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
17.3ºC, annual average maximum of 25.7ºC,
and annual mean minimum of 10.4ºC. 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 (mag-
nification 10X) and digital calipers. The crite-
rion 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 ac-
cumulation has begun to create an opaque and
milky aspect, indicating the finalization of devel-
opment (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 ± 0.41g, body mass of 155.9 ± 8.06g and SVL of 109.4 ± 1.6mm while males had an average fat body mass of 1.69 ± 0.19g, body mass of 100.7 ± 3.7g and SVL of 94.5 ± 1.1mm. 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 post-reproductive periods for fat body mass (ANOVA: $F_{1,37} = 21.68; P < 0.00004; \text{cov.} = \text{SVL}$) (Fig. 1), body mass (ANOVA: $F_{1,37} = 3.80; P < 0.005; \text{cov.} = \text{SVL}$) (Fig. 2), and number of mature ova (ANOVA: $F_{1,24} = 9.73; P < 0.004; \text{cov.} = \text{SVL}$) (Fig. 3). There were no significant differences in egg size (ANOVA: $F_{1,26} = 1.98; P > 0.17; \text{cov.} = \text{SVL}$).

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

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

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

**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.](image-url)
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, opportunist 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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