

Relationships between body growth indices and environmental factors on the reproductive cycle of the *Gymnodactylus geckoides* Spix, 1825 (Squamata, Gymnophthalmidae) in Northeast Brazil

Leonardo P. C. Oitaven¹, Beatriz B. M. R. Silva¹, Simone A. Gavilan², Eduardo J. R. Dias³, Marcio F. Chaves⁴, Luis M. Lobo⁵, Juan S. M. Gonzalez^{6,7}, Daniel O. Mesquita⁸, Geraldo J. B. de Moura^{1,9}

¹Laboratório 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.

²Laboratório de Morfofisiologia de Vertebrados, Centro de Biociências, Departamento de Morfologia, Universidade Federal do Rio Grande do Norte, Natal, RN.

³Departamento de Biociências, Universidade Federal de Sergipe – UFS, Campus Prof. Dr. Alberto Carvalho de Aracajú, Itabaina, SE

⁴Centro de Saúde e Educação, Universidade Federal de Campina Grande, Rua Olho D'Água da Bica s/n, CEP 58175-000, Cuité, PB, Brazil.

⁵Laboratório de Ecologia e Evolução, Instituto Butantan, Av. Vital Brasil, 1500, CEP 05503-900, São Paulo, SP, Brazil.

⁶Laboratori d'Ecologia de Vertebrats Terrestres, Institut "Cavanilles" de Biodiversitat i Biologia Evolutiva, Universitat de València, C/ Catedrático José Beltrán nº 2 E-46980, Paterna, València, Spain.

⁷Departament de Microbiologia i Ecologia, Universitat de València, C/ Dr. Moliner 50 E-46100, Burjassot, València, Spain.

⁸Laboratório de Herpetologia, Universidade Federal da Paraíba (UFPB), 58059-900 João Pessoa, Paraíba, Brazil.

⁹Directorate of Environmental Monitoring and Innovation of the State Agency for the Environment, Government of the State of Pernambuco, Brazil.

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ABSTRACT

In this study, we analyzed the energy and reproductive cycles of female and male *Gymnodactylus geckoides* in the Caatinga area of northeast Brazil. We investigated whether these proxies of body condition, such as reproductive cells maturation and cellular structures changed in response to variation in abiotic and biotic factors (i.e., humidity, temperature, seasonality, body temperature, growing rate and gonad volume), using individuals stored under scientific conditions collected between September 2018 and December 2021. The condition factor showed an isometric growth pattern in the population studied. Meanwhile, the lipid, hepatic, and gonad factors correlated with body growth and showed monthly and seasonal variations, as well as reproductive cell maturation and cellular structure morphology. The cycles displayed constant replacement of energy reserves and mature reproductive cells, indicating constant and acyclic reproduction in *G. geckoides*. Energy reserves appear to be used for many reproductive activities, including meeting, gestation, and egg laying that occur at different frequencies during different periods in the dry and rainy seasons. Therefore, the reproductive cycle is likely to be strongly controlled by biotic factors, which are modeled using abiotic factors and environmental conditions (environmental patterns which proportionate greater resource availability). Our study is the first to investigate energy cycles and reproductive strategies in *G. geckoides*. It has shown that this

species stores greater amounts of energy during the rainy season and then depletes these reserves during the dry period, since the rainy season correspond to the increase in energy consumption, mainly because of gestation and egg laying.

Key words: Brood; Cell Development; Endogenous Factors; Energy Cycle; Exogenous Factors; Gecko; Reproductive Tasks.

Introduction

Terrestrial animals, especially ectotherms such as Squamata, use biochemical mechanisms to build (Pinilla, 1995; Schaffner, 1998) and accumulate energy reserves within a relatively short time span (Derickson, 1976; Norval *et al.*, 2019). In lizards, the accumulation and use of energy reserves, that is, the energy cycle, vary temporally and ontogenetically. They are mostly directed toward vital activities, including body growth, survival, and reproductive activity (Derickson, 1976; Cheng, 1987; Galdino *et al.*, 2003; Ramirez-Bautista *et al.*, 2006; Norval *et al.*, 2019; Paz *et al.*, 2019). Although lizards have efficient feeding strategies that facilitate energy storage, the energy cycle is ultimately influenced by abiotic factors including temperature, precipitation, and photoperiod (Benabib, 1994; Ramirez-Bautista and Vitt, 1997; Norval *et al.*, 2019).

Environmental variations, mainly seasonal and annual, often correlate with variations in the natural traits of a population (Sexton and Brown 1977; Benabib, 1994). Food availability and abiotic factors, such as temperature, precipitation, and humidity, can affect growth rates and reproductive strategies. This includes the time and duration of the reproductive event, brood size and frequency, and the size at which individuals reach sexual maturity (Ramirez-Bautista and Vitt, 1997; Oitaven *et al.*, 2022). Lizard populations and individuals can also show variation in different proxies for energy reserves, including the growth investment comparing morphology and weight, condition factor (K1) (Le Cren, 1951; Galdino *et al.*, 2003; Oitaven *et al.*, 2022), as well as many index, which represents association of individuals' weight with some energetical reserves stored in the body, like: the lipid index (RFB) (Derickson, 1976; Sexton and Brown, 1977; Paz *et al.*, 2019), the hepatic index (RWL) (Schaffner, 1998; Ramírez-Bautista *et al.*, 2006; Norval *et al.*, 2019), and the gonadosomatic index (RGS) (Pinilla, 1995; Ramirez-Bautista and Vitt, 1997; Sánchez-Hernández *et al.*, 2013).

Previous studies on Neotropical lizards have shown that energy cycles are primarily regulated

by food availability, patterns of reproductive investment, and variations in abiotic factors (Derickson, 1976; Cheng, 1987; Benabib, 1994; Galdino *et al.*, 2003; Ramirez-Bautista *et al.*, 2003; Ramírez-Bautista *et al.*, 2009; Norval *et al.*, 2019; Paz *et al.*, 2019; Guedes *et al.*, 2020; Oitaven *et al.*, 2022). In sexually mature individuals, energy reserves tend to diminish as reproductive activities demand high investments in spermatogenesis, oogenesis, gestation, and gonadal development (Ramírez-Bautista and Vitt, 1997; Ramirez-Bautista *et al.*, 2006; Paz *et al.*, 2019).

The reproductive cycle of lizards is associated with several factors, such as photoperiod, precipitation, temperature, growth rate, and resource availability (Ferreira *et al.*, 2002; Oitaven *et al.*, 2022). Therefore, delineation of the reproductive cycle could be associated with many factors that influence several processes in this cycle, including gametogenesis, follicular growth, mating, sperm storage, ovulation, gestation, and egg hatching (Newlin, 1976; Moodley y Van-Wyk, 2007).

Mathies (2011) analyzed the reproductive cycle of squamata groups in females and males, at the individual and population levels. At the individual level, the cycle can be classified as discontinuous with total gonad regression and interruption of the gamete production process, continuous with partial gonad regression and regression of the gamete production process, or acyclic without gonad regression and continuous gamete production process. At the population level, continuous and non-continuous reproductive cycles could display a synchronic and/or semi-synchronic seasonal pattern. This can occur with reproductive events concentrated during a season or a time range, being synchronized or not between females and males, or in a non-seasonal pattern with the reproductive events distributed throughout the year, without synchrony between females and males (Sherbroke, 1975; Mathies, 2011).

Given the high diversity of lizards in the tropical region, information on indices of energy cycles, as well as reproductive cycles, is scarce for most

species, especially in dry tropical forests. This is the case for *Gymnodactylus geckoides* Spix, 1825, an endemic lizard that is widely distributed in the Caatinga domain (Alcântara *et al.*, 2019; Oitaven *et al.*, 2022b), with LC status, regarding IUCN list (IUCN, 2023), as well as local population from Pernambuco state (SEMAS, 2017; Costa and Bérnils, 2018). Given that the Caatinga domain has a marked seasonal climate, we hypothesized that body indices representing different energy reserves, as well as the reproductive cycle and reproductive cell maturation, vary seasonally with precipitation, humidity, and air temperature. We further hypothesized that they are associated with several vital activities and bodily structures in *G. geckoides*. In this study, we aimed to describe the morphological cellular structures and the energetic and reproductive cycles of *G. geckoides*. We then related the patterns and cycles of females and males to biotic and abiotic factors.

Materials and methods

Study area

The study was conducted in the Catimbau National Park (692781.38E; 9050296.71S), which is a remnant of the Caatinga. This park is located in the Ipanema Valley in the central region of Pernambuco, Brazil. It encompasses an area of approximately 62,300 ha in the municipalities of Buíque, Sertânia, Ibimirim, and Tupanatinga (Fig. 1) (Ferreira, 2010; Oitaven *et al.*, 2022b). The local climate receives rainfall ranging from 0 to 256 mm. We defined the dry and rainy seasons as constant processes. Dry season (September to February) had reduced rainfall levels (4–94 mm) and a slightly air temperature variation (24–33 °C) compared to rainy season. The rainy season (March to August), in turn, had higher precipitation levels (38 – 188 mm) with low air temperature variations (26–30 °C). However, air temperatures seem not display higher variance between both seasons (see Supplementary Material 1). We obtained data on the monthly average air temperature (°C) and rainfall (mm) levels from the Pernambuco Water and Climate Agency database (APAC) based on meteorological stations near (less than 70 Km) the study site (APAC, 2019).

Data collection

We captured lizards monthly between September 2018 and August 2019 using the active search method. This involved searching for individuals on

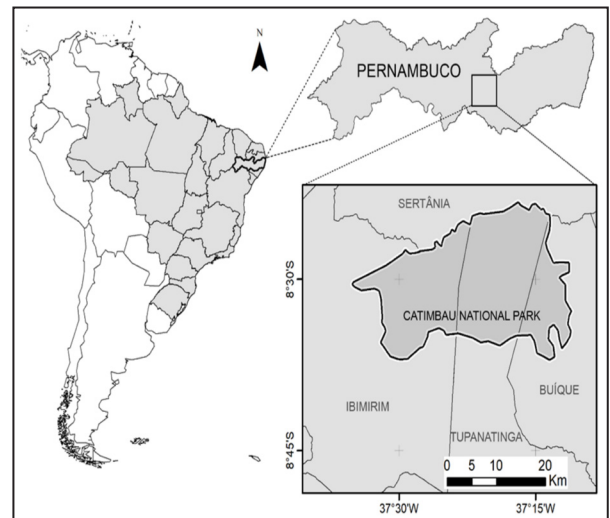


Figure 1. Map of Brazil depicting the state of Pernambuco and the location of the Catimbau National Park (study area), a conservation unit in the Caatinga biome whose area extends over four municipalities.

different substrates and collecting them manually. Surveys were carried out during hourly intervals over three days, being conducted during the day (from 9:00 h to 14:00 h) and at night (from 16:00 h to 20:00 h), with collections and observations made by a pair of observers, totalizing 360h/men of sampling effort. Since *G. geckoides* did not display external morphological differences (Oitaven *et al.*, 2022b), we were just able to classify individuals as sexually analyzing the sexual gonads. Females were considered sexually mature when having either vitellogenic follicles or eggs in the oviduct. We considered vitellogenic follicles to be those follicles showing a yellowish color and diameter > 0.3 mm. Follicles with diameter < 2.1 mm were arbitrarily regarded as small, whilst those with diameter > 2.1 mm were regarded as large. Males were sexually mature when having enlarged testes, contorted epididymis, and sperm in the testes and/or epididymis (Colli *et al.*, 2003). To estimate gonad volume, we obtained the length and width of the ovaries, testes and eggs using a digital caliper (± 0.01 mm).

During the study period, 77 sexually mature individuals were captured. Given that the region of Picuí, Paraíba, has climatic conditions similar to those of our study area, with limited amounts of rainfall and constant high temperatures, we added 31 individuals captured in 2021 from the Picuí region to our sample size. We also included 54 specimens collected in 2014 in our sample from Catimbau National Park. This increased the sample size to

162 individuals, comprising 66 females and 96 males. The mean number of individuals collected per month from 2018 to 2021 was eight (range = 4 – 15) (see Supplementary Material 2). We euthanized the individuals collected using intramuscular anesthesia liquid ketamine, a barbituric composed by Ketamine Hydrochloride (10 mg/Kg) and Methedomidine (0.1-0.3mg/Kg).

To evaluate the influence of abiotic factors at a current situation, we measured, the air temperature and humidity at the time of collection of individuals with a digital thermos-hygrometer with accuracy to the nearest $\pm 0.1^{\circ}\text{C}$ (Colli *et al.*, 2003). We weighed each individual collected using a digital scale with accuracy to the nearest ± 0.001 g and measured the snout–vent length (SVL) using a digital caliper with accuracy to the nearest ± 0.01 mm. Given that geckos accumulate lipid reserves in the caudal region (Vitt *et al.*, 2007), the caudal region of each specimen was moved through a cross-section and the lipid reserves were weighed separately, including the tail mass bonded to the abdominal region of some individuals, with the aim to calculate the lipid index (RFB). We made the same procedure, through a ventromedial incision to remove the liver and gonads and calculate the hepatic (RWL) and gonadosomatic index (RGS), respectively. We weighed all structures using the same digital scale (± 0.001 g).

We dissected the individuals to examine their lipid, hepatic, and gonad reserves and compared to total weight (WT) of the individual. The energy reserves were estimated using the following formulas: to RFB ($\text{RFB} = \text{WFB} (100) / \text{WT}$), where “WFB” is the lipidic bodies weight, to RWL ($\text{RWL} = \text{WL} (100) / \text{WT}$), where “WL” is the liver weight, and to RGS ($\text{RGS} = \text{WG} (100) / \text{WT}$), where “WG” is the gonad weight (Ramirez-Bautista and Vitt, 1997; Schaffner, 1998; Wootton, 1999).

Reproduction

To analyze the influence of biotic and abiotic factors, regarding females and males, as well as seasonality, we sorted the individuals according to collection period and sex. For both female and male, the gonad volume was estimated using the ellipsoid formula ($V = 4/3\pi a^2b$), where “V” is the gonad volume, “a” is half the smallest gonad diameter, and “b” is half the longest gonad diameter (Selby, 1965). The volume of eggs was measured using the formula for an ovoid sphere ($V = \pi w^2l/6$), where “V” is the egg volume, “w” is the egg width, and “l” is the egg length

(Ramirez-Bautista y Vitt, 1997). We calculated the condition factor (K1) using the Allometric Method, following the expression $K1 = w/Lb$, where “W” is the total mass and “L” is the specimen standard length. To estimate the “b” coefficient, we performed a simple mass–length ratio equation ($W = aLb$) (Le Cren, 1951; Galdino *et al.*, 2003).

Histological procedures

The gonads were fixed in Bouin’s solution (71% picric acid, 24% formaldehyde, and 5% glacial acetic acid) for 24 h and dehydrated in a series of increasing alcohol solutions for 30 min at each of six stages: 70%, 80%, 90%, 95%, 100%, and 100% (Hopwood, 1990). Subsequently, we embedded the samples in paraffin and sectioning the gonads to obtain 5 μm thick histological sections. These were colored in Hematoxylin and Eosin and prepared on slides for analysis (Robinson y Gray, 1990; Aguilar-Morales *et al.*, 1996). We analyzed both cuts, as well as quantitative sliders, using a Nikon E800 microscope, taking digital pictures with a Nikon DS-Ri camera, with all cellular measurements, as well as the measure of dispersion, using the NIS Elements System ver. 4.60 (Laboratory Imaging, 2016) program. We performed these procedures on 77 sexually mature individuals, of which 39 were females and 38 males.

Regarding females, we identified reproductive cell types and cellular structures according to Gribbins (2011), Lozano *et al.* (2014), and Santos *et al.* (2020). We analyzed the reproductive cells and cellular structures of the internal and peripheral layers of the ovary, including granulosa, oocyte, and germinal bed diameters. The male spermatogenic cycle was based on the Mandarin-de-Lacerda (1995) and Weibel (1979) precepts. We calculated the volume density (Vv) of primary and secondary spermatocytes and sperms as reproductive cell types, representing the individual’s reproductive maturation state (Torki, 2007). We also measured the following cellular structures: seminiferous tube diameter (STD), seminiferous epithelium height (SEH), diameter of ductus deferens (DEF), diameter of epididymal ductus (DEP) and epididymal ductus height (DEH) (N = 8 by structure, 40 individuals) (Hernández-Franyutti y Uribe, 2012; Lozano *et al.*, 2015). We calculated the sample using Hally’s formula (1964) and corrected the data according to Mandarin-de-Lacerda (1995) to provide an accurate stereological precept.

This study has shown histological changes in both sexes, taking into account the catch period, that is, the onset of the dry season (ODS), the end of the dry season (EDS), the onset of the rainy season (ORS), and the end of the rainy season (ERS). Finally, to determine the reproductive period of the species, we quantified the profile density (Qa) by counting several testing grounds in the testing area (TA) for each individual. We obtained the results using the mean of their respective profiles with the following formula: $Qa = \frac{\sum \text{profiles}}{TA} \text{ (mm}^2\text{)}$ (Mandarim-de-Lacerda, 1995), analyzing reproductive cellular types of seminiferous tubes, using 40× magnification microscopic lenses (Santos y Oliveira, 2007). We then analyzed the cellular structures and randomly counted the cells between individuals (Lozano *et al.*, 2015).

Data analysis

Prior to statistical analysis, we checked the data for normal distribution using the Shapiro–Wilk test, with the data of males and females combined. Given that the data were not normally distributed, we used the Kruskal–Wallis test to test for monthly variation in the indices used as proxies for energy reserves. This was then followed by post-hoc pairwise comparisons (Tukey) to check which samples differed significantly (Ramirez-Bautista and Vitt, 1997; Norval *et al.*, 2019). To control for the effects on the response variables of variation in body condition between individuals, we performed linear regressions (r^2) between SVL and body mass, and each body variable, including lipid, hepatic, and gonadal masses. We used linear regression not just to verify the investment of growth in *G. geckoides*, as well as the growth of energetical reserves and gonads (Ramírez-Bautista y Vitt, 1997; Paz *et al.*, 2019).

To analyze monthly variation, we only used data from months in which three or more individuals of each sex were captured (Ramirez-Bautista *et al.*, 2006; Ramirez-Bautista *et al.*, 2009). To test for seasonal variations in body indices and gonad volume, we performed an analysis of variance (ANOVA) with dry and rainy periods as predictors (Zar, 1999; Ramirez-Bautista *et al.*, 2006).

We tested the influence of abiotic factors and body indices on the presence of individuals in each period using generalized linear models (GLM) in the *DHARMA*, *readxl*, and *mice* packages (Zuur *et al.*, 2009; Villamar-Duque *et al.*, 2019). We modeled generalized linear “mixed” models (GLMM) to control

for the influence of sampling periods and between-individual variation. Since the reproduction process, as well as the arrival of lizards are associated with abiotic factors (Paz *et al.*, 2019), we used body indices and gonad volume as predictor variables in a model fitted to verify the absence or presence of individuals through the seasons. Air temperature, humidity, and precipitation were used as predictor variables in models fitted to test the influence of abiotic factors on body indices (Zuur *et al.*, 2009).

We compared differences/variation in cellular density values and cellular structure morphology using the Kruskal–Wallis test and, if necessary, the Dunn test, for females and males. In females, we compared the relationship between environmental factors including temperature, humidity, and precipitation and body factors including body temperature, SVL, gonad volume, and K1 using generalized linear models (GLM), with environmental and body factors as predictors. For the GLM analysis, we used the *DHARMA*, *readxl*, and *mice* packages. In males, we first used the ANOVA test to analyze variations in the reproductive cellular types and structures according to annual, as well as seasonal cycles. We then used GLM analysis to compare cellular structure measurements and reproductive cells at different stages, that is, primary and secondary spermatocytes and sperm, with abiotic and biotic factors (Zar, 1999). We considered the effect to be statistically significant when $p < 0.05$. All the analyses were performed using the R program (R Development Core Team 2019) (Zar, 1999; Zuur *et al.*, 2009).

Results

Growth rate and energy cycles

The angular regression coefficient (b) of the mass–length ratio equation was 2.85. This value, which is close to the Condition Factor (K1), indicates a similar investment in body mass and length, that is, isometric growth in the population of *Gymnodactylus geckoides* sampled (Fig. 2). The mean SVL of females was 41.40 ± 4.43 mm (range: 28.55 – 47.25 mm, $n = 66$). Meanwhile, the mean SVL of males was 40.22 ± 3.63 mm (range: 29.50 – 45.74 mm, $n = 96$). The minimum female and male SVL at sexual maturity were 32.84 mm and 32.11 mm, respectively. The mean gonad volume was 4.67 ± 4.52 mm³ for females (range: 0.3 – 22.42 mm³, $n = 64$), and 7.82 ± 5.96 mm³ for males (range: 1.49 – 32.78 mm³, $n = 95$). Large follicles were found in females

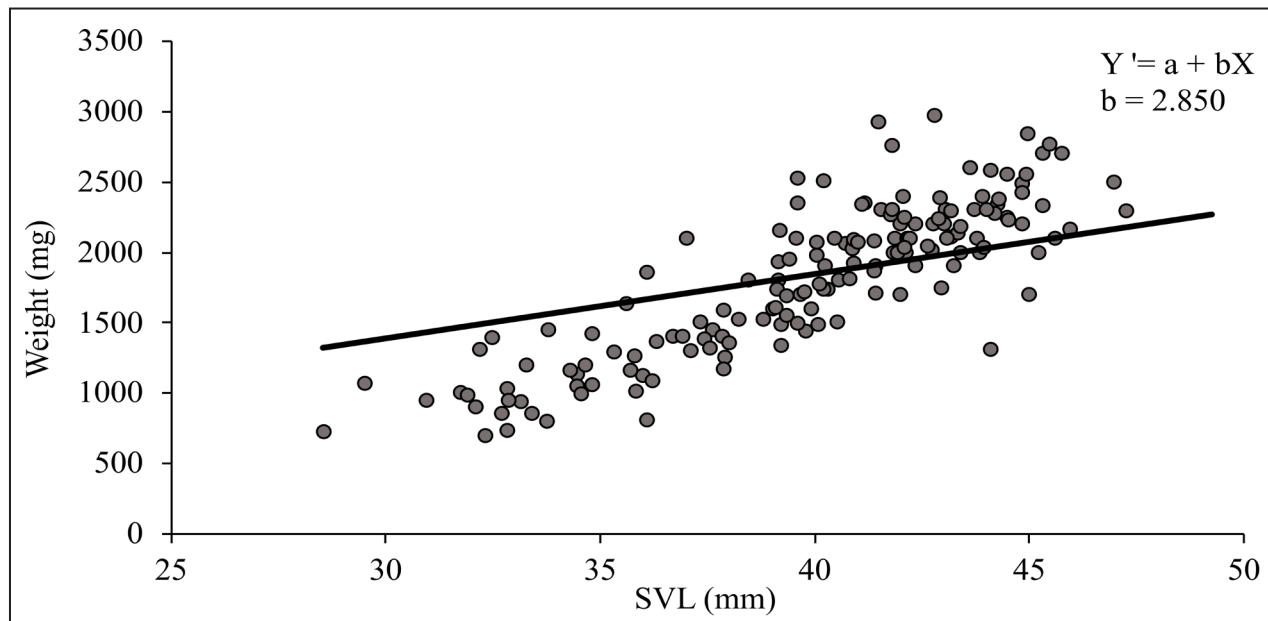


Figure 2. Relationship between the snout-vent length (SVL) and the body weight of individuals of *G. geckoides* Spix, 1825 collected in Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, between September 2018 and September 2021.

throughout the year, particularly during dry periods (September–February). Eggs were found during both seasons, but predominantly in rainy periods (March–August) (Fig. 3A). They were always in a fixed brood of one egg per female, with a mean volume of $295.40 \pm 120.12 \text{ mm}^3$ (range: $112.33 - 434.19 \text{ mm}^3$, $n = 6$). Males presented similar gonad cycles, with larger testes during the dry period (September–December), reduced testes at the end of the dry season (January–February), and growing testes during the rainy season, except in May and June, without recrudescence (Fig. 3B).

Body length had a positive, significant effect on gonad volume in females ($\beta \pm \text{SE} = 0.074 \pm 0.027$, $z = 2.723$, $p < 0.01$) and males ($\beta \pm \text{SE} = 0.091 \pm 0.020$, $z = 4.488$, $p < 0.001$). This indicated that gonad growth is proportional to the SVL of the individuals. In females, SVL was positively and significantly correlated with lipid and hepatic reserves, but not with gonadal conditions. Similarly, the body mass of females was positively and significantly correlated with lipid and hepatic reserves but not with gonadal conditions. In males, SVL was positively and significantly correlated with lipid, hepatic, and gonadal reserves. Body mass of males also showed a positive and significant correlation with lipid, hepatic, and gonadal reserves (See supplementary material 3).

Females showed a reduction in lipid reserves (RFB) prior to the beginning of the rainy season

(Fig. 4A). However, they did not show significant seasonal variation in RFB (Kruskal–Wallis = 2.32; $p = 0.50$). In contrast, the males showed an increase in RFB prior to the beginning of the rainy season and showed a significant variation in RFB between the ending of rainy season and onset of dry season (Kruskal–Wallis = 22.33; $p = 0.02$). Hepatic reserves (RWL) decreased slightly at the end of the dry season and the beginning of the rainy season, followed by an increase at the end of this period, especially in females (Fig. 4B). In females, the main variation in RWL occurred at the end of the period of abundant rainfall, especially in June (Kruskal–Wallis = 17.01; $p = 0.02$) and August (Kruskal–Wallis = 17.01; $p < 0.01$). Males, in turn, showed no significant variation in RWL (Kruskal–Wallis = 4.86; $p = 0.85$). Regarding gonadal reserves (RGS), both sexes experienced a decrease at the end of the dry season and beginning of the rainy season. This was then followed by an increase at the end of the dry season (Fig. 4C). Females showed a significant decrease in RGS by November for both years (Kruskal–Wallis = 20.63; $p < 0.01$), and males showed a significant decrease in RGS in the driest months (October and February) (Kruskal–Wallis = 17.22; $p = 0.03$).

ANOVAs showed higher concentrations of energy reserves in females and males in periods coinciding with the end of abundant rainfall and the beginning of the dry season. The volume of sexual

gonads in females did not show significant seasonal variation. In contrast, the males had enlarged, more robust gonads during periods with high precipita-

tion levels (Table 1). The generalized linear models showed no influence from body indices or gonad volume on the seasonal appearance of individuals of either sex (Supplementary Material 4). Regarding abiotic factors, the models showed significant negative and positive effects of temperature and precipitation on the RFB of females, with negative and positive effects of these variables, respectively. However, humidity showed a significant negative effect on RGS, which was associated with a reduction in gonadal reserves (Table 2).

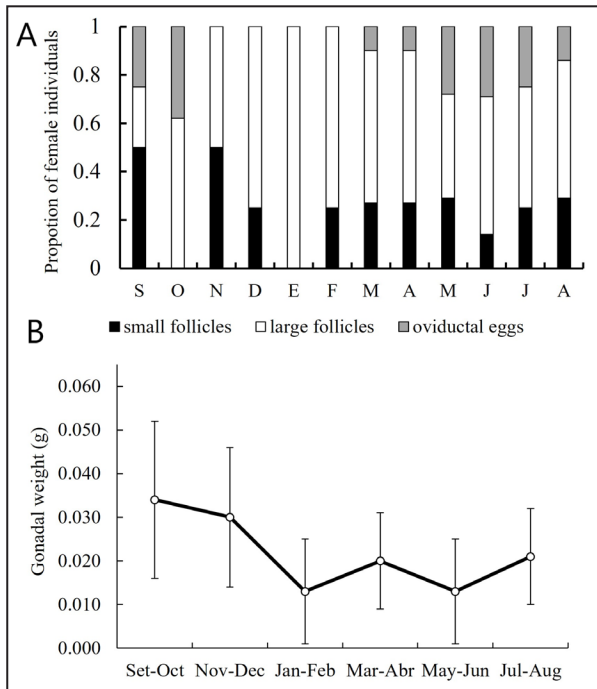


Figure 3. Annual variation in gonads of *G. geckoides* from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, between 2018 and 2021. A) Monthly proportions of females presenting small follicles (diameter < 2.10 mm), large follicles (diameter > 2.10 mm), and oviductal eggs. B) Monthly variation in the weight of testes (in g); the white circles are the adjusted means, and the vertical bars denote the associated standard error.

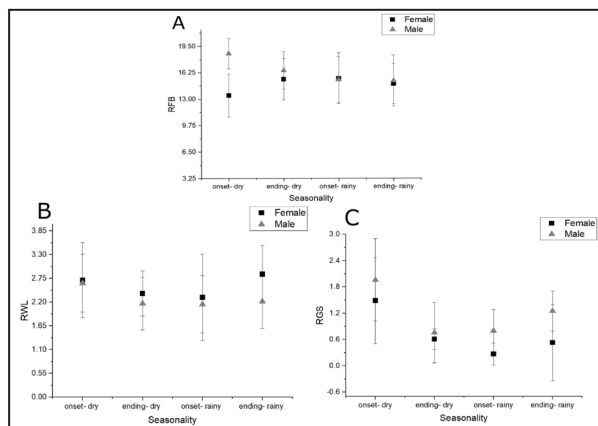


Figure 4. Seasonal variation in different types of energetic reserves in female and male *G. geckoides* Spix, 1825 from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021. A) Liposomal ratio (RFB); B) Hepatosomatic ratio (RWL); C) Gonadosomatic ratio (RGS). Triangles and squares are the adjusted means, and the vertical bars denote the associated standard error.

Cellular variation: females

In *G. geckoides*, the ovary comprises oocytes, granulosa cells, thecal cells, and germinal tissue. Ovaries in the previtellogenic and vitellogenic stages were simultaneously recorded during all sampling months (Figs. 5 and 6). During pre-vitellogenic stages, III and IV, oocytes displayed average diameters of $290 \pm 45.6 \mu\text{m}$ and $835 \pm 75.0 \mu\text{m}$ respectively. In those stages, the thickness of the yolk membrane and granulosa layer reached average diameters of $4.75 \pm 0.7 \mu\text{m}$ and $50.45 \pm 10.7 \mu\text{m}$ respectively. Regarding the granulosa layer cells, small cells were more abundant at the periphery of follicles (Figs. 5A–B). The germinal bed was substantially thicker, mainly during the earlier stages, with germinal cells having an ovoid or spherical shape with hyaline cytoplasm. During vitellogenesis, the germinal bed tended to become homogeneous and reduced (Figs. 5A–B).

Follicular growth continues to advance during the vitellogenic stage. At earlier stages of vitellogenesis, V and VI, *G. geckoides* displayed ovaries with a mean oocyte diameter of $1100 \pm 318 \mu\text{m}$. The granulosa layer, as well as the germinal bed, displayed reductions in thickness, reaching diameter averages of $21.1 \pm 3.5 \mu\text{m}$ and $40.8 \pm 6.6 \mu\text{m}$, respectively. The granulosa was modified into a single layer containing only small cells with small cores (Fig. 6B). Meanwhile, the thecal region was composed of fibroblasts, collagen fibers, and blood vessels (Figs 6A–B). At these stages, changes in the zonapellucida were recorded, which displayed two layers, that is, a homogeneous layer known as the hyaline band and an internal striated layer known as the radiata zone. A mean oocyte diameter of $3.98 \pm 0.94 \mu\text{m}$ was recorded at these stages, with lipid storage maximized and occupying almost the entire ooplasmic space (Fig. 6A). The zona pellucida zone was highly reduced, as well as the granulosa, consisting of a single layer composed of only spherical

Table 1. Summary of results of the Analysis of Variance (ANOVA) performed to test for seasonal differences in body indices and gonad volume, according to precipitation levels, in female and male *G. geckoides* Spix, 1825 from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021. Body indices: liposomal ratio (RFB), hepatosomatic ratio (RWL), gonadosomatic ratio (RGS). An asterisk (*) indicates a statistically significant difference.

	Seasonal variation (Precipitation level)				
	Df	Sum Sq	Mean Sq	F-value	P-value
Females					
RFB	36	170.7	56.90	2.79	0.05*
RWL	36	5.98	1.99	2.97	< 0.01*
RGS	36	12.94	4.31	3.21	0.03*
Gonadal volume	36	8.30	2.75	0.10	0.95
Males					
RFB	91	30.3	10.08	1.43	0.23
RWL	91	1.74	0.58	1.35	0.26
RGS	91	3.88	1.29	4.58	< 0.001*
Gonadal volume	91	161.62	4.44	2.58	< 0.001*

cells, displaying a mean diameter of $4.68 \pm 0.56 \mu\text{m}$. Meanwhile, the thecal layer showed an increase in vascularization (Fig. 6B).

The abiotic factors seemed to be strongly influenced by the germinal bed. This structure appeared to display a greater size during periods of higher humidity and precipitation, indicating increased cellular activity, mainly during periods of stronger rainfall. Despite the slight range of variance between seasons, germinal bed also seems to be positively influenced while temperature increase (Table 3). Biotic factors, in turn, have had a strong influence on oocyte diameter and germinal bed. Significant positive effects were recorded regarding the increase in oocyte and germinal bed diameters with an increase in body temperature. The germinal bed also had a significant positive influence on condition factors (K1) such as gonad volume (Table 3). The granulosa layer was not significantly influenced by any biotic or abiotic factors. *G. geckoides* displayed constant ovary maturation, with reproductive activities increasing or decreasing according to the season (Supplementary Material 5).

Cellular variation: males

The testes of *G. geckoides* consisted of paired, symmetric, and ovoid organs located in the celomatic

cavity, whereas the hemipenis had a horn shape, also in pairs (Fig. 7A–B). Seminiferous tubes remained spermatogenetically active throughout the year without quiescence. Reproductive cells during the primary stage (Spt1) were located at the periphery of the seminiferous tubes, whereas cells in the secondary stage (Spt2) were located closer to the lumen (Fig. 8B). At the end of the maturation stage, most sperms (SPZ) occupied the lumen region in the seminiferous tubes (Fig. 8B). Spermatids (SEP) were located comprising SPZ at the lumen region (Fig. 8C). Deferens ducts lacked sexually mature reproductive cells in the lumen (Figs. 8D–E). The epididymal ducts, in turn, always contained mature reproductive cells in the lumen (Figs. 8D–F).

Regarding seasonality, significant increases in STD and SEH were recorded during the rainy season, whereas at the end of the rainy season, there was a significant reduction in DEF and an increase in DEP and DEH (Table 4). Mature reproductive cells (SPZ) also showed a significant increase at the end of the rainy season and at the onset of the dry season, with the opposite trend observed in the earlier stages of reproductive cell maturation (Spt1 and Spt2) (Table 4). Despite constant reproductive cell maturation, the ANOVA test showed significant variations in cellular structure measurements and the number of reproductive cells in different stages. This indicated variations in reproductive activity in *G. geckoides* males for seasonal and annual cycles (Table 4). GLM analysis revealed a significant increase in diameter of DEP associated to the increase of mature cells (SPZ) at the seminiferous tubes ($\beta \pm \text{SE} = 0.065 \pm 0.009$, $z = 6.638$, $p < 0.001$).

According to our correlation analysis, biotic and abiotic factors seem to be strongly related to reproductive cell maturation and cellular structure measurements including diameter and height (Table 5). Regarding biotic factors, gonad volume and body temperature revealed a rather range of significant correlation. Considering significant correlation, a positive relationship was recorded between gonad volume and STD, as well as between gonadal volume and cell types analyzed (Spt1, Spt2, and SPZ), indicating a range of structures, as well as many mature reproductive cells, as the gonad volume increases. Body temperature, in turn, showed a positive relationship to Spt1 and Spt2, whereas it was recorded negative relationship with several cellular structures (SEH, DEF, DEP and DEH), as well as SPZ. K1, in turn, just revealed a significant negative relationship

Table 2. Summary of results of the Generalized Linear Model (GLM) fitted to test the influence of abiotic environmental factors on different body indices and gonad volume of female and male *G. geckoides* Spix, 1825 from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021. Body indices: liposomal ratio (RFB), hepatosomatic ratio (RWL), gonadosomatic ratio (RGS).

	Females (n = 61)				Males (n = 97)			
	Estimate	Std. Error	z-value	p-value	Estimate	Std. Error	z-value	p-value
RFB								
(Intercept)	4.333	0.926	4.679	<0.001	18.926	6.887	2.748	<0.001
Temperature	-0.067	0.034	-1.983	0.047	-0.148	0.214	-0.694	0.489
Humidity	-0.016	0.017	-0.949	0.342	-0.014	0.030	-0.460	0.646
Precipitation	0.001	0.019	2.774	<0.001	0.019	0.056	0.339	0.735
RWL								
(Intercept)	3.271	1.593	2.053	0.040	4.341	1.146	3.789	<0.001
Temperature	-0.013	0.049	-0.273	0.785	-0.043	0.029	-1.460	0.147
Humidity	-0.007	0.007	-1.035	0.305	-0.014	0.008	-1.760	0.081
Precipitation	0.004	0.013	0.035	0.972	-0.003	0.005	-0.644	0.520
RGS								
(Intercept)	1.297	0.629	2.059	0.044	1.861	0.784	2.372	<0.01
Temperature	-0.001	0.017	-0.085	0.932	-0.015	0.002	-0.751	0.454
Humidity	-0.009	0.004	-2.044	0.045	-0.006	0.005	-1.061	0.291
Precipitation	-0.004	0.003	-1.367	0.177	-0.004	0.003	-1.059	0.292
Gonadal Volume								
(Intercept)	17.442	9.733	1.792	0.050	-4.888	1.744	-2.802	<0.01
Temperature	-0.328	0.301	-1.089	0.280	0.103	0.050	2.032	0.042
Humidity	-0.067	0.047	-1.410	0.164	0.047	0.025	1.917	0.050
Precipitation	-0.117	0.079	-1.477	0.145	0.003	0.001	3.165	<0.001

with DEH, with a significant and positive correlation, mainly regarding the final stage of sexually mature cells (SPZ), indicates constant maturation (Table 5).

Regarding abiotic factors, temperature, and humidity significantly influenced cellular structure and reproductive cell maturation, respectively. Our results displayed a reduction in reproductive cell maturation rates during periods of higher temperature and humidity, despite the constant reproductive cell maturation process, which indicates an optimization of reproductive activity during certain periods at the study site (Table 5).

Discussion

Our results have shown that individuals of *Gymnodactylus geckoides* maintain relatively constant values of indices, displaying variations throughout the year. Females and males have shown similar patterns of investment in energy reserves after attaining

sexual maturity. Both cycles could be attributed to the different reproductive roles of each sex, mainly regarding reproductive process like mating, gestation and laying eggs, and to different sexual responses to abiotic factors such as temperature, humidity, and precipitation (e.g. Sexton and Brown, 1997; Norvalet *al.*, 2019).

There was no significant variation in the total mass of the population sampled of *G. geckoides* throughout the year. This indicated that individuals constantly gained mass, a pattern confirmed by the condition factor (K1). Isometric growth was characterized by similar energy investment in both the gonads and body size (Cox *et al.*, 2009). Given that gonad growth is accompanied by an increase in SVL, investing in body growth is the key for an individual to reach sexual maturity (Sexton and Brown, 1977; Ramirez-Bautista *et al.*, 2006; Oitaven *et al.*, 2022). The lack of lipid reserves in body parts other than the tail indicates that the tail region stores the most important lipid reserves in this species (Paz *et al.*,



Figure 5. Ovarian follicles on maturation process at previtellogenic (A) and vitellogenic phase(B) in *G.geckoides*: (A) Polymorphic and multilayered epithelium, with granulosa layer (G), with double layers, containing pyriform (PyC), intermediate (Ic) and small cells (Sc) containing spherical nucleus and a nucleolus; (B) the epithelium is composed by a single layer of small cuboidal cells (SC), with vacuole zones (asterisk) during the previtellogenic phase progress. GB: germinal bed, YM: vitelline membrane, Oop: ooplasm. H-E-stained Scale bar: 50 µm.

2019). Given that all types of reserves analyzed showed a direct relationship with SVL and weight, it can be inferred that the availability of food resources in Catimbau National Park is sufficient to allow *G. geckoides* to accumulate the energy reserves necessary to sustain vital activities.

Abiotic factors and seasonality are significantly related to reproductive cell maturation, mainly in the earlier stages. The body temperature in *Gymnodactylus* genus is strongly associated with environmental temperatures (Vitt, 1986; Colliet *et al.*, 2003).

Therefore, since there was a positive and significant relationship between Spt1 and body temperature, the reproductive cell maturation process tended to be optimized at higher temperatures within certain limits. This corresponds to pattern temperature in the Caatinga domain. The epididymal and deferential ducts also seem to increase their composites production, which is responsible for storage and sperm maturation, probably led by optimized cell maturation. This situation facilitates the meeting process during the dry season, whereas gestation

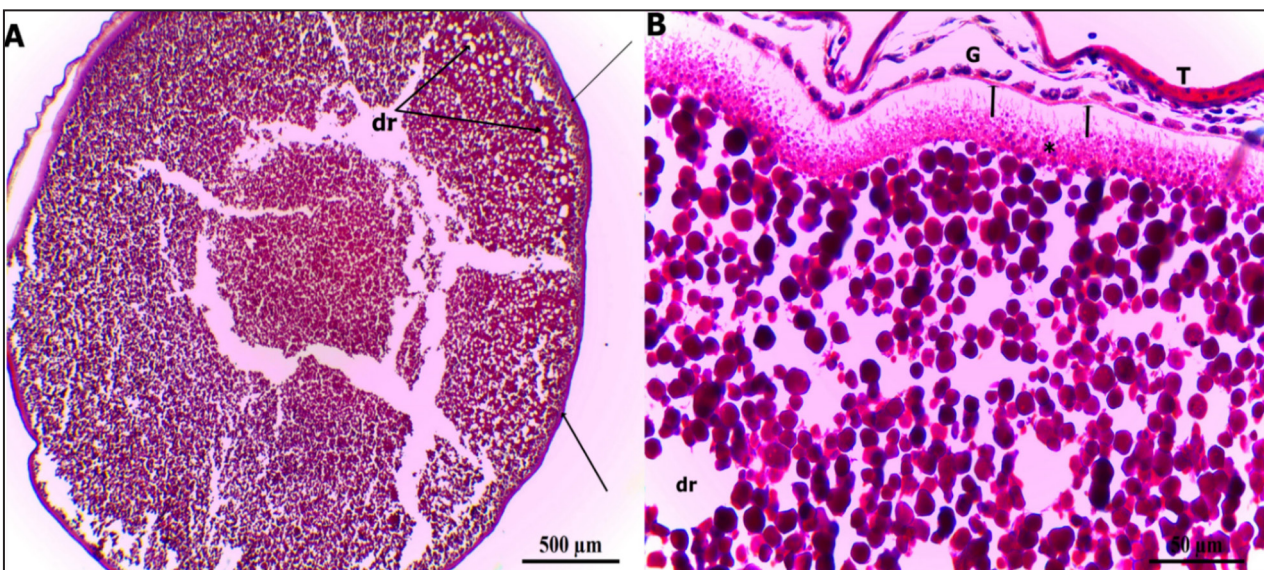


Figure 6. Ovarian follicle of *G. geckoides* from Catimbau National Park on vitellogenic stage. (A) Small and intermediate lipid droplets (dr) and defined yolk membrane (arrows) (Bar = 500 µm). (B) Detail of the Figure A. The yolk membrane (arrows) exhibits a hyaline band and a zonaradiata. Abundant small yolk platelets (*) in the periphery of the ooplasm merge as they move toward the central area of oocyte. The granulosa is reduced to small and cubical cells. Granulosa (G), and theca (T) (Bar = 50 µm).

Table 3. Influence of abiotic and biotic factors analyzed under the reproductive cellular structures modifications.

Celular structures (Females)	Biotic factors				Celular structures (Females)	Abiotic factors			
	Estimate	Std. Error	z-value	p-value		Estimate	Std. Error	z-value	p-value
Oocyte diameter					Oocyte diameter				
(Intercept)	8.672	4.518	1.920	0.050	(Intercept)	5.009	1.087	4.605	< 0.001
Precipitation	-0.032	0.018	-1.759	0.078	K1	-21.320	11.933	-1.787	0.074
Temperature	-0.051	0.125	-0.408	0.683	Gonadal Volume	0.010	0.021	0.469	0.638
Humidity	-0.005	0.019	-0.270	0.787	Body Temperature	0.107	0.045	2.376	0.017
Granulosa diameter					Granulosa diameter				
(Intercept)	4.972	3.027	1.642	0.028	(Intercept)	3.504	1.920	1.824	0.05
Precipitation	-0.001	0.001	-0.786	0.432	K1	-1.499	9.912	-0.146	0.883
Temperature	-0.015	0.062	-0.247	0.805	Gonadal Volume	0.227	0.278	0.815	0.415
Humidity	-0.044	0.132	-0.335	0.738	Body Temperature	0.015	0.063	0.24	0.81
Germinal bed diameter					Germinal bed diameter				
(Intercept)	-9.843	3.780	-2.604	< 0.001	(Intercept)	-1.589	1.836	-0.866	0.021
Precipitation	0.003	0.001	2.613	< 0.001	K1	22.667	9.204	2.463	0.013
Temperature	0.432	0.126	3.427	< 0.001	Gonadal Volume	0.728	0.265	2.738	< 0.001
Humidity	0.174	0.059	2.950	< 0.001	Body Temperature	0.151	0.060	2.491	0.012

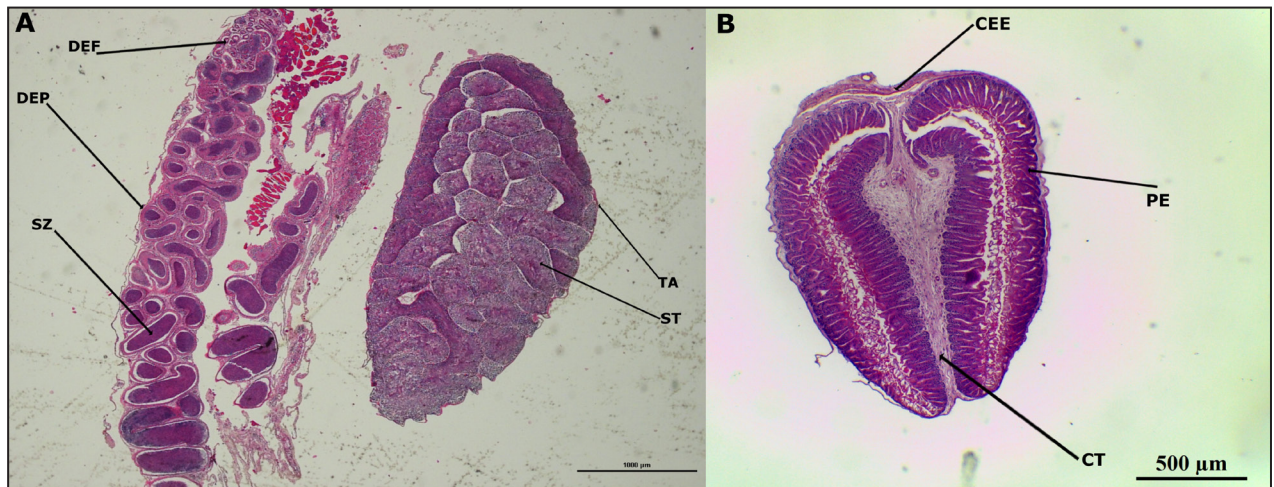


Figure 7. Panoramic view of testis and anterior testicular ducts (A) and hemipenis (B) of *G. geckoides* sexually matures individuals from Catimbau National Park. The tests displayed seminiferous tubules with active spermatogenesis, with seminiferous tubules (ST) surrounded by tunica albuginea (TA), also containing reproductive cells in all maturation stages. The ductuliefferentes (DEF) have not spermatozoa (Sz), and the ductus epididymis (DEP) is full of spermatozoa. Hemipenis displayed connective tissue (CT) in central region, surrounded by squamousepithelium (PE) and stratifiedcolumnar epithelium (CEE). Scale bars: A = 1 mm; B = 0.5 mm.

occurs more frequently during the rainy season. Eggs are normally laid at the end of the rainy season (Ferreira *et al.*, 2002; Mamouet *et al.*, 2017).

An endogenous mechanism appears to be triggered in *G. geckoides* after individuals reach sexual maturity. This can make them sensitive to environmental variations and affect their body indices and gonadal development in different ways. According to our results, RFB accumulation differed between sexes. Females experienced a constant reduction

in RFB until the end of the rainy season, whereas males accumulated RFB until the end of this period, with the reduction in females associated with the gestation process. These results indicate that lipid reserves in females increases as precipitation increases and the air temperature decreases, while increasing humidity is associated with the reduction of gonadal reserves.

In contrast, RWL and RGS showed synchronized activity, with both indices showing a reduction

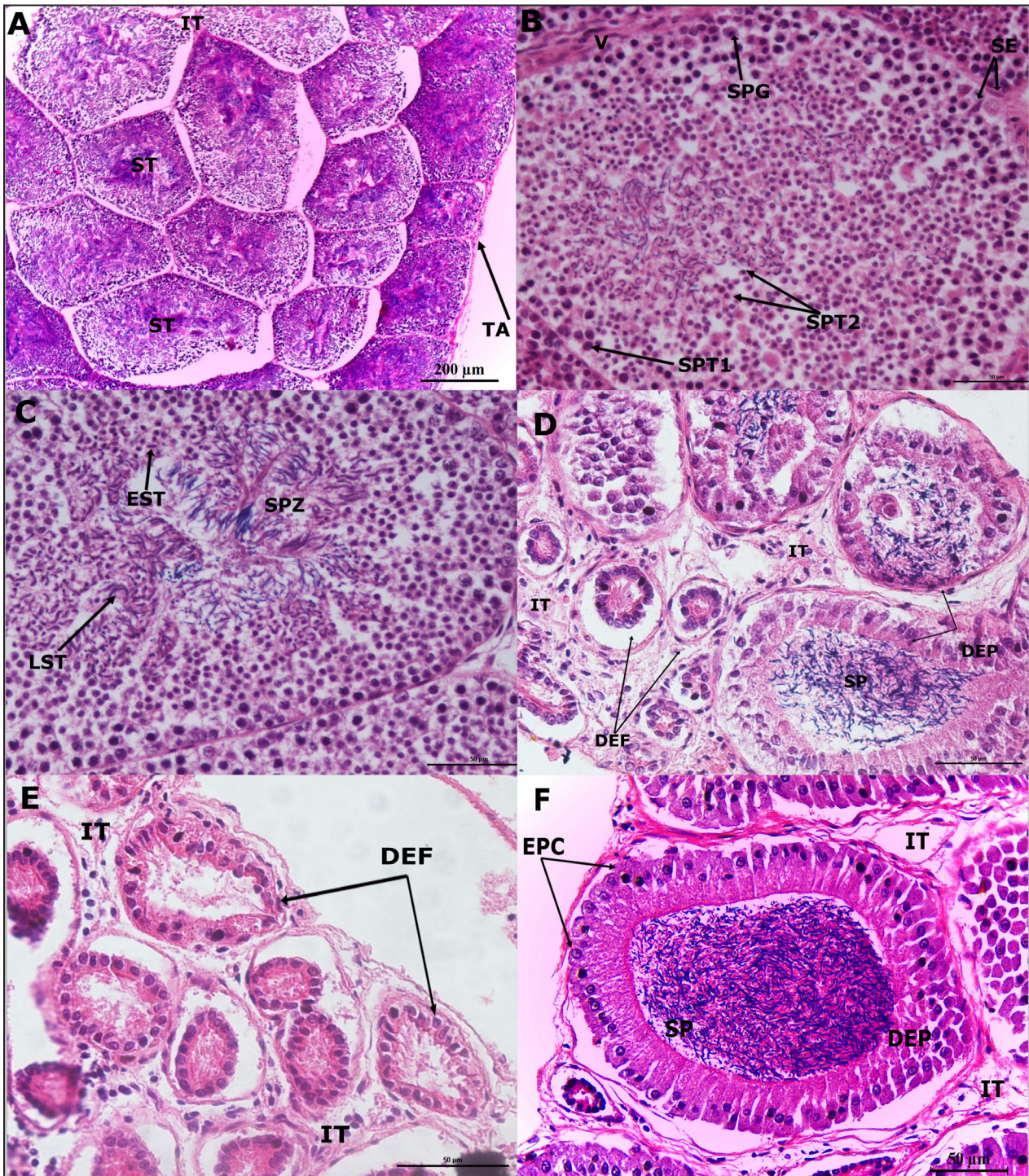


Figure 8. Tests structures of *G. geckoides* from Catimbau National Park. All the seminiferous tubules (ST) display constant spermatogenesis, evolved by tunica albuginea (TA), varying the maturation activity from a tubule to another (A). Seminiferous tubule under spermiogenesis process, with the presence of blood vessels (v) and Sertoli cells (SE) on the periphery, and abundance of spermatogonia (SPG), primary spermatocytes (SPT1) and secondary spermatocytes (SPT2) (B). Seminiferous tubule under espermiation process, with high abundance of early (EST) and late spermatids (LST), as well as sperms (SPZ) (C). Ductuliefferentes (DEF) and ductus epididymis (DEP), surrounded by interstitial tissue (IT) during spermatogenesis process (D). The ductuliefferentes have not spermatozoa, and they are surrounded by interstitial tissue (IT) (E). The ductuliefferentes is lined by a columnar epithelium (EPC), and its lumen contains spermatozoa (SP). The interstitial tissue (IT) is observed around the epididymal duct. The cuboidal epithelium of the ductuliefferentes possesses large stereocilia (arrowhead) in the apical region (F). Scale bars: A = 0.2 mm; B, C, D, E, F = 50 µm.

Table 4. ANOVA's tests for differences throughout the annual cycle (months) and across the phases of the spermatogenic cycle in selected structures, as well as reproductive cell stages, of the testis and anterior seminiferous tubes of *G. geckoides*, for Catimbau National Park, Pernambuco, Brazil northeastern. the reproductive cellular structures modifications.

Analyzed pattern	Model	F	P
Seasonality			
	Seminiferous tubule diameter	4.54	< 0.001
	Seminiferous epithelium height	10.94	< 0.001
	Ductuli efferentes diameter	11.40	< 0.001
	Ductus epididymis diameter	13.09	< 0.001
	Ductus epididymis heighth	21.98	< 0.001
	Primary spermatocytes	9.41	< 0.001
	Secondary spermatocytes	12.02	< 0.001
	Sperms	18.94	< 0.001
Annual cycle			
	Seminiferous tubule diameter	2.15	< 0.01
	Seminiferous epithelium height	4.04	< 0.001
	Ductuli efferentes diameter	12.48	< 0.001
	Ductus epididymis diameter	3.44	< 0.001
	Ductus epididymis heighth	10.89	< 0.001
	Primary spermatocytes	5.77	< 0.001
	Secondary spermatocytes	5.62	< 0.001
	Sperms	10.88	< 0.001

until the beginning of the rainy period in females and males. However, females showed substantially greater variation in body indices than males. Such sexual differences in energy reserves are likely because of the high energy costs faced by females during vitellogenesis and gestation process (Ramirez-Bautista y Vitt, 1997; Schaffner, 1998). In males, abiotic factors influenced gonad volume only, which increased with increasing temperature, humidity, and precipitation, indicating a constant increase in gonad volume. Variation in gonad volume indicates that males in *G. geckoides* show variation in mating activity during each period, although the production of sperm occurs throughout the year without

gonadal quiescence. Finally, reduced RGS levels indicate reduced mating activity during periods of abundant rainfall, potentially because most females in the population are gestating (Galdino *et al.*, 2003; Ramirez-Bautista *et al.*, 2009).

High humidity increased hatching success and brood survival (Derickson, 1976; Norvalet *et al.*, 2019). In a population of *G. geckoides* from the Catimbau National Park, females had a fixed single egg brood in their oviducts (Vitt, 1986; Alcântara *et al.*, 2019). In geckos, low investment in egg number is compensated for by the production of more than one brood per year, with females potentially investing similar amounts of their energy reserves in consecutive gestations (Guedes *et al.*, 2020; Oitaven *et al.*, 2022). The dominance of gravid females during periods of high precipitation and minor variation in air temperature suggests that this combination of abiotic conditions is favorable for gestation and egg deposition. This is potentially because increased rainfall and more stable temperatures reduce the risk of egg desiccation and brood mortality (Colli *et al.*, 2003; Norval *et al.*, 2019). Consequently, energy reserves are significantly reduced as the rainy season progresses, especially in reproductively active females.

The alternations between lipid reserves and gonad conditions in females, and the fact that some females simultaneously had ovaries at different stages, that is, pre-vitellogenic and vitellogenic, provide evidence that throughout the annual cycle of *G. geckoides*, more than one vitellogenesis process may take place simultaneously in some individuals. This process causes a marked reduction in lipid reserves, followed by the appearance of yolk follicles and oocytes. The process is characterized by an increase in lipid and hepatic reserves. An increase in liver volume correlates with an increase in lipids in this organ, which are then used in the second vitellogenesis process (Aun and Martori, 2010; Paz *et al.*, 2019). In the Caatinga, these stages coincide with late periods of the dry and rainy seasons. This is likely to be an adaptive reproductive strategy, because producing multiple broods maximizes the probability that some broods will have favorable environmental conditions for their development and survival. This ultimately increases their reproductive success (Vitt, 1986; Colli *et al.*, 2003). In contrast, the lack of variation in the energy reserves of males suggest that sperm production and copulation do not impose high energy costs to males (Guillete and Casas-Andreu, 1980; Ramírez-Bautista *et al.*, 2006),

Table 5. Summary results of GLM models between the selected structures, as well as reproductive cells of the testis and seminiferous tubes of *G. geckoides* and abiotic factors (Temperature, precipitation and humidity), such as biotic factors (body temperature, growth rate and gonadal volume) in Catimbau National Park, Pernambuco, Northeastern Brazil.

Model Analyzed	Abiotic factors					Biotic factors				
	Effect	Estimate	Std. Error	t	P	Effect	Estimate	Std. Error	t	P
Seminiferous tubule diameter	Intercept			1.62	0.1	Intercept			2.66	< 0.001
	Precipitance	0.22	0.15	1.45	0.14	Body temperature	-0.11	2.67	-0.04	0.94
	Temperature	0.7	5.98	0.11	0.9	K1	125.17	153.89	0.81	0.41
	Humidity	0.41	0.88	0.47	0.63	Gonadal Volume	8.00	1.82	4.39	< 0.001
	Overall model: F = 0.87; r = 0.01, P = 0.45									
Seminiferous epithelium height	Intercept			2.49	< 0.01	Intercept			3.38	< 0.001
	Precipitance	0.15	0.07	2.1	0.03	Body temperature	-5.00	1.31	-3.81	< 0.001
	Temperature	-2.11	2.86	-0.73	0.46	K1	-39.94	75.06	-0.53	0.59
	Humidity	0.05	0.42	0.13	0.89	Gonadal Volume	-0.51	0.89	-0.57	0.56
	Overall model: F = 2.05; r = 0.02, P < 0.01									
Ductuli efferentes diameter	Intercept			5.02	< 0.001	Intercept			2.99	< 0.001
	Precipitance	-0.01	0.04	-0.18	0.85	Body temperature	-3.4	0.93	-3.66	< 0.001
	Temperature	-8.27	1.87	-4.4	< 0.001	K1	4.55	52.79	-0.08	0.93
	Humidity	-0.15	0.27	-0.57	0.56	Gonadal Volume	-0.74	0.63	-1.17	0.24
	Overall model: F = 15.98; r = 0.18, P < 0.001									
Ductus epididymis diameter	Intercept			-0.14	0.88	Intercept			1.37	0.17
	Precipitance	0.04	0.13	0.36	0.71	Body temperature	-5.76	2.44	-2.35	< 0.01
	Temperature	6.62	5.26	1.25	0.2	K1	-132.28	139.52	-0.94	0.34
	Humidity	1.55	0.78	1.98	0.04	Gonadal Volume	1.90	1.65	1.15	0.25
	Overall model: F = 1.54; r = 0.02, P = 0.20									

Ductus epididymis height	Intercept			1.01	0.31	Intercept		0.20	0.83
	Precipitance	0.07	0.06	1.16	0.24	Body temperature	-3.67	-3.27	< 0.001
	Temperature	0.42	2.45	0.17	0.86	K1	64.79	-2.6	< 0.001
	Humidity	0.36	0.36	0.99	0.32	Gonadal Volume	0.28	0.37	0.70
	Overall model: F = 1.19; r = 0.01, P = 0.31				Overall model: F = 4.48; r = 0.05, P < 0.001				
Primary Spermatozoocytes	Intercept			-1.2	0.22	Intercept		1.03	0.30
	Precipitance	-0.15	0.14	-1.17	0.24	Body temperature	0.38	4.78	< 0.001
	Temperature	0.55	0.19	2.91	< 0.01	K1	5.50	0.99	0.32
	Humidity	0.01	0.02	0.47	0.63	Gonadal Volume	-0.24	-3.89	< 0.001
	Overall model: F = 6.00; r = 0.06, P < 0.001				Overall model: F = 13.85; r = 0.13, P < 0.001				
Secondary Spermatozoocytes	Intercept			2.34	< 0.01	Intercept		2.24	0.02
	Precipitance	0.17	0.17	0.99	0.32	Body temperature	0.24	1.88	0.06
	Temperature	-0.18	0.3	-0.61	0.54	K1	8.21	0.90	0.36
	Humidity	-0.04	0.04	-0.97	0.33	Gonadal Volume	-0.26	-2.62	< 0.001
	Overall model: F = 0.64; r = 0.07, P = 0.58				Overall model: F = 3.91; r = 0.04, P < 0.001				
Espermatozoa	Intercept			6.83	< 0.001	Intercept		1.55	0.12
	Precipitance	0.02	0.01	1.9	0.05	Body temperature	-0.56	-2.06	0.03
	Temperature	-3.05	0.57	-5.33	< 0.001	K1	-10.05	-0.54	0.58
	Humidity	-0.32	0.08	-3.99	< 0.001	Gonadal Volume	0.38	1.90	0.05
	Overall model: F = 10.26; r = 0.10, P < 0.001				Overall model: F = 2.94; r = 0.03, P = 0.03				

maintaining constant levels in their energy reserves (Sexton and Brown, 1977; Galdino *et al.*, 2003; Ramirez-Bautista *et al.*, 2006; Oitaven *et al.*, 2022).

The population of *G. geckoides* analyzed displayed continuous and acyclic activity in their reproductive patterns. Despite the marked seasonality in the Caatinga area, temperatures tended to be adequate, allowing these cycles to be maintained for females and males. This study represents the first examination of energy cycles and reproductive strategies in *G. geckoides* in the Caatinga area, describing the cycles of different body indices and gonads, while also testing the effects of environmental factors and morphological traits. Despite seasonal variations in energy reserves, *G. geckoides* are constantly active throughout the year and reproduces more than once in a single year. The energy required to sustain continuous activity and related vital processes, that is, maintenance and reproduction, in *G. geckoides* is dependent on diet. This indicates that prey availability seems to be sufficient at the Caatinga domain.

Acknowledgments

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Supplemental online material legends

Supplementary material 1. Climatological variations at Catimbau National Park, regarding data recorded by the municipalities of Buíque, Pernambuco state, Northeastern Brazil, and Picuí, Paraíba state, Northeastern Brazil, from September 2018 to September 2021. Total rainfall, between 0.5 to 188 (mm–line) and average monthly temperature, between 24 to 33(°C–bars). Climatological variations recorded by Pernambuco Water and Climate Agency database (APAC), with many meteorological, and information recorded from 2018 to 2019 according to many municipalities of Pernambuco state,

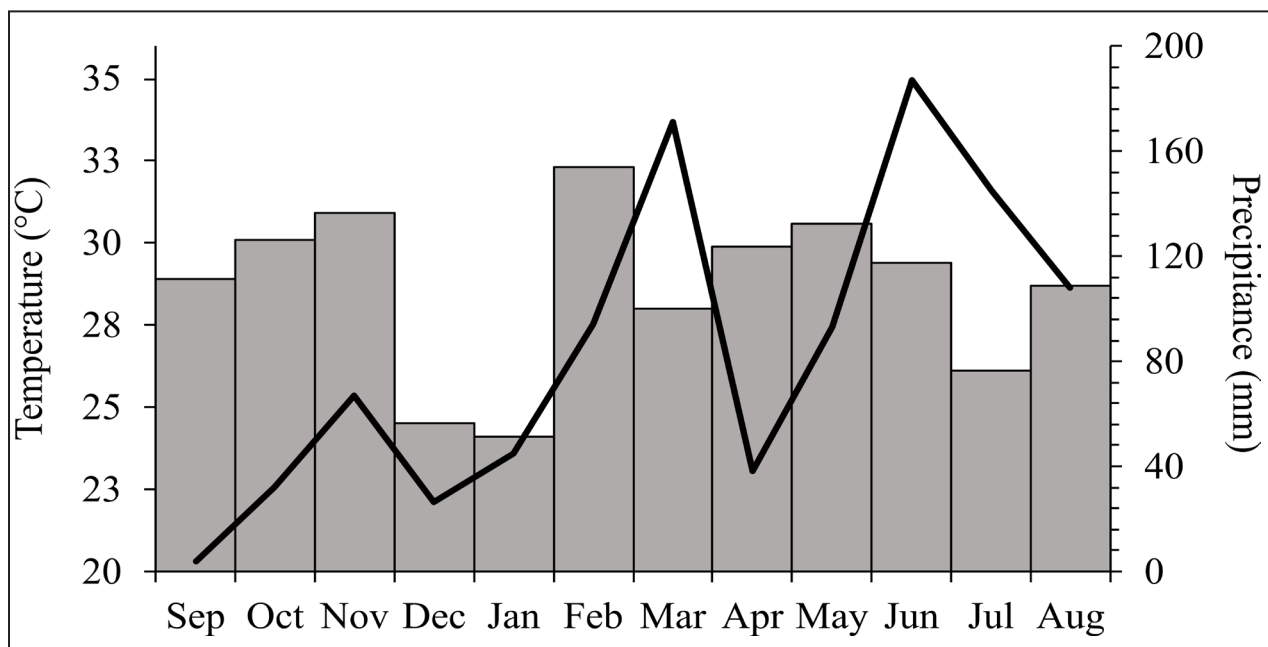
Northeastern Brazil.

Supplementary material 2. Summary of the total numbers of female and male *G. geckoides* collected by month and year in Catimbau National Park, municipality of Buíque, Pernambuco state, and Picuí municipality, Paraíba state, Brazil. The quantity of asterisks (*) denotes how many gravid females were found in that month.

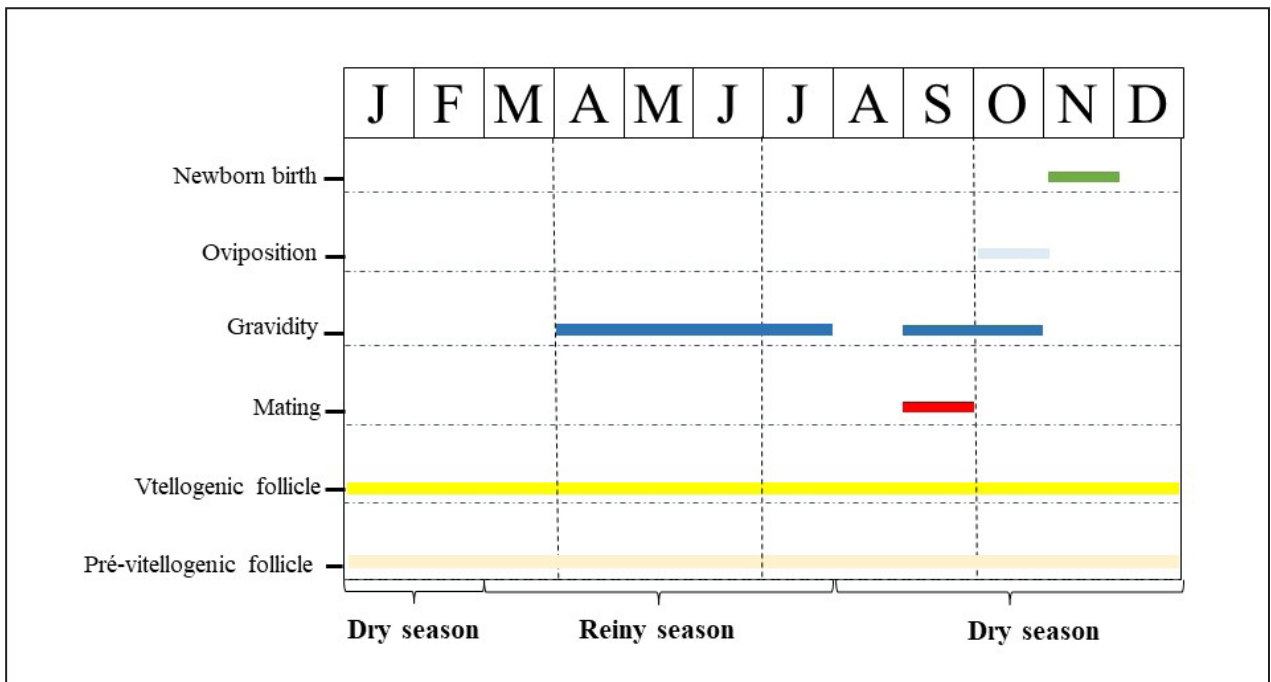
Supplementary material 3. Correlation analysis comparing body structures (SVL and Weight), as independent variables, and body reserves (lipids, hepatic and gonadal), as dependent variables, for both female and male of *G. geckoides* Spix, 1825 individuals from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021.

Supplementary material 4. Summary of results of the Generalized Linear Model (GLM) fitted to test the influence of body indices and gonad volume, according to seasonal variations (precipitation levels), on the appearance of individuals of *G. geckoides* Spix, 1825 in Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021. Body indices: liposomal ratio (RFB), hepatosomatic ratio (RWL), gonadosomatic ratio (RGS).

Supplementary material 5. Reproductive cycle from female of *G. geckoides* at Catimbau National Park, including follicles maturation, mating, gestation, as well as eggs laying and hatchling.



Sampling month	2018 – 2021		Monthly total
	Females	Males	
September	03	03	06
October	02	02	04
November	06	02	08
December	03	03	06
January	03	03	06
February	03	08	11
March	04	06	10
April	03*	11	14
May	04**	05	09
June	04**	04	08
July	03*	08	11
August	06*	09	15
Total			108



Predictors	Low precipitation levels (n = 66)			High precipitation levels (n = 63)		
	Estimate ± SE	z-value	p	Estimate ± SE	z-value	p
Intercept	3.305 ± 4.718	0.701	0.484	-3.446 ± 4.709	-0.732	0.464
RFB	-0.001 ± 0.060	-0.022	0.982	0.004 ± 0.060	0.083	0.934
RWL	-0.115 ± 0.317	-0.363	0.717	0.136 ± 0.321	0.426	0.670
RGS	0.446 ± 0.478	0.933	0.351	-0.533 ± 0.499	-1.069	0.285
Gonadal volume	-0.028 ± 0.060	-0.467	0.640	0.024 ± 0.060	0.398	0.691

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