Reproduction of a whiptail lizard (*Ameivula ocellifera*, Teiidae) from a coastal area in northeastern Brazil

DJan ZANCHI-SILVA$^{1,2}$, DivA M. BORGES-NOJOSA$^{1,2}$ and Conrado A.B. GALDINO$^3$

$^1$Núcleo Regional de Ofiologia da Universidade Federal do Ceará (NUROF-UFC), Campus do Pici, Bloco 905, 60455-760 Fortaleza, CE, Brasil

$^2$Programa de Pós-Graduação em Ecologia e Recursos Naturais, Universidade Federal do Ceará, Campus do Pici, Centro de Ciências, Bloco 902, 60455-970 Fortaleza, CE, Brasil

$^3$Programa de Pós-Graduação em Zoologia de Vertebrados, Pontifícia Universidade Católica de Minas Gerais, Avenida Dom José Gaspar, 290, Bairro Coração Eucarístico, 30535-901 Belo Horizonte, MG, Brasil

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ABSTRACT

The reproductive ecology of *Ameivula ocellifera* was studied from September 2009 to August 2010 in a coastal area of the state of Ceará, Brazil. Females reproduced continuously throughout the year, with a peak at the end of the rainy season. Even though there was a predominance of pre-reproductive individuals in the sample, gonadal activity of males peaked synchronously to female reproduction. Mean clutch size was 1.98 ± 0.56 and positively associated with female body size, while mean egg volume was 510.54 ± 84.29 mm$^3$ and unrelated to female body size. We did not find any association between clutch size and average egg volume.

Key words: *Ameivula*, *Cnemidophorus*, life history, reproductive cycle.

INTRODUCTION

Lizards are known to have diverse reproductive tactics, and in the neotropics, rainfall is a factor that plays an important role in the regulation of their time of reproduction (Rezende-Pinto et al. 2009, Balestrin et al. 2010, Van Sluys et al. 2010). Hence, for neotropical species, cyclic reproduction is found mainly in lizard populations from environments with a marked seasonality of rainfall (Wiederhecker et al. 2002, Miranda and Andrade 2003, Van Sluys et al. 2010) although cyclic reproduction may also be found in species from environments where rains are evenly distributed throughout the year (Magnusson 1987, Rezende-Pinto et al. 2009). However, in neotropical environments where rains are unpredictable, continuous reproduction might be selected (Vitt and Goldberg 1983).

Widely distributed lizard species often present inter-populational variation in reproductive activity as well as in other life histories attributes such as clutch size and volume whose variations might be related to responses of individuals to proximal cues (*Ameiva ameiva*, Vitt and Colli 1994, *Ameivula ocellifera*, Mesquita and Colli 2003b). Therefore, information regarding the reproductive ecology of a population of widely distributed species is valuable for understanding the effects of geographic variations in the attributes of the life history of lizards.
The speciose genus *Ameivula* comprises species which occur in Cis-Andean lowlands habitats from South America (Harvey et al. 2012). Reproduction in this genus was evaluated for *A. ocellifera* (formerly *Cnemidophorus ocellifer*) (Vitt 1983, Mesquita and Colli 2003a, b, Menezes et al. 2011) and *A. nativo* (formerly *C. nativo*) (Menezes et al. 2004). In both species, reproduction was continuous, in spite of studies having occurred in seasonal environments (Vitt 1983, Menezes et al. 2004). Clutch sizes ranged from one to six eggs (*A. ocellifera*, Menezes et al. 2011), while egg volumes varied from 381.2 mm$^3$ to 538.1 mm$^3$ (*A. ocellifera*, Menezes et al. 2011).

The whiptail lizard *Ameivula ocellifera* is a widespread species throughout the open formations in South America, being found in Brazil, Bolivia, Paraguay, and Argentina (Harvey et al. 2012). In Brazil, *A. ocellifera* occurs in biomes as diverse as the Cerrado (Mesquita and Colli 2003a, b), the northeastern Caatinga (Vitt 1983, 1995, Menezes et al. 2011), and in the coastal “restinga” of the Atlantic Forest (Santana et al. 2010).

The reproductive ecology of this iteroparous lizard species has been studied in populations from the Caatinga (Vitt 1983, Mesquita and Colli 2003a, Menezes et al. 2011), and from the Cerrado (Mesquita and Colli 2003a, b). To the best of our knowledge, up to now, studies on life history traits of the species are biased towards inland populations. Thereby, the present study provides the first information on the reproductive ecology of a coastal population of *A. ocellifera*.

Because the studied population inhabits a seasonal environment regarding rainfall, we predicted that *A. ocellifera* reproduction would be cyclical, with an increase in the frequency of reproductive individuals occurring in response to the rains. We also expected that female body size would positively affect their reproductive output (i.e. clutch size), as has been previously reported for other reptiles (King 2000).

**MATERIALS AND METHODS**

**STUDY AREA**

Fieldwork was conducted in two areas (ca. 5 km apart from each other) in the municipality of São Gonçalo do Amarante (SGA), state of Ceará, northeastern Brazil. The region’s original vegetation is typical of the littoral zone (see details in Nogueira et al. 2005), characterized by arboreal vegetation covering the edge behind the coastal strip of dunes. However, the studied sites correspond to altered open areas dominated by herbs and shrubs and under constant management due to farming of subsistence crops. The region’s climate is tropical, hot and semi-arid (Ipece 2010) with an average annual temperature of 27°C and an average annual rainfall of 1026.4 mm. The rains extend from February to May with May having the most rainfall (Figure 1).

**LIZARDS SAMPLING**

Lizards were sampled monthly from September 2009 to August 2010 by shooting with an air gun or manually caught. All procedures were carried out according to the ethical requirements of the Guidelines for the Euthanasia of Animals from American Veterinary Medical Association (AVMA 2013). Animals were preserved by the usual techniques (Auricchio and Salomão 2002), and deposited in the Herpetological Collection from Núcleo Regional de Ofiologia of the Universidade Federal do Ceará.

**LABORATORIAL PROCEDURES**

In the laboratory, the snout-vent length (SVL) of the lizards was measured using digital callipers (to the nearest 0.01 mm) before being dissected. Females were considered reproductive when carrying eggs in their oviducts or when they had enlarged vitellogenic follicles and/or corpora lutea. Clutch size was estimated by counting the number of vitellogenic follicles or eggs in the oviducts. Production of multiple clutches was considered to
have occurred by the simultaneous occurrence of eggs in the oviducts and vitellogenic follicles or by the presence of vitellogenic follicles and corpora lutea (Galdino et al. 2003). Oviductal eggs were measured for their length and width with digital callipers (to the nearest 0.01 mm) and egg volume was estimated by use of the formula for the volume of a prolate spheroid:

\[ V = \frac{4}{3}\pi \times (L/2) \times (W/2)^2, \]

where \( V \) is the egg volume, \( L \) is the egg length and \( W \) is the egg width. Females were considered as adults when their sizes were equal or larger than the smallest reproductive female from the sample (Diehl 2007).

For males, the left testis was removed and measured for the length and width. Testicular volume (TV) was estimated using a similar procedure to the one used to estimate the eggs’ volume. Testis were sectioned at 5 μm, mounted on slides and stained with hematoxylin-eosin. Additionally, seminiferous tubule diameters (STD) and germinative epithelium height (GEH) were measured using Axiosvision® software, connecting an optical microscope to a personal computer. Following Wiederhecker et al. (2002), STD and GEH measurements were taken from ten different fields in each slide (one slide per individual lizard). For the analysis, we used the mean values of STD and the mean of GEH per individual. Male reproductive states were based on spermatogenic stages, following Licht (1967), and individuals were considered reproductive when found above Licht’s Stage 5.

Male and female fat bodies were weighed using an electronic scale (nearest 0.001 g) after being drained on a towel paper.

**Statistical Analyses**

The data were checked for conformity with normal distribution using the Shapiro-Wilk test, and homoscedasticity was checked with F test. When necessary, we loge-transformed the data to meet normality assumptions. In cases where the assumptions of parametric tests were not met, even after performing data transformation, we used the corresponding non-parametric tests. Statistical analyses were performed using R (R Development Core Team 2010) with the significance level set at 0.05.

Differences in body sizes between adult males and females were analyzed using Student’s t-test. The effect of female SVL on clutch size was evaluated using Spearman rank correlation while the relationship between female body size and egg volume was evaluated with a simple linear regression. The association between females’ mean egg volume and clutch size was evaluated using the Spearman rank correlation. The effect of SVL was removed from variables (fat body mass,
testis volume, STD and GEH) by regressing SVL against each variable (one regression model per dependent variable) and the residuals were used in further analyses.

The effect of short-term (i.e., one year of study) mean monthly rainfall (one-month time lag) and temperature upon the frequency of reproductive females (arcsine-transformed) was evaluated by multiple regression analysis. Multiple regression was also used to evaluate the effect of long term (i.e., average of 20 years) mean monthly rainfall and temperature on the frequency of reproductive females (arcsine-transformed).

**RESULTS**

We collected 385 *A. ocellifera* individuals, 193 males (171 adults, 14 juveniles, and 8 without age determination), 175 females (105 adults, 64 juveniles, and 6 without age determination), and 17 juveniles whose sex was not identified. The mean adult female SVL was 62.89 ± 7.02 mm (range 51.46 – 81.83; n = 107), while the mean adult male SVL was 60.70 ± 10.79 mm (range 43.39 – 98.35; n = 174). Adult females were larger than adult males (t-test, \( t = -2.60, P = 0.009 \)).

In September 2009 we found the only male in reproductive stage 6 in our sample, which was the smallest reproductive male (43.39 mm SVL). We found one male at reproductive stage 5 in each of the following months: January, June and July 2010 (Figure 2a). Considering overall males, 82% were found in stage 4, and such males predominated during all sampled months (Figure 2a). Values of TV, STD and GEH varied over the study period (Figure 3), with the highest values of TV observed between May and July 2010 (Figure 3a), the highest values of STD were found in March, May, June and July 2010 (Figure 3b), and the highest values of GEH were found from March to May 2010 (Figure 3c). For both sexes, fat body mass was nearly invariable during the study period with a mean 0.0053 ± 0.0233.

![Figure 2 - a) Monthly frequencies of males at different reproductive stages and b) reproductive activity of females of *Ameivula ocellifera* from September 2009 to August 2010 in a coastal tabuleiro area from northeastern Brazil. I = Seminiferous tube involuted with only spermatogonia; II = Primary spermatocytes appearing; III = Secondary spermatocytes and spermatids abundant; IV = Transforming spermatids with few spermatozoa; V = Spermatids and spermatozoa abundant; VI = Spermatozoa abundant. NR = non-reproductive females; VOE = simultaneous presence of vitellogenic follicles and oviductal eggs; O = presence of oviductal eggs; VF = presence of vitellogenic follicles. Numbers above each bar indicate sample sizes.](image)

Female reproduction occurred throughout the period of September 2009 to August 2010 (Figure 2b), with a peak in May 2010 when 71% of females were reproductive. Females with oviductal eggs occurred in September and December 2009, January 2010, and from April to July 2010 (Figure 2b). The smallest reproductive female was collected in October 2009, measuring 51.46 mm SVL. The monthly frequency of reproductive females was not related to short and long term effects of precipitation and temperature (multiple regression analysis, \( F_{2,9} = 0.73; P = 0.50 \) and \( F_{2,9} = 0.83; P = 0.47 \), respectively).
The simultaneous presence of vitellogenic follicles and oviductal eggs was observed in a unique female (59.70 mm SVL). We found three females with a simultaneous presence of vitellogenic follicles and corpora lutea (SVLs: 59.70; 65.28; 67.69). Clutch size mode was two eggs (range 1 – 3, mean = 1.98 ± 0.56, n = 72), which corresponded to 68.05% of the females, while 16.66% had a clutch of one egg and 15.27% had a clutch of three eggs. Clutch size was positively associated to female body size (Spearman rank correlation, \( P < 0.001; r_s = 0.43; n = 72 \)). Mean egg volume was 510.54 ± 84.29 mm\(^3\) (range 333.84 – 698.55 mm\(^3\), n = 22), and clutch volume was related to female body size (\( F_{1,9} = 9.48; P = 0.01; r^2 = 0.51 \)). However, no association was found between clutch size and mean egg volume (Spearman rank correlation, \( P = 0.11; r_s = 0.48; n = 12 \)).

**DISCUSSION**

The majority of sampled males were found to be at stage 4, which is considered a pre-reproductive stage (see Licht 1967). We are not able to explain the predominance of pre-reproductive males in the population, although the analysis of STD and GEH suggests some variation in males’ gonadal activity throughout the year. Taking the values of STD and GEH, an elevation of testis cellular activity coincides with the period in which female reproduction peaks, despite it is not resulting in sperm production.

Females *A. ocellifera* from SGA are able to reproduce continuously, although the proportion of reproductive females varied markedly during the year, with a peak of reproduction at the end of the rainy season. For months not coinciding with the reproductive peak, the frequency of reproductive females was always less than 50%. Female *A. ocellifera* from SGA were found carrying eggs in September and December 2009, January 2010, and from April to July 2010, and it is important to note that the frequency of females with multiple reproduction was low. The pattern of egg production of females from SGA contrasts with that found for females of other *A. ocellifera* population by Vitt (1983). He showed that females of this species inhabiting a semi-arid (Caatinga) site - c.a. 400km south of SGA in the municipality of Exú, state of Pernambuco, Brazil - were found with eggs throughout the year. Vitt (1983) also found that many female *A. ocellifera* were apt to produce short term multiple clutches, which might be an efficient strategy to increase reproductive output when females produce small clutches (Tinkle et al. 1969, Manríquez-Morán et al. 2005, Vitt and Zani 2005). However, contrary to females from Exú, the low

![Figure 3 - Variation in a) SVL-independent testis volume, b) SVL-independent seminiferous tubule diameter, and c) SVL-independent germinative epithelium height in males of *Ameivula ocellifera* from September 2009 to August 2010 in a coastal tabuleiro area from northeastern Brazil.](image-url)
number of females investing in short time multiple reproduction in SGA might constitute an evidence that they benefit from inter-spacing reproduction over a relatively large time period during the year.

Because the rains pattern creates a seasonal and predictable environment in SGA, we had expected that the reproductive pattern of the studied population would be cyclical as found for other lizards from seasonal environments (Ameiva ameiva, Cnemidophorus lemniscatus, and Kentropyx striata, Magnusson 1987, Tropidurus torquatus, Wiederhecker et al. 2002, Eurolophosaurus nanuzae, Galdino et al. 2003, Pediplanis burchelli, Nkosi et al. 2004, Sceloporus formosus scitulus, Sceloporus omiltemanus, and Mesaspis gadovii, Ramírez-Pinilla et al. 2009). However, contrary to our expectations, the reproductive activity of females from SGA occurred continuously throughout the year. Nonetheless, one might consider that, in contrast to the Caatingas’ population studied by Vitt, reproduction of females in SGA shows a marked concentration at the end of the rainy period, characterizing a continuous reproduction with variations in reproductive activity.

We found no effect of temperature and rainfall upon the frequency of reproductive A. ocellifera females. The occurrence of reproductive females, irrespective of the time of the year, should lead to statistical non-significance. However, the increase in the frequency of reproductive females at the end of rainy season suggests some effects of rainfall upon female reproduction. Thus, despite the statistical non-significance, we cannot discard the evidence that the rains may possibly influence females’ reproduction in SGA, as evidenced for other lizards’ species from seasonal habitats (Liolaemus lutzae, Rocha 1992, Eurolophosaurus nanuzae, Galdino et al. 2003). Menezes et al. (2004) did not find significant effects of rainfall upon reproduction of the parthenogenetic lizard Ameivula nativo, formerly Cnemidophorus nativo, in a seasonal habitat, and argued that “the occurrence of more than 50% of reproductive females during almost all months of the year indicates that reproduction is extended and relatively independent of habitat seasonality”. On the other hand, females from SGA increased their frequency of reproduction only in the peak of rains at the wet season, evidencing that seasonality in environmental conditions may play a role regulating reproduction of A. ocellifera from SGA.

In SGA, clutch size and clutch volume of A. ocellifera was positively associated to female body size, a trend usually common in teiid lizards (Ameiva plei, Censky 1995, Aspidoscelis lineattissima, formerly Cnemidophorus lineattissimus, Ramírez-Bautista et al. 2000, Ameivula nativo, formerly Cnemidophorus nativo, Menezes et al. 2004, Ameiva ameiva, Rocha 2008, Contomastix lacertoides, formerly Cnemidophorus lacertoides, Balestrin et al. 2010). Only larger females were capable of producing a clutch of three eggs. Since size is a proxy of age in lizards (Andrews 1982), our results suggest that A. ocellifera females from SGA have made differential reproductive investments according to their age as argued by Galdino and Van Sluys (2011). Clutch size was not associated with egg volume for the studied females, and this result might be due to the fact that the mean clutch size of A. ocellifera from the coastal tabuleiro area was small (on average two eggs) and with small variance. Kiefer et al. (2008) reported differences in clutch size among populations of Tropidurus torquatus to the effects of distinct female body sizes among populations, where those populations of larger females produced larger clutches. Similarly, two populations of Xenosaurus platyceps from distinct habitats differed in litter size produced by females, although larger females produced larger litters in both populations (Rojas-González et al. 2008). The results obtained in the present study reinforce the capability of some lizards species of adjusting their fecundity to respond to local selective pressures.

Small clutch size has also been observed in other teiid lizard species, such as Aspidoscelis inornata, formerly Cnemidophorus inornatus, and
A. neomexicana, formerly C. neomexicanus, who produce clutches of two eggs in average (Christiansen 1971). Similarly, populations of Cnemidophorus murinus presented a clutch size of one single egg, produced by the majority of females (Dearing and Schall 1994), while populations of C. lemniscatus and Ameiva parecis, formerly Cnemidophorus parecis, had clutches of up to two eggs (Mesquita and Colli 2003a). Production of larger clutches may imply in higher predation risks for females of active forager lizards (Shine 1980) since they depend on running speeds to escape from predators (Vitt 1990). Therefore, small clutches are expected to be selected in active forager species (C. graminivagus, C. lemniscatus, and Ameiva parecis, formerly Cnemidophorus parecis, Mesquita and Colli 2003a).

In the present study, fat body mass was near zero for most of the sample period, providing evidence of the high energetic costs experienced by A. ocellifera individuals in SGA. Moreover, the production of a smaller clutch by females of this population might also be related to limited energy stocks available to invest in larger clutches.

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REFERENCES


