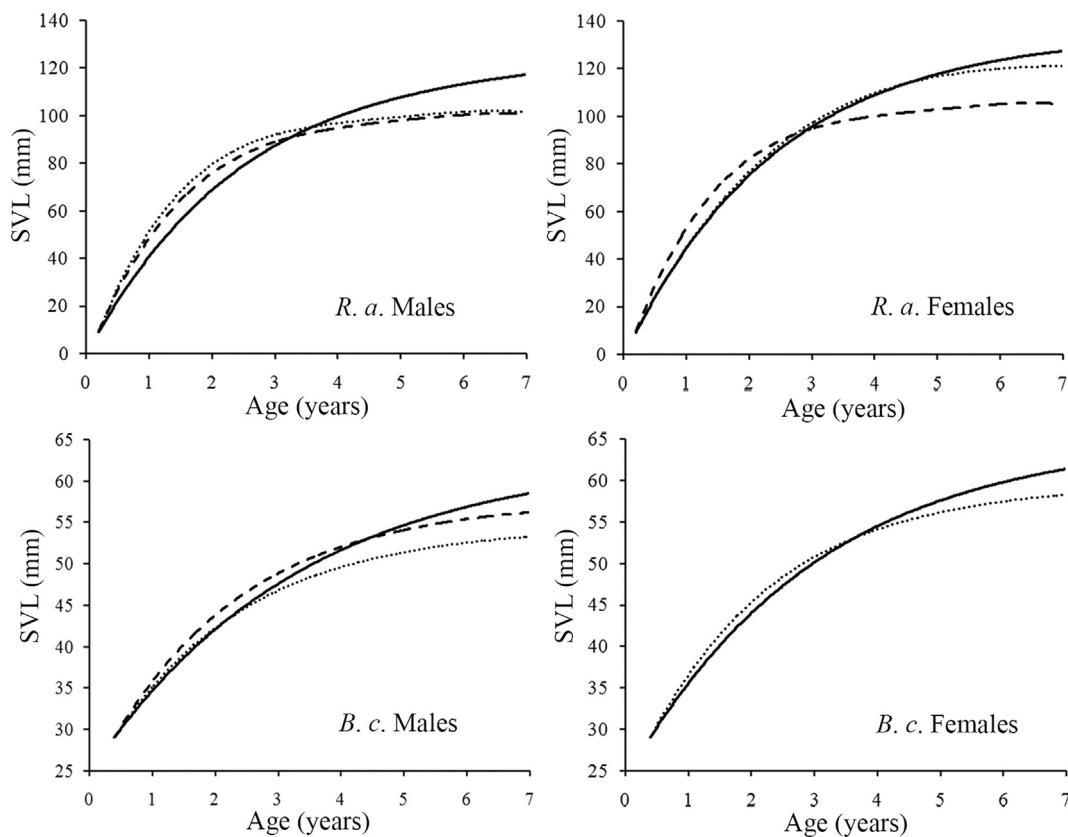
Fig. 3. Relationship between body length (SVL) and age in *Rhinella arenarum* and *Boana cordobae*. Lines represent the von Bertalanffy growth model for each site (LV: solid line; CN: dashed line; and DP: dotted line).

the value suggested by Camargo (2003) for the protection of aquatic organisms (0.5 mg/L) and the suggested by the Argentinean government (1.4 mg/L – Law 24051, 1992). Mean fluoride content registered at DP was 13.8 mg/L. This concentration was elevated, considering that inhibited growth and decreased metamorphosis rates in amphibians have been observed at 10 mg/L NaF and above (Goh and Neff, 2003; Chai et al., 2016). Moreover, preliminary results of microcosm assays show inhibition of growth and lower rate of metamorphosis in *R. arenarum* tadpoles from DP (Otero, unpublished results).

Toxicity levels of many contaminants depend mainly on pH and temperature (Hoffman et al., 2003). In our study, the values of pH differed among sites, thus showing the most basic values (8.61) at DP. This value is near to optimal limits for growth, survival, and normal development reported for amphibians (6 < pH < 9) (García and Fontúrbel, 2003; Addy et al., 2004; Gauthier et al., 2004). It is documented that abrupt exposure of amphibians to basic pH (≈9) produce mortality, whereas slight variations in pH (7 < pH 9 <) generate sublethal effects such as embryonic abnormalities, malformations, and delay in tadpole development (Henao Muñoz and Bernal Bautista, 2011). Moreover, acid or alkaline stress could cause physiological and genetic disorders (Pough and Wilson, 1977). Furthermore, DP showed average temperatures higher than streams. This may be due to the fact that during the industrial process of fluorite flotation, the water is heated to 28 °C. In amphibians, temperature is known not only to affect behavioral performance but also to affect life history traits including growth rates, developmental rates, and adult body size (Berven, 1982).

On the other hand, dissolved oxygen was lower in DP than in the other sites. This is because DP is a lentic environment with no flow, in which the eutrophication process intensifies, thereby causing physical, chemical, and biological changes in water quality (Esteves, 2011). Hypoxic conditions have been related to tadpole mortality and to alter tadpole behavior in low concentrations of dissolved oxygen (Noland

and Ultsch, 1981). However, dissolved oxygen showed normal values for the development of amphibians in all sites (> 60%; Gauthier et al., 2004).

DP also showed an increase in major ions (Na^+, Ca^{++}, Mg^{++}, K^+, Cl^-, $SO_4^=$, and HCO_3^-) and, consequently, an increase in the salinity and conductivity. A high salinity directly impacts freshwater organisms because they require holding an internal osmotic pressure relative to that in the medium. Therefore, when the salt concentration becomes very high, it may result in cellular damage and likely death (Cañedo Argüelles et al., 2013). All ion concentrations exceeding legal standards (CCME, 2002) showed that DP has poor water quality. However, DP is an artificial and isolated site, and if there is no leakage of wastewater, then the impact of mining activity on the natural streams could be limited.

Comparing biometric measures, size did not differ between sites, whereas adult individuals from DP weighed significantly heavier than individuals from streams. BC index has two components, weight and SVL. Only the first component can vary rapidly in response to environmental stress, which may be due to multiple factors such as competition, food shortage, unfavorable climatic conditions, or a poor quality of environment (Reading and Clarke, 1995). Therefore, individuals from DP may have had a higher mean weight because the environment presents a high availability of food resources and warmer temperatures that increase metabolic rate and promote digestion. Thus, the selection of this site could be explained by the reproductive and ecological characteristics of these species. The choice of DP by *R. arenarum* is possibly related to their reproductive strategy, thus preferring lentic ponds with vegetated margins and with direct sunlight incidence as oviposition sites (Bionda et al., 2011). On the other hand, *B. cordobae* could choose DP because it prefers vegetated ponds as reproduction sites, thus favoring the calling site availability (Barrio, 1962). Spear et al. (2009) and Zhelev et al. (2017) used BC as an index of

physiological health and found higher values in less disturbed environments. Similarly, in our study, *R. arenarum* showed a higher BC index in LV. However, *B. cordobae* showed conflicting results with high values of BC in the least and highest disturbed sites. These results are in accordance with those of Polo Cavia et al. (2010) and Pollo et al. (2016), which suggest that an increased BC index does not necessarily imply that individuals are in a better state of health. A habitat could lead to phenotypic variations in natural populations, thereby resulting in apparently healthy features such as a bigger body size that may not directly correlate with the true population's health state. Consequently, the BC of individuals in these sites may not be a good endpoint from which the response of anurans to fluorite mining pollution can be assessed.

Several studies from disturbed environments confirmed modifications of some demographics traits in anuran populations, principally a reduction in their average age (Attademo et al., 2014; Zhelev et al., 2014; Zamora-Camacho and Comas, 2017). The average age recorded in this study was not different between sites in *R. arenarum*, but it varied between sites in *B. cordobae*. However, as reported by Spear et al. (2009), Attademo et al. (2014) and Zhelev et al. (2014) in disturbed sites, delayed sexual maturity, and reduced longevity were observed in both species. These results support the idea that characteristics of anthropically modified environments can affect the reproductive behavior and generational age of anurans (Kutrup et al., 2011), which could lead to long-term population declines.

The timing and conditions at which organisms reach a fixed switch point between stages (e.g., hatching and metamorphosis) in species with complex life cycles are crucial in determining subsequent survival, development, and fitness (Rowe and Ludwig, 1991, Orizaola et al., 2010). Studies conducted on *B. cordobae* (Pollo et al., 2016) in the same study sites reported increased genotoxicity and lower developmental rates of tadpoles in DP. However, our study showed that growth coefficient (K) estimated at the terrestrial stage in both species was higher in altered environments, whereas SVLmax was slightly higher in populations from less disturbed sites. Therefore, populations that inhabit the less disturbed site (LV) maintain a prolonged growth period and consequently reach a larger body size at older ages than populations from sites disturbed by mining activity (DP and CN). These populations reach their maximum length more rapidly and are smaller in size at older ages. These results support the idea that organisms frequently grow at submaximal rates, and that growth can be increased after periods of unfavorable growth, what is known as compensatory growth (Metcalfe and Monaghan, 2001, Mangel and Munch, 2005). Compensatory growth mechanisms require balancing the advantage of rapid growth or development early, with the costs that a faster lifestyle can have on survival and fitness later in the ontogeny (Orizaola et al., 2010). Thus, the delayed sexual maturity, reduced longevity, and lesser RP recorded in the disturbed environment (DP) for both species, but mainly in *B. cordobae*, could be a direct effect of energy spending related to compensatory growth at juvenile stages to compensate for fewer breeding events with a higher reproductive effort in each attempt.

5. Conclusions

Differences obtained in life history traits may be explained by the lack of survival of larger/older individuals or compensatory growth, required to balance the benefits of fast growth with the costs that can have to inhabit high disturbed sites on individual fitness and survival. This plasticity in ontogeny can be the main mechanism by which some species might thrive in unfavorable environments.

The most affected species was *B. cordobae*. This tendency was mainly reflected in the variation of morphometric variables, reduced SVLmax, and the lower RP of their populations in disturbed sites. Therefore, for future studies, it is extremely important to know the biology of sentinel species because not all amphibian species may

respond in the same way to similar disturbances.

This study expands the limited number of studies *in situ* and explores the impacts of fluoride mining on anuran amphibian life history traits. Nevertheless, more long-term research is needed to evaluate the significance of observed effects on future population trends.

Conflict of interest

The authors declare that they have no conflicts of interest.

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