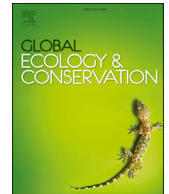




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## Original Research Article

Impact assessment of agriculture and livestock over age, longevity and growth of populations of common toad *Rhinella arenarum* (anura: Bufonidae), central area of ArgentinaClarisa de L. Bionda <sup>a, b, \*</sup>, Selene Babini <sup>a, b</sup>, Adolfo L. Martino <sup>a</sup>, Nancy E. Salas <sup>a</sup>, Rafael C. Lajmanovich <sup>c</sup><sup>a</sup> Ecología-Educación Ambiental, Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional N° 36-km 601, Río Cuarto, Córdoba, Argentina<sup>b</sup> Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina<sup>c</sup> Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional Del Litoral, Ciudad Universitaria El Pozo S/n (3000) Santa Fe, Argentina

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## ABSTRACT

The central area of Argentina has been greatly affected mainly due to urbanization and intensive agriculture (corn, soybean and cattle). Age, longevity and growth were determined in populations of the common toad, *Rhinella arenarum*, from urban and agricultural sites in the central area of Argentina. Five sites in Río Cuarto (33°05'52.95" S - 64°26'02.99" W, 471 m a.s.l.; Córdoba Province, Argentina) with different degree of anthropic disturbance were selected to evaluate the impact of urbanization and agriculture on common toad populations as sentinel species. The selected sites were chosen according to intensive to moderate agricultural and livestock activity, low influence of agricultural and an intensive to moderate urban influence. A total of 114 adults (males and females) were sampled. Skeletochronology was used to estimate toad's growth and age, which relies on the analysis of the annual Lines of Arrested Growth or LAGs in bones. Size, age and growth reductions occurrence in intensive agricultural systems could suggest long term impacts on fitness. In addition, intensive urban activity also affects life history of the population of *R. arenarum*, although to a lesser extent than the agricultural-livestock activity.

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## 1. Introduction

Degradation and fragmentation of natural habitats due to agriculture and urbanization has been shown to have deleterious effects on amphibian populations (Young et al., 2004; Collins and Crump, 2009; Hamer and McDonnell, 2008, 2010; Collins and Fahrig, 2017). The central area of Argentina has been greatly affected by livestock and agricultural practices (Rossi, 2006). Recent evidence has revealed that deterioration of agricultural ponds in this region could have altered the richness and abundance of amphibian communities as well as have affected the mean morphological characteristics and population demographics of several representative amphibian anuran species (Peltzer et al., 2006; Bionda et al., 2012a,b; 2013a,b; Babini

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et al., 2015a,b; 2016). In addition, cellular abnormalities (Caraffa et al., 2013; Pollo et al., 2015) and morphological abnormalities in adults and tadpoles (Peltzer et al., 2011; Bionda et al., 2012b; Babini et al., 2015a) were recorded.

Aquatic systems in urban landscapes generally receive contaminants from different sources such as drainage of sewage, waste disposal and or agricultural runoff. Natural habitats modification by anthropogenic activities could have a negative impact over amphibian species that may result into local extinction of populations (Mckinney, 2002; Löfvenhaft et al., 2004; Bionda et al., 2013a; Babini et al., 2015a; Green and Bailey, 2015; Zhelev et al., 2017). However, some amphibian species are relatively resilient to urbanization as a consequence of habitat generalism, or have specific life history traits that render them less vulnerable to habitat modification in urbanized areas (Rubbo and Kiesecker, 2005; Hamer and McDonnell, 2008, 2010).

The common South American toad, *Rhinella [Bufo] arenarum* (Hensel, 1867), belongs to the family Bufonidae and has been assessed at Least Concern (LC) according to IUCN Red List of Threatened Species (IUCN, 2015). This toad has a wide distribution in South America and inhabits in a wide range of habitats. In addition, it is a domestic species, due to their frequent presence in the surroundings of houses. Thus, habitat diversity and large population size make this toad a particularly conspicuous species. Also, sensitivity of this anuran native species was proven in several studies (Herkovits and Pérez-Coll, 1993; Howe et al., 1998; Vera Candiotti et al., 2010; Bosch et al., 2011; Lajmanovich et al., 2014) and therefore it is used as a bioindicator organism, especially for aquatic ecosystems management and biomonitoring (Venturino et al., 2003). Several studies suggest that this species is sensitive to the particular conditions and environmental quality given in intensive agricultural systems, whether in their aquatic or terrestrial stages of life cycle (Bionda et al., 2011; Bionda et al., 2012a; 2013a; Babini et al., 2015a,b; 2016; Pollo et al., 2015). However, information about the potential impact that agriculture practices will have over growth and longevity of anuran amphibian still remain largely unknown. In addition, the demographic life-history traits of common South American toad have been little investigated (Echeverria and Filipello, 1990; Bionda et al., 2015). Demographic life-history traits tend to evolved in response to environmental conditions, in addition, some parameters as longevity and age at first reproduction often interact via trade-offs (Sinsch et al., 2007a b; Spear et al., 2009). In this sense, we could ask the following questions ¿Demographic life-history traits of common South American toad such as morphometry, growth rate, longevity and size at sexual maturity can be indices of population health in sites with anthropic disturbance? ¿There is trade-off between such life-history traits in sites with anthropic disturbance?

Therefore, the aim of this study was to determine life-history characteristics: individual age and growth, size and age at maturity, longevity and age structure of *R. arenarum* populations inhabiting agroecosystems and urban sites with varying degrees of alteration by using skeletochronological analysis.

## 2. Material and methods

### 2.1. Study areas

The study area is located in the pampa plains of central Argentina. Moderately undulating plains and a temperate climate are characteristics of the area. Mean temperature is 23 °C in January and 6 °C in July. Mean annual temperature is 18 °C. Absolute maximum and minimum temperatures are 40 °C in summer and –8 °C in winter (Bionda et al., 2015; Babini et al., 2017). The region is characterized by rainy and dry seasons, with rains typically starting in October and continuing through the warm months until March with a mean annual rainfall of 800–1000 mm (Gatica et al., 2012).

Sampling was carried out in the surroundings of the city of Río Cuarto (33°07'25.71''S, 64°20'56.52''W; 434 m elevation, Córdoba province, Argentina). Five sites were selected with different degree of anthropic disturbance. Three of these sites are used for agricultural-livestock activity placed on east Río Cuarto city: **C1** (33°05'52.95' S - 64°26'02.99' W, 471 m elevation): 9 ha, rural landscape, the reproduction area consists in pond close to the crop (50 m distanced), used daily by cattle to drink water (11.3 ± 3.1 livestock per hectare per month, Bionda et al., 2011); **C2** (33°06'12.83' S - 64°25'30.26' W, 467 m elevation): 10 ha, rural landscape, the reproduction area consists in a pond to the crop (60 m distanced), not used by cattle to drink water; **C3** (5 ha, 33°05'39.21' S - 64°25'56.93' W, 468 m elevation): 43 ha, rural landscape, amphibian reproduction area consists in a pond away from the crop (300 m distanced), used by livestock to drink water discontinuously (26.3 ± 3.5 livestock per hectare per month, Bionda et al., 2011). The last two sites are urban sites with different degrees of anthropic perturbation: **UL** (33°06'21.00' S - 64°22'36.83' W, 467 m elevation): 20 ha, an urban lake used for fishing and recreation activities (Mancini et al., 2000; Bionda et al., 2012 b); **SM** (33°06'41.86' S - 64°18'15.83' W, 428 m elevation): 10 ha, consists in a pond not affected by crops or cattle, located in the National University of Río Cuarto campus. Productive activities are not practiced in a radius of 5 km. All ponds in the study are temporary, with the exception of UL that is a permanent pond. During sampling season the C1, C2 and C3 sites were surrounded by soybean crops.

We classified the sites according to land use degree by agricultural and livestock activity. Thus, we refer to C1 as intensive agricultural and livestock activity with the highest disturbance degree, followed by C3 subject to a moderate agricultural and livestock activity, and C2 with intensive agricultural and no cattle. Finally, for SM and UL, urban influence in UL was considered more exhaustive than in SM site.

### 2.2. Environmental parameters

We recorded in each site: water temperature (TA<sub>w</sub>; half of the water column and with a mercury thermometer) and air temperature (TA<sub>a</sub>; 100 cm soil with a mercury thermometer), pH water (approximately 5 cm in depth and with pH indicator

strips) and depth of the ponds (PL, with tape measuring). The water samples are analyzed for standard water quality parameters as levels of nitrate (N) and phosphorus (P) using the colorimetric methods and visible spectrophotometry, respectively (USEPA, 1994).

### 2.3. Data collection

We surveyed all five sites from September 2008 to April of 2009. During these months, all sites were surveyed on the same day, with a frequency of two weekly visits, preferably after registering rainfall. Individuals of *R. arenarum* are hand-captured during surveys at the pond shore. Snout-vent length (SVL; mm) we measured for each individual with a Vernier caliper (0.01 mm precision) and mass (P; g) with a digital balance scale (0.01 g precision). Also for each individual captured we estimated the body condition (BC) according to [Bagenal and Tesch \(1987\)](#). Adults are distinguished from juveniles by secondary sexual characters: presence of vocal sacs and nuptial pads. Sex is determined by secondary sexual characters as vocal sacs and nuptial pads, as well using coloration: males with brownish or greenish back; females greyish or light brown back, scattered with large dark or dark brown blotches ([Cei 1980](#)). Moreover, only males when taken in amplexus produce a release call ([Di Tada et al., 2001](#)). A toe of the forelimb is clipped at the level of the penultimate phalanx and stored in 70% ethanol until being processed for skeletochronological analysis. Antifungal or antibacterial and healing agent is added at the puncture to prevent infections and each individual is released 2 h later at the site of its capture. The toe clip is used as a batch mark to prevent resampling ([Bionda et al., 2011](#); [Bionda et al., 2013 a](#)). Skeletochronology method relies on the analysis of annual growth zones alternating with dense lines of arrested growth (LAGs) in bones. LAGs formation is ultimately controlled by a genetically based, circannual rhythm which is synchronized with and reinforced by seasonal cycles such as hibernation in temperate zone species ([Smirina 1994](#); [Alcobendas and Castanet 2000](#); [Morrison et al., 2004](#); [Sinsch et al., 2007ab](#); [Marangoni et al., 2009](#)).

### 2.4. Laboratory procedures

Followed the standard methods of skeletochronology ([Sinsch et al., 2001](#); [Bionda et al., 2015](#); [Sinsch, 2015](#); [Otero et al., 2017](#)): (1) fixation in formol 4% (at least 12 h), (2) decalcification of bones (5–10% formic acid, 24 h), (3) paraffin embedding, (4) cross sectioning of the diaphysis at 8–10  $\mu\text{m}$  using a Leyca rotation microtome, (5) staining with Ehrlich's haematoxylin (2 min, sample), (6) light microscopic count of the number of lines of arrested growth (=LAG) using a Zeiss Axiophot-Axiolab, (7) documenting the most informative cross sections with an AxiocamHRc Zeiss digital camera using Axio Vision 4.3. The number of lines of arrested growth (LAGs) in each section is counted in the periosteal part of the bone by at least two authors (CB, AM). We defined age as the number of LAGs counted. The criteria for incomplete or faint lines, or double lines (two very closely adjacent growth marks), as well as LAGs potentially lost due to endosteal resorption is applied according to [Tsiora and Kyriakopoulou-Sklavounou \(2002\)](#), [Guarino et al. \(2008\)](#), [Ma and Lu \(2009\)](#), [Li et al. \(2010\)](#) and [Bionda et al. \(2015\)](#).

Growth is estimated using the [von Bertalanffy's \(1938\)](#) equation ([Üzüm and Olgun, 2009](#); [Liao and Lu, 2010a,b](#); [Marangoni et al., 2012](#); [Bionda et al., 2015](#); [Otero et al., 2016, 2017](#)). We used the following equation:  $SVL_t = SVL_{max} - (SVL_{max} - SVL_{met}) e^{-K(t-t_{met})}$ , where  $SVL_t$  = average SVL at age  $t$ ,  $SVL_{max}$  = average maximum SVL or asymptotic size (mm),  $SVL_{met}$  = average SVL at metamorphosis,  $t$  = number of growing season experienced (age),  $t_{met}$  = proportion of the growing season until metamorphosis (age at metamorphosis), and  $K$  = growth coefficient (shape of the growth curve). The von Bertalanffy growth model is fitted to the empiric age-size data using the least square procedure (e.g. [Cogălniceanu and Miaud, 2002](#); [Çiçek et al., 2011](#); [Bionda et al., 2015](#); [Otero et al., 2016, 2017](#)).  $SVL_{max}$  and  $K$  are given with the corresponding 95% confidence interval. The average SVL at metamorphosis ( $SVL_{met}$ ) for each sites is obtained from the hand-captured of recently metamorphosed ( $n = 20$ ) at the pond shore. In addition, half-life as  $\text{Ln}2/K$ , that is to say, in this time the toad will be halfway between  $SVL_{met}$  and  $SVL_{max}$ , and the time needed for a toad to have reached the 0.99% of  $SVL_{max}$  as  $t_{0.99} = 1/k \ln((SVL_{max} - SVL_{met}) / ((1 - 0.99)SVL_{max}))$  are estimated following to [Cailliet et al. \(2006\)](#). In addition, annual growth rates (R) by derivation the von Bertalanffy equation as  $R = dSVL/dt = K*(SVL_{max} - SVL_t)$ , where  $SVL_t$  is body size at age ( $t$ ) is estimated following to [Liao and Lu \(2012\)](#), [Otero et al. \(2016, 2017\)](#).

Demographic variables are calculated according to [Leskovar et al. \(2006\)](#), [Marangoni et al. \(2012\)](#), [Bionda et al. \(2015\)](#) and [Otero et al. \(2016, 2017\)](#): (1) age at maturity: minimum number of LAGs counted in breeding individuals; (2) longevity: maximum number of LAGs counted in reproductive individuals; (3) potential reproductive lifespan: difference between longevity and age at maturity; (4) size at maturity: average snout-vent length of all first breeders with the minimum number of LAGs; (5) modal lifespan: mode of age distribution.

We used the significance level of  $P < 0.05$  in all tests. Descriptive statistics are given as mean  $\pm$  standard deviation. We used parametric tests for sets SVL data because the data met the assumptions (normality and homogeneity of variance). Moreover, related to age data differed from a normal distribution; we used the non-parametric Mann-Whitney  $U$  test or Kruskal-Wallis test for significant differences between the sexes and among sites, respectively. Growth parameters are statistically compared based on the range of the  $CI_{95\%}$  interval. Tests are performed using the statistical packages STATISTICA 6.0 (Statsoft Inc. USA, 2001).

### 3. Results

#### 3.1. Water quality parameters

There were no significant differences in habitat variables between sites ( $P > 0.05$ ; Table 1). The nitrate median concentrations were the greater at the three agro-ecosystems; mainly at C2 site recording an extremely high value of  $52.5 \pm 53.1$  mg/L. The highest mean concentration of phosphorus was detected in C2 ( $3 \pm 3.1$  mg/L), in the rest of the sites reported similar values, although slightly above the value admitted. Values for pH at the UL and SM sites showed neutrality though certain variation, while in C1 and C3 sites varied into the alkaline range and even exceeded the 9.0. Values for pH with relatively small variation around neutrality were at C2 site.

#### 3.2. Body length

We captured a total of 24 individuals of *R. arenarum* at C1 (6 juveniles, 15 males and 3 females), 20 individuals at C2 (1 juvenile, 16 males and 3 females), 23 individuals at C3 (5 juvenile, 15 males and 3 females), 17 individuals at UL (14 males and 3 females) and 43 individuals at SM (1 juvenile, 28 males and 14 females). Due to the fact that the field sampling method relied upon male mating calls, a greater number of males than females were captured at all sites. Sample numbers for females was 3 individuals for all sites, with the exception of SM with 14 females. Hence statistical comparisons were not deemed appropriate. Body condition indices varied significantly between sites for males ( $t$ -test = 13.7;  $P < 0.01$ ). The population at C3 had the lowest index (BC = 0.0100) suggesting that they were underweight related to body length. The C1 population also had a low condition index (BC = 0.0106) whereas C2 and UL presented indices with intermediate values (BC = 0.0108 and 0.0109, respectively). Finally, SM present the best index values of all sites (BC = 0.0111). The SVL of the individuals in all sites differed significantly between sexes ( $t$ -test,  $P < 0.05$ ), with the exception of C1 and UL ( $t$ -test,  $P > 0.05$ ); females were larger than males in all sites. The SVL of males in SM differs significantly from other sites ( $t$ -test,  $P < 0.05$ ) except with C2 ( $t$ -test,  $P > 0.05$ ). The average SVL for females in C1 differs significantly from SVL recorded in C2 and C3 ( $t$ -test,  $P < 0.01$ ). Regarding the sexual maturity SVL, differences are found between C1 and C2 ( $t$ -test,  $P < 0.05$ ) and, C2 and C3 ( $t$ -test,  $P < 0.05$ ). The size distribution of males and females did not differ from a normal distribution (Shapiro-Wilk test,  $P > 0.05$ ).

There was a highly significant correlation between SVL and age in males of agroecosystem sites (C1:  $r = 0.86$ ; C2:  $r = 0.60$ ; C3:  $r = 0.70$ ;  $P < 0.005$  all cases). Whereas in females, there was only a highly significant correlation between SVL and age in the individuals of SM site ( $r = 0.71$ ;  $P < 0.01$ ).

#### 3.3. Age structure

All adults studied showed well-defined lines of arrested growth (LAGs) in the periosteal bone layer, which allowed assessing the individual age (Fig. 1). Endosteal resorption was present in 18% of the sample (e.g. Fig. 1 E), but the resorption did not hamper age determination because the first LAG was never completely reabsorbed. In many cases the outer most lines were closely adjacent, but in these cases, at the insertion site of the phalangeal ligament allows to discern the peripheral LAGs and to reliably count them (Fig. 1 F). Double LAGs were rarely observed and counted as a single LAG (Fig. 1 D). Juveniles captured for all sites, reported between 45 and 70 mm of SVL and were captured between the months of February to April 2009. For all of these individuals not LAGs in the periosteal bone were observed (Fig. 1 A and B).

The demographic life history traits are summarized in Table 2. Mean age significantly differ among sites for males (Chi-Square = 19.1,  $df = 4$ ,  $P = 0.0008$ ), but did not significant difference for the mean age of females (Chi-Square = 5.2,  $df = 4$ ,  $P = 0.2664$ ). The mean age was higher in both sexes at the sites of UL and SM, and the lowest average age was recorded in agroecosystem sites, being C1 the youngest them (Table 2). Similarly, the highest modal age was registered in UL and SM, and the lowest modal age was recorded in agroecosystem sites (Table 2). The greater longevity and reproductive potential was recorded in SM, followed by UL and C2. The minimum number of LAGs counted in reproductive individuals for all sites was 1

**Table 1**

Average and standard deviation of the environmental variables by site.

	TA <sup>a</sup>	Tag <sup>b</sup>	pH <sup>c</sup>	PL <sup>d</sup>	N <sup>e</sup>	P <sup>f</sup>
C1	22.7 ± 9.5	20.7 ± 6.8	8.3 ± 1.0	17.5 ± 6.5	13 ± 3.5	2 ± 0.3
C2	21.3 ± 6.5	22.3 ± 4.4	7.2 ± 0.4	17.9 ± 9.3	52.5 ± 53.1	3 ± 3.1
C3	20 ± 4.4	22.2 ± 3.9	8.3 ± 1.1	16.5 ± 9.9	15 ± 0.0	2 ± 0.6
UL	22 ± 6.5	24 ± 2.5	6.5 ± 1.2	14.8 ± 6.5	10 ± 0.1	1.7 ± 0.1
SM	22.5 ± 2.4	25.5 ± 2.4	7 ± 2.4	25.2 ± 8.0	10 ± 0.0	1.8 ± 0.1

<sup>a</sup> Air temperature (C).

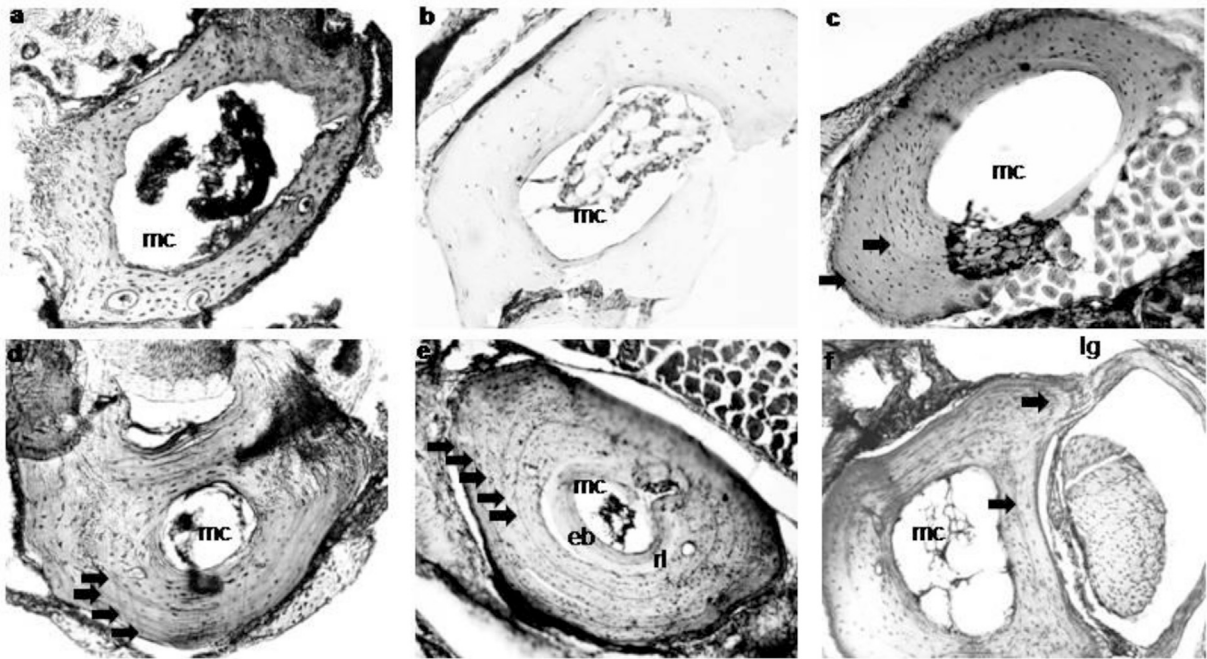
<sup>b</sup> Water temperature (C).

<sup>c</sup> Water pH.

<sup>d</sup> Water depth (cm).

<sup>e</sup> Nitrate (mg/L).

<sup>f</sup> Phosphorus (mg/L).



**Fig. 1.** Examples of cross sections of phalanges of adult *R. arenarum*. (a–b) Juveniles, 0 LAGs, SVL = 64.24 mm and SVL = 64.76 mm, respectively; (c) male, 2 LAGs, SVL = 103.62 mm; (d) female, 4 LAGs, SVL = 107 mm; (e) male, 5 LAGs, SVL = 96.88 mm; (f) female, 2 LAGs, SVL = 100.74 mm. Arrows indicate line of arrested growth (LAG). eb: endosteal bone. mc: medullary cavity. rl: resorption line. lg: ligament.

LAG in both, males and females, except for females C2, C3 and UL. However, the males of the C1 and C3 sites, reach sexual maturity at size lower than other males. C2 males are larger to reach maturity, followed by males of UL and SM. Since single value from size at maturity in SM and UL could not be performed statistical comparisons with other sites. For the rest, there were significant differences in the size at sexual maturity between males C2 and C3, and between males C1 and C2 ( $P < 0.05$  in both cases).

### 3.4. Growth pattern

The SVL mean of recently metamorphosed ( $SVL_{met}$  for von Bertalanffy's model) was: C1:  $12.07 \pm 1.51$  mm; C2:  $12.86 \pm 1.02$  mm; C3:  $13.8 \pm 1.51$  mm; UL:  $12.55 \pm 0.52$  mm and SM:  $11.45 \pm 0.59$  mm. The [Von Bertalanffy \(1938\)](#) growth

**Table 2**  
Demographic life history traits of *R. arenarum* for the sampling sites.

Sites	Sex	n	Age mean (LAGs)	Mode (frequency)	Age at sexual maturity (LAGs)	Longevity (LAGs)	Potential reproductive lifespan (years)	SVL at sexual maturity (mm)	SVL Mean (mm)
C1	Male	15	$1.6 \pm 0.7$ (1–3)	1 (50%)	1	3	2	$80.6 \pm 12.7$	$93.8 \pm 11.9$ (71.3–110.4)
	Female	3	$1.7 \pm 0.6$ (1–2)	2 (66.7%)	1	2	1	97.2	$98.3 \pm 1.8$ (97.2–100.4)
C2	Male	16	$2.2 \pm 0.9$ (1–4)	2 (50%)	1	4	3	$97.2 \pm 8.8$	$101.5 \pm 7.9$ (88–117)
	Female	3	$2.7 \pm 1.2$ (2–4)	2 (66.7%)	2	4	2	$112.1 \pm 4.5$	$112.9 \pm 3.5$ (108.9–115.3)
C3	Male	15	$1.9 \pm 0.8$ (1–3)	1 (40%)	1	3	2	$83.1 \pm 9.1$	$92.3 \pm 12.1$ (72.4–111.2)
	Female	3	$2 \pm 1.4$ (2–3)	3 (66.7%)	2	3	1	110	$110.5 \pm 4.28$ (106.5–115)
UL	Male	14	$2.9 \pm 0.9$ (1–4)	3 (50%)	1	4	3	90	$93.5 \pm 5.5$ (85.1–103.4)
	Female	3	$3.3 \pm 1.2$ (2–4)	4 (66.7%)	2	4	2	100.4	$100.8 \pm 6.0$ (95–107)
SM	Male	28	$2.7 \pm 0.7$ (1–5)	3 (60.7%)	1	5	4	90.8	$100.6 \pm 7.7$ (89.4–111.9)
	Female	14	$2.9 \pm 0.9$ (1–4)	3 (50%)	1	4	3	82.6	$108.4 \pm 9.9$ (82.6–123.3)

model correctly fitted the age/body-length relationship (Fig. 2;  $R^2$ : C1 = 0.89, C2 = 0.94, C3 = 0.90, UL = 0.96, SM = 0.97). The growth coefficient (K) was greater in the SM site, whereas C2 and C3 presented coefficient with intermediate values and, C1 and UL sites had the lowest coefficients (Table 3). However, these differences were not statistically significant because the 95% confidence intervals of K were overlapped in all sites (Table 3). SM with the higher K reaches faster to  $\ln 2/K$  and  $t_{0.99\%}$ ; while that C1 was the lowest K (Table 3). The growth curve for SM is different from the other sites, rapid growth in the early years is recorded, and later the growth slows down, reaching its asymptote. In C2 the growth curve is similarly to SM, while C1, C3 and UL a slower growth is observed (Fig. 2). In Table 4, the annual growth rates (R) for each sites is showed. Coinciding with that observed for other parameters annual growth rates (R) of SM and C2 indicates that most of the growing occurs in the first year of life; something similar to although less pronounced is observed for C3. In all cases, a slowing in growth rates year after year is observed, except for SM, with a slight increase in growth rates in the fourth and fifth year (Table 4). The modeled maximum SVL ( $SVL_{max}$ ) were higher than the maximum SVLs recorded in this study for all sites (Table 2 and 3). However, is worth mentioning that at sites C3 and SM, the estimated maximum asymptotic SVL were narrowly higher than the maximum SVLs recorded in this study. The modeled maximum SVL of males did not vary significantly among sites because the 95% confidence intervals of  $SVL_{max}$  were overlapped in all sites (Table 3).

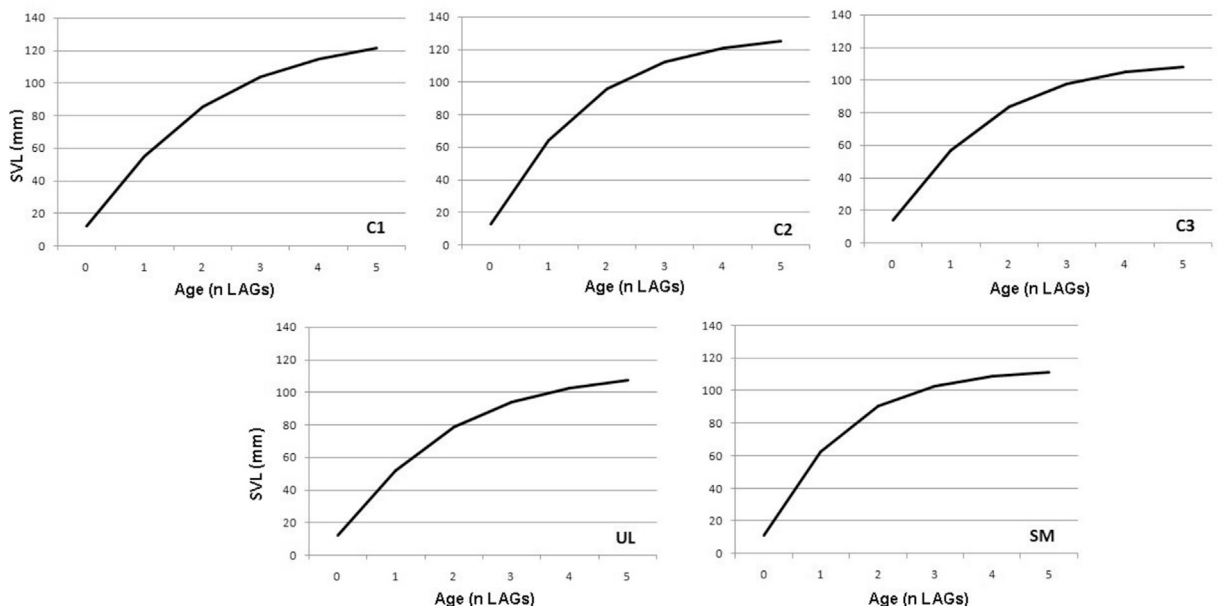
#### 4. Discussion

On C1 and C3, alkaline pH values (8.3–8.4) were recorded. The pH values for 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 physiological and genetic disorders in early stages (Freda and Dunson, 1984; Pough and Wilson, 1977). Median concentrations of total dissolved phosphorus and nitrate were greater at the agroecosystem ponds. Additionally, these values were similar or

**Table 3**

Growth parameters (mean  $\pm$  SE) in *Rhinella arenarum* estimated by von Bertalanffy's growth model for all sites.

	C1	C2	C3	UL	SM
Asymptotic size ( $SVL_{max}$ in mm) ( $CI_{95\%}$ )	131.47 $\pm$ 13.97 (103.86–159.08)	129.61 $\pm$ 9.87 (109.83–149.39)	111.98 $\pm$ 8.84 (94.42–129.53)	113.39 $\pm$ 8.47 (96.25–130.52)	113.57 $\pm$ 5.05 (103.43–123.71)
Growth coefficients (K) ( $CI_{95\%}$ )	0.50 $\pm$ 0.12 (0.27–0.74)	0.66 $\pm$ 0.14 (0.38–0.94)	0.67 $\pm$ 0.13 (0.42–0.93)	0.57 $\pm$ 0.14 (0.28–0.87)	0.78 $\pm$ 0.15 (0.48–1.1)
r	0.89	0.94	0.90	0.97	0.97
Variance Explained ( $r^2$ )	78%	89.6%	80.6%	93.6%	94.6%
$\ln 2/K$	1.38	1.10	1.08	1.24	0.94
$t_{0.99\%}$	9.02	6.82	6.63	7.79	5.77



**Fig. 2.** Age-size relationship of *R. arenarum* in five sites sampled. Lines represent the von Bertalanffy models of growth: (C1)  $SVL [mm] = 131.47 \text{ mm} - (131.47 \text{ mm} - 12.07 \text{ mm}) \cdot e^{(-0.5088 \cdot (LAGs - 0.128))}$ ; (C2)  $SVL [mm] = 129.61 \text{ mm} - (129.61 \text{ mm} - 12.86 \text{ mm}) \cdot e^{(-0.6653 \cdot (LAGs - 0.126))}$ ; (C3)  $SVL [mm] = 111.98 \text{ mm} - (111.98 \text{ mm} - 13.8 \text{ mm}) \cdot e^{(-0.6752 \cdot (LAGs - 0.149))}$ ; (UL)  $SVL [mm] = 113.39 \text{ mm} - (113.39 \text{ mm} - 11.91 \text{ mm}) \cdot e^{(-0.5767 \cdot (LAGs - 0.127))}$ ; (SM)  $SVL [mm] = 113.57 \text{ mm} - (113.57 \text{ mm} - 11.45 \text{ mm}) \cdot e^{(-0.7875 \cdot (LAGs - 0.113))}$ .

**Table 4**  
The Annual Growth Rates (R) in *Rhinella arenarum* for all age class and for all sites.

Age (year)	R (mm/year)				
	C1	C2	C3	UL	SM
0	59.70	77.05	66.27	58.55	79.65
1	23.5	21.41	19.48	13.49	17.75
2	15.4	20.51	10.51	13.93	9.86
3	11.17	13.38	7.37	10.24	9.40
4		13.79		11.22	12.26
5					13.01

higher than those reported previously in other studies at agricultural systems (Spear et al., 2009; Babini et al., 2015 a; 2017). Also, at C2 site was recorded an extremely high value in phosphorus and nitrate. Ecological and toxicological effects of phosphorus and nitrogen pollution in aquatic ecosystems have been tested in several studies (Carpenter et al., 1998; Camargo and Alonso, 2006). Levels above 1 mg/L of phosphorus in water at rivers and streams, can consider this element as a contaminant for human by World Health Organization (WHO, 2006). For Spear et al. (2009) phosphate criterion for the protection of aquatic life should be < 1  $\mu$ M. Nitrate and phosphorous are the main components of the agrochemicals utilized on crops and are indicators of livestock activity (Perdomo et al., 2001; Spear et al., 2009). For Spear et al. (2009) level of excess of some compounds generally is associated with increased application of chemical, in addition to increased production of legume crops (e.g. soy). Therefore, the elevated nitrate levels reported in the present paper may be associated with a mixed agricultural due to extensive or intensive practice. Although this site has no cattle around its ponds and fields only are dedicated to culture, it should be mentioned that these ponds receive runoff inputs from various crops systems, which can increase the concentration of certain compounds if there have been recent rainfall.

Skeletochronological age assessment is an essential tool for investigations on demographic and growth traits (e.g. Marunouchi et al., 2000; Martino and Sinsch, 2002; Sinsch et al., 2007a b; Sinsch, 2015). It has been successfully used on a variety of species from temperate, tropical and subtropical areas (Sinsch, 2015). Moreover, it is a nonlethal destructive technique that can be performed on the phalanges, without sacrificing the animals (Guarino et al., 2008; Bionda et al., 2015; Sinsch, 2015). Assessment the age structure of populations and longevity of individuals might be very useful for understanding the species dynamics and to determine the causes of population instability (Driscoll, 1999).

Several studies confirmed the formation of one LAG per year equivalent to the number of hibernations of each individual (Smirina, 1994; Driscoll, 1999; Guarino et al., 2008; Matsuki and Matsui, 2009; Marangoni et al., 2012). In addition, annual periodicity of LAG formation is more pronounced when the climate in which inhabit the organisms studied is characterized by a marked seasonal variation and the organisms has seasonal activity pattern (Guarino et al., 2011). Although *R. arenarum* are potential continuous breeders (Quiroga and Sanabria, 2012), the monitoring of these populations since 2006 indicates a seasonal activity pattern (Bionda et al., 2011, 2013a; 2015; Babini et al., 2018). However, the accuracy in the counting of LAGs decreases considerably in individuals older than 8 years (Sinsch, 2015). This toad species has longevity less than 8 years (Echeverria and Filipello, 1990; Bionda et al., 2015); however, *R. arenarum* reproduces when winter ends, and therefore the last (outermost) LAG and the perimeter of the phalange were very close together due to the absence of substantial bone growth. In agreement with Spear et al. (2009) in a study with *L. catesbeianus* and Bionda et al. (2015) for this same species, in most individuals analyzed, distance between two successive LAGs decreased from the ones in the deepest cortex to the ones at the phalange periphery. This appears as a normal sequence in conformity with a gradual decrease of growth rate in amphibians (Sinsch, 2015). In accordance with some authors (Caetano and Castanet, 1993; Guarino et al., 2008, 2011; Sinsch, 2015) a marked decrease of growth rate occurs after the presumed attainment of sexual maturity. Thus, the individual allocates more energy into reproduction instead of bone growth; therefore the successive LAGs tend to be closely to the periphery of periosteal bone. Besides, for these authors the age at which the LAGs tend to approach, is an estimator age of sexual maturity. In these cases and for a more accurately estimate of the age of individuals, for closely adjacent LAGs, at the insertion site of the phalangeal ligament is possible to discern the peripheral LAGs, as well as the identification of double and false LAGs in others (Bionda et al., 2015).

LAGs in the periosteal bone were not recorded for juveniles captured (SVL 45–70 mm), for all sites. Driscoll (1999) in a study with frog species *Geocrinia alba* and *G. vitellina*, juveniles appear not to form their first LAG in the dry season or winter following metamorphosis. Instead, ossification in phalanges may occur later but before their second spring. In our case, periosteal bone is observed, but not LAGs. These were surely recruited from the same breeding season of 2008 but they were captured between February to April of 2009. Variable SVL registered in juvenile (about 70 mm in some cases), is consistent with the fact that in general, populations are growing between 60 and 80 mm (R, Table 4) in its first year.

In our study, sexual size dimorphism is female-biased in all sites. It is consistent with the studies of Echeverria and Filipello (1990), Bionda et al. (2011) and Bionda et al. (2015), also conducted with populations of *R. arenarum*. Female-biased sexual size dimorphism is commonly observed in amphibians (Shine, 1979), it would be expected an increase of egg production, either to produce larger eggs, or to lay more eggs (Cummins, 1986). In addition, Echeverria and Filipello (1990) and Bionda et al. (2015) found that the size at maturity of males is about 90–100 mm, which agrees with our results except as noted in C1 and C3 with considerably lower SVL values (see Table 2).

Several studies suggested that longevity is an important factor accounting for differences in body size between the sexes (Huang et al., 2013). However, our results are not consistent with those authors, since in some cases the females were larger than males but long-lived equal than them. Also noteworthy is that the modal age was higher in females in some sites; however, in such sites no difference in size between the sexes was noted. Therefore, we could say that in our samples, longevity is not an important factor accounting for differences in body size between the sexes.

Females often delay maturity up to one year compared with the males and allocate the energy to somatic growth, thus reaching sexual maturity at larger sizes (Marangoni et al., 2012). Because of that a threshold size could be mandatory for attaining maturity (Hemelaar, 1988). Camargo et al. (2008) also suggest that there is a trade-off growth vs reproduction in females. They hypothesized that younger females allocate energetic resources in growth instead of reproduction (an investment to increase fitness in future reproduction); whereas older females use all the available resources in egg maturation, which is the growth slows down. To that respect, minimum age at sexual maturity was the same for males and females (1 LAG), except for females C2, C3 and UL (2 LAGs). In fact on such females was registered a greater mean on it SVL. Nevertheless, absence of females of one LAG in C2, C3 and UL would not allow us to ensure that females are sexually immature at this age. In this regard, early maturity often implies a short lifespan, while late maturity can be frequently observed in long-lived amphibian species (Smirina, 1994; Guarino et al., 2010; Oromi et al., 2012). This principle could correspond with our samples, considering that in those sites where females mature at the same age than males (C1 and SM), are short-lived compared to them. Whereas if they mature later (C2, C3 and UL), females have a longevity equal to that of males on the same site. However, sexual size dimorphism in anurans is thought to be largely a function of distinct life-history strategies of males and females (Monnet and Cherry, 2002; Cogălniceanu et al., 2014; Fattah et al., 2014; Green and Bailey, 2015), still vary among populations of the same species (Arantes et al., 2015) and it could be difficult to establish a direct or linear relationship between only a few parameters. In agreement with Echeverria and Filipello (1990) and Bionda et al. (2015), our study demonstrates that age at maturity and long-lived did not differ significantly between the sexes, but the size threshold for attaining sexual maturity of females is greater, with exception of SM. Females may show considerable growth pre or post maturity compared with males (Erişmiş et al., 2009; Ma and Lu, 2009; Liao and Lu, 2010 b; Guarino et al., 2011) and, females may continue to grow a few months longer than males at the same rate. Therefore, as the temporal unit detectable with skeletochronology is one year and females growth period a few months, prolonged female growth cannot be detected by a difference in LAG number (Bionda et al., 2015). Thus, evidence for a possible trade-off between age at maturity, growth and longevity requires a larger dataset from a larger number of populations.

Von Bertalanffy's growth models were in good agreement with empirical values ( $R^2$ : C1 = 0.89, C2 = 0.94, C3 = 0.90, UL = 0.96, SM = 0.97). The form of the growth curves and the values of K and  $SVL_{max}$  demonstrate that SM has a faster growth and occurred mainly in the early years and reaches its maximum SVL faster (less  $\ln 2/k$  and  $t_{0.99\%}$ ). C2 and C3 recorded slow growth (with intermediate values for K,  $\ln 2/k$  and  $t_{0.99\%}$ ), and finally, C1 y UL with slowest growth (lower K and higher  $\ln 2/k$  and  $t_{0.99\%}$ ). Thus, von Bertalanffy's growth models showed that there are differences in growth rates mainly in the early years. The age mean and potential reproductive lifespan reported low values for both males and females at agroecosystems sites, mainly in C1 and C3, since most individuals do not reproduce more than once or twice in a lifetime. The sites showing most long-lived values were SM, followed by UL and C2. According to Spear et al. (2009) in a study with *L. catesbeianus*, high population longevity makes them more susceptible to chronic exposure to aquatic contaminants. Also this can be increased by the entirely/partiality aquatic life cycle, and high trophic level which could lead to bioaccumulation effects. This effect could possibly be reflected on the individual health, for example in low values of BC. This was not observed in our results. The best value of BC was registered in SM, followed by intermediate values at UL and C2, and finally C1 and C3 sites showing the worst BC indexes. Also, chronic exposure to certain aquatic contaminants could be analyzed through hematological health biomarkers that reflect exposure to contaminants or environmental stressors. In this sense, some studies conducted with *R. arenarum* at the same sites, have shown genetic damage through blood biomarkers such as micronucleus and nuclear abnormalities frequencies, mainly in UL and C1, followed by C2 and C3, and low or absent values for SM (Caraffa et al., 2013; Babini et al., 2015 a; Pollo et al., 2015). There have also been registered changes in the leukocyte formula in *R. arenarum* adults of C1 and UL sites, would be indicating stress by unfavorable conditions in their environment (Salinas et al., 2015). Therefore, high population longevity at sites UL and C2, could lead to chronic exposure to contaminants in their environments. However that is not the case of SM. Although this site has greater population longevity, blood biomarkers do not reflect to exposure to contaminants, probably due to the absence of xenobiotics in the environment.

The largely unfavorable demographic parameters were recorded in C1 and C3, mainly C1. The populations in these agroecosystems appear to be comprised to younger and smaller individuals. These populations report the lowest values for reproductive potential, longevity, growth coefficient (K) and BC, and generally need more time to reach their average sizes ( $\ln 2/k$ ). Similar results were recorded by Spear et al. (2009), with younger and smaller individuals of *Lithobates catesbeianus* in intensive agriculture sites. In this regard, several hypotheses could be formulated, including the movement of larger/older adults to other sites or a lack of survival of larger/older, or the case of an unstable population maintained by smaller/younger adults. Furthermore, it could be due to a decreased of the growth rate of adults, or genetic differences between the agricultural and livestock activity sites and the other sites (Spear et al., 2009). Hypotheses about the movement of larger/older adults or an unstable population or the occurrence of genetic differences between populations require additional studies to that performed in this study. Instead, the lack of survival of larger/older is highly probable. In fact, at C1 and C3, mode of age is the lowest, and this corresponds to the age of half of sampled population in each site. In addition, regarding the longevity, we register the lowest values. Population stability can be influenced by the longevity of individuals, because populations



comprised of short-lived individuals may be prone to size fluctuations because the impact of failed recruitment in a single year can be severe (Driscoll, 1999). In this sense, the croplands produced the modification of aquatic environments or deterioration in the quality of them, particularly can affect the duration of hydroperiod with the effect of the early drying, reducing eggs and larval survivorship for amphibians (Carey and Bryant, 1995; Tejado, 2003; Taylor et al., 2005; Peltzer et al., 2008). In addition, the presence of livestock can negatively affect the different populations (Schmutzer et al., 2008; Burton et al., 2009; Bionda et al., 2011). Intense trampling by the cattle in and around the ponds may also contribute directly to increased mortality of amphibians eggs and tadpoles (Bécart et al., 2007; Jofré et al., 2007; Burton et al., 2009) and may have direct or indirect negative effects by decreasing water quality through deposition of nitrogenous waste, causing eutrophication and grazing shoreline vegetation that contributes to detrital cover and food (Schmutzer et al., 2008) affecting the successful metamorphosis. In studies conducted by Bionda et al. (2011) and Bionda et al. (2013a) the proportion of recruitment in tadpoles of *R. arenarum* was smaller in the C1 and C3. This could be caused by several factors, but mainly due to the reduced hydroperiod in these sites, where larval mortality of *R. arenarum* was recorded. Moreover, a study on the health of the larvae of C1, C2 and C3 sites, analyzing morphological and growth abnormalities, indicated that C1 has the less healthy tadpoles (Babini et al., 2015 a).

Similarly, the hypothesis of a low growth rate also is highly probable, considering that all sites with disturbances, whether with influence of agricultural, livestock or urban, showed lower values of K, mainly C1; and the same occurs with the other growth parameters ( $\ln 2/K$  and  $t_{0.99\%}$ ). The low level of survival of larger or older individuals as well as low growth rate in these age classes for sites with disturbances is consistent with the results obtained in other investigations on these same populations. Bionda et al. (2013 a) recorded long-term population projections less favorable in C1, C2 and C3. In addition, tadpoles that inhabit these same agroecosystems showed that consume less food and had lower body condition index and survival rate (Bionda et al., 2012 a). Mouth abnormalities have been reported in larvae of sites C1, C2 and C3, mainly in C1, which affect feeding activity (Babini et al., 2015 a). Food quantity and quality below the optimum value can have a significant influence in the tadpoles on the metamorphosis time and size (Carey and Bryant, 1995; Taylor et al., 1995), which could eventually affects the size reached by adults, time and size to reach maturity and ultimately survival or reproductive fitness (Ryser, 1988).

In C2, the demographics parameters are favorable for mean age and longevity, as well as their growth parameters such as K,  $\ln 2/K$  and  $t_{0.99\%}$ , despite the poor water quality (level of nitrate and phosphorus) in their ponds. Our data do not agree with Spear et al. (2009) in *L. catesbeianus*, because lower population longevity was recorded in those sites where higher phosphorus level was found. Furthermore, the mean SVL of the population of C2 was the highest, as well as the size at sexual maturity. Although in regard to BC index, C2 presents intermediate values, indicated lower rate of mass gain despite length growing. According to Reading and Clarke (1995), of the two component variables of body condition only mass, and not length, can both increase and decrease rapidly in response to a change in stress. This may be due to multiple factors as food shortage, competition, unfavorable climatic conditions or indeed a poor quality environment.

A high nitrate and phosphorus level at C2 site, may be associated with a mixed agricultural practice, and due to an uncommon event. Moreover, among the agroecosystem sites, C2 is the lower intensity agricultural site, since no have alteration by livestock. Although due to these uncertain results, populations of *R. arenarum* of this site should be subjected to further investigation in order to better comprehend their demographic.

UL has intermediate values for the mean ages, BC, reproductive potential and longevity. However, in regard to growth parameters, K,  $\ln 2/K$  and  $t_{0.99\%}$ , have low values, indicating a lower rate of growth gain despite of age increase. Villa Dalcar Lake, is used for fishing and recreation activities (Mancini et al., 2000), thus it is considered an environment with high anthropic disturbance. Several studies were conducted in Villa Dalcar Lake, in order to asses mass mortality of fish (Mancini et al., 2000), or algae growth (Novoa et al., 2006). These events may have occurred by the presence of xenobiotics in the lake (Mancini et al., 2000), due to urban and agricultural runoffs, since that could be only one that flows into the lake after heavy rains. As mentioned above, several studies (Caraffa et al., 2013; Babini et al., 2015 a; Pollo et al., 2015; Salinas et al., 2015) have shown genetic damage and stress in populations of *R. arenarum* of UL. In addition, Bionda et al. (2012 b) already recorded a high rate of morphological abnormalities (13.64% of the total sample) of toads from this urban lake and although it cannot be assure the proximate cause(s) of abnormalities, the chemical pollutants or presence of aquatic predators present themselves as the most likely causes. This uncertain result in demographic and growth parameters also suggests the need to continue monitoring these populations.

In other words, according to demographic and growth parameters, C1 consisting in intensive agricultural and livestock activity represents the least favorable site by amphibian population development. Followed by C3, and then C2 and UL, which consists in sites with a moderate influence of agricultural and livestock, or urban activities. This is indicating unfavorable and other favorable demographic and growth parameters. Finally, we could refer to SM as the one showing the more favorable parameters, which consists in a moderate urban activity site.

## 5. Conclusion

Size, age and growth reductions at the intensive agricultural systems, suggest long term impacts on fitness, as population projections have shown in these same populations (Bionda et al., 2013a). An intensive agricultural system, with crop and livestock combined activity (e.g. C1 and C3 sites) or intensive anthropic activity (UL), appears to have a significant negative impact on common toad *R. arenarum* in the central area of the Córdoba Province, Argentina. Our results showed the impact

that agriculture practices have over growth and longevity on common toad populations suggesting thus from the demographic life-history traits to *R. arenarum* specie as bioindicator organism.

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