

## Variation in reproductive parameters of *Rhinella arenarum* (HENSEL, 1867) (Anura: Bufonidae) between the reproductive and post-reproductive periods

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**ABSTRACT.** We compared reproductive parameters of *Rhinella arenarum* in a wetland of the Monte in Argentina during reproductive and post-reproductive seasons. Individuals were collected at random, monthly from November 2001 to October 2002. August through November was considered the reproductive period, and December through April was considered the post-reproductive period. Of the 116 adults, 75 were males and 41 were females. The reproductive parameters measured included body mass, fat body mass, number of mature ova, ova size, and testicular volume. There were significant differences between the reproductive and post-reproductive periods in females for body mass, fat body mass, and number of mature ova. Likewise, males also had significant differences between these periods for body mass and fat body mass. Apparently, *R. arenarum* has an opportunistic and continuous reproductive strategy. Entering dormancy with large fat bodies and testes in apparent spermatogenesis allows males to reproduce immediately after emerging in the spring. However, females have mature but fewer ova during this period, which is a reproductive feature shared by most temperate amphibians. Our data, and the primarily tropical distribution of *R. arenarum*, suggest that this species recently invaded the temperate region wherein males retained acyclic reproductive activity and females, owing to their higher reproductive costs, have evolved cyclic reproduction.

**KEY WORDS:** Argentina, Reproduction, *Rhinella arenarum*, San Juan, Seasonal variation.

### INTRODUCTION

Amphibians exhibit a great diversity of reproductive patterns (DUELLMAN & TRUEB, 1986). Anurans that live in tropical areas, where temperatures do not show large seasonal fluctuations, have continuous reproductive patterns. By contrast, anurans of temperate and cold climates, where ambient temperatures show large variations, both daily and seasonal, breed discontinuously (CRUMP, 1974; TSIORA & KYRIAKOPOULOU-SKLAVOUNOU, 2001; WELLS, 2007).

LAVILLA & ROUGES (1992) described the reproductive mode of *R. arenarum*, in which eggs are laid in gelatinous strings at the

bottom of water bodies, where hatching and embryonic development also occur. Studies of reproductive parameters of a species allow us to better understand the reproductive modes and their ecological and evolutionary significance (CRUMP, 1974). Previous research has found a relationship between the size of females and fertility; thus it is expected that females of larger size have more eggs for each clutch than females of smaller size (BASSO, 1990; PEROTTI, 1997; PERALTA DE ALMEIDA-PRADO & UETANABARO, 2000; DÍAZ-PÁEZ & ORTIZ, 2001; CASTELLANO et al., 2004; SANABRIA et al., 2007a; SANABRIA et al., 2007b).

Also, there are annual variations in the size of the fat body, which correlates with the functional

status of the gonads (MARTORI et al., 2005). DÍAZ-PÁEZ & ORTIZ (2001) found that in both sexes of *Pleurodema thaul* (LESSON, 1826) the increase in size of fat body is positively correlated with temperature, but not with rainfall. Furthermore, the size of the fat body is reduced (sometimes to zero grams) during the breeding season, as the stored fat is used as an energy source during and after winter aestivation. DUELLMAN & TRUEB (1986) suggested that the amount of energy devoted to reproduction depended on the season, age, and sex of the frog. Many amphibians accumulate energy reserves to survive long periods of dormancy (FITZPATRICK, 1976). Therefore, species with a short period of activity, in sites with little precipitation and short summer seasons, should partition the energy between reproduction and reserves (WELLS, 2007).

The distribution of energy for reproduction in a year can affect the amount of energy available for future growth or reproduction (RYAN et al., 1983). The aim of this investigation was to compare reproductive parameters (fat body mass, body mass, number of mature ova, size of ova, and testicular volume) between the reproductive and post-reproductive periods of *R. arenarum* in the arid region of San Juan, Argentina.

## MATERIALS AND METHODS

The study area is located 25 km west of San Juan, Zonda Department (31.52716 S, 68.69580 W; Datum: WGS 84; elevation 724m). In winter, the wetland is reduced to two small bodies of water and in summer it increases in size until it becomes a large, flooded area (VICTORIA, 1999).

It is part of the Monte phytogeographical province (CABRERA, 1994) at 800 m asl, an arid region with an average annual temperature of 17.3°C, annual average maximum of 25.7°C, and annual mean minimum of 10.4°C. Rainfall is concentrated in the summer with an annual average of 84 mm. Individuals were collected via haphazard monthly sampling from November 2001 to October 2002. The herpetological

assemblage of this region is composed of 12 species, of which only three are anuran (*R. arenarum*, *Leptodactylus latrans* (STEFFEN, 1815), *Pleurodema nebulosum* (BURMEISTER, 1861)) (SANABRIA & QUIROGA, 2010).

The months of August, September, October and November were considered the reproductive period, as they are the months during which amplexus and male songs are reported (SANABRIA et al., 2005). The months of December, January, February, March and April constitute the post-reproductive period in which the species generally is found foraging. The individuals were euthanized with an injection of 2.5ml of xylocaine 2% placed in the lymph sac, fixed with 10% formaldehyde and preserved in 70% alcohol.

All individuals were measured from snout to the cloaca (SVL) using digital calipers (Essex; China. Accuracy 0.01mm) and weighed with a digital scale (Denver; Boemia, NY, USA. accuracy 0.1 g).

In the laboratory, specimens were dissected and their gonads and fats bodies removed for further analysis. To determine the reproductive status of gravid females, the ovarian mass was weighed on a digital scale (Denver; Boemia, NY, USA. Accuracy 0.1g). In addition, we calculated the ovarian complement (number of mature ova) through a sample taken from the ovarian mass. The ova from a fraction of the ovarian mass were weighed and counted, and then we extrapolated the data to the total weight of the ovarian mass (CRUMP, 1974). We measured the diameter of mature ova with a binocular microscope (magnification 10X) and digital calipers. The criterion used to define mature ova was the degree of pigmentation. Immature ova resemble an undifferentiated mass where the ova has not begun to accumulate yolk in the cytoplasm, whereas in mature ova that are black in color, the yolk accumulation has begun to create an opaque and milky aspect, indicating the finalization of development (MARTORI et al., 2005).

To find the testicular volume of males, we measured the length and width of the testicles and calculated the volume using the spheroid formula (DUNHAM, 1983). In both sexes we extracted fat bodies and weighed them on a digital scale (Denver, Boemia, NY, USA. Accuracy 0.0001g). This method was used because the fat bodies have irregular form and are difficult to measure (VITT & OHMART, 1975). Means and standard errors were calculated for all data, and an ANCOVA was used to test differences between reproductive and post-reproductive periods using body weight and SVL as covariates.

### RESULTS

We gathered data from 116 individuals of which 75 were males and 41 females. Females had an average fat body mass of  $2.28 \pm 0.41$ g, body mass of  $155.9 \pm 8.06$ g and SVL of  $109.4 \pm 1.6$ mm while males had an average fat body mass of  $1.69 \pm 0.19$ g, body mass of  $100.7 \pm 3.7$ g and SVL of  $94.5 \pm 1.1$ mm. Females exhibited larger size than males for all of the measured variables. Table 1 shows the variables of both sexes for the reproductive and post-reproductive periods.

Females of *R. arenarum* showed significant differences between the reproductive and

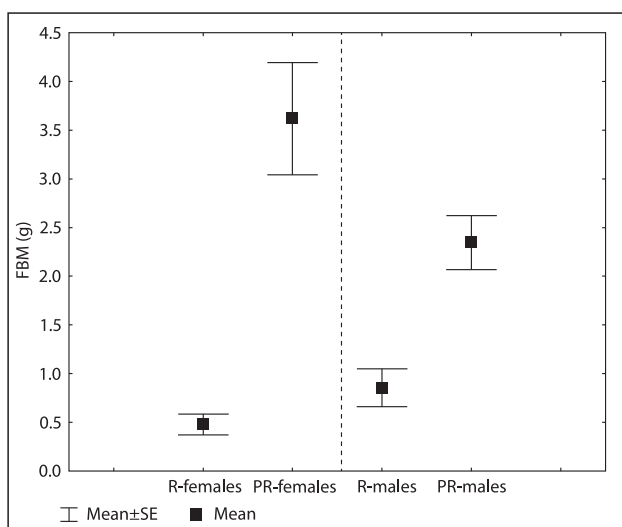


Fig. 1 – Variation in fat body mass (FBM) of the sexes between the reproductive (Rfemales, Rmales) and post-reproductive (PRfemales, PRmales) periods.

TABLE 1

Variables of females (♀) and males (♂) in the reproductive and post-reproductive periods for fat body mass (FBM), body mass (BM), number of mature ova (NMO), size ovules (SO), and testicular volume (TV).

Variables	Periods	
	Reproductive	Post-reproductive
FBM ♀	0.48 ± 0.11	3.78 ± 0.57
BM ♀	138.56 ± 7.7	168.8 ± 12.2
NMO ♀	33219 ± 6304.6	25063.79 ± 2011.8
SO ♀	1.06 ± 0.09	1.16 ± 0.02
FBM ♂	0.85 ± 0.19	2.34 ± 0.27
BM ♂	90.09 ± 4.74	109.02 ± 5.21
TV ♂	80.34 ± 7.42	94.14 ± 7.83

post-reproductive periods for fat body mass (ANCOVA:  $F_{1,37} = 21.68$ ;  $P < 0.00004$ ; cov. = SVL) (Fig. 1), body mass (ANCOVA:  $F_{1,37} = 3.80$ ;  $P < 0.005$ ; cov. = SVL) (Fig. 2), and number of mature ova (ANCOVA:  $F_{1,24} = 9.73$ ;  $P < 0.004$ ; cov. = SVL) (Fig. 3). There were no significant differences in egg size (ANCOVA:  $F_{1,26} = 1.98$ ;  $P > 0.17$ ; cov. = SVL).

Males also showed differences between the reproductive and post-reproductive periods

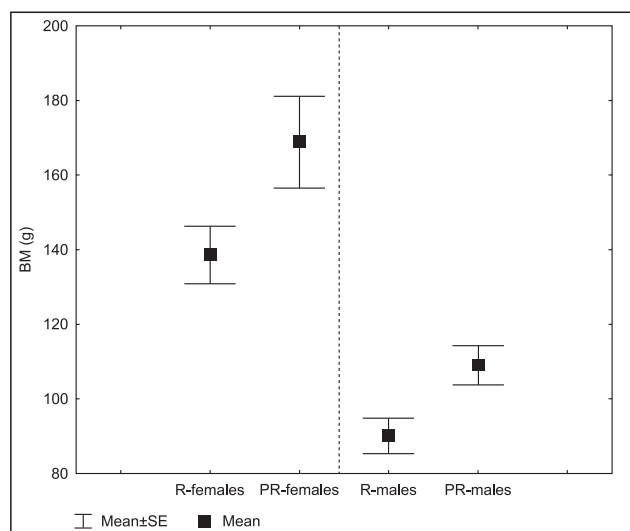


Fig. 2 – Variation in body mass (BM) of the sexes between the reproductive (Rfemales, Rmales) and post-reproductive (PRfemales, PRmales) periods.

for fat body mass (ANCOVA:  $F_{1,72} = 21.17$ ;  $P < 0.00002$ ; cov. = SVL) (Fig. 1) and body mass (ANCOVA:  $F_{1,72} = 17.74$ ;  $P < 0.00007$ ; cov. = SVL) (Fig. 2). There was no significant difference in testicular volume (ANCOVA:  $F_{1,72} = 0.53$ ;  $P > 0.46$  cov. = SVL).

## DISCUSSION

As do most anurans, *Rhinella arenarum* exhibits sexual dimorphism, with females larger than males (DUELLMAN & TRUEB, 1986; QUIROGA et al., 2004). Moreover, gender variation exists in the storage organs (fat bodies) with females storing more fat than males. According to SHINE (1979), females are larger than males in 90% of anuran species; this appears to be related to the ability of females to produce large numbers of ova (CRUMP, 1974; PEROTTI, 1994). There are other hypotheses, however, to explain larger female size, such as predation pressure on larger males because of the risks involved in territorial defense and mating (HOWARD, 1981; PERALTA DE ALMEIDA-PRADO & UETANABARO, 2000). However, both sexes show lower body mass during the reproductive period, which suggests that both males and females spend their energy in reproduction (BRATTSTROM, 1979).

Consequently, fat bodies also exhibit minimum size during the spawning period (DÍAZ-PÁEZ & ORTIZ, 2001).

Males have restrictions on their growth due to the energy demands during reproductive activity, where the major energy expenditure relates to sperm production, calls, and defense of breeding territory (DUELLMAN & TRUEB, 1986; WELLS, 2007; NAVAS et al., 2008). In addition, they consume only a small amount of food during the calling period (WOOLBRIGHT, 1989), and empty stomachs have been observed during this period (QUIROGA, unpubl. data).

On other hand, development of the fat bodies and increases in body mass have been observed during the post-reproductive period enabled by the large quantity of food ingested between the months of December and February (QUIROGA et al., 2009). Thus, *R. arenarum* can store enough energy and nutrients for the next period of hibernation and for reproduction during the following year (BRATTSTROM, 1979; WHITFORD, 2002). Energy acquisition in both sexes is an important factor in reproductive events, and thus gonadal function depends on the contribution of fat bodies (WELLS, 2007).

In females, the size of mature ova does not differ between the reproductive and post-reproductive periods. Presumably growth ceases once the ovum matures. However, the number of mature ova is significantly higher in the reproductive period. During this period, the ova are being deposited at any time (MARTORI et al., 2005). In contrast, during the post-reproductive period, the number of mature ova diminishes. It is likely these remaining mature ova will be deposited in the first clutches after aestivation, as observed for *Pleurodema thaul* by DÍAZ-PÁEZ & ORTIZ (2001). Also, SANABRIA et al. (2005) found that reproduction in *R. arenarum* begins in mid-August, coinciding with the end of winter hibernation. This strategy would allow the species to avoid or delay the predation of their eggs by invertebrate predators (HEYER et al., 1975).

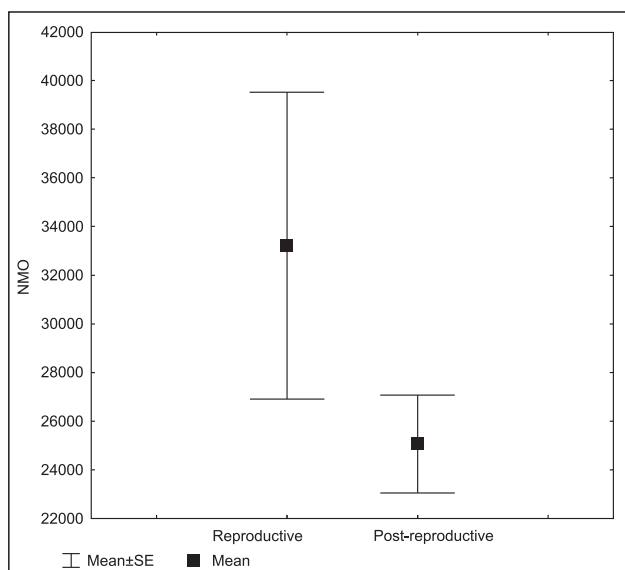


Fig. 3 – Differences in the number of mature ova (NMO) between the reproductive and post-reproductive periods.

Males of *R. arenarum* did not differ in testicular volume between the reproductive and post-reproductive periods. It is likely that during the activity period the testes are active and individuals potentially ready to breed (LAVILLA & ROUGE, 1992), as observed for *Rhinella fernandezae* by MARTORI et al. (2005). Thus, *R. arenarum* would exhibit continuous reproduction, agreeing with the observations of TSIORA & KYRIAKOPOULOU-SKLAVOUNOU (2001) for *Rana epeirotica*, where the weight of the testis was not related to spermatogenic activity, indicating potentially continuous spermatogenesis.

Males of *Rhinella arenarum*, appear to exhibit a continuous, opportunistic reproductive strategy, as they maintain large fat bodies and mature sperm, allowing them to reproduce immediately after emerging in spring. By contrast, females have fewer mature ova during this period, reflecting a reproductive cycle shared by amphibians in temperate zones. Our reproductive data, and the primarily tropical distribution of *R. arenarum* (CEI, 1980), suggest that this species recently invaded the temperate region wherein males retained acyclic reproductive activity and females, owing to their higher reproductive costs, have evolved cyclic reproduction.

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