Trabajo

Cuad. herpetol. 36 (1): 21-32 (*2022*)

Lipidic cycle, condition factor and reproductive cell maturation in *Gymnodactylus darwinii* Gray, 1845 (Squamata, Phyllodactylidae) from a fragment of Atlantic Forest in northeastern Brazil

Leonardo P. C. Oitaven[1,3], Márcio F. Chaves[2], José L. de Araújo[2], Felipe S. Ribeiro[3], Geraldo J.B. de Moura[3]

[1] Laboratório de Estudos Herpetológicos e Paleoherpetológicos, Departamento de Biologia, Universidade Federal Rural de Pernambuco – UFRPE. Graduate Program in Animal Bioscience, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE, Brasil.

[2] Laboratório de Biologia de Anuros, Centro de Saúde e Educação, Universidade Federal de Campina Grande, Rua Olho D´Agua da Bica s/n, CEP 58175-000, Cuité, PB, Brasil.

[3] Laboratório de Estudos Herpetológicos e Paleoherpetológicos, Departamento de Biologia, Universidade Federal Rural de Pernambuco – UFRPE, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE. Graduate Program in Animal Bioscience-UFRPE. Graduate Program in Biodiversity-UFRPE. Graduate Program in Environmental Sciences-UFRPE, Brasil.

Recibido: 23 Junio 2021
Revisado: 05 Agosto 2021
Aceptado: 02 Mayo 2022
Editor Asociado: N. Ibargüengoytía

doi: 10.31017/CdH.2022.(2021-049)

ABSTRACT

In this study, we describe a variation in fat bodies (liposomatic relationship), condition factor (the welfare degree of a population against the environment, K1), and male and female reproductive cells of the gecko *Gymnodactylus darwinii* (Gray, 1845) collected in two contiguous protected areas (Tapacurá and Mata do Camucim forests) in the municipality of São Lourenço da Mata, Pernambuco state, Brazil. We assessed seasonal variation and the influence of biotic (body temperature and K1 condition factor) and abiotic (air temperature and precipitation) factors on the lipidic cycle and reproductive cells' maturation. As typical in geckos, fat bodies in *G. darwinii* decreased markedly during the high temperature and low precipitation periods. A slight variation in K1 suggests similar levels of energetic investment in growth and sexual maturation. Different reproductive cells matured similarly in sexually mature individuals, indicating a continuous, synchronised maturation cycle. Nonetheless, while fat bodies decreased and the number of mature reproductive cells increased in dry months, the opposite pattern was observed in rainy months. Our results indicate that *G. darwinii* does not show reproductive seasonality but instead peaks of reproductive activity (reproductive cell maturation, mating, lay eggs) synchronised in females and males, regulated by body temperature and climatic variables, including air temperature and precipitation.

Key Words: Gender Environmental Changes; Gametogenesis; Gonadal Histology; Lipid Storage; Lizards

Introduction

Studies concerning energetic cycles and reproductive cell maturation provide important insights for testing hypotheses about lizards' life-history evolution strategies (Ramírez-Bautista *et al.*, 2009; Norval *et al.*, 2019; Resendiz, 2020). In these animals, the lipidic cycle, including condition factor (a population degree of well-being against the environment) and gametogenesis, tends to be regulated by the interaction of abiotic (Serrano-Cardozo *et al.*, 2007; Sánchez-Hernández *et al.*, 2013; Lozano *et al.*, 2015)

Author for correspondence: geraldo.jbmoura@ufrpe.br

and biotics factors (Galdino *et al.*, 2003; Garda *et al.*, 2014). The influence of these factors can be assessed through several parameters, including temperature, precipitation, and photoperiod (Cheng, 1987; Norval *et al.*, 2019; Díaz-Vega *et al.*, 2020), and through correlations between body conditions, such as reproductive cells maturation and the condition factor (K1), body temperature, and variation in energy reserves (Derickson, 1976; Galdino *et al.*, 2003, Salvador, 2011).

The condition factor is measured as the lizards' lipids reserves. It provides valuable insights concerning the individuals' recent nutritional patterns and energetic investment into cyclical, vital activities, including feeding, thermoregulation, and breeding, therefore inferring a population's general well-being (Ballinger, 1977; Cheng, 1987; Autumn y De Nardo, 1995). In addition, identifying morphological characteristics and seasonal changes in cellular structures of reproductive cell maturation in different sexes is key to determining the reproductive strategies employed by the species (Newlin, 1976; Trauth, 1979; Uribe et al., 1995). Therefore, data on energetic cycles and reproductive cell maturation allow inferences on the population reproductive strategies, the prevailing environmental conditions, and the availability of local resources, contributing to the development of more effective conservation and management programs (Uribe et al., 1995; Torki, 2007; Norval et al., 2019).

The reproductive dynamics, body growth patterns, and lipidic cycles of most Neotropical lizards are unknown or scarcely documented so far, as is the case of the naked-toed gecko, *Gymnodactylus darwinii* Gray, 1845. This lizard is a typical ground-dwelling, sit-and-wait forager (Oitaven *et al.*, 2019; Guedes *et al.*, 2020) endemic to the Brazilian Atlantic Forest (Costa y Bérnils, 2018; Oitaven *et al.*, 2019). *Gymnodactylus darwinii* presents a generalist diet (Almeida-Gomes *et al.*, 2012), a continuous reproductive strategy (Guedes *et al.*, 2020), and rupicolous habits, inhabiting mainly rocky outcrops (Huey *et al.*, 2009; Oitaven *et al.*, 2019). Given the great diversity of Neotropical lizards and the environments they inhabit, different reproductive patterns and strategies might be expected to be used by different populations, especially in environments with intense climatic seasonality (Ferreira *et al.*, 2002; Galdino *et al.*, 2003; Ferreira *et al.*, 2009; Migliore *et al.*, 2017).

Several studies have reported that abiotic factors, such as precipitation and air temperature, do not play a clear role in increasing or decreasing lipid storage in individuals' bodies (i.e., in the lipidic cycle). Such factors seem not to influence the investment put by individuals into bodyweight and length gain (i.e., in the growth cycle) (Ramírez-Bautista *et al.*, 2009; Norval *et al.*, 2019). Likewise, the reproductive cells' maturation in lizards also lacks a clear relationship with abiotic factors (Uribe *et al.*, 1995; Lozano *et al.*, 2014; Lozano *et al.*, 2015). However, there is evidence that biotic factors influence the cell maturation process and the lipid cycle (Derickson, 1976; Cooper *et al.*, 1987; Galdino *et al.*, 2003).

In this study, we described the condition factor (K1), the fat body cycle, and the temporal variation in the lizard *G. darwinii* gamete production from an Atlantic Forest remnant in the state of Pernambuco, northeastern Brazil. We use these data to 1) infer how geckos manage their energetic resources over the year, i.e., whether seasonally or not, and 2) evaluate whether and to what extent abiotic and biotic factors influence the reproductive cell maturation process and the lipidic cycle. Specifically, we expected variation in the *G. darwinii* lipidic cycle and gamete production to be driven by biotic factors, including body temperature and K1 condition factor, and abiotic factors, including air temperature and precipitation.

Materials and methods

Study site

We performed this study in two Brazilian Protected Areas (PAs): Mata do Camucim (200 ha; -8.041513S, -35.1923423W, Datum WGS84) and Mata do Tapacurá (100 ha; -8.044166S, -35.201388W, Datum WGS84), which are geographically contiguous and inserted within another protected area, the Tapacurá Ecological Station (Fig. 1). Once both areas constitute interconnected, close-located PAs that do not present significant environmental differences, the collected *Gymnodactylus darwinii* individuals were analysed together. Both PAs comprise seasonal Atlantic Forest remnants surrounded by sugarcane crops in the municipality of São Lourenço da Mata, part of the metropolitan region of Recife city, in the Pernambuco state, northeastern Brazil (Carneiro-Moura *et al.*, 2014; Oitaven *et al.*, 2019). The altitude gradient in the study area ranges from 120 m to 150 m above sea level.

The local climate is tropical, and rainfall is concentrated between March and August, typical of

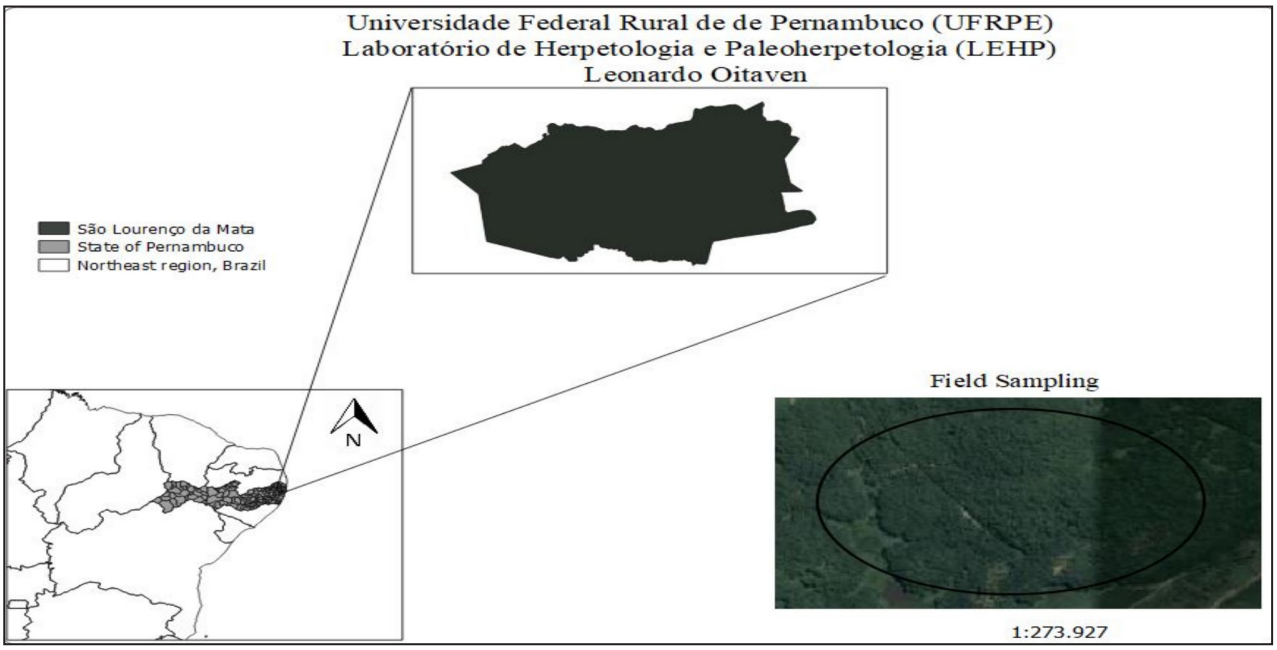

Figure 1. Map of the state of Pernambuco (PE), northeastern Brazil, depicting the municipality of São Lourenço da Mata and the study areas in Mata do Camucim and Mata do Tapacurá, both located within the Tapacurá Ecological Station.

the Brazilian Northeast Rainforest Zone. The annual precipitation is 1900 mm, but precipitation amounts may be less than 100 mm in the driest months (APAC, 2017; Oitaven *et al.*, 2019). Because precipitation and the air temperature vary considerably in the study area, we defined the dry and rainy seasons according to climatic data collected during the study (Fig. 2). The dry season was characterised by low monthly precipitation (0.5–20.7 mm) and relatively high temperatures (30.3–34.9°C). In contrast, we recorded a drastic increase in precipitation levels (13.4–211.4 mm) during the rainy season, although temperatures decreased slightly (32.4–34°C). We obtained data on monthly air temperature (°C) and rainfall (mm) levels from the Pernambuco Water and Climate Agency database (APAC, 2017).

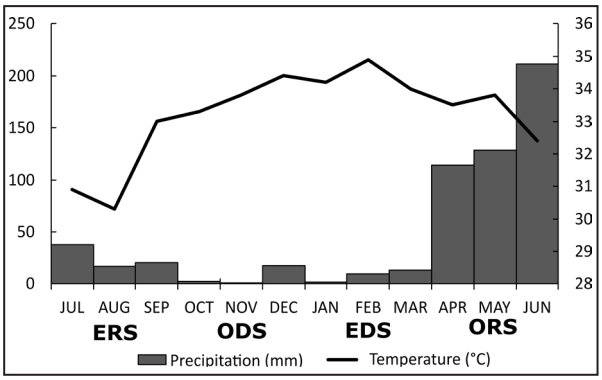

Figure 2. Climatogram from the Tapacurá Ecological Station (Açude de Tapacura, APAC) region, municipality of São Lourenço da Mata, state of Pernambuco, Brazil. The climatogram shows the mean air temperature (°C) and precipitation (mm) monthly between July 2016 and June 2017, according to the onset of dry season (ODS), ending of dry season (EDS), onset of rainy season (ORS), and ending of rainy season (ERS).

Field and laboratory procedures

We collected up to six individuals per month (three males and three females) between July 2016 and June 2017 through active searching, following the recommendation of the collection license available and applying the same effort from previous studies (e.g., Lozano *et al.*, 2015; Hernández-Gallego *et al.*, 2018). Even with a limited number of specimens, the monthly sample size of collected individuals was enough to conduct all analyses in this study.

Immediately after capturing the individuals, we measured the body temperatures using a digital thermohygrometer with precision to the nearest 0.01°C (Colli *et al.*, 2003), weighed the body masses with a digital scale (to the nearest 0.1 g), and then euthanised the specimen with a Ketamine (10 mg/Kg) and Medetomidine (0.1–0.3 mg/Kg) lethal injection. We measured each specimen's snout-vent length (SVL) using a digital calliper (to the nearest 0.01 mm). After the measurements, we removed the specimen's tail and weighed its mass (w) separately on a digital scale (to the nearest 0.001 mm) (Galdino *et al.*, 2003). Because most of the individuals captured presented the

entire tail, showing no evidence of earlier autotomy, we also measured the tail length and diameter using a digital calliper (to the nearest 0.01 mm). Then, we calculated their tail volume (π(D/2)2H), where D represents the diameter and H is the extension of the tail. In addition, because abdominal fat bodies were absent in all *G. darwinii* individuals collected, we used tail mass to calculate the lipidic reserves index in the population (Derickson, 1976).

We fixed and preserved the collected specimens in 10% formaldehyde and 70% ethyl alcohol, respectively, and stored them in the UFRPE Herpetological and Paleoherpetological collection (catalogue numbers 4843–4907). The procedures were authorised by the Federal Environment Agency (SISBIO #54374-1) and the UFRPE Ethics Committee on Experimental Use of Animals (CEUA UFRPE 098/2016).

We removed each specimen's gonads through a longitudinal incision from the throat to the cloaca for reproductive cells analysis and conserved the gonads in 70% ethyl alcohol. Then, we fixed the material in Bouin's solution (71% picric acid, 24% formaldehyde, 5% glacial acetic acid) for 24 hours and dehydrated it in an increasing alcohol series for 30 minutes at each of the six stages: 70%, 80%, 90%, 95%, 100%, and 100% (Hopwood, 1990). Finally, we embedded the samples in paraffin to obtain 5 μm thick histological sections, placed them on microscope slides, and stained them with Hematoxylin and Eosin for analysis (Robinson y Gray, 1990).

Reproductive cells and structures analyses and the liposomatic relationship (LR) index and the condition factor (K1) estimation

We analysed the histological slides qualitatively using a conventional, trinocular bench microscope (Olympus AX70, Tokyo, Japan) attached to a digital image acquisition system (ERc 5s camera with Axiovision 6.3, Carl Zeiss, Jena, Germany). Cell types were identified according to Gribbins (2011). The seminiferous tubules were analysed based on a random selection of 27 tubules from each male specimen at a 400x magnification (Mayhew y Wright, 1970; Lozano *et al.*, 2015).

We analysed the male testicle slides stereologically by following Mandarim-de-Lacerda (1995) and Weibel (1979). We calculated the Volume Density (Vv) of the primary and secondary spermatids and the spermatozoa to obtain the reproductive maturation index for each specimen (Torki, 2007). We calculated the Volume Density using Hally's (1964) formula to represent the stereological parameters (Mandarim-de-Lacerda, 1995). We described the morphology of the male reproductive cells following Gribbins (2011) and quantified the density profiles (Qa: primary and secondary spermatids and spermatozoa, which are the best-differentiated structures). We also counted a different number of fields within the Test Area (AT) for each analysed specimen. The results (profiles/mm²) were obtained from the profiles' average, based on QA = ∑profiles/AT (Mandarim-de-Lacerda, 1995).

We also analysed the female ovaries microscopically. We used the follicles described by Uribe *et al.* (1995) and Santos *et al.* (2020) as references for histological descriptions. Because the thickness of the follicular wall modifies during the previtellogenic and vitellogenic phases, we quantified different cell types of this region using the population density approach, considering a test area of 88 mm2 within which we identified small and intermediate ovarian cells and pyriform cells (Uribe *et al.*, 1995; Santos *et al.*, 2020). We also quantified the number of oviductal eggs in each female over the sampling months to describe temporal variation in mature reproductive structures in the studied population. We measured the length of the oviductal eggs using a digital calliper (to the nearest 0.01 mm) and estimated the eggs' volume using the formula of an ellipsoid (Mesquita *et al.*, 2015).

We calculated the specimens' liposomatic relationship (LR) using the following formula: LR = WT (100)/w, where WT is the tail mass, and w is the total mass. Because abdominal fat bodies were absent in the specimens collected, the LR index was applied to tail measurements, and weight was used to proxy the individuals' lipid reserves (Vazzoler, 1982; Norval *et al.*, 2019). We then used the Allometric Method to calculate the overlap of the individuals' condition factor (K1), based on the equation K1 = W/Lb, where W is the specimen's total mass, L is the specimen's snout-vent-length, and b is a coefficient determined by each specimen mass-length ratio (W = aLb) (Lima-Junior *et al.*, 2002).

Data analysis

To analyse the monthly patterns, we calculated the mean values and the standard deviation of the condition factor (K1), the liposomatic relationship (LR) indices, the number of male reproductive cells (primary and secondary spermatocytes and sperms),

and the number of oviductal female eggs in each collecting month. We assessed seasonal variation (regarding the onset and dry and rainy seasons end) in reproductive cell types and K1 and LR indices values using a non-parametric Kruskal-Wallis' analysis of variance (Zar, 1999; Galdino *et al*., 2003). Due to the limited monthly sample size and the variation in the number of individuals collected throughout the study, only samples with at least four individuals were included.

We modelled linear regressions (R2) using the *Iswr* package, considering conditions (reproductive cell types and LR) as dependent variables and the abiotic (air temperature and precipitation) and biotic factors (body temperature and K1) as independent variables to verify the correlation on reproductive cell types and LR. Finally, we tested the relationship between LR and tail volume using simple linear regression ($r2$) (Zar, 1999). We considered a significance level of $P < 0.05$ For all statistical analyses, and the analyses were performed in the R program v3.6.1 (R Development Core Team, 2019).

Results

Sixty-one adult individuals were used in the present study, corresponding to 81.3% of the total individuals sampled (n = 83). No adults or males were sampled in August and September 2016, respectively. The SVL of gravid females varied from 48.32 mm to 59.23 mm. Based on the ellipsoid formula, the mean egg volume was 63.27 ± 49.5 mm^3 (20.06–185.17 mm^3; n = 14). Eggs were always found in the oviducts, and the ovaries were adhered to the dorsal wall by the mesovarium, showing fixed clutch size (always two eggs).

Follicles in both previtellogenic and vitellogenic phases were observed in the ovaries' histological sections. The previtellogenic phase is characterised by a large number of vitellus granules, with earlier signs of the vitellogenic process and the ooplasm forming the yolk platelets with evident layers (Fig. 3A). The granulosa layer is extremely thick in this phase, presenting a well-developed theca and yolk membrane (Fig. 3B) with small, cuboid, intermediate, and pyriform cells (Fig. 3C). In the vitellogenic phase, the ooplasm is characterised by yolk granules in the cytoplasm (Fig. 3D) and a significant reduction in the granulosa layer, yolk membrane, and theca, with only small cells present (Fig. 3D). Some individuals showed corpus luteum, a hypertrophied theca containing "whorl" cells and lutein mass (Fig. 3E).

The microscopic analysis of testicles' histological sections revealed the presence of germinal cells at all development stages, including spermatogonia, spermatocytes, and sperms, indicating that males are reproductively active throughout the year. The spermatogonia (SPG) mainly occupied the seminiferous tubules' periphery and were ovoid-shaped and dark-coloured (Fig. 4A). The central region of the seminiferous tubules is filled up as the cell maturation process develops (mitotic process), becoming a rod-shaped spermatocyte (SPT), which may be either primary (following meiosis 1) or secondary after meiosis 2 (Fig. 4A). At the final spermatogenesis stage, the cells are fully developed and termed spermatozoa (SPZ) (Fig. 4B), being stored in the epididymis and presenting structures that allow them to move towards and survive in the female's reproductive organ.

Condition factor (K1)

According to the Allometric Method, the body condition factor of the studied *G. darwinii* population had a K1 value of 2.707, based on the positive correlation between the individuals' body mass and body length ($r^2 = 0.81$; $P < 0.001$) (Fig. 5). K1 values in males increased at the beginning of the dry and rainy seasons (Fig. 6), whereas in females, they were higher at the end of the rainy season (Fig. 7). Lower LR values were recorded in males during the end of the dry and rainy seasons (Fig. 6), whilst in females, the lowest value was recorded during the rainy season, even though the highest value was recorded at the beginning of this season (Fig. 7). Mean tail length did not differ between males and females ($t = -1.14$; df = 44.2; $P = 0.25$), nor did tail volume ($t = 1.55$; df = 55.23; $P = 0.12$). Despite the lack of a significant difference between males and females, there was a positive and significant correlation between tail weight and volume (r2 = 0.12; df = 57; p < 0.001), indicating that lipid reserves in tail regions increase according to tail volume.

There was significant seasonal variation in the K1 values obtained for males, with higher K1 values recorded at the beginning of the dry and rainy seasons (Table 1). In contrast, LR values did not vary significantly between seasons for either sex (Table 1). In males, primary (Spt1) and secondary (Spt2) spermatocytes varied significantly between seasons (Kruskal-Wallis: $p < 0.05$), with a peak of Spt1 production at the end of the rainy season and the

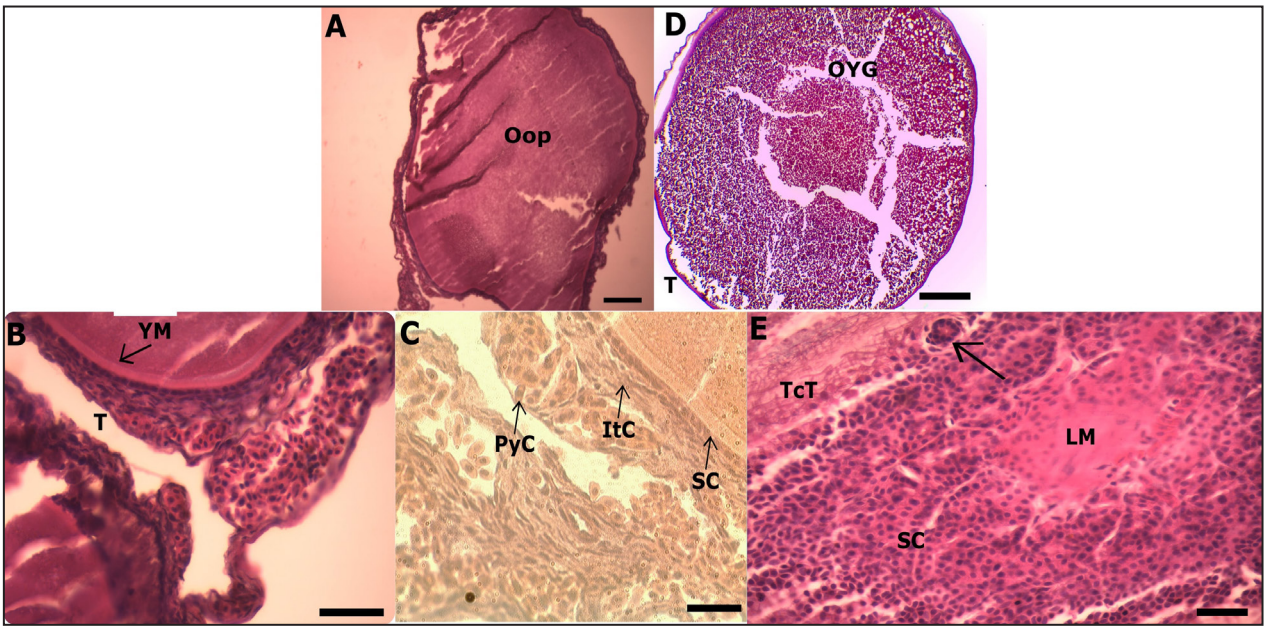

Figure 3. Histological sections of follicles of *G. darwinii* specimens collected in the Tapacurá forest, São Lourenço da Mata, Pernambuco, Brazil. (A) Previtellogenic follicle in early vitellogenic process, with the ooplasm (Oop) forming yolk platelets. H-E Stained. Bar = 150 µm. (B) Ooplasm showing few, small yolk platelets, with yolk membrane (YM) exhibiting a hyaline band and zona radiata. Theca (T) well defined. H-E Stained. Bar = 150 µm. (C) Follicular wall with poly stratified epithelium and cells under apoptosis. Granulosa containing small cells (SC), theca interna with intermediate cells (ItC), and theca externa with pyriform cells (PyC). H-E Stained. Bar = 400 µm. (D) Vitellogenic follicle exhibiting ooplasm with yolk granules (OYG) in their cytoplasm. Thinner theca (T) with blood vessels. H-E Stained. Bar = 500 µm. (E) Hypertrophied theca with lutein mass (LM) within the central cavity of the follicle, connective tissue (TcT), and cell "whorl" (arrow). At the vitellogenic stage, only small cells (SC) are found at theca layer. H-E Stained. Bar = 400 µm.

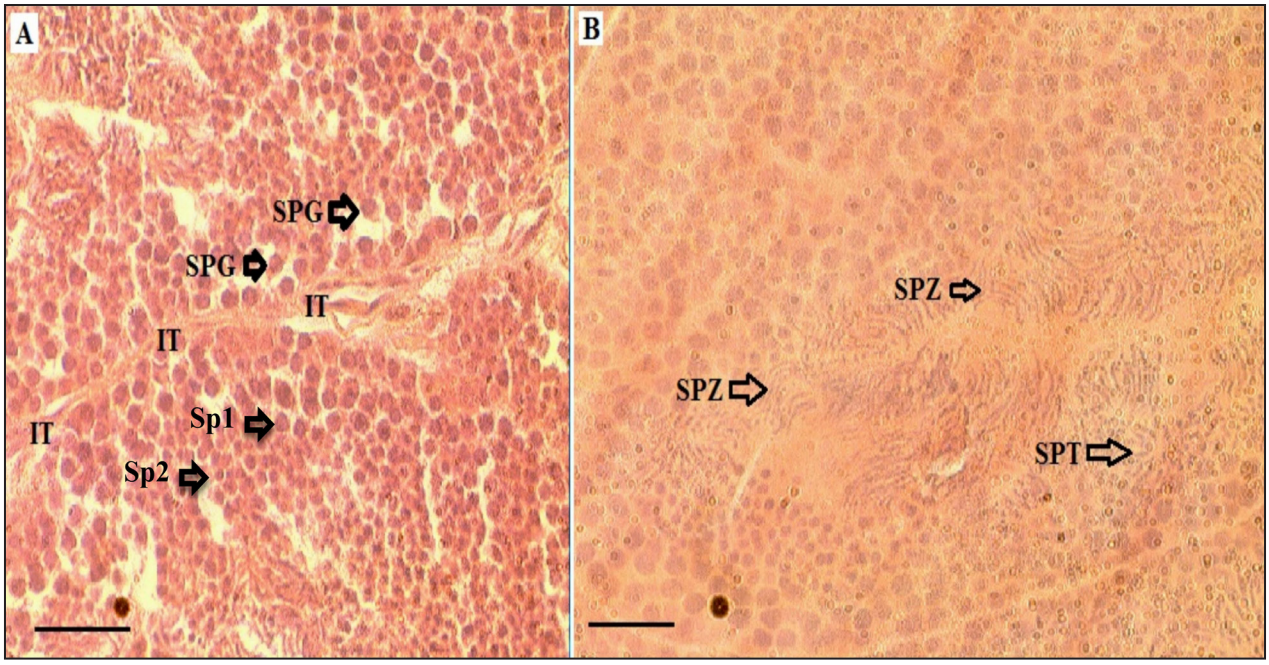

Figure 4. Histological sections of seminiferous tubes of *G. darwinii* specimens collected in October and November 2016 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil. IT = interstitial tissue; SPG = spermatogonia; SPT = Spermatids; Spt1 = primary spermatocytes; Spt2 = secondary Spermatocytes; SPZ = sperms; (A) October; (B) November. H-E Stained. Bar = 50 µm.

beginning of the dry season, while Spt2 production only peaked at the beginning of the dry season, with no seasonal effects on the stages of cell maturation (Table 1). Females did not present seasonal variation in small cells (Table 1). Males produced reproductive cells year-round, which peaked during the driest

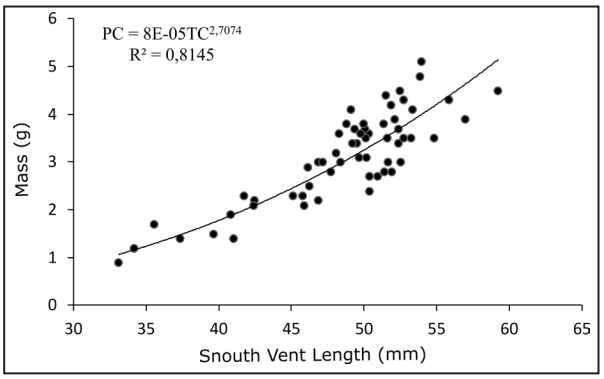

Figure 5. Ratio between body mass (g) and snout-vent length (SVL; mm) of *G. darwinii* specimens collected between July 2016 and June 2017 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil.

months (October and November), followed by a progressive decrease in the number of spermatozoa during the rainy months (April and May), shifting again and growing during the end of the rainy season (Fig 6). In seven females, fixed clutches size of two eggs were observed both during the dry and the rainy seasons (Fig 7).

The linear regression between the liposomatic relationship (LR), environmental temperatures, and precipitation revealed negative and positive correlations. The number of small cells (SC) in females did not covary with precipitation or air temperature. On the other hand, the number of Spt1 cells in males covaried negatively with both precipitation and air temperature (Table 2). LR covaried positively with K1 and negatively with body temperature, particularly in females. In contrast, small cells in females did not covary with any biotic factor, whereas there was a negative correlation between the number of spermatozoa and body temperature in males (Table 2).

Discussion

Our study showed that reproductive cells of sexually mature individuals of *G. darwinii* were continuously produced over a year, indicating continuous reproductive activity. Energetic reserves, in turn, declined at the end of the dry season and during the rainy season, especially in females, a process associated with precipitation levels. During the rainy season, primary production rates are higher in tropical environments, proportionating a food resource for many lizard populations inhabiting these domains. Those species tend to accumulate energetical reserves constantly, which are used in many phases of the continuous reproductive activity (mating, gestation, and egg-laying) after the investment in growth to reach the sexual maturity, i.e., K1 (Ballinger 1977; Galdino *et al.*, 2003).

Histological changes include the oogenesis in females, with the modifications displayed by the ovary and their structures between previtellogenic and vitellogenic phases, such as the drastic reduction

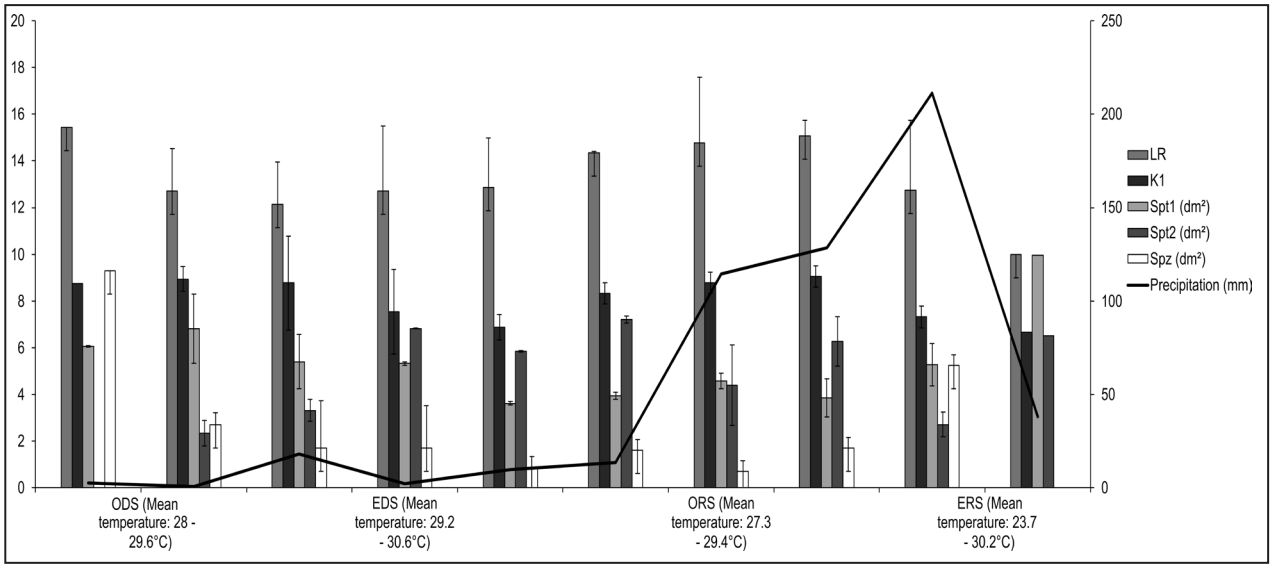

Figure 6. Monthly variation and standard deviation of the condition factor (K1), liposomatic relationship (LR), primary spermatocytes (Spt1), secondary spermatocytes (Spt2), and sperms (SPZ) recorded in males of *G. darwinii*, with the mean air temperature (°C) and precipitation (mm) registered in the same months. Specimens were collected between July 2016 and June 2017 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil. ODS = onset of dry season (September – November); EDS = ending of dry season (December – February); ORS = onset of rainy season (March – May); ERS = ending of rainy season (June – August).

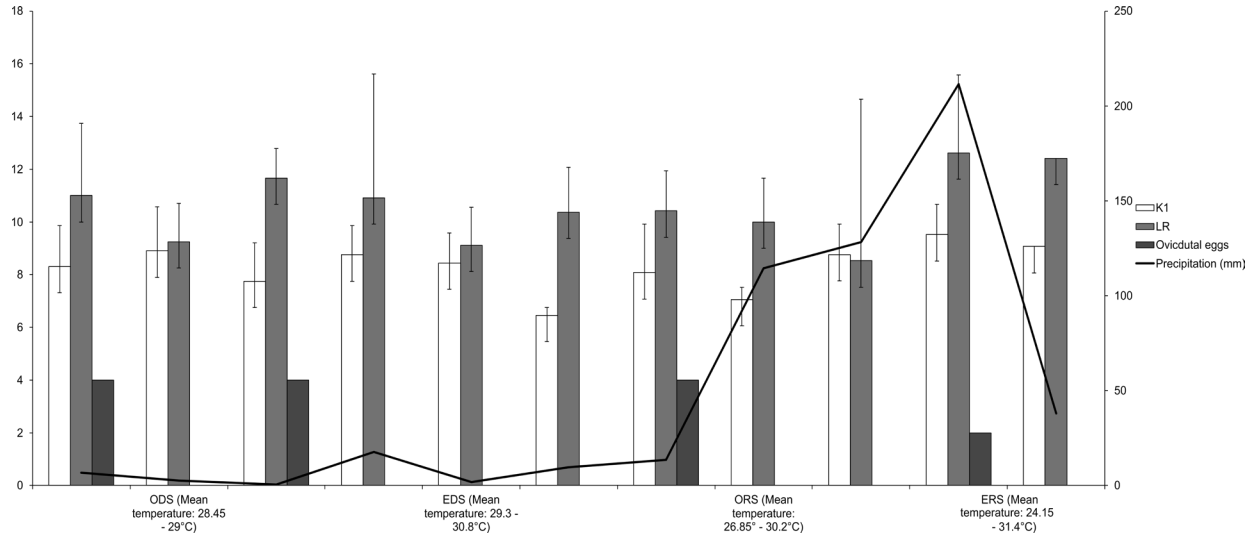

Figure 7. Monthly variation and standard deviation of the condition factor (K1), liposomatic relationship (LR), and number of oviductal eggs recorded in females of *G. darwinii*, with the mean air temperature (°C) and precipitation (mm) registered in the same months, according to seasonality. Specimens were collected between July 2016 and June 2017 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil. ODS = onset of dry season (September – November); EDS = ending of dry season (December – February); ORS = onset of rainy season (March – May); ERS = ending of rainy season (June – August).

of the Granulosa thickness (Moodley y Van-Wyk, 2007; Santos *et al.*, 2020). Regarding males, these changes include different arrangements of reproductive cells on seminiferous tubes along the maturation stages (Primary and secondary spermatids and sperms). Most reproductive cells at the initial maturation stages (Primary and secondary spermatids) were found in the periphery of the seminiferous epithelium. In contrast, at the final maturation stages (Sperms), most reproductive cells were located in the seminiferous epithelium centre, without reclusion stages on reproductive cell maturation. Histological changes in the shape and arrangement of the reproductive cells in both females and males were similar to patterns documented for other reptiles but displayed some differences compared to other vertebrates (Tumkiratiwong *et al.*, 2012; Sánchez-Ospina *et al.*, 2014; Machado-Santos *et al.* 2015). In other ectothermic vertebrates (amphibians and fishes), male Germ lineage cells forming the seminiferous tubule are grouped into cysts probably due to the strict water dependency for reproduction and the lack of amniotic protection (Sylva y Brender, 1997; Santos y Oliveira, 2008). On the other hand, in endothermic vertebrates (mammals), the Sertoli cells, containing complex folds in their plasmalemma and

Table 1. Results of the Kruskal-Wallis nonparametric analysis of variance applied to the different parameters of the physiology and reproductive biology of the *G. darwinii* specimens (female and male) collected in Tapacurá forest in São Lourenço da Mata, Pernambuco (Brazil), between July 2016 and June 2017. * Significant difference ($p < 0.05$).

Group	H	df	P
Female			
Small Cells	5.50	3	0.13
Liposomatic Relationship	3.98	3	0.26
Condition Factor (K1)	3.70	3	0.29
Male			
Primary Spermatocytes (Spt1)	7.41	3	0.04*
Secondary Spermatocytes (Spt2)	9.28	3	0.02*
Sperms	2.43	3	0.48
Liposomatic Relationship	4.58	3	0.20
Condition Factor (K1)	7.64	3	0.05*

Table 2. Results of the linear regression analyses (r^2) using exogenous (Temperature and Precipitation) and endogenous factors (Body temperature and the K1 Condition Factor) as the independent variables for the comparisons with the following variables: the Liposomatic Relation (LR) and, in the case of the reproductive cells, the Small Cells (SC) in the females, and the Primary (Spt1) and Secondary Spermatocytes (Spt2), and Spermatozoa (SPZ) in the male *G. darwinii* specimens collected in the Tapacurá forest in São Lourenço da Mata, Pernambuco (Brazil), between July 2016 and June 2017. * Significant difference ($p < 0.05$).

	Group analyzed	Temperature (°C)		Precipitation (mm)		K1		Body Temperature (°C)	
		r^2	p	r^2	p	r^2	p	r^2	p
LR	Female and Male	-0.32	0.01*	0.32	0.01*	0.17	0.02*	-0.17	0.02*
SC	Female	-0.12	0.11	0.09	0.16	-0.03	0.58	-0.03	0.61
Spt1	Male	-0.15	< 0.001*	-0.04	< 0.01*	-0.14	< 0.001*	0.01	0.07
Spt2	Male	0.02	0.88	0.01	0.89	0.01	0.09	0.03	0.64
SPZ	Male	-0.09	0.18	0.01	0.22	0.02	0.75	-0.14	< 0.001*

junctions of occlusion in their lateral membranes, subdivides the seminiferous epithelium into the basal compartment, containing spermatogonia and primary spermatocyte; and the adluminal compartment, containing secondary spermatocytes and sperms (Takashiba *et al.*, 2011).

The reproductive cells' maturation in lizards tends to be influenced by extrinsic and intrinsic processes regarding the studied species. (Abu-Zinadah, 2008; Mamou *et al.*, 2017). In *G. darwinii*, reproductive cell maturation seems constant, with sexually mature individuals showing mature reproductive cells regardless of the period of the year (Moodley y Van-Wyk, 2007; Torki, 2007). Current studies have reported that environmental conditions, such as appropriate rainfall and optimal temperatures, also contribute to a constant oogenesis and gametogenesis process in neotropical lizards (Salvador, 2011; Migliore *et al.*, 2017; Díaz-Vega *et al.*, 2020). However, the lack of any significant seasonal variation in the reproductive cells' production in *G. darwinii* suggests that reproductive cell maturation is likely to be firstly determined by the species' biotic rhythm, optimised by suitable environmental conditions. Since *G. darwinii* usually maintain low body temperatures, adopting similar temperatures to the environment (range = 22.4 – 34.6° C), and considering the low-temperature variation in the Atlantic Forest, the resource availability in the environment, such as adequate microhabitats and feeding, seems to display stronger determinant factors for individuals' sexual maturation, also regarding the reproductive cells (Guedes *et al.*, 2020; Lara-Resendiz, 2020). Individuals of *G. darwinii* seems to maintain optimised vital activities, including reproduction, growth, and digestion, since it keeps a slightly constant body temperature and display sit-wait behaviour (Autumn and De Nardo, 1995; Lara-Resendiz, 2020).

Spermatozoa were found in both the testes and the males' epididymis, a pattern associated with high testosterone levels in some lizard species (Cooper *et al.*, 1987; Galdino *et al.*, 2003). This hormone is well known to modulate aggressive, territorial behaviour in males (Galdino *et al.*, 2003). Unfortunately, no behavioural data is available for *G. darwinii* so far (Stamps, 1983), which precludes further discussion on whether this species is territorial. On the other hand, the seasonal variation observed in primary and secondary spermatocyte levels seems to be associated with a peak in gonadal activity coinciding with the period in which reproductively active females are more abundant in the population and when copula occurs (Jenssen *et al.*, 2001; Widerhecker *et al.*, 2002). This scenario also suggests that selective pressures from abiotic environmental factors (such as precipitation and temperature) acting on cell maturation are weak, for both females and males, despite the climatic seasonality observed in the study area (Galdino *et al.*, 2003). Nevertheless, our results do not preclude the role of abiotic factors such as air temperature and precipitation on the reproductive biology and gamete production in this species, especially in females (Galdino *et al.*, 2003; Norval *et al.*, 2019).

It was recorded that females with oviductal eggs during both dry and rainy seasons, despite the importance of precipitation and environmental temperature for the development of reptilian eggs (Ramírez-Bautista *et al.*, 2009; Norval *et al.*, 2019. Therefore, our results reinforce that *G. darwinii* seems to offset the fixed and/or small clutch size conditions, increasing the frequency of clutches per year (Doughty, 1997, Guedes *et al.*, 2020). In addition, due to the rigid-shelled eggs and the low-

temperature variance at the study site, eggs seem to be laid at any moment, despite the seasonality and the variation of precipitation levels (Pike et al., 2012; Guedes *et al.*, 2020). Due to those conditions and the constant availability of feeding resources in the environment, the energy invested probably did not affect gravid females in different gestation periods (Norval *et al.*, 2019; Guedes *et al.*, 2020).

Our results indicate that *G. darwinii* increased in weight proportionally to the increase in body length (isometric coefficients of 2.5–4.0), suggesting that individuals invested similarly in accumulating body mass and body length (Vazzoler, 1996; Migliore *et al.*, 2017). Tail fat bodies and body size tend to grow at similar rates when lacking previous caudal autotomy (Derickson, 1976). As males and females did not differ in tail length, variation in caudal fat body accumulation in *G. darwinii* is likely related to differences in energetic requirements between the sexes and variation in air temperature and precipitation (Pinilla, 1991). Indeed, caudal fat body accumulation correlated negatively with temperature and positively with precipitation, reflecting the higher temperatures effect during the most favourable period for mating in the study area (i.e., the dry season; Pinilla, 1995; Norval *et al.*, 2019). Previous studies have also shown that caudal fat bodies are used in activities other than reproduction, which implies that tail fat loss during the dry season can be regulated by additional factors such as decreasing resource availability and increasing energy-demanding territorial contests (Derickson, 1976; Ramirez-Bautista *et al.*, 2009; Norval *et al.*, 2019). On the other hand, the tail fat bodies' negative relationship with air temperature and positive relationship with precipitation indicate a high energetic buildup during the rainy season (Ramírez-Bautista *et al.*, 2009).

Although there was seasonal variation in the lipid reserves of the studied population, our data indicates that individuals of *G. darwinii* do not accumulate energy reserves for long periods, which suggests that the local availability of food resources is adequate for sustaining other vital individuals' activities (Pinilla, 1991; Serrano-Cardozo *et al.*, 2007). As *G. darwinii* is a sit-and-wait forager (Almeida-Gomes *et al.*, 2012), little energy is required for foraging (Colli *et al.*, 2003; Norval *et al.*, 2019). Our results are consistent with other Neotropical lizards, such as *Liolaemus huacahuasicus* Laurent, 1984 (Pinilla, 1991), *Liolaemus bitaeniatus* Laurent, 1984 (Pinilla, 1995) *Iguana iguana* Linneaus, 1785 (Ferreira *et al.*, 2002) and *Eurolophosaurus nanuzae* Rodrigues, 1981 (Galdino *et al.*, 2003).

Our study presents the first dataset about seasonal variation in energetic and growth cycles and the reproductive cells' maturation of male and female *G. darwinii*. Overall, our findings support that the energetic cycle and reproductive cell maturation of *G. darwinii*'s spermatogenesis, oogenesis, and egg development are influenced firstly by biotic factors and slightly by abiotic factors (Watling *et al.*, 2005). The fat body reserves seem to be affected by abiotic factors, probably also sustaining the reproduction during the year, which can be related to the lack of relationship between the gonadal activity and the environment. In conclusion, our study reinforces the continuous gamete production over the year and its optimised production with increased air temperature and precipitation to an acceptable degree. Finally, we suggest that future studies investigate whether the continuous production of reproductive cells in *G. darwinii* modulates other fitness-related behaviours, such as feeding, thermoregulation, mating, and territory defence.

Acknowledgments

We are grateful to Alexandre Marques for helping in statistical analyses. We also thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial support to this study (0287-2.05114), and the federal environmental agency, Instituto Chico Mendes (ICMBio), and the Comitê de Ética e Uso de Animais (CEUA-UFRPE), for providing the study licenses and authorizations. To Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, for the research productivity grant awarded to G.J.B.Moura (1D).

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