



Reproductive biology of *Odontophrynus americanus* females (Duméril & Briçon, 1841) (Anura, Cycloramphidae) from Uruguay

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Abstract. This paper describes the female reproductive pattern in a population of the frog *Odontophrynus americanus* from Uruguay. It is a species with explosive reproductive dynamics; such strategy has been little studied in temperate environments. Seventyfive adult females collected for one year were used, belonging to the Scientific Collection of the Facultad de Ciencias (Montevideo, Uruguay). We analyzed ovary mass, fecundity, reproductive investment, oocytes size and fat bodies. Also we studied the association between reproductive activity and abiotic variables. Larger size females have greater potential reproductive, and also invest in both the number and the size of oocytes proportionally. Reproductive investment did not show a clear pattern of temporal variation, and relative abundance of fat bodies remains unchanged over year. Reproductive activity of females of *O. americanus* is not found associated photoperiod and neither with temperature, but if with rainfall. These results are congruent with an explosive-type reproductive dynamic.

Key words: reproductive investment, fecundity, fat bodies, Uruguay

Resumen. Biología reproductiva de las hembras de *Odontophrynus americanus* (Duméril & Briçon, 1841) (Anura, Cycloramphidae) de Uruguay. En este trabajo se describe el patrón reproductivo de las hembras en una población de *Odontophrynus americanus* de Uruguay. Es una especie con dinámica reproductiva explosiva, dicha estrategia ha sido poco estudiada en ambientes templados. Se utilizaron 75 hembras adultas colectadas durante un año, que pertenece a la Colección Científica de la Facultad de Ciencias (Montevideo, Uruguay). Se analizó la masa del ovario, la fecundidad, la inversión reproductiva, el tamaño de los ovocitos y los cuerpos grasos. También se estudió la asociación entre la actividad reproductiva y las variables climáticas. Las hembras de mayor tamaño tienen mayor potencial reproductivo, además invierten tanto en el número como en el tamaño de los ovocitos de forma proporcional. La inversión reproductiva no mostró un claro patrón de variación temporal, y la abundancia relativa de los órganos de grasa se mantiene sin cambios con respecto al año. La actividad reproductiva de las hembras de *O. americanus* no se encontró asociada al fotoperiodo ni a la temperatura, aunque sí con las precipitaciones. Estos resultados son congruentes con una dinámica de tipo explosiva.

Palabras claves: inversión reproductiva, fecundidad, cuerpos grasos, Uruguay.

Introduction

The diversity of reproductive strategies among amphibians is associated with a variety of activity patterns and reproductive modes (Duellman & Trueb, 1994). Both are components intrinsically related with species reproductive cycles, which are

interpreted as responses to environmental and climatic factors, photoperiod, internal cycles and biotic interactions (Prado & Haddad, 2005; Both *et al.*, 2008; Canavero *et al.*, 2008). In tropical regions where the temperature is more constant the anurans exhibit continuous breeding, but breed when the

rainfall provide site of oviposition (Prado & Haddad, 2005). Furthermore, in temperate and subtropical or seasonal environments anurans have cyclical reproduction, the temperature stimulates spermatogenesis and determines the duration breeding season (Duellman and Trueb, 1994) while rainfall trigger the start of the reproductive event (Stebbins & Cohen, 1995). However a combination of factors (humidity and photoperiod) may have greater predictive power (Both *et al.*, 2008), which can determine reproductive strategies for anurans.

Wells (1977, 2007) proposed two types of reproductive strategies. In seasonal breeders the activity period extends for several months and both sexes arrive asynchronously to the breeding site. Males calling from fixed positions to attract females (Wells, 1977). The explosive reproduction can occur in days or hours and the arrival to the site is synchronous for both sexes. Moreover, there is an active search for partners and physical combat between males for females (Wells, 2007). Furthermore, seasonal breeders and explosive breeders represent extremes of a continuum where there are intermediate strategies that combine characteristics of both (Prado & Haddad, 2005; Rodrigues *et al.*, 2004; Wells, 2007) like the period of reproduction, territorial behavior in males, etc.

The strategies chosen by females must balance the amount of energy allocated to reproduction, growth and survival, in addition to providing the tradeoff between quantity and quality of offspring (Castellano *et al.*, 2004). In fact, the reproductive effort has been defined as the proportion of resources invested in reproduction over a defined period (Gadgil & Bossert, 1970). One way to estimate this investment in anurans is measuring the size, mass, or volume of the gonads in relation to the body (Crump, 1974; Prado *et al.*, 2000). Studies conducted by Camargo *et al.* (2005, 2008) with populations of *Physalaemus gracilis* and *Physalaemus biligonigerus* (two seasonal breeders) as those by Rodrigues *et al.* (2003, 2004) with populations of *Elachistocleis bicolor* and *Eupemphix nattereri* (two species with explosive reproductive activity) demonstrated the existence of a positive relationship between fecundity and body size in females. Regardless of the reproductive strategy, adult females with larger body sizes are those with higher reproductive success because they can accommodate more quantity or improve a size-mediated quality of oocytes (Howard, 1988; Castellano *et al.*, 2004; Rodrigues *et al.*, 2004; Prado and Haddad, 2005).

However, fat bodies may also be related to energy investment, since anurans utilized such structures to store lipids for growth, survivorship and reproduction (Jørgensen, 1992). Indeed, several studies suggest for some species that there is an association between the abundance of fat bodies and reproductive status of the animals (Brenner & Brenner, 1969; Diaz-Paez & Ortiz, 2001; Prado & Haddad, 2005). It is suggested that these lipids are used for vitellogenesis in females, since in some species decrease in volume during the spawning period, while oocytes size increases (Díaz-Páez & Ortiz, 2001). Although lipid reserves are also used as an energy source during aestivation (Hillman *et al.*, 2009) and in temperate regions are used during hibernation (Prado & Haddad, 2005). However this theoretic framework was developed based in seasonal breeders.

There are very few studies on the reproductive biology of anurans with explosive reproductive dynamics in seasonal environments. The genus *Odontophrynus* generates special interest by having species with explosive dynamics (Canavero *et al.*, 2008; Santos *et al.*, 2008) as *Odontophrynus americanus* (Duméril & Bibron, 1841). This specie inhabits throughout Uruguay, occupying a variety of habitats, mainly in meadows and marshes, as well as urban environments (Maneyro & Carreira, 2012). *Odontophrynus americanus*, although of being a fossorial anuran, is an active forager with a highly diverse diet, therefore has a more generalist diet (Isacch & Barg, 2002). This species is breeds almost all year with exception of the coldest months, the breeding sites consist of lentic and temporary water bodies (Maneyro & Carreira, 2012), characteristic explosive breeders (Wells, 1977). According to a study by Bertoluci, 1998 in Atlantic Forest anurans, *O. americanus* was one of the species who responded directly to rainfall, showing large aggregations of calling males during the first heavy rains of the period.

The aim of this paper is to describe the features of the reproductive biology of females in a population of *Odontophrynus americanus*. The specific objectives are: 1) to check a possible association between fecundity and body size, and analyze the variation of reproductive investment in mature females along the year; 2) to explore associations between climatic factors and reproductive activity; and 3) to investigate associations between the abundance of fat bodies and reproductive activity. Our hypothesis is that reproduction in explosive breeders reproduction is

related to rainfall (an unpredictable abiotic factor), and therefore, the timing of reproductive events is unpredictable, and females have continuous availability of fat bodies and mature eggs to breed at any time of year.

Materials and Methods

Specimens of *Odontophrynus americanus* used in this work were deposited in the Collection of Vertebrate Zoology, Facultad de Ciencias, Universidad de la República (Montevideo, Uruguay). They were caught monthly between February 2007 and January 2008 in the Department of Tacuarembó (32° 26' 06" S, 55° 31' 59" W) (Fig. 1). Snout-vent length (SVL) and body mass (BM) data of 75 specimens of adult females were obtained from the collection records.

The ovaries were removed to measure their mass (OM). We considered mature females those frogs with post-vitellogenic oocytes, with different hemispheres (germinal pole dark brown colored and vegetal pole ivory colored) (Hermosilla *et al.*, 1986). Fecundity (F) was then recorded as the total number of mature oocytes and oocytes size (OS) as the average diameter of the oocytes in each specimen (on the basis of ten measurements in each ovary) (Camargo *et al.*, 2005), macroscopically from a loupe with ocular scale.

We studied the association between reproductive activity and abiotic variables

(temperature, rainfall and photoperiod), an linear regression analysis between the monthly absolute frequency of mature females and abiotic variables was performed. Climatic data (temperature and rain fall) were provided by the Dirección Nacional de Meteorología (National Meteorology Office) (INE, 2009) (Fig. 2). The photoperiod data were obtained from Sun Earth Tools .com (http://www.sunearthtools.com/dp/tools/pos_sun.php).

The individual Reproductive Investment (RI) was estimated as the percentage of OM relative to BM ($IR = OM \times 100 / BM$) (Prado & Haddad, 2005, Rodrigues *et al.*, 2004, 2005). We performed linear regression analysis to study the association between BM and reproductive variables (OM, F) and we also studied the association between F and OS (Camargo *et al.*, 2005, 2008). Fat bodies were extracted and their mass was measured, then a relationship index (FBI) was calculated as the ratio between the fat bodies mass and the BM. Linear regressions between FBI and reproductive variable F were performed.

Non-parametric Kruskal-Wallis tests were used to evaluate the seasonal variation of SVL, RI and FBI was used nonparametric Kruskal-Wallis test. Analyses were performed using the program XLSTAT version 7.5.3 (Addinsoft, 1995) and the Statistica program version 6.1 (StatSoft Inc., 2004). Mass measurements were taken with a Sartorius balance with 0.1 mg precision.

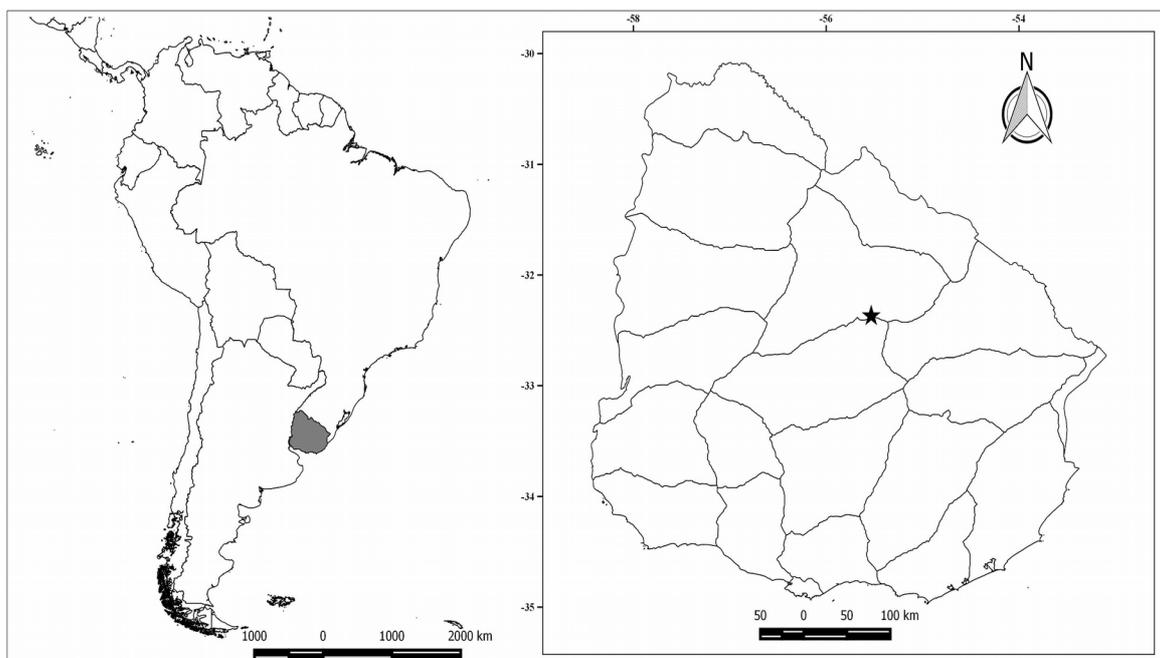


Figure 1. Location map of the locality where the specimens of *Odontophrynus americanus* were collected in the department of Tacuarembó, Uruguay.

Results

There were 75 adult females in the sample, 69 of which were mature (38% were captured in March in summer). The minimum SVL for a mature female was 27.9 mm, and therefore, above this value, were considered adult females.

Mature females were found in all months of study, except in June (when only an immature female was found), with a peak in sample size in March (in summer) (37.7%) (Fig. 2). This month coincides with the greatest accumulation of precipitation and is one of the months with the highest average temperature. Although mature female frequency was not correlated with the photoperiod ($R^2 = 0,012$; $p = 0,780$) and neither with average temperature ($R^2 = 0.087$; $p = 0.391$), there was a positive correlation with rainfall ($R^2 = 0.390$; $p < 0.05$).

Snout-vent length (SVL) for the mature females ($n = 69$) ranged between 27.9 and 53.9 mm (mean \pm SD = 43.5 ± 4.9 mm) and there was no significant seasonal variation ($H = 5, 793$; $p = 0.122$) (Table 1). Among them, BM in the sample ($n = 69$) was 20.31 ± 7.3 g (mean \pm SD) and SVL 43.8 ± 4.1 mm (mean \pm SD). The number of mature oocytes for both ovaries ranged between 28 and 7860 (mean \pm

SD = 2029 ± 1799). The diameter of mature oocytes for both ovaries ranged between 0.59 and 1.13 mm (mean \pm SD = 0.97 ± 0.11 mm); and the reproductive investment ranging from 0.83% in April to 20.5% in March (mean \pm SD = 6.03 ± 5.0 %). The RI showed significant differences among seasons ($H = 15.27$; $p < 0.05$) (Table 1), being the highest values in February, March and December, but without a predictable pattern of variation. The frequency of distribution of the RI shows that the majority of the females have low RI and a few of them exhibit high value of RI (Fig. 3).

It was found a significant positive correlation between OM and BM adjusted for log – log regression ($R^2 = 0.16$; $p < 0.05$; slope < 1 , therefore a negative allometric relationship, i.e., there is a disproportionately low growth of the variable OM, Fig. 4). Also it was found a significant positive correlation between F and BM ($R^2 = 0.11$; $p < 0.05$), OS and F ($R^2 = 0.55$; $p < 0.0001$, Fig. 5). The results show no significant variation of FBI among seasons in females of *O. americanus* ($H = 2,133$; $p = 0.545$) (Table 1), and there was not significant correlation between FBI and F ($R^2 = 0.0012$; $p = 0.789$).

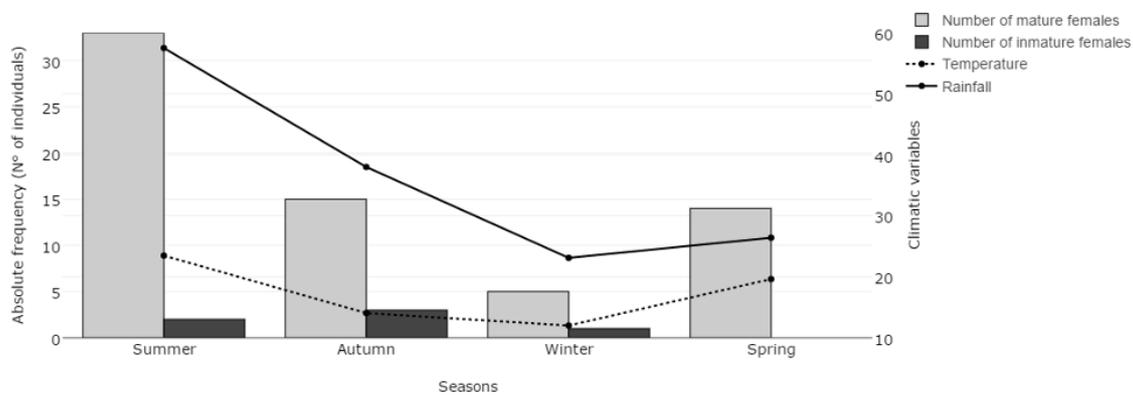


Figure 2. Seasonal mean temperature (C°) and accumulated rainfall (cm). Seasonal frequency distribution of absolute number of mature (grey bars) and immature (black) females of *Odontophrynus americanus*.

Table 1. Means and standard deviation of snout-vent length (SVL), reproductive investment (RI) and fat bodies index (FBI) of *Odontophrynus americanus* females for each season.

Seasons	SVL	RI	FBI
Summer	43,343 \pm 3,892	8,167 \pm 5,350	0,0058 \pm 0,0116
Autumn	43,535 \pm 3,588	3,453 \pm 3,734	0,0047 \pm 0,0070
Winter	46,123 \pm 3,202	5,350 \pm 4,738	0,0076 \pm 0,0049
Spring	44,038 \pm 5,238	4,021 \pm 3,541	0,0049 \pm 0,0041

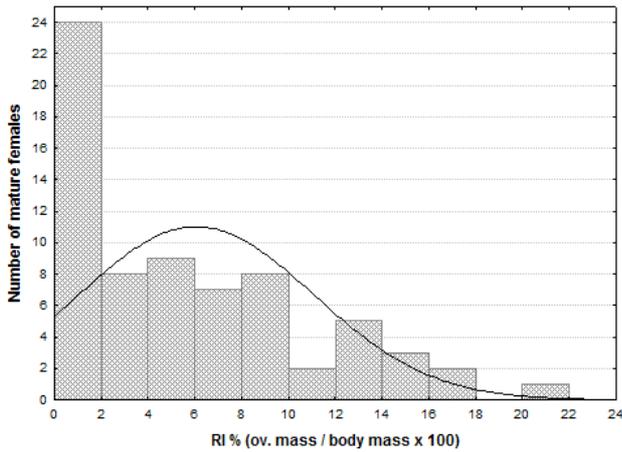


Figure 3. Absolute frequency of reproductive investment for mature females.

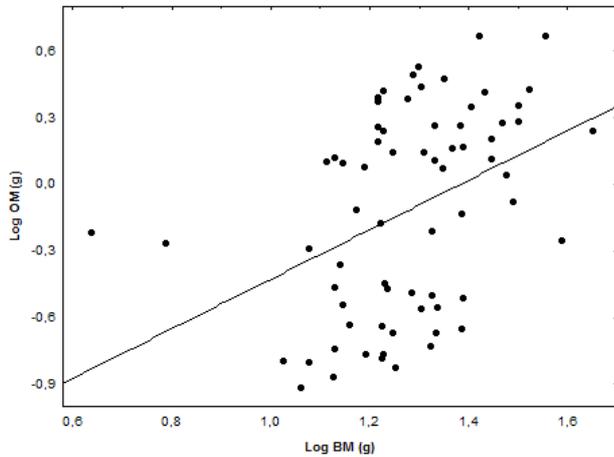


Figure 4. Linear regression between ovary mass (OM) and body mass (BM).

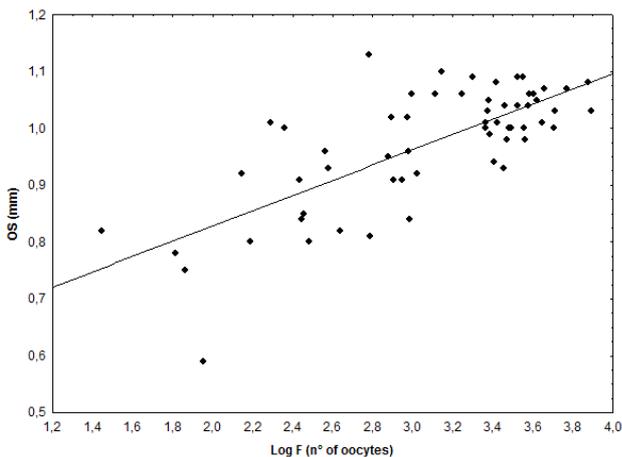


Figure 5. Linear regressions between oocytes size (OS) and fecundity (F).

Discussion

In this study mature females were every month except in June, unlike immature female were

absent in several months. These facts can be interpreted at the light of the paradigm of Wells (1977), as these individuals can breed at any time of year, a feature that is expected for explosive breeders. In studies with populations of *Physalaemus biligonigerus* and *P. riograndensis*, a seasonal pattern of reproductive activity was proposed (Camargo *et al.*, 2008; Pereira & Maneyro, 2012). With the highest number of mature females (November), the ratio did not exceed 65% of adult females in *P. biligonigerus* (Camargo *et al.*, 2008), whereas in *P. riograndensis* there were two peaks of abundance (March and December) (Pereira & Maneyro, 2012), in both cases, mature females were absent in several months. Therefore, *O. americanus* it can breed at any time of year, which is consistent with the explosive dynamics.

A wide range in fecundity (number of oocytes) has been observed in other anurans, like *Pleurodema thaul* (Díaz-Páez & Ortiz, 2001), and could be explained as a consequence of multiple oviposition events (e.g., female retains some mature oocytes for use in a subsequent oviposition). In an explosive species it is unlikely to occur, however in a population of the explosive breeder *Melanophryniscus montevidensis*, mature oocytes were found in both ovaries in a female dissected after a spawning in amplexus (Pereira, 2014). As in the case of *O. americanus*, the more intuitive explanations for the observed fecundity range, as the physical restriction (*i.e.* the female cannot expel all the eggs). An alternative explanation may be behavioral, as a result of male finishing the amplexus before female releases all oocytes.

According to literature *O. americanus* shows activity throughout the year mainly in the summer months (Gallardo, 1963), but always after heavy rainfall (Maneyro & Carreira, 2012). It has been suggested that during winter, the adults of this species are often hibernating (Gallardo, 1963), which explains the few specimens collected in this season. The reproductive activity of females had no correlation with the photoperiod and neither with average temperature, but a significant positive correlation with accumulated rainfall was observed. This was also observed in *Melanophryniscus montevidensis*. While in species with seasonal dynamics, it is expected that reproductive activity would be synchronized with a cyclic variable at an annual scale such as the temperature (e.g. *P. riograndensis*, Pereira & Maneyro, 2012); in explosive breeders, reproduction would be synchronized with an unpredictable variable, such a

rainfall, that facilitates the formation of water bodies as breeding sites for reducing the risk of desiccation of the eggs and aquatic larvae, because the duration of the metamorphosis it is prolonged (Vitt & Caldwell, 2008). In a study by Rodrigues *et al.*, 2003 with tropical populations of *Elachistocleis bicolor*, these authors found that the explosive breeder species have a seasonal reproductive pattern marked by rain because the particular climatic conditions of tropical regions. Another factor that may be considered is the photoperiod, which has a strong association with the reproductive activity of anurans. It acts as a signal suitable for breeding environmental conditions, controlling biological rhythms through endogenous mechanisms by activation of hormonal stimuli associated with reproduction, gametogenesis and calling activity (Both *et al.*, 2008).

A study realized with frogs from a Neotropical assemblage in the Pantanal by Prado & Haddad (2005) describes that explosive breeders have higher values of RI compared to species that exhibit prolonged and seasonal reproduction. Explosive females generally have less possibility to release many clutches during a reproductive event, which could lead to a high RI; in contrast, the prolonged breeder females that reproduce for several consecutive months may put several clutches in a single season. Our study showed the ratio between average fecundity and SVL in *O. americanus* (46.5) is higher than in *P. biligonigerus* (34), *P. cuvieri* (20.13), *P. gracilis* (10.3), *P. henselii* (12.8) (Pupin *et al.*, 2010), *P. riograndensis* (16.01) (Pereira and Maneyro 2012) and, *P. albonotatus* (30.6) (Prado & Haddad, 2005) that have prolonged reproductive activity. Only in *P. centralis* (54.2) this ratio is higher because this species shows seasonal reproductive activity, although the studied population has reproductive activity restricted to the rainy season (Brasileiro & Martins, 2006). On the other hand *P. venulosa* (51.43) and *L. chaquensis* (69.23) (Prado & Haddad, 2005) have an explosive reproductive pattern and their values are higher than *O. americanus*.

Only few gravid females showed high reproductive investment (RI), similar characteristics were observed in several species of the genus *Physalaemus* in seasonal temperate environments (e.g. *P. gracilis*, Camargo *et al.*, 2005; *P. riograndensis*, Pereira & Maneyro 2012). It was found a positive relationship between ovary mass and body mass, and also between fecundity and SVL. This result is consistent with several studies

showing that body size limits the accommodation of oocytes; therefore, adult females with larger sizes have greater potential reproductive success. They invest more in gametes than small females, which must still invest in growth to ensure their future reproductive success (e.g. Castellano *et al.*, 2004; Rodrigues *et al.*, 2004; Prado & Haddad, 2005; Camargo *et al.*, 2005, 2008). Possibly females with low RI are young and still spend part of their energy to grow. Therefore this delayed reproduction in females (Marangoni *et al.*, 2011; Cajade *et al.*, 2013). This fact may explain the sexual dimorphism observed in this population and other species in the genus (Quiroga *et al.*, 2015) where females are heavier and larger than males.

Previous results have shown that there is a tradeoff between the number and size of oocytes, i.e. quantity vs. quality (e.g. Wells, 2007; Prado & Haddad, 2005; Pupin *et al.*, 2010). Among different taxa, the number of eggs produced by a female can vary depending of the species reproductive modes. Oocyte quantity tends to decrease and the size tends to increase from aquatic to terrestrial species (Salthe & Duellman 1973; Crump, 1974). Species with terrestrial modes or both endotrophic larvae and direct development require more yolk for development (Salthe & Duellman, 1973). Our results do not show evidence that there is a tradeoff between the number and size of oocytes, as oocytes size was positively correlated with fecundity. Larger females, invests proportionally more in oocytes (big females have both more and bigger oocytes than small females). The same result was obtained in a population of *P. riograndensis* (Pereira & Maneyro, 2012) and *P. gracilis* (Camargo *et al.*, 2008). In those works, a negative association was observed when the variation in the acquisition of resources was lower than the variation in the distribution of investment, suggesting that the species differ in the levels of resource acquisition (Camargo *et al.*, 2008). In the case of a positive relationship this model suggests that it may be derived from variations in the acquisition of resources among individuals, which can be explained by differences between individual strategies among females (van Noordwijk & de Jong, 1986).

In the present study, no correlation between F and relative abundance of FBI was found. Moreover, the absence of temporal variation in this parameter would be consistent with the reproductive strategy of *O. americanus*, as they have little time to redirect the energy stored and must be prepared to breed at any time of year. In seasonal breeders, e.g. *Pleurodema*

thaul (Díaz-Páez & Ortiz, 2001) and *P. riograndensis* (Pereira & Maneyro, 2012) fat bodies decrease gradually as gonads increases in size. Possibly it is also necessary for this species have fat bodies throughout the year, to use that energy in hibernation during winter (Gallardo, 1963) and catching prey as it is an active predator (Isacch & Barg, 2002), addition to investing in reproduction.

This study confirms that *Odontophrynus americanus* is a species with an explosive reproductive dynamics. This is consistent with the dynamics of fat bodies and reproductive investment that does not vary along the year. It also could be associated with the relationship detected with rainfall. Larger females, have higher reproductive investment because they can accommodate more number of larger oocytes, probably due to the existence of a variation in resource acquisition among individuals. The results suggest that these individuals are ready to breed at any time of the year, and the reproductive activity seems to be influenced by rainfall. This study answers important questions about the species, but it could complete microscopy studies reproductive organs to corroborate the macroscopic results.

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