

Reproductive biology in a Uruguayan population of *Elachistocleis bicolor* (Guérin-Meneville, 1838) (Anura, Microhylidae)

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Recibido: 08 Enero 2016

Revisado: 30 Agosto 2016

Aceptado: 21 Octubre 2016

Editor Asociado: M. Tejedo

ABSTRACT

This paper describes ecological features of the reproductive biology of a population of *Elachistocleis bicolor* from Uruguay. From a monthly sample obtained throughout a year, we measured mass of the fat bodies and the ovaries, testicular volume, and estimate fecundity. We found evidence of a relationship between reproductive activity and air temperature, although we cannot rule out photoperiod as a regulator mechanism of the reproductive cycle. The dynamics of fat bodies are independent of the reproductive cycle, since their mass does not vary significantly along the year. We found evidence of sexual dimorphism and dichromatism, expressed by females being largest than males and by dark colored vocal sacs of males, although only present in some of them. Fecundity in females increases with body size, however larger females do not invest most of resources in reproduction.

Key words: Sexual dimorphism; Reproductive investment; Fat bodies.

RESUMEN

Este trabajo describe aspectos de la biología reproductiva de una población uruguaya de *Elachistocleis bicolor*. En una muestra mensual obtenida durante un año, se midió la masa de los cuerpos grasos y ovarios, el volumen testicular y se estimó la fecundidad. Se encontró evidencia que la actividad reproductiva se encuentra correlacionada con la temperatura, sin embargo no podemos descartar al fotoperiodo como regulador del ciclo reproductivo. La dinámica de los cuerpos grasos es independiente del ciclo reproductivo, ya que su masa no varió significativamente durante el año. Se encontró evidencia de dimorfismo y dicromatismo sexual, expresado por el mayor tamaño de las hembras y en la coloración oscura del saco vocal de los machos, aunque sólo presente en algunos de ellos. La fecundidad de las hembras se incrementó con el tamaño corporal; sin embargo, las hembras más grandes no invierten una mayor proporción de sus recursos en la reproducción.

Palabras claves: Dimorfismo sexual; Inversión reproductiva; Cuerpos grasos.

Introduction

Reproductive biology is an important component in organisms' life history (Gadgil and Bossert, 1970). Amphibians possess a great diversity of species and several types of reproductive strategies (Pough *et al.* 1999; Haddad and Prado, 2005). Each species exhibits a group of reproductive characteristics which mainly respond to a combination of both its evolutionary history and the environment in which it inhabits (Basso, 1990; Bevier, 1997). Wells (1977) proposes two basic types of amphibian reproductive strategies: prolonged and explosive breeders. The former are characterised by lasting several weeks

or months, during which there is high competition among territorial males for females, along with the formation of stable leks. On the other hand, explosive breeders are characterised by a reproductive period that extends for few days (or even few hours), and by a synchronized arrival of both genders to the breeding site. The strategies described are two extremes of a continuum, and most species show intermediate strategies (Prado and Haddad, 2005; Wells, 2007).

Reproductive investment may be defined as the proportion of resources that an organism invests

in the reproduction, in relation with the resources that are used for its maintenance and growth (Castellano *et al.*, 2004). Previous studies have proposed different explanations to such energetic trade-offs, but generally showing a positive correlation between body size and reproductive investment, observed for both explosive and prolonged breeders (Castellano *et al.*, 2004; Camargo *et al.* 2005; Rodrigues *et al.*, 2003; Rodrigues *et al.*, 2004).

Fat bodies are structures used for lipid storage, which reflect the nutritional condition of the individuals (Rastogi, 1976). A greater portion of the reproductive effort depends on such structures, and an inverse relationship has been observed between gonadal development and abundance of fat bodies (Fitzpatrick, 1976; Jørgensen, 1992). Nonetheless, some species have shown independence between the dynamics of fat bodies and reproductive activity, suggesting that, in some cases, the reproduction is not the main target of these resources (Jørgensen *et al.*, 1979; Jørgensen, 1986).

The pattern of sexual dimorphism, originated as a product of natural or sexual selection, is another characteristic of reproductive biology (Andersson, 1994). In amphibians, differences between sexes can include divergences in body size, colour, muscular structures, presence of vocal sacs, nuptial pads, among others (Wells, 2007).

This study deals about the natural history of *Elachistocleis bicolor* (Guérin-Meneville, 1838), a South American microhylid, which it is found from southern Bolivia to Uruguay, including a large part of Argentina and Paraguay and southeast Brazil (Frost, 2015). *Elachistocleis bicolor* has fossorial habits and it is considered an explosive breeder with a reproductive period lasting from October until February (Maneyro and Carreira, 2012). The aim of this study is a description of the reproductive patterns in a population of *Elachistocleis bicolor* from Uruguay, including the seasonal variations of several reproductive parameters (fecundity, reproductive investment and sexual dimorphism) and their relationships with climatic variables.

Materials and methods

The toad samples were stored in the Vertebrate Zoology Collection of Facultad de Ciencias, Universidad de la República, Montevideo (Uruguay), under the acronym ZVC-B. The specimens correspond to animals that were captured, measured and weighted

monthly, between January and December 2007, in Tacuarembó, Uruguay (32°26'06'S; 55°31'59'W). Climatic data were provided by the Dirección Nacional de Meteorología (INE, 2009).

A total of 405 individuals were dissected and then sexed via gonadal examination. In females, the ovaries were extracted and weighed (OM), and the mature (post-vitellogenic) oocytes were counted to determine fecundity (F) of each individual (Crump, 1974). In males, testicular length and width were measured in order to calculate testicular volume (TV), using an ellipsoid formula: $V = 4/3\pi (\text{length}/2) (\text{width}/2)^2$ (Dunham, 1981).

In all the specimens were extracted both fat bodies, and measure their mass (FB) with a Sartorius® digital scale, with 0.1 mg precision. Snout-vent length (SVL) and body mass (BM) of the sample was gathered from Vertebrate Zoology Collection database. Subsequently, the female reproductive investment (RI) was estimated as the percentage of OM relative to BM (Rodrigues *et al.*, 2004).

The smallest mature female and the smallest male with dark gular region were used to establish a discrimination rule of juveniles from adults (Maneyro and Carreira, 2012). The coloration of vocal sac was evaluated as one of the feature of sexual dimorphism.

Mean monthly variation in RI, F, TV and FB was analyzed via Kruskal-Wallis test and pos hoc Bonferroni test. In order to exclude females that already ovulated or have not completed vitellogenesis, for the regression analysis of SVL we considered individuals which fecundity was higher than 180 eggs (F'), since this was the minimum number of eggs per spawn that has been previously reported for the species (Cacciali, 2010). For data analysis, F, TV and FB were standardized dividing by body mass (reproductive variable/BM), except for the linear regressions with SVL.

Linear regressions were performed between: RI and SVL, TV and SVL, F' and SVL, FB and F, and FB and TV. Linear regressions were also applied between environmental variables (monthly temperature and monthly rainfall) and fat bodies abundance, RI, F and TV. Differences in SVL between sexes and between males with and without coloured vocal sacs were tested via U Mann-Whitney's nonparametric test.

Spearman's nonparametric rank correlation test was performed to verify if the variations in frequency of males and females followed a similar

pattern throughout the study, as to analyse a possible synchronization between frequencies of males with dark gular region and mature females.

Results

Of the total of specimens examined, 199 were females (128 adults and 71 juveniles) and 206 were males (161 adults and 45 juveniles). Among the adult females, 59 (46.1%) presented post-vitellogenic oocytes.

Reproductive seasonal variation and climatic variables

There was monthly variation of reproductive variables RI ($H=15.906$, $p=0.026$), F ($H=18.563$, $p=0.010$) and TV ($H=40.355$, $p<0.001$) (Fig. 1). Mature females were only found between October and March. On the other hand, the mass of fat bodies (FB) showed no significant monthly variation in adult females ($H=9.202$, $p=0.163$) and marginally significant variation in adult males ($H=13.865$, $p=0.054$).

We found significant associations between monthly temperatures and RI ($R^2=0.532$, $p=0.007$) and F ($R^2=0.504$, $p=0.010$) in adult females; and with TV ($R^2=0.650$, $p=0.002$) in adult males. However, monthly temperatures showed a better adjustment to the monthly number of mature females, through exponential model ($R^2=0.910$, $p<0.001$) than linear model ($R^2=0.522$, $p=0.008$). Monthly rainfalls were not significantly correlated to any of the tested variables.

Body size and fat bodies

Female SVL had a weaker relationship with RI ($R^2=0.137$, $p<0.001$), than with F' ($R^2=0.419$, $p<0.0001$). In adult males, TV was positively correlated to SVL ($R^2=0.281$, $p<0.001$). No correlation was found between female FB and F ($R^2=0.010$, $p=0.261$). However, in adult males, we found a weak relationship between TV and FB ($R^2=0.091$, $p=0.0001$).

Females were significantly larger than males, in both adults ($U=16349$, $p<0.001$) and juveniles ($U=2214$, $p<0.001$). On the other hand, 38 out of the 161 adult males presented dark gular region (18 specimens were found in January, one in April, five in November, and 14 in December). There was no significant correlation between the number of males with dark gular region and monthly rainfalls amounts ($R^2=0.047$, $p=0.497$). Nonetheless the frequency of males with dark gular regions was found related to the frequency of mature females

($R=0.658$, $p=0.020$). Additionally, males with dark gular regions were larger ($U=837.00$, $p<0.001$) and have heavier fat bodies ($U=1447.500$, $p=0.001$) than non pigmented males. However no significant differences were detected in TV between these two groups ($U=2693.00$, $p=0.156$).

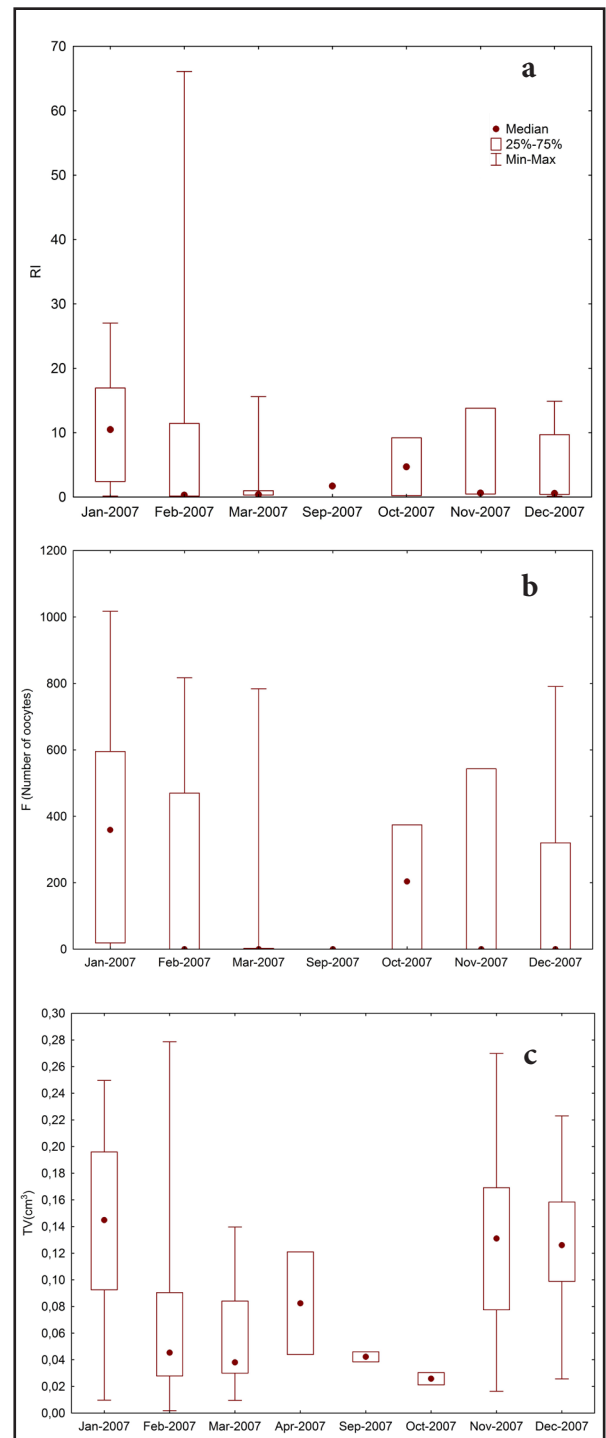


Figure 1. (a) Monthly variation of the reproductive investment (RI) (ovary mass/body mass) of adult females of *Elachistocleis bicolor*; (b) Monthly variation in adult females' fecundity (F); (c) Monthly variation in testicular volume (TV) of adult males.

Discussion

Reproductive seasonal variation and climatic variables

Based on the presence of mature females, reproductive period for the studied population of *E. bicolor* may be estimated from October to March, in agreement with descriptions for the species in Uruguay and Argentina (Martori *et al.*, 2005; Achaval and Olmos, 2007; Maneyro and Carreira, 2012). That it is also supported by the monthly variation found in reproductive investment, fecundity and testicular volume.

No significant monthly mass variation was found in the adult fat bodies (FB), which indicates that dynamics of FB does not follow gonadal variations and reproductive cycles, as found in other species from temperate environments (Díaz-Páez and Ortiz, 2001; Pereira and Maneyro, 2012). This might be due to an energetic allocation of fat bodies, in which gonadal development is not the main target of these resources.

The reproductive cycle seems to be mainly regulated by monthly temperatures, which agrees with the findings on most anurans from temperate regions (Wiest, 1982; Pombal, 1997). Another possibility for this result can be an internal regulation determined by the photoperiod, which would be masked by temperature, since both covary during the year (Both *et al.*, 2008). Even though reproductive activity of *E. bicolor* have been observed to trigger after heavy rainfalls (Martori *et al.*, 2005; Achaval and Olmos, 2007; Maneyro and Carreira, 2012), no relationship was found between monthly rainfalls and gonadal parameters. This is possibly due to the temporal scale of this study. We consider that daily analyses would help to detect the explosive breeding pattern reported for this species (Martori *et al.*, 2005; Maneyro and Carreira, 2012).

Body size and fat bodies

The lack of covariation between female SVL and either RI or F, suggests that other factors, such as the season and the individual nutritional conditions, are more determinant than size for the reproductive status. As in other studies, the results obtained with maximum fecundity proved that larger body size generate greater abdominal capacity, which allow by females to produce either higher numbers of oocytes, or larger oocytes (Camargo *et al.*, 2008; Rodrigues *et al.*, 2004; Rodrigues *et al.*, 2005). However, when analyzing RI, no trend was found

that larger females invest greater proportions of their resources in reproduction, compared to smaller specimens. This could be due to the small size (or perhaps short lifespan) of the species, and the possibility of greater advantage in full investment in each reproductive event, rather than prioritizing growth for the maximization of future reproductive investments. A similar association was observed in a Neotropical species of the same family, which have a short reproductive lifespan and higher reproductive investment in comparison with others anurans of the same region (Stănescu *et al.*, 2016).

Contrary to our expectations, there was no relationship between reproductive variables and FB in adult females. The relationship between the sizes of fat bodies and ovaries shows non-significant relationship, therefore reproduction could be understood as non-priority for the allocation of these reserves. Such result has been also found in the temperate species *Bufo bufo* (Jørgensen *et al.*, 1979); although a determinant role of fat bodies has been proved in the gametogenesis (Jørgensen, 1992). The same happens regarding TV in adult males, which indicates that possibly the dynamics of fat bodies is not determined by gametogenesis for this species. Neither effects on other aspects of reproductive activity in fat bodies was recorded, as could be reproductive movements or vocalization.

Among amphibians, females use to be larger than males (Rodrigues *et al.*, 2003; Martori *et al.*, 2005). Although this difference is often interpreted as a consequence of different growth rate between sexes, there might be other ecological causes that explain it. Wells (2007) proposes that such difference might be due to differential predation, since adult males are more exposed during reproductive events. The observations that female size exceeds male size in juvenile specimens support the idea of intrinsic causes of sexual dimorphism, such as divergent growth rates between gender, or late sexual maturity in females (Wells, 2007). Such size difference may be adaptive for females since, greater size increases fecundity (although there was not a parallel increase in relative reproductive investment by increasing the size of the abdominal cavity) (Kuramoto, 1978; Camargo *et al.*, 2008; Prado and Haddad, 2005; Rodrigues *et al.*, 2005).

For females, an investment in growth greater than that of males, or age procrastination of their first reproduction, seems more beneficial for them reaching larger sizes. Nail *et al.* (2014) have recently

found evidence that species which have larger females are those of small body size and explosive reproduction strategy; conditions that this species meets.

The dark gular region in males is considered a feature of sexual dimorphism in *E. bicolor* and related species (Rodrigues *et al.*, 2003; Martori *et al.*, 2005; Thomé and Brasileiro, 2007; Maneyro and Carreira, 2012). Nevertheless, our results indicates that only a small fraction of the adult males presented such characteristic, thus, even though the secondary sexual character is confirmed, it cannot be used as a distinctive feature to distinguish males from females. Visual signals can be important and complementary to male vocalizations in reproductive events, even in nocturnal species (Rosenthal *et al.*, 2004). Given that the males call in a semi-erect position, with the black vocal sac fully inflated contrasting with the yellow ventral coloration (Maneyro and Carreira, 2012), it is possible that gular pigmentation in males develops during reproductive events, which agrees with the association found between males with gular pigmentation and frequency of mature females. Larger body size and great amount of fat bodies found in the males showing dark gular region, could be biased by greater permanence of large males in the reproductive sites, or by the possibility of participation in more reproductive events due to greater reserves. Histological and physiological studies could complete these results and contribute to a better understanding of pigmentation mechanisms and their functions.

In conclusion, we found a reproductive period for *E. bicolor* from October to March, regulated by temperature or photoperiod. Contrary to our expectations, reproductive activity does not seem to determine the dynamics of the fat bodies. Sexual dimorphism seems to be expressed by dark gular region in males (even though it is not present in all males) and by greater body size in females. These differences could be promoted by the reproductive advantage that higher fecundity implies. However, female reproductive investment was independent from body size.

Acknowledgments

We are grateful to S. Carreira, I. Lombardo, E. da Costa and A. Nuñez for collecting the sample and give us biological information. To C. Fernandez for his help in laboratory work. The authors acknowledge the financial support of Comisión Sectorial de Investigación Científica (CSIC) and

Agencia Nacional de Investigación e Innovación (ANII).

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