Ecological Indicators 93 (2018) 36–44

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

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

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ARTICLE INFO

Keywords:
Mining
Anurans
Indicators
Life history traits
Environment

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),

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https://doi.org/10.1016/j.ecolind.2018.04.065
Received 22 February 2018; Received in revised form 24 April 2018; Accepted 25 April 2018

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