



Research paper

Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard *Salvator merianae*



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ABSTRACT

The tegu lizard *Salvator merianae* is a large, widely distributed teiid lizard endemic to South America that exhibits annual cycles of high activity during the spring and summer, and hibernation during winter. This pattern of activity and hibernation is accompanied by profound seasonal changes in physiology and behavior, including endothermy during the austral spring. The unusual combination of seasonal endothermy, hibernation and oviparity, in a non-avian, non-mammalian species, makes *S. merianae* an interesting subject for study of comparative aspects of endocrine regulation of seasonal changes in physiology. In the present study, we first validated commercially available immunoassay kits for quantification of hormone concentrations of the reproductive (testosterone, estradiol and progesterone), adrenal (corticosterone), and thyroid [thyroxine (T_4) and triiodothyronine (T_3)] axes in plasma of an outdoor, captive adult male and female *S. merianae* in southeastern Brazil. All assays exhibited parallelism and accuracy with *S. merianae* plasma. We next assessed patterns of concentration of these hormones across the annual cycle of *S. merianae*. Testosterone in males and estradiol in females peaked in spring coincident with the peak in reproductive behavior. Progesterone in females was significantly elevated in October coincident with putative ovulation when gravid females build nests. Thyroid hormones, known for regulating energy metabolism, varied seasonally with some sex-dependent differences. T_4 gradually increased from an annual nadir during pre-hibernation and hibernation to high concentrations during spring in both sexes. In contrast, T_3 did not vary seasonally in males, but females showed a two-fold increase in T_3 during the spring reproductive season. T_3 may be involved in energy investment during the seasonal production of large clutches of eggs. Corticosterone was significantly elevated during the active season in both sexes, suggesting its involvement in mobilization of energy stores and modulation of behavior (territoriality) and physiology. Ours is the first investigation of concurrent changes in reproductive, thyroid and adrenal hormone concentrations in this endemic and physiologically unique South American lizard. Our findings set the stage for future investigations to determine the extent to which these hormones influence activity and thermoregulation in *S. merianae*.

1. Introduction

Seasonal adjustments in behavior and physiology are often driven by changes in hormones, which, among vertebrates, coordinate timing of breeding and energy allocation in environments that undergo cycles of scarcity annually (Wilsterman et al., 2015). Hormonal correlates of seasonal physiological and behavioral adjustments have been extensively documented in endothermic vertebrates, in which the high

energetic cost of endothermy often drives those adjustments to survive phases of food and/or water scarcity (McNab, 1974; Richter et al., 2017). Study of other vertebrates (especially if they show endothermy, partial or seasonal), would be valuable for illuminating general endocrine mechanisms of seasonal modulation of physiology and behavior (Davenport et al., 1990; Korsmeyer and Dewar, 2001; Tattersall et al., 2016; Slip and Shine, 1988).

The seasonally endothermic tegu lizard *Salvator merianae*, a large-

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bodied oviparous species that undergoes hibernation annually, has the widest geographic distribution of the three species of the *Salvator* genus (the other two are *Salvator duseni* and *Salvator rufescens*, also known as “teguus”; Harvey et al., 2012) and can be found from tropical to subtropical and temperate zones in most biomes of Brazil, Uruguay, Paraguay and northern Argentina (Lanfri et al., 2013). *S. merianae* has been the subject of numerous investigations of physiology and behavior (Abe, 1995, 1983; Andrade et al., 2004; Chamut et al., 2012; Chani et al., 1993; Da Silveira et al., 2013; do Nascimento et al., 2016; Klein et al., 2006; Milsom et al., 2008; Sanders et al., 2015; Tattersall et al., 2016; Toledo et al., 2008; Zena et al., 2016) and has been shown to be endothermic during the breeding season (Tattersall et al., 2016), exhibiting profound seasonal shifts in metabolic rate and body temperature (Lopes and Abe, 1999; Sanders et al., 2015; Tattersall et al., 2016). Highest resting metabolic rates are observed during spring (reproductive season) and summer, while lowest levels occur during its approximately three-month-long hibernation in winter in Southeastern Brazil (Lopes and Abe, 1999; Sanders et al., 2015; Tattersall et al., 2016). At this geographic region, dormancy in *S. merianae* occurs seasonally during the winter, which is characterized by mild ambient temperatures (Abe, 1995; Andrade et al., 2004; Sanders et al., 2015) and low precipitation. The word aestivation has been used for this dormancy, but we follow Milsom et al., 2012 and use the term hibernation hereafter.

During the reproductive season, *S. merianae* exhibit territoriality, courtship, and copulation, as well as nest construction and incubation of the eggs by females (Chamut et al., 2012; García-Valdez et al., 2016), even in captive populations (L. Zena, pers. obs.). Interestingly, the seasonal cycle of activity and hibernation in *S. merianae* seems not to involve direct temperature-induced metabolic changes, but rather is a temperature-independent phenomenon (Abe, 1995; Milsom et al., 2008; Sanders et al., 2015). Because of this unique combination of traits among lizards — thermogenic capacity and seasonal metabolic adjustments, with a regularly occurring hibernation phase — *S. merianae* constitutes an interesting species for exploring basic features of annual cycles in hormones and behavior.

Sex steroids, including estrogens, progesterone, and androgens, are well-documented players in the regulation of season-specific behaviors, physiology and maintenance of secondary sex characteristics in reptiles (Chamut et al., 2012; Crews et al., 1978; García-Valdez et al., 2016; Hamlin et al., 2014; Hews and Moore, 1995). Similarly, the glucocorticoids (corticosterone and cortisol) also commonly exhibit seasonal changes that correlate with energetic requirements and exposure to social, nutritional or physiological stress (Anderson et al., 2014; Welcker et al., 2014). Though not classically considered reproductive hormones, glucocorticoids frequently show predictable seasonal elevations of baseline during reproduction, possibly due to increased energetic requirements as well as increased stress of social interactions (Romero, 2002). Finally, thyroid hormones (THs; thyroxine [T_4] and triiodothyronine [T_3]) are of particular interest, especially in seasonally endothermic species. They have been best studied in mammals and birds, where they play a major role in energy metabolism and metabolic regulation, maintaining body temperature and influencing a wide variety of physiological processes (Mullur et al., 2014). In ectotherms, circulating TH concentrations positively correlate with aerobic energetic capacity, nutritional status, and ambient temperature (Dunlap, 1995; John-Alder, 1990; Little and Seebacher, 2014; Moon et al., 1999; Rivera and Lock, 2008), and also often exhibit relationships with photoperiod and reproductive events (Boggs et al., 2011; El-Deib, 2005; John-Alder, 1984; Kohel et al., 2001; Moon et al., 1999; Southwood et al., 2003).

The seasonal patterns of the steroid and thyroid hormones are widely observed in mammals and birds, but comparable data from the few studied reptiles are scarce. For *S. merianae* in particular, endocrine data are limited to one study on seasonal testosterone patterns in males (Chamut et al., 2012). Progesterone and estradiol profiles have been

described for the related *S. rufescens* (García-Valdez et al., 2016) but not for *S. merianae*, while glucocorticoids and thyroid profiles have not been described for any member of the *Salvator* genus. As a next step in exploring seasonal changes in gonadal, adrenal and thyroid hormones in this seasonally endothermic, hibernating lizard, we set out to (1) validate immunoassays for *S. merianae* plasma for all hormones of interest, and (2) document seasonal changes in circulating levels of the major steroid and thyroid hormones. We focused on three reproductive steroids (testosterone, estradiol and progesterone), the primary reptilian adrenal glucocorticoid (corticosterone), and two THs (T_4 and T_3). Validations employed tests of assay parallelism and accuracy of *S. merianae* plasma to verify that commercial immunoassay kits can accurately quantify these six hormones (Ellsworth et al., 2014; Grotjan and Keel, 1996; Hunt et al., 2017). For our second goal, we re-sampled *S. merianae* housed under semi-natural conditions across the year, and assayed plasma of males for testosterone, of females for progesterone and estradiol, and of both sexes for adrenal and thyroid hormones, assessing resulting data for significant seasonal patterns.

2 Materials and methods

2.1 Animals

Twenty adult captive-bred *S. merianae* tegu lizards of both sexes (10 males and 10 females) were obtained from Jacarezário, São Paulo State University-UNESP, Rio Claro, São Paulo state, Brazil (IBAMA permit no. 02001-000412/94-28 and SISBIO-ICMBio/n. 26677-1/transport and maintenance permit: ICMBio #52085-1). Males and females did not significantly differ in their initial body mass (2.29 ± 0.23 kg for males and 1.88 ± 0.09 kg for females; $P = 0.17$; $F_{1, 158} = 1.989$). All individuals were housed communally in a single 42 m² outdoor enclosure with natural exposure to ambient fluctuations in temperature, daylight, humidity and rainfall at the campus of UNESP in Jaboticabal, SP (21° 14' 05" S and 48° 17' 09" W; ~160 km from Rio Claro). Tegus were moved into this enclosure in March 2016 and remained there for the duration of the study, which was from October 2016 to September 2017). The enclosure contained common shelters for the animals to retreat to and to use during hibernation (tegu hibernation in southeastern Brazil occurs from late May or June to mid-August). In addition, the shelters provided protection from direct sunlight and precipitation. Animals had free access to a shared open area, providing options for thermoregulatory, ambulatory, and social behavior. Animals were fed 2–3 times/week with cooked chicken, eggs, mice, fruits and captive bred cockroach (*Nauphoeta cinerea*) supplemented with calcium and vitamin D₃ (Zoo Med Reptivite; San Luis Obispo, CA, USA). Water was provided *ad libitum*. Even in captivity, tegus voluntarily stop eating at the end of the austral autumn in late May (de Andrade and Abe, 1999; de Souza, 2004; Zena et al., 2016) and begin eating again upon emergence from hibernation (“emergence” hereafter) in early August; during hibernation water, but no food, was provided. All experimental protocols were approved by a local ethical committee of São Paulo State University (CEUA; # 7.434/16).

2.2. Sample collection

Although tegus are individually recognizable due to variable color patterns, all animals were additionally marked with a subcutaneous microchip (transponder ISO FDX-B 134.2 KhZ, AnimallTAG, São Carlos, Brazil) to further confirm individual identification. Blood was collected from all animals in October and December of 2016, and February, April, May, June, August and September of 2017. One male was injured by other animals in November 2016 and therefore was not sampled during recovery in December 2016. For blood sampling, animals were individually captured by hand and had their heads covered with a cloth bag. Blood was collected (up to 5% of total blood volume; Stahl, 2006) from the ventral coccygeal vein using a heparinized 5 mL syringe and a

21 G needle; whole blood samples were immediately transferred to plastic tubes (2 mL) on ice. Total handling time was ~3 min to minimize potential effects of handling stress (Romero and Reed, 2005) and all handling was performed between 8:00 and 10:00 a.m. to eliminate time of day effects (Jessop et al., 2002; Jones and Bell, 2004). Body mass was measured to the nearest ± 1 g on a digital scale (Bel Equipamentos Analíticos, Piracicaba, São Paulo, Brasil). After collection, blood samples were held on ice for a maximum of 2.5 h, centrifuged at 9,391g (speed: 10,000 rpm; radius of the rotor: 84 mm) for 10 min at 4 °C, and plasma was aliquoted into four 0.5 mL microcentrifuge tubes and frozen at -80 °C for future analyses.

2.3. Reproductive hormone extraction

Based on manufacturer's recommendations and previous experience, estradiol, testosterone and progesterone were extracted using solid phase extraction of 500 μ L plasma (detailed in Newman et al., 2008). Samples were then eluted in 90% methanol, dried in a ThermoSavant SpeedVac Concentrator (model SDP121P; Thermo Fisher Scientific, Waltham, MA, USA) at 35 °C and stored at -80 °C. One day prior to assay, samples were resuspended in 1 mL assay buffer (1:2 dilution), shaken on a multi-tube vortexer (Glas-Col Large Capacity Mixer, speed set on 50; Glas-Col, Terre Haute, IN, USA) for 1 h and then stored at 4 °C overnight. Before assays, the tubes were shaken again for 1 h. Corticosterone, T₄ and T₃ assays were performed using unextracted plasma.

2.4. Hormone assays and validation

Commercially available enzyme immunoassay (EIA) kits (Arbor Assays, Ann Arbor, MI, USA) were used to quantify plasma testosterone (kit #K032), estradiol (kit # KB30), progesterone (kit #K025), corticosterone (kit #K014), and T₄ (kit #K050), while a coated-tube ¹²⁵I radioimmunoassay (RIA) kit was used to quantify plasma T₃ (MP Biomedicals, Solon, OH, USA; kit #06B-254215). Two additional low-dose standards (created by mixing equal volumes of previous standard + assay buffer) were added to the T₃ standard curve to improve assay precision for low concentrations of hormone. Similarly, one additional low-dose standard was added to the testosterone standard curve. Following the manufacturer's protocols, the T₄ and corticosterone assays included addition of a dissociation reagent to dissociate hormones from plasma binding proteins. All hormone assays were validated for *S. merianae* plasma by conducting tests of parallelism and accuracy, using standard methods described in Grotjan and Keel (1996). To test for parallelism, a pooled plasma extract (testosterone, estradiol, and progesterone assays) or pooled unextracted plasma (corticosterone, T₄ and T₃) was serially diluted (1:2 – 1:128) and assayed. The slope of percentage of antibody bound vs. relative dose was compared to the slope of the known concentration standards. Parallelism of the two lines indicates that the antibody binds well to the hormone of interest with similar affinity as to pure parent hormone; this is considered evidence that the hormone is in fact present (Grotjan and Keel, 1996). From the parallelism results, a dilution was selected for each hormone assay to keep results as near as possible to 50% bound, the area of greatest assay precision. The dilutions selected were 1:2 for testosterone and estradiol, and 1:4 for progesterone (all diluted with Arbor Assays assay buffer X065), while corticosterone, T₄ and T₃ were assayed with undiluted plasma.

Assay accuracy (aka “matrix effect test”, “interference test”; Grotjan and Keel, 1996) was determined by spiking each standard with pooled tegu plasma, with assay of the spiked curve as unknowns alongside a second standard curve that was spiked only with buffer. Slopes of apparent hormone concentration vs. known concentration were assessed; a slope close to 1.0 indicates the ability of the assay to correctly quantify the hormone concentration across the desired range without interference from the sample matrix (i.e., tegu plasma).

Following validations for all assays, plasma samples from both sexes were assayed for corticosterone, T₄ and T₃. The reproductive steroids were each studied in only one sex, with samples from males assayed for testosterone and samples from females assayed for estradiol and progesterone. All assays included a full standard curve. Standards and unknowns were run in duplicate. Any sample that exceeded 10% coefficient of variation between duplicates was re-analyzed. Intra-assay and inter-assay variations were < 10%. For antibody cross-reactivities, assay sensitivities and other methodological details, see Hunt et al., 2017 (all EIAs) and Wasser et al., 2010 (T₃ RIA).

2.5. Statistical analysis

Parallelism results for all hormones were plotted as percentage of antibody bound vs. log[relative dose]. Differences between slopes of the binding curve for the serially diluted pool and the standard curve were assessed with an F test. Accuracy results were plotted as the apparent concentration vs. the standard concentration, and assessed by linear regression, with acceptable accuracy defined as $r^2 \geq 0.99$ and slope within 0.7–1.3 (ideal slope = 1.0). Monthly changes in sex steroid concentrations (testosterone, estradiol and progesterone) were assessed using a one-way repeated-measures ANOVA (i.e., independent variable of month). Corticosterone and the thyroid hormones (T₄ and T₃) were assessed with two-way repeated-measures ANOVA (i.e., independent variables of month and sex). Differences among means were further determined by Tukey post-hoc tests. Due to preliminary indications of possible relationships of testosterone with body size in males, we regressed testosterone and male body mass in each month separately and all months together using the false-discovery-rate (FDR) correction for multiple comparisons (Curran-Everett, 2000; Curran-Everett et al., 2004). When data were not normally distributed, logarithmic transformations were performed. Statistical analyses were carried out using SigmaPlot version 11 (ANOVA; Systat Software Inc.) and GraphPad Prism 6 (parallelism, accuracy, regressions, and multiple comparisons corrections; GraphPad Software). All data are presented as means \pm SEM and all differences considered significant at $P < 0.05$.

3. Results

3.1. Enzyme immunoassay (EIA) and radioimmunoassay (RIA) validations

All hormone assays of serially diluted tegu lizard plasma yielded displacement curves parallel to the respective standard curves (Figs. 1 and 2). There were no significant differences in slopes between standards and serially diluted tegu plasma pools ($P > 0.05$) for testosterone ($F_{(1, 10)} = 0.0799$; $P = 0.7831$), estradiol ($F_{(1,7)} = 0.2813$; $P = 0.6123$), progesterone ($F_{(1,8)} = 0.6258$; $P = 0.4517$), corticosterone ($F_{(1,6)} = 0.0739$; $P = 0.7949$), T₄ ($F_{(1,6)} = 0.3073$; $P = 0.5994$), or T₃ ($F_{(1,6)} = 0.0010$; $P = 0.9754$). Accuracy was acceptable for all hormones tested, as indicated by a linear relationship between observed and expected hormone concentration ($r^2 \geq 0.99$ for all hormones), and a slope within the desired range of 0.7–1.3, as follows (Fig. 3): testosterone (slope = 0.7), estradiol (slope = 1.2), progesterone (slope = 1.1), corticosterone (slope = 1.2), T₄ (slope = 0.7) and T₃ (slope = 0.9).

3.2. Seasonal variation in endocrine profiles of *Salvator merianae*

3.2.1. Sex steroid hormones

Male tegus started emerging from hibernation in mid-August, and displayed reproductive behaviors in September. Plasma concentrations of testosterone varied seasonally ($F_{(7,71)} = 21.660$; $P < 0.001$; Fig. 4A), with a single prominent peak in September coincident with the reproductive season for this species in southeastern Brazil (September; range: 0.0063–8.21 ng.mL⁻¹; mean: 4.82 \pm 0.83 ng.mL⁻¹). The smallest male had the lowest testosterone in September (0.0063 ng.

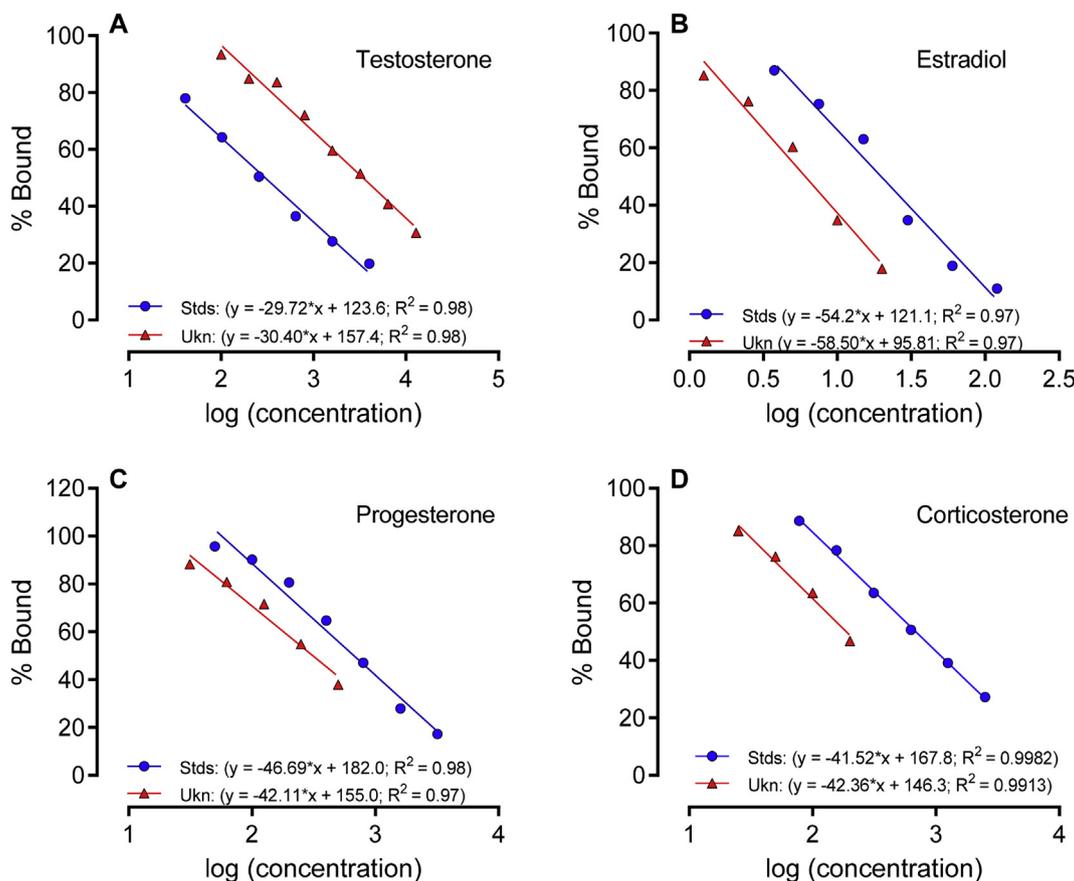


Fig. 1. Parallelism results for steroid hormones. (A) testosterone, (B) estradiol, (C) progesterone and (D) corticosterone in the plasma of the tegu lizard *Salvator merianae*; serial dilutions of pooled plasma (red triangles) are parallel to the standard curve (blue circles). Best-fit regression equations given at bottom. Stds: standard; Ukn: unknown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mL^{-1}) as well as relatively low circulating testosterone concentrations across the other months. Subsequent analysis of relationships of testosterone with body mass revealed a significant positive relationship in the months of May ($r^2 = 0.6753$, $P = 0.0035$; compare P to FDR multiple-comparisons correction criterion d of 0.0063, where $P < d$ indicates significance); and October ($r^2 = 0.6198$, $P = 0.0069$; compare P to d of 0.0125), but no significant relationship in any of the other six months ($P \gg d$ for all remaining tests).

All hormone concentrations were quantified for nine of ten females; however, we were unable to collect sufficient plasma from the tenth

female in October to assay for estradiol. Female tegus exhibited significant seasonal changes in plasma estradiol concentration ($F_{(7,71)} = 9.652$; $P < 0.001$; Fig. 4B), again peaking in September coincident with the reproductive season (September range: 0.020–0.103 ng.mL^{-1} ; mean: $0.041 \pm 0.007 \text{ ng.mL}^{-1}$). Progesterone also varied seasonally in females ($F_{(7,71)} = 11.565$; $P < 0.001$; Fig. 4C) with two distinct peaks, one in October (range: 0.51–1.24 ng.mL^{-1} ; mean: $0.856 \pm 0.085 \text{ ng.mL}^{-1}$) and another one in April (range: 0.33–1.64 ng.mL^{-1} ; mean: $1.05 \pm 0.14 \text{ ng.mL}^{-1}$)-May (range: 0.42–1.99 ng.mL^{-1} ; mean: $0.87 \pm 0.18 \text{ ng.mL}^{-1}$).

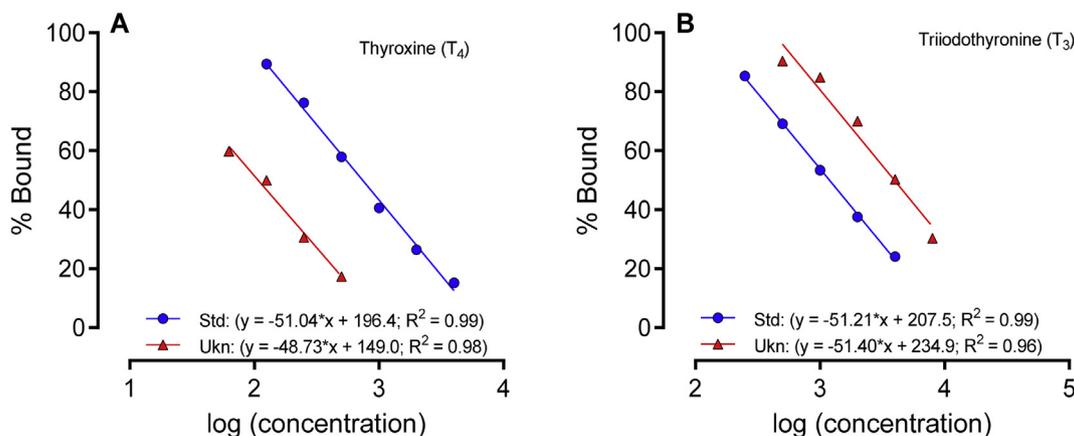


Fig. 2. Parallelism results for thyroid hormones. (A) thyroxine (T_4) and (B) triiodothyronine (T_3) in the plasma of the tegu lizard *Salvator merianae*; serial dilutions of pooled plasma (red triangles) are parallel to the standard curve (blue circles). Best-fit regression equations given at bottom. Stds: standard; Ukn: unknown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

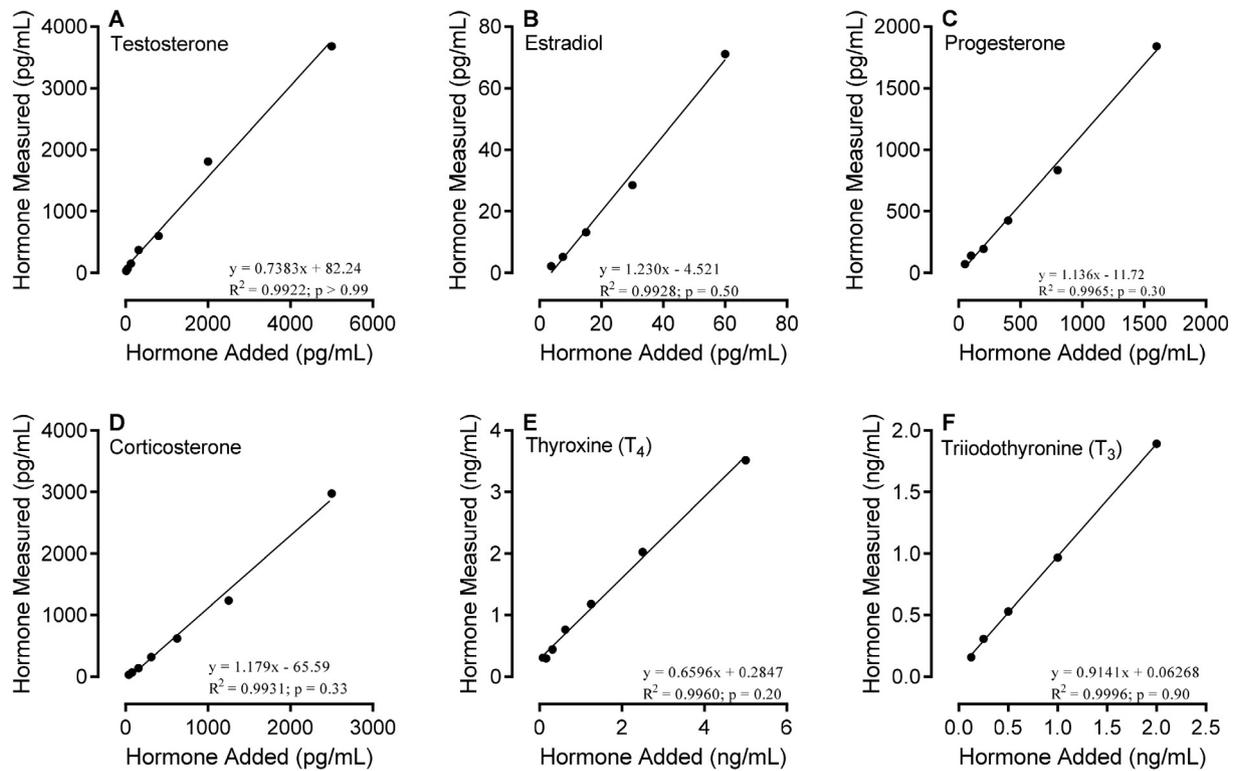


Fig. 3. Accuracy results. (A) testosterone, (B) estradiol, (C) progesterone, (D) corticosterone, (E) thyroxine (T_4), and (F) triiodothyronine (T_3) in the plasma of the tegu lizard *Salvator merianae*. Pooled plasma spiked with known amounts of hormone show good accuracy and little to no matrix effect. Best-fit regression equations given at bottom.

3.2.2. Corticosterone

Marked monthly variation in plasma corticosterone levels was observed for all lizards ($F_{(7,150)} = 43.441$; $P < 0.001$; Fig. 5). There were no significant differences between the sexes ($P = 0.99$) so male and female data were combined for all analyses. The lowest concentrations of corticosterone occurred during hibernation (June: 29.97 ± 2.86 ng.mL $^{-1}$) and early emergence (August: 28.59 ± 2.58 ng.mL $^{-1}$). Corticosterone initially rose in September (67.68 ± 6.84 ng.mL $^{-1}$), coincident with the onset of the reproductive season. Peak concentrations occurred in December (125.68 ± 11.71 ng.mL $^{-1}$) followed by a decline over the months preceding to hibernation.

3.2.3. Thyroid hormones

Thyroid hormones (T_4 and T_3) were quantified in nine males and nine females. The tenth male was not sampled for T_4 in October (detailed in Materials and Methods), and T_3 for October and December (insufficient plasma), while data for one female are missing T_3 in October due to insufficient plasma. There were no significant sex differences in plasma T_4 concentration ($P = 0.500$) and therefore data were pooled for all analyses. Monthly levels of plasma T_4 changed across the year ($F_{(7,151)} = 19.559$; $P < 0.001$; Fig. 6A), increasing gradually from the lowest values during hibernation (May: 1.80 ± 0.21 ng.mL $^{-1}$ and June: 1.98 ± 0.23 ng.mL $^{-1}$) to peak concentrations in October (3.68 ± 0.15 ng.mL $^{-1}$) that remained elevated through December (3.64 ± 0.15 ng.mL $^{-1}$) and February (3.50 ± 0.12 ng.mL $^{-1}$).

Concentrations of T_3 exhibited a significant interaction between sex and month ($F_{(7,149)} = 2.923$; $P = 0.007$; Fig. 6B), with a considerable difference between males and females ($F_{(1,155)} = 9.630$; $P = 0.006$; Fig. 6B). Male T_3 concentrations remained fairly constant across the year whereas plasma T_3 levels of females increased two-fold from the annual low during hibernation in June (0.38 ± 0.03 ng.mL $^{-1}$) to a peak in October (0.89 ± 0.07 ng.mL $^{-1}$). Despite the decrease in plasma T_3 levels of females from December to February, female T_3

levels were significantly elevated relative to the males for the same months ($P < 0.05$).

4. Discussion

This is the first study to characterize the integrated seasonal profile of reproductive (testosterone in males, estrogen and progesterone in females), adrenal (corticosterone) and thyroid (T_4 and T_3) hormones in captive adult male and female *S. merianae*, using repeat sampling across an entire year. More importantly, the present study is one of the most complete datasets in any lizard or reptile. All six hormones fluctuated seasonally in a manner consistent with published seasonal patterns of metabolism, behavior and reproduction of *S. merianae* (Abe, 1983; Andrade et al., 2004; Tattersall et al., 2016). Elevated levels of gonadal sex steroid hormones occurred in synchrony with specific reproductive behaviors in both males and females. In addition, an important sex difference in the seasonal thyroid (T_3) hormone profiles in *S. merianae* may underlie important aspects of reproductive and metabolic physiology and behavior.

4.1. Reproductive hormones

Male tegu lizards had lowest levels of plasma testosterone during hibernation followed by a gradual increase starting in August, late winter in southeastern Brazil. Testosterone peaked in September, during the reproductive season for *S. merianae* in this region (Lopes and Abe, 1999). The highest levels of testosterone coincided with the observation of male-typical reproductive behaviors such as scent marking (femoral gland secretion), courtship and mating. This is also the time of the year when spermatogenesis is known to occur (Chamut et al., 2012). These events are therefore temporally linked, i.e., male tegus express an associated reproductive pattern, similar to the majority of vertebrates (Crews, 1984). In contrast, some species of snakes, turtles and bats display a dissociated reproductive pattern, where mating

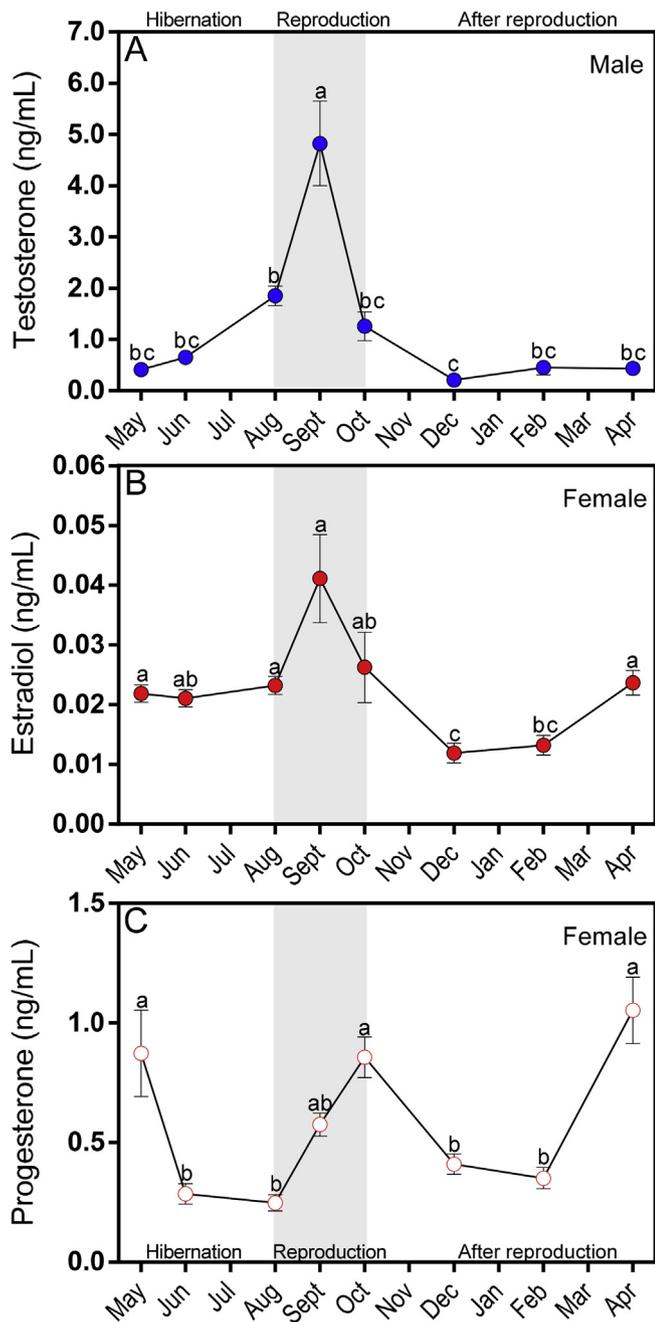


Fig. 4. Mean monthly plasma gonadal steroid concentrations in tegu lizards *Salvator merianae*. Testosterone concentrations from adult males (A; n = 9–10) and estradiol (B; n = 9–10) and progesterone (C; n = 9–10) concentrations from adult females kept in an outdoor enclosure in southeastern Brazil. Different letters denote significant differences. Data are presented as means ± SEM. Data collection began in Oct 2016 and concluded in Sep 2017; data are presented with Sep 2017 adjacent to Oct 2016 for visual comparison of the Sep–Nov breeding season months.

behavior occurs when gonads are quiescent and levels of plasma sex steroid hormones are low. A striking example of a dissociated reproductive pattern is found in the red-sided garter snake (*Thamnophis sirtalis parietalis*), the northernmost reptile. These animals exhibit a pronounced seasonal cycle of hibernation and activity but have a strong seasonally restricted breeding period (Woolley et al., 2004; Joy and Crews, 1987). In contrast, *S. merianae* in the subtropical South America have long and predictable phases of food availability, potentially favoring associated and longer seasonal reproduction.

In late-winter and early spring (August), *S. merianae* emerge from

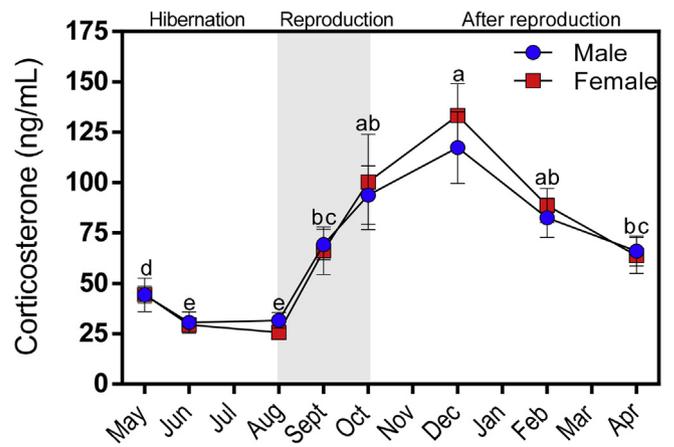


Fig. 5. Mean monthly plasma corticosterone concentrations in tegu lizards *Salvator merianae*. Corticosterone concentrations from adult males (n = 9–10) and females (n = 10) kept in an outdoor enclosure in southeastern Brazil. Different letters denote significant differences. Data are presented as means ± SEM. Data collection began in Oct 2016 and concluded in Sep 2017; data are presented with Sep 2017 adjacent to Oct 2016 for visual comparison of the Sep–Nov breeding season months.

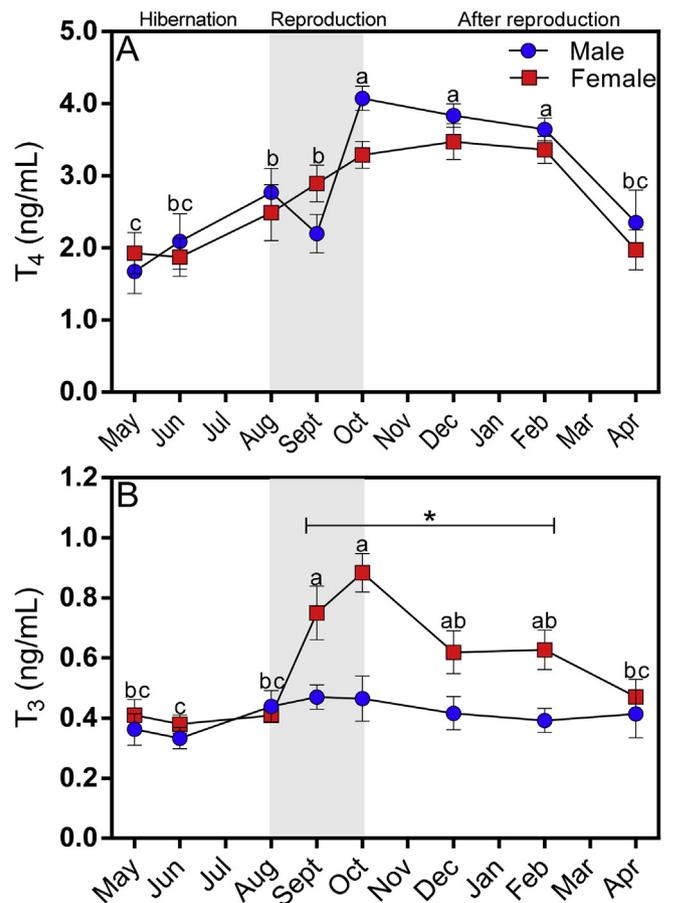


Fig. 6. Mean monthly plasma thyroid hormone concentrations in tegu lizards *Salvator merianae*. Concentrations of plasma thyroid hormones (thyroxine; T₄, A, and triiodothyronine; T₃, B) from adult males (n = 9–10) and females (n = 10) kept in an outdoor enclosure in southeastern Brazil. Different letters denote significant differences among months; asterisk in panel B represents significant differences between sexes. Data are presented as means ± SEM. Data collection began in Oct 2016 and concluded in Sep 2017; data are presented with Sep 2017 adjacent to Oct 2016 for visual comparison of the Sep–Nov breeding season months.

hibernation, shed their skins, forage for food and initiate their first reproductive behaviors some weeks after emergence (~around 20–30 days after emergence; Lopes and Abe, 1999). Male red-sided garter snakes emerge from hibernation and immediately exhibit intense breeding activity, mating within an hour of emergence regardless of their quiescent gonads and low plasma androgen concentration (Crews et al., 1984; Mendonça and Crews, 1996; Lutterschmidt and Mason, 2009). While male red-sided garter snake breeding activities require a long phase of low temperatures during hibernation followed by warming (Lutterschmidt and Mason, 2009; Krohmer, 2004), seasonal changes in physiology and behavior of *S. merianae* are temperature independent (Zena et al., 2016; Abe, 1995; Sanders et al., 2015). It is still unknown whether rhythms in physiology and behavior of *S. merianae* are driven by an endogenously generated program or triggered by reflexive responses to environmental changes, such as the photoperiod.

Another study of testosterone concentrations in *S. merianae* comes from an investigation by Chamut et al. (2012) on captive tegus from the province of Tucumán, Argentina (26°51'S and 65°17'W). They reported that male *S. merianae* in that region peak in testosterone in September (Chamut et al., 2012). Although the Argentine population and ours have similarly timed peaks in testosterone, their behaviors appear temporally offset. Specifically, tegus from Argentina emerge from hibernation in September, nearly a month after the tegus in southeastern Brazil. Further, the Brazilian tegus display sexual behavior soon after emergence in September, whereas the Argentinian tegus delay sexual behavior until October. Interestingly, sexual behavior among the Brazilian tegus coincided with their peak in testosterone whereas Argentinian tegus displayed sexual behavior after their initial testosterone peak. These differences in timing suggest that the annual cycle may be modulated by environmental differences along the latitudinal gradient of South America, similar to the plasticity in hibernation and reproductive timing exhibited by mammalian hibernators at different sites (Williams et al., 2014) and when exposed to different weather events (Sheriff et al., 2017).

Among mammalian hibernators, elevated testosterone is typically incompatible with expression of torpor or hibernation (Darrow et al., 1988; Goldman et al., 1986; Lee et al., 1990; Smit-Vis, 1972; Vitale et al., 1985) and testosterone has been hypothesized to trigger the end of hibernation in males (Richter et al., 2017). The gradual increase in plasma testosterone levels in male tegus from hibernation to emergence in August (present study; Chamut et al., 2012), may have an inhibitory effect in dormancy. In contrast, male red-sided garter snakes undergo hibernation with plasma testosterone which gradually declines across winter dormancy until the lowest annual levels are attained at emergence in spring (Lutterschmidt and Mason, 2009). Clearly, there are species differences among seasonally breeding reptiles, which might be associated with their reproductive strategies.

Estradiol peaked in September in our female tegus, concurrent with the testosterone peak in males and the onset of courtship and mating behaviors. Peak levels of estrogens are usually associated with follicular development and vitellogenesis in female reptiles (Edwards and Jones, 2001; Hamlin et al., 2014; Lance et al., 2009; Taylor et al., 2004) and further serve to promote sexual receptivity and pheromone production (Mendonça and Crews, 1996; Parker and Mason, 2012). After peaking in September, estradiol in *S. merianae* decreased to minimum levels in December, rose again and remained constant from April–August (pre-hibernation and hibernation). Stable levels of estradiol suggest that the immature follicles persist throughout hibernation, with some degree of steroidogenesis. Comparably, in the closely related *S. rufescens*, development of follicles occurs at emergence in association with high plasma levels of estradiol (García-Valdez et al., 2016).

In our female tegus, progesterone peaked in October, nearly a month later than estrogen. Progesterone is associated with ovulation in many reptiles (Currylow et al., 2017; García-Valdez et al., 2016; Rostal and Robeck, 1998; Taylor et al., 2004; Weiss et al., 2002). Similar to male tegus, timing of the female reproduction exhibits plasticity across

populations according by latitude. For example, gravid females from our captive population oviposit in October coinciding with a progesterone surge, while the onset of ovipositioning in females *S. merianae* from a southernmost wild population in Espinal and Pampa regions (30°55'S, 63°40'W to 31°55'S, 62°15'W) occurs in mid-November, when corpora lutea can be observed in the ovaries (Naretto, et al., 2016).

In other oviparous vertebrates (e.g., birds), progesterone is associated with nest-building behavior and early stages of nest attendance, often in combination with permissive or priming effects of estradiol (Buntin, 1996; Rosenblatt et al., 1985; Silver, 1978; Wood-Gush and Gilbert, 1973). In October, some female tegus were seen building nests by gathering dried grass. Intriguingly, progesterone peaked again in April and May (pre-hibernation). Two peaks in progesterone have also been reported in female Northern Pacific rattlesnakes (*Crotalus oreganus*) and male house geckos (*Hemidactylus flaviviridis*). In these cases, other sex steroids follow the same pattern, possibly due to progesterone being upstream in steroid synthesis (Al-Amri et al., 2013; Lind et al., 2010). The nature and function of the non-reproductive peak for progesterone in our female tegus is presently unknown and needs further investigation.

4.2. Glucocorticoids

In most vertebrates, glucocorticoid secretion fluctuates seasonally as well as in response to stressful stimuli (McEwen and Wingfield, 2003; Romero et al., 2009) to elicit a variety of physiological and behavioral responses that presumably help animals cope with stressors (French et al., 2007; Meylan et al., 2010). Stimulation of the hypothalamus–pituitary–adrenal (HPA) axis in response to unpredictable events (e.g., injury, storms, starvation) can elicit dramatic elevations in glucocorticoids that are frequently associated with suppression of reproduction (Buck et al., 2007; Love et al., 2004). Nevertheless, the predictable and energy demanding process of reproduction is often associated with elevated baseline (i.e., not acute-stress-induced) corticosterone. Reproduction in female *S. merianae* is associated with the energy demanding processes of nesting building and incubation (Manes et al., 2003; García-Valdez et al., 2016). Although baseline corticosterone is elevated during the breeding season, some females reproduced and those with developing follicles engaged in reproductive behaviors such as construction of the nest. Therefore, it appears that corticosterone may have a role in mobilizing energy without suppressing reproduction (Moore and Jessop, 2003; Romero, 2002; Williams et al., 2008) in *S. merianae*.

The association between elevated baseline corticosterone and reproduction is known as the 'cort-adaptation hypothesis' (Bonier et al., 2009). To test this hypothesis, a more detailed study of tegu reproduction, including variables such as clutch size, eclosion rate, body condition etc., along with endocrine data will be necessary. Besides that, corticosterone levels of free-living tegus would offer an interesting comparison with captive animals since home range and available space are less constrained in natural environments, perhaps reducing opportunity for agonistic behaviors and subsequent stress (Winck et al., 2011).

4.3. Thyroid hormones

Thyroid hormones exhibited a complex seasonal pattern in adult male and female tegus. T₄ of both sexes was elevated from October to February, encompassing spring and summer seasons, when energy expenditure and activity are high (Abe, 1995, 1983; Klein et al., 2006; Sanders et al., 2015). These patterns of T₄ are consistent with well-documented changes in physiology (energy expenditure) and behavior in captive and free-living *Salvator* lizards (Abe, 1995, 1983; Andrade et al., 2004; Lopes and Abe, 1999; Montaña et al., 2013; Tattersall et al., 2016; Zena et al., 2016). In contrast, T₃ patterns differed by sex; males

had little fluctuation in plasma T_3 throughout the year, while females exhibited profoundly elevated plasma T_3 during the reproductive and post-reproductive seasons, slightly earlier than their T_4 peak.

Elevated T_3 levels during the reproductive season in female tegus may reflect increased energetic demand related to egg production, in addition to recovery of body condition after oviposition. Female tegus invest heavily in large clutches of eggs once a year, which vary from 7 (young females in their first reproductive season) to ~50 eggs (Lopes and Abe, 1999; pers. obs.). Thyroid hormones have been implicated in vitellogenesis in some oviparous species. In the goldfish, high levels of T_3 are associated with early gonadal recrudescence and enhance production of vitellogenin, the egg yolk precursor protein, by upregulating estradiol receptors in hepatocytes (Nelson and Habibi, 2016). If similar phenomena occur in lizards, peak circulating estradiol (Fig. 4) coinciding with the initial surge in T_3 levels (Fig. 6) suggests a cross-talk between hypothalamus-pituitary-gonadal and -thyroid axes during the development of follicles and subsequent vitellogenesis in female *S. merianae*. Similarly, seasonal regulation of reproduction in birds has been associated with activation of the hypothalamic-pituitary-thyroid axis (Dawson, 2015; Nakane and Yoshimura, 2010; Shinomiya et al., 2014).

Thyroid hormones are also involved in non-breeding season modulation of physiology, metabolism and behavior. In birds, thyroid hormones have been associated with mass gain, fattening, and muscle hypertrophy prior to migration (Pant and Chandola-Saklani, 1993; Pérez et al., 2016). Possibly, thyroid hormones in female *S. merianae* serve similar somatotrophic functions when animals are actively foraging before undergoing hibernation.

Although our captive population is located in a humid subtropical climate zone (hot and humid summers, dry and mild winters), tegus nonetheless have a strong annual cycle in metabolic rate (Abe, 1995, 1983; Sanders et al., 2015). The thyroid hormone data presented here resemble thyroid hormone cycles of female arctic ground squirrels (Wilsterman et al., 2015), an obligate seasonal hibernator that inhabits the extreme and highly seasonal Arctic (Buck and Barnes, 1999), suggesting some common patterns underlying regulation of metabolic rate in hibernating species from highly divergent taxa and environments.

5. Conclusions

Many aspects of an animal's behavior and physiology are seasonally modulated by changes in endocrine secretion. *S. merianae* is known to seasonally vary metabolic rate, which is high in spring and summer coincident with reproduction, and low during hibernation in winter (Lopes and Abe, 1999; Sanders et al., 2015). Building upon these observations, our study represents the first step in describing annual patterns and interrelationships among the major steroid, thyroid and glucocorticoid hormones that likely influence predictable changes in physiology in this unique lizard from a subtropical region in South America. Peaks in sex steroid hormones (testosterone and estradiol/progesterone) temporally coincide with observed reproductive behavior. Regardless the sex, corticosterone surge may be related to costs of reproduction and activity during spring. Finally, sex-dependent seasonal changes in T_3 secretion may underlie reproductive physiology of female *S. merianae* by supporting increased metabolism associated with modulation of energy allocation during annual cycles of folliculogenesis, nest building and oviposition. Useful follow-up studies could evaluate seasonal interrelationships among environmental conditions, body temperature, activity, metabolic rate, and hormone levels, in both captive and natural environments.

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References

- Abe, A.S., 1995. Estivation in South American amphibians and reptiles. *Braz. J. Med. Biol. Res.* 28, 1241–1247.
- Abe, A.S., 1983. Observations on dormancy in tegu lizard, *Tupinambis teguixin* (Reptilia, Teiidae). *Naturalia* 8, 135–139.
- Al-Amri, I.S., Mahmoud, I.Y., Waring, C.P., Alkindi, A.Y., Khan, T., Bakheit, C., Al-Mawali, K.M., 2013. The reproductive cycle of the male house gecko, *Hemidactylus flaviviridis*, in relation to plasma steroid concentrations, progesterone receptors, and steroidogenic ultrastructural features, in Oman. *Gen. Comp. Endocrinol.* 187, 23–31.
- Anderson, L., Cree, A., Towns, D., Nelson, N., 2014. Modulation of corticosterone secretion in tuatara (*Sphenodon punctatus*): evidence of a dampened stress response in gravid females. *Gen. Comp. Endocrinol.* 201, 45–52.
- Andrade, D.V., Sanders, C., Milsom, W.K., Abe, A.S., 2004. Overwintering in Tegu Lizards. In: Barnes, B.M., Carey, H.V. (Eds.), *Life in the Cold: Evolution, Mechanisms, Adaptation, and Application*, Twelfth International Hibernation Symposium. Institute of Arctic Biology, Fairbanks, AK, pp. 339–348.
- Boggs, A.S.P., Hamlin, H.J., Lowers, R.H., Guillette, L.J., 2011. Seasonal variation in plasma thyroid hormone concentrations in coastal versus inland populations of juvenile American alligators (*Alligator mississippiensis*): influence of plasma iodide concentrations. *Gen. Comp. Endocrinol.* 174, 362–369.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* 24, 634–642.
- Buck, C.L., Barnes, B.M., 1999. Annual cycle of body composition and hibernation in free-living arctic ground squirrels. *J. Mammal.* 80, 430–442.
- Buck, C.L., O'Reilly, K.M., Kildaw, S.D., 2007. Interannual variability of Black-legged Kittiwake productivity is reflected in baseline plasma corticosterone. *Gen. Comp. Endocrinol.* 150, 430–436.
- Buntin, J.D., 1996. Neural and hormonal control of parental behaviour in birds. *Adv. Study Behav.* 25, 161–213.
- Chamut, S., Jahn, G., Arce, O., Manes, M., 2012. Testosterone and reproductive activity in the male tegu lizard, *Tupinambis merianae*. *Herpetol. Conserv. Biol.* 7, 299–305.
- Chani, J.M., Cruz, F., Perotti, G., Aguirre, M., Rufino, S., 1993. Rol de la hembra *Tupinambis teguixin* (Teiidae), durante la nidificación. *Acta Zool. Lilloana* 42, 295–299.
- Crews, D., 1984. Gamete production, sex hormone secretion, and mating behavior uncoupled. *Horm. Behav.* 18, 22–28.
- Crews, D., Traina, V., Wetzel, F.T., Muller, C., 1978. Hormonal control of male reproductive behavior in the lizard, *Anolis carolinensis*: role of testosterone, dihydrotestosterone, and estradiol. *Endocrinology* 103, 1814–1821.
- Crews, D., Camazine, B., Diamond, M., Mason, R., Tokarz, R.R., Garstka, W.R., 1984. Hormonal independence of courtship behavior in the male garter snake. *Horm. Behav.* 18, 29–41.
- Curran-Everett, D., 2000. Multiple comparisons: philosophies and illustrations. *Am. J. Physiol.* – Regul. Integr. Comp. Physiol. 279, R1–R8.
- Curran-Everett, D., Benos, D.J., American Physiological Society, 2004. Guidelines for reporting statistics in journals published by the American Physiological Society. *Am. J. Physiol. Endocrinol. Metab.* 287, E189–E191.
- Currylow, A.F.T., Rafeliasoa, T.H., Louis, E.E., Stanford, C.B., Randrianjafizana, S.T., Chinn, S.M., Crocker, D.E., 2017. Characterization of seasonal reproductive and stress steroid hormones in wild Radiated Tortoises, *Astrochelys radiata*. *Gen. Comp. Endocrinol.* 253, 70–78.
- Da Silveira, L.C., Do Nascimento, L.F.R., Colquhoun, A., Abe, A.S., De Souza, S.C.R., 2013. Cardiac hypertrophy and structural and metabolic remodeling related to seasonal dormancy in the first annual cycle in tegu lizards. *Comp. Biochem. Physiol. – Mol. Integr. Physiol.* 165, 371–380.
- Darrow, J.M., Duncan, M.J., Bartke, A., Bona-Gallo, A., Goldman, B.D., 1988. Influence of photoperiod and gonadal steroids on hibernation in the European hamster. *J. Comp. Physiol. A* 163, 339–348.
- Davenport, J., Holland, D.L., East, J., 1990. Thermal and biochemical characteristics of the lipids of the leatherback turtle *Dermochelys coriacea*: Evidence of endothermy. *J. Mar. Biol. Assoc. United Kingdom* 70, 33–41.
- Dawson, A., 2015. Annual gonadal cycles in birds: modeling the effects of photoperiod on seasonal changes in GnRH-1 secretion. *Front. Neuroendocrinol.* 37, 52–64.
- de Andrade, D.V., Abe, A., 1999. Gas exchange and ventilation during dormancy in the tegu lizard *Tupinambis merianae*. *J. Exp. Biol.* 202 (Pt 24), 3677–3685.
- de Souza, S.C.R., 2004. Seasonal metabolic depression, substrate utilisation and changes

- in scaling patterns during the first year cycle of tegu lizards (*Tupinambis merianae*). *J. Exp. Biol.* 207, 307–318.
- do Nascimento, L.F.R., da Silveira, L.C., Nisembaum, L.G., Colquhoun, A., Abe, A.S., Mandarim-de-Lacerda, C.A., de Souza, S.C.R., 2016. Morphological and metabolic adjustments in the small intestine to energy demands of growth, storage, and fasting in the first annual cycle of a hibernating lizard (*Tupinambis merianae*). *Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol.* 195, 55–64.
- Dunlap, K.D., 1995. Hormonal and behavioral responses to food and water deprivation in a lizard (*Sceloporus occidentalis*): implications for assessing stress in a natural population. *J. Herpetol.* 29, 345–351.
- Edwards, A., Jones, S.M., 2001. Changes in plasma progesterone, estrogen, and testosterone concentrations throughout the reproductive cycle in female viviparous blue-tongued skinks, *Tiliqua nigrolutea* (Scincidae), in Tasmania. *Gen. Comp. Endocrinol.* 122, 260–269.
- El-Deib, S., 2005. Serum catecholamine and hormonal titers in the hibernating snake *Naja haje haje*, with reference to the annual climatic cycle. *J. Therm. Biol.* 30, 580–587.
- Ellsworth, A., Buck, C.L., Atkinson, S., Hollmén, T., 2014. Longitudinal monitoring of sex steroid hormones in excrement of spectacled eiders (*Somateria fischeri*). *Gen. Comp. Endocrinol.* 198, 59–65.
- French, S.S., McLemore, R., Vernon, B., Johnston, G.I.H., Moore, M.C., 2007. Corticosterone modulation of reproductive and immune systems trade-offs in female tree lizards: long-term corticosterone manipulations via injectable gelling material. *J. Exp. Biol.* 210, 2859–2865.
- García-Valdez, M.V., Chamut, S., Jahn, G.A., Arce, O.E.A., Manes, M.E., 2016. Plasmatic estradiol and progesterone variations during the reproductive cycle of captive female Argentine red tegu lizards, *Tupinambis rufescens*. *Herpetol. Conserv. Biol.* 11, 519–526.
- Goldman, B.D., Darrow, J.M., Duncan, M.J., Yoyeg, L., 1986. Photoperiod, reproductive hormones, and winter torpor in three hamster species. In: Heller, H.C., Musacchia, X.J., Wang, L.C.H. (Eds.), *Living in the Cold: Physiological and Biochemical Adaptations*. Elsevier, New York, pp. 341–350.
- Grotjan, H.E., Keel, B.A., 1996. Data interpretation and quality control. In: Diamandis, E.P., Christopoulos, T.K. (Eds.), *Immunoassay*. Academic Press, San Diego, CA, pp. 51–95.
- Hamlin, H.J., Lowers, R.H., Kohno, S., Mitsui-Watanabe, N., Amano, H., Hara, A., Ohta, Y., Miyagawa, S., Iguchi, T., Guillette, L.J., 2014. The reproductive hormone cycle of adult female American alligators from a barrier island population. *Reproduction* 147, 855–863.
- Harvey, M.B., Ugueto, G.N., Gutberlet, R.L., 2012. Review of teiid morphology with a revised taxonomy and phylogeny of the Teiidae (Lepidosauria: Squamata). *Zootaxa* 3459, 1–156.
- Hews, D.K., Moore, M.C., 1995. Influence of androgens on differentiation of secondary sex characters in tree lizards, *Urosaurus ornatus*. *Gen. Comp. Endocrinol.*
- Hunt, K.E., Lysiak, N.S., Moore, M.J., Seton, R.E., Torres, L., Buck, C.L., 2017. Multiple steroid and thyroid hormones detected in baleen from eight whale species. *Conserv. Physiol.* 5, 1–14.
- Jessop, T.S., Limpus, C.J., Whittier, J.M., 2002. Nocturnal activity in the green sea turtle alters daily profiles of melatonin and corticosterone. *Horm. Behav.* 41, 357–365.
- John-Alder, H.B., 1990. Thyroid regulation of resting metabolic rate and intermediary metabolic enzymes in a lizard (*Sceloporus occidentalis*). *Gen. Comp. Endocrinol.* 77, 52–62.
- John-Alder, H.B., 1984. Seasonal variations in activity, aerobic energetic capacities, and plasma thyroid hormones (T3 and T4) in an iguanid lizard. *J. Comp. Physiol. B* 154, 409–419.
- Jones, S.M., Bell, K., 2004. Plasma corticosterone concentrations in males of the skink *Egernia whitii* during acute and chronic confinement, and over a diel period. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* 137, 105–113.
- Joy, J.E., Crews, D., 1987. Hibernation in garter snakes (*Thamnophis sirtalis parietalis*): seasonal cycles of cold tolerance. *Comp. Biochem. Physiol. A* 87, 1097–1101.
- Klein, W., Perry, S.F., Abe, A.S., Andrade, D.V., 2006. Metabolic response to feeding in *Tupinambis merianae*: circadian rhythm and a possible respiratory constraint. *Physiol. Biochem. Zool.* 79, 593–601.
- Kohel, K., MacKenzie, D., Rostal, D., Grumbels, J., Lance, V., 2001. Seasonality in plasma thyroxine in the desert tortoise, *Gopherus agassizii*. *Gen. Comp. Endocrinol.* 121, 214–222.
- Korsmeyer, K.E., Dewar, H., 2001. Tuna metabolism and energetics. In: Block, B.A., Stevens, E.D. (Eds.), *Tuna: Physiology, Ecology and Evolution*, Fish Physiology. Academic Press, San Diego, CA, pp. 35–78.
- Krohmer, R.W., 2004. The male red-sided garter snake (*Thamnophis sirtalis parietalis*): reproductive pattern and behavior. *ILAR J.* 45, 54–74.
- Lance, V.A., Rostal, D.C., Eelsey, R.M., Trosclair, P.L., 2009. Ultrasonography of reproductive structures and hormonal correlates of follicular development in female American alligators, *Alligator mississippiensis*, in southwest Louisiana. *Gen. Comp. Endocrinol.* 162, 251–256.
- Lanfri, S., Di Cola, V., Naretto, S., Chiaraviglio, M., Cardozo, G., 2013. Understanding the ecological niche to elucidate spatial strategies of the southernmost *Tupinambis* lizards. *Amphibia-Reptilia* 34, 551–565.
- Lee, T.M., Pelz, K., Licht, P., Zucker, I., 1990. Testosterone influences hibernation in golden-mantled ground squirrels. *Am. J. Physiol.* 259, R760–R767.
- Lind, C.M., Husak, J.F., Eikenaar, C., Moore, I.T., Taylor, E.N., 2010. The relationship between plasma steroid hormone concentrations and the reproductive cycle in the Northern Pacific rattlesnake, *Crotalus oreganus*. *Gen. Comp. Endocrinol.* 166, 590–599.
- Little, A.G., Seebacher, F., 2014. The evolution of vernal hibernation is explained by thyroid hormone-mediated responses to cold in early vertebrates. *J. Exp. Biol.* 217, 1642–1648.
- Lopes, H.R., Abe, A.S., 1999. Biología reproductiva e comportamiento do teiú, *Tupinambis merianae*, em cativeiro (Reptilia, Teiidae). In: Fang, T.G., Montenegro, O.L., Bodmer, R.E. (Eds.), *Manejo y conservación de fauna silvestre en América Latina*. Editorial Instituto e Ecología, La Paz, Bolivia, pp. 259–272.
- Love, O.P., Breuner, C.W., Vézina, F., Williams, T.D., 2004. Mediation of a corticosterone-induced reproductive conflict. *Horm. Behav.* 46, 59–65.
- Lutterschmidt, D.L., Mason, R.T., 2009. Endocrine mechanisms mediating temperature-induced reproductive behavior in red-sided garter snakes (*Thamnophis sirtalis parietalis*). *J. Exp. Biol.* 212, 3108–3118.
- Manes, M.E., Ibáñez, M.A., Manilla, A., 2003. Factores físicos y conductas de nidificación de lagartos *Tupinambis merianae* en cautiverio. *Rev. Arg. de Prod. Anim.* 23, 119–126.
- McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43, 2–15.
- McNab, B.K., 1974. The energetics of endotherms. *Ohio J. Sci.* 74, 370–380.
- Mendonça, M.T., Crews, D., 1996. Effects of ovariectomy and estrogen replacement on attractivity and receptivity in the red-sided garter snake (*Thamnophis sirtalis parietalis*). *J. Comp. Physiol. A* 178, 373–381.
- Meylan, S., Haussy, C., Voituron, Y., 2010. Physiological actions of corticosterone and its modulation by an immune challenge in reptiles. *Gen. Comp. Endocrinol.* 169, 158–166.
- Milsom, W.K., Andrade, D.V., Brito, S.P., Toledo, L.F., Wang, T., Abe, A.S., 2008. Seasonal changes in daily metabolic patterns of tegu lizards (*Tupinambis merianae*) placed in the cold (17°C) and dark. *Physiol. Biochem. Zool.* 81, 165–175.
- Milsom, W.K., Sanders, C., Leite, C., Abe, A.S., Andrade, D.V., Tattersall, G., 2012. Seasonal changes in thermoregulatory strategies of tegu lizards. In: Ruf, T., Biebrer, C., Arnold, W., Millesi, E. (Eds.), *Living in a Seasonal World*. Springer, Berlin, Heidelberg, pp. 317–324.
- Montaño, R.R., Cuéllar, R.L., Fitzgerald, L.A., Mendoza, F., Soria, F., Fiorello, C.V., Deem, S.L., Noss, A.J., 2013. Activity and ranging behavior of the red tegu lizard *Tupinambis rufescens* in the bolivian chaco. *South Am. J. Herpetol.* 8, 81–88.
- Moon, D.-Y., Owens, D.W., MacKenzie, D.S., 1999. The Effects of fasting and increased feeding on plasma thyroid hormones, glucose, and total protein in sea turtles. *Zool. Sci.* 16, 579–586.
- Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Horm. Behav.* 43, 39–47.
- Mullur, R., Liu, Y.-Y., Brent, G.A., 2014. Thyroid hormone regulation of metabolism. *Physiol. Rev.* 94, 355–382.
- Naretto, S., Cardozo, G., Blengini, C.S., Chiaraviglio, M., 2016. Importance of reproductive biology of a harvest lizard, *Tupinambis merianae*, for the management of commercial harvesting. *Wildl. Res.* 42, 697–704.
- Nakane, Y., Yoshimura, T., 2010. Deep brain photoreceptors and a seasonal signal transduction cascade in birds. *Cell Tissue Res.* 342, 341–344.
- Nelson, E.R., Habibi, H.R., 2016. Thyroid hormone regulates vitellogenin by inducing estrogen receptor alpha in the goldfish liver. *Mol. Cell. Endocrinol.* 436, 259–267.
- Newman, A.E.M., Chin, E.H., Schmidt, K.L., Bond, L., Wynne-Edwards, K.E., Soma, K.K., 2008. Analysis of steroids in songbird plasma and brain by coupling solid phase extraction to radioimmunoassay. *Gen. Comp. Endocrinol.* 155, 503–510.
- Pant, K., Chandola-Saklani, A., 1993. A role for thyroid hormones in the development of premigratory disposition in redheaded bunting, *Emberiza bruniceps*. *J. Comp. Physiol. B* 163, 389–394.
- Parker, M.R., Mason, R.T., 2012. How to make a sexy snake: estrogen activation of female sex pheromone in male red-sided garter snakes. *J. Exp. Biol.* 215, 723–730.
- Pérez, J.H., Furlow, J.D., Wingfield, J.C., Ramenofsky, M., 2016. Regulation of vernal migration in Gambel's white-crowned sparrows: Role of thyroxine and triiodothyronine. *Horm. Behav.* 84, 50–56.
- Richter, M.M., Barnes, B.M., O'Reilly, K.M., Fenn, A.M., Buck, C.L., 2017. The influence of androgens on hibernation phenology of free-living male arctic ground squirrels. *Horm. Behav.* 89, 92–97.
- Rivera, S., Lock, B., 2008. The reptilian thyroid and parathyroid glands. *Vet. Clin. North Am. - Exot. Anim. Pract.* 11, 163–175.
- Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128, 1–24.
- Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model – a new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* 55, 375–389.
- Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: Is under 3 min good enough? *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* 140, 73–79.
- Rosenblatt, J.S., Mayer, A.D., Siegel, H.I., 1985. Maternal Behavior among the Nonprimate Mammals. In: dler, N., Pfaff, D., Goy, R.W. (Eds.), *Reproduction. Handbook of Behavioral Neurobiology*. Springer, Boston, MA.
- Rostal, D., Robeck, T., 1998. Seasonal reproductive cycle of the Galapagos tortoise (*Geochelone nigra*) in captivity. *Zoo Biol.* 17, 505–517.
- Sanders, C.E., Tattersall, G.J., Reichert, M., Andrade, D.V., Abe, A.S., Milsom, W.K., 2015. Daily and annual cycles in thermoregulatory behaviour and cardio-respiratory physiology of black and white tegu lizards. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 185, 905–915.
- Sheriff, M.J., Boonstra, R., Palme, R., Buck, C.L., Barnes, B.M., 2017. Coping with differences in snow cover: the impact on the condition, physiology and fitness of an arctic hibernator. *Conserv. Physiol.* 5, 1–12.
- Shinomiya, A., Shimmura, T., Nishiwaki-Ohkawa, T., Yoshimura, T., 2014. Regulation of seasonal reproduction by hypothalamic activation of thyroid hormone. *Front. Endocrinol.* 5, 1–7.
- Silver, R., 1978. The Parental Behavior of Ring Doves The intricately coordinated behavior of the male. *Am. Sci.* 66, 209–215.
- Slip, D.J., Shine, R., 1988. Reptilian endothermy: a field study of thermoregulation by brooding diamond pythons. *J. Zool.* 216, 367–378.

- Smit-Vis, J.H., 1972. The effect of pinealectomy and of testosterone administration on the occurrence of hibernation in adult male golden hamsters. *Acta Morphol. Neerl. Scand.* 10, 269–282.
- Southwood, A.L., Darveau, C.A., Jones, D.R., 2003. Metabolic and cardiovascular adjustments of juvenile green turtles to seasonal changes in temperature and photoperiod. *J. Exp. Biol.* 206, 4521–4531.
- Stahl, S.J., 2006. Reptile hematology and serum chemistry. In: NAVC Conference 2006. North American Veterinary Community, Gainesville, pp. 1672–1676.
- Tattersall, G.J., Leite, C.A.C., Sanders, C.E., Cadena, V., Andrade, D.V., Abe, A.S., Milsom, W.K., 2016. Seasonal reproductive endothermy in tegu lizards. *Sci. Adv.* 2 e1500951 e1500951.
- Taylor, E.N., Denardo, D.F., Jennings, D.H., 2004. Seasonal steroid hormone levels and their relation to reproduction in the Western Diamond-backed Rattlesnake, *Crotalus atrox* (Serpentes: Viperidae). *Gen. Comp. Endocrinol.* 136, 328–337.
- Toledo, L.F., Brito, S.P., Milsom, W.K., Abe, A.S., Andrade, D.V., 2008. Effects of season, temperature, and body mass on the standard metabolic rate of tegu lizards (*Tupinambis merianae*). *Physiol. Biochem. Zool.* 81, 158–164.
- Vitale, P.M., Darrow, J.M., Duncan, M.J., Shustak, C.A., Goldman, B.D., 1985. Effects of photoperiod, pinealectomy and castration on body weight and daily torpor in Djungarian hamsters (*Phodopus sungorus*). *J. Endocrinol.* 106, 367–375.
- Wasser, S.K., Azkarate, J.C., Booth, R.K., Hayward, L., Hunt, K., Ayres, K., Vynne, C., Gobush, K., Canales-Espinosa, D., Rodríguez-Luna, E., 2010. Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. *Gen. Comp. Endocrinol.* 168, 1–7.
- Weiss, S.L., Jennings, D.H., Moore, M.C., 2002. Effect of captivity in semi-natural enclosures on the reproductive endocrinology of female lizards. *Gen. Comp. Endocrinol.* 128, 238–246.
- Welcker, J., Speakman, J.R., Elliott, K.H., Hatch, S.A., Kitaysky, A.S., 2014. Resting and daily energy expenditures during reproduction are adjusted in opposite directions in free-living birds. *Funct. Ecol.* 250–258.
- Williams, C.T., Barnes, B.M., Kenagy, G.J., Buck, C.L., 2014. Phenology of hibernation and reproduction in ground squirrels: Integration of environmental cues with endogenous programming. *J. Zool.* 292, 112–124.
- Williams, C.T., Kitaysky, A.S., Kettle, A.B., Buck, C.L., 2008. Corticosterone levels of tufted puffins vary with breeding stage, body condition index, and reproductive performance. *Gen. Comp. Endocrinol.* 158, 29–35.
- Wilsterman, K., Buck, C.L., Barnes, B.M., Williams, C.T., 2015. Energy regulation in context: Free-living female arctic ground squirrels modulate the relationship between thyroid hormones and activity among life history stages. *Horm. Behav.* 75, 111–119.
- Winck, G.R., Blanco, C.C., Cechin, S.Z., 2011. Population ecology of *Tupinambis merianae* (Squamata, Teiidae): home-range, activity and space use. *Anim. Biol.* 61, 493–510.
- Wood-Gush, D.G.M., Gilbert, A.B., 1973. Some hormones involved in the nesting behaviour of hens. *Anim. Behav.* 21, 98–103.
- Woolley, S.C., Sakata, J.T., Crews, D., 2004. Evolutionary insights into the regulation of courtship behavior in male amphibians and reptiles. *Physiol. Behav.* 83, 347–360.
- Zena, L.A., Dantonio, V., Gargaglioni, L.H., Andrade, D.V., Abe, A.S., Bicego, K.C., 2016. Winter metabolic depression does not change arterial baroreflex control of heart rate in the tegu lizard *Salvator merianae*. *J. Exp. Biol.* 219, 725–733.