Journal of Comparative Physiology B (2022) 192:127–139
https://doi.org/10.1007/s00360-021-01397-3

ORIGINAL PAPER

Seasonal changes in steroid and thyroid hormone content in shed skins of the tegu lizard *Salvator merianae*

Lucas A. Zena[1,2,3] · Danielle Dillon[3] · Kathleen E. Hunt[4] · Carlos A. Navas[1] · Kênia C. Bícego[2] · C. Loren Buck[3]

Received: 10 January 2021 / Revised: 30 June 2021 / Accepted: 2 August 2021 / Published online: 11 August 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Sampling blood for endocrine analysis from some species may not be practical or ethical. Quantification of hormones extracted from nontypical sample types, such as keratinized tissues, offers a less invasive alternative to the traditional collection and analysis of blood. Here, we aimed to validate assays by using parallelism and accuracy tests for quantification of testosterone, corticosterone, progesterone, and triiodothyronine (T_3) in shed skins of tegu lizards. We assessed whether hormone content of sheds varied across one year similar to what was previously detected in plasma samples. In addition, we aimed to identify the phase relationship between hormone levels of shed skin and plasma levels obtained from the same animals. High frequency of shedding occurred during the active season for tegus (spring/summer), while shedding ceased during hibernation (winter). All hormones measured in shed skins exhibited seasonal changes in concentration. Levels of testosterone in shed skins of male tegus correlated positively with plasma testosterone levels, while corticosterone in both males and females exhibited an inverse relationship between sample types for the same month of collection. An inverse relationship was found when accounting for a lag time of 3 and 4 months between sheds and plasma testosterone. These results indicate that endocrine content of sheds may be confounded by factors (*i.e.*, seasons, environmental temperature, thermoregulatory behavior, among others) that affect frequency of molting, skin blood perfusion, and therefore hormone transfer from the bloodstream and deposition in sheds of squamates.

Keywords Testosterone · Progesterone · Corticosterone · Thyroid hormone · Hibernation · Non-invasive

Introduction

Hormones coordinate and integrate animal physiology and behavior. These regulatory molecules are produced by specialized groups of cells, secreted into the bloodstream and circulate throughout the body before binding to receptors at target cells. While circulating, many hormones permeate a variety of tissue types, which allow alternative sample types to be used for inferring animals' physiological status. Hormone concentrations have been quantified in a diversity of sample types besides blood, among them, saliva, urine, feces, tissue biopsies (*e.g.*, skin, adipose tissue), and whole-body homogenate, as well as cornified epithelial tissues such as skin, nail, hair, feather, horn and whale baleen (Lattin et al. 2011; Berkvens et al. 2013; Baxter-Gilbert et al. 2014; Gardell et al. 2015; Fernández Ajó et al. 2018).

Cornified epithelial tissues incorporate hormones into the keratin matrix as the tissue grows and then retain the incorporated hormone over weeks, months or even years (Hunt et al. 2016). These tissues therefore may enable retrospective analysis of hormone secretion, which can reveal aspects of an animal's physiological status over time (Fernández Ajó et al. 2018; Hunt et al. 2018). For example, quantification of hormones from keratinized tissues may identify reproductive events or nutritional stress in

Communicated by K.H. Dausmann.

✉ Lucas A. Zena
lucaszena@yahoo.com.br; lucaszena@gmail.com

[1] Department of Physiology, Institute of Biosciences, University of São Paulo, Rua Do Matão, 14 - Butantã, São Paulo, SP 05508-090, Brazil

[2] Department of Animal Morphology and Physiology, College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, SP 14884-900, Brazil

[3] Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA

[4] Department of Biology and Smithsonian-Mason School of Conservation, George Mason University, Fairfax, VA, USA

prior months or years, or facilitate identification of periods of chronic stress in species where collection of blood is not recommended or practical (Hunt et al. 2014, 2016). For instance, corticosterone content of a bird feather is thought to represent hormone that circulated during the period of tissue growth during feather molt (Bortolotti et al. 2008; Jenni-Eiermann et al. 2015) while continuously-growing keratinized tissues, such as whale baleen, can provide a continuous time series of endocrine information spanning months or years (Hunt et al. 2018). Hormone content of such tissues may or may not correlate with plasma hormone content due to the variable "lag time" and the different time frames and temporal integration of hormone in the tissue as compared to plasma.

Shed skin (sheds) of reptiles is a tissue that has not been fully investigated for its utility as a sample type for retrospective analysis of hormone content. Episodic shedding of the epidermis associated with growth is common among the Squamata (snakes and lizards) (Chang et al. 2009). The frequency and pattern of shedding differs among species and environments and is influenced by nutritional status and growth rate (Semlitsch 1979; Gibson et al. 1989; Maas 2013; Carlson et al. 2014). Shedding in lizards like tegus is asynchronous across the body, with shedding occurring in a patchwork fashion (*e.g.*, small and large pieces of shed), while snakes typically shed a single contiguous piece (Ling 1972; Vitt and Caldwell 2008). Generally, squamates have an episodic molting cycle with two phases, resting and renewal. Depending on species, the resting phase lasts for a few days to many months and is characterized by a reduction or absence of mitosis in the epidermis. The renewal phase requires about 14 days and is characterized by epidermal proliferation and differentiation as a new epidermal layer is laid down underneath the existing layer (Vitt and Caldwell 2008; Chang et al. 2009). In keratinized epidermal tissues of other vertebrates, hormones are incorporated over the time that the living tissue is growing, through diffusion from the bloodstream (Jenni-Eiermann et al. 2015; Hansen et al. 2016; Erickson et al. 2017). Assuming reptilian skin functions similarly, circulating hormones are most likely deposited into keratinized layers of reptilian epidermis during the renewal phase. Thus, reptilian sheds could be useful as a sample type for retrospective evaluation of physiological status, since its collection is minimally or noninvasive, avoids capture stress, and might be useful for species from which blood sampling is not advisable or permitted. However, because few studies have used sheds as a potential matrix for assessments of a reptile's endocrine history (Berkvens et al. 2013; Carbajal et al. 2018), it remains unclear whether sheds accumulate and retain hormones at physiologically meaningful levels. Thus, the value of sheds as a sample type for determining longitudinal profiles of reproduction, nutritional status, exposure to stressors, etc., is not known.

The tegu (*Salvator merianae*) is a large, widely distributed lizard endemic to South America (Ribeiro-Júnior 2015; Jarnevich et al. 2018) that exhibits a pronounced seasonal cycle of metabolism, reproduction, activity and hibernation, which occurs during the austral winter (*i.e.*, from end-May to early-August) (Abe 1983, 1995; Sanders et al. 2015; Zena et al. 2019, 2020). We have previously shown that plasma levels of testosterone peak in males in September, during the reproductive season for *S. merianae* in southeastern Brazil (Lopes and Abe 1999), coinciding with male-typical reproductive behaviors such as scent marking (femoral gland secretion), courtship and mating (Zena et al. 2019). Regarding females, progesterone surges in October and is associated with ovulation when gravid females exhibit nest-building behavior before oviposition. Interestingly, the thyroid hormone T_3 (triiodothyronine), known for regulating energy metabolism, varies seasonally in tegus and exhibits sex-dependent differences, with females showing a two-fold increase in plasma T_3 levels during the spring reproductive season. This suggests that T_3 may be involved in energy investment during the seasonal production of large clutches of eggs by females. Finally, corticosterone, the primary reptilian adrenal glucocorticoid, gradually increases from the lowest values during hibernation to peak concentrations around December in both sexes, suggesting its involvement in energy mobilization during the active season of tegus. Tegus shed their skins in late winter and early spring (August) when they emerge from hibernation, forage for food, and initiate their first reproductive behaviors some weeks after emergence (Lopes and Abe 1999). However, data regarding how often tegus shed throughout the annual cycle is missing (Lopes and Abe 1999; Zena et al. 2018, 2020).

The goals of this study were threefold. First, we sought to validate hormone immunoassays for tegu sheds via tests of assay parallelism and accuracy for quantification of: (i) reproductive steroid hormones (testosterone in males and progesterone in females); (ii) the primary reptilian adrenal glucocorticoid (corticosterone; both sexes), and iii) the active form of thyroid hormone, triiodothyronine (T_3; both sexes). Second, we explored whether hormone content of sheds opportunistically collected from individual tegus varies across the year, thereby encompassing at least one hibernation and one reproductive cycle. Finally, we sought to identify the phase relationship between plasma hormone levels and shed skin hormone levels.

Material and methods

Animals

Adult tegu lizards of both sexes [10 males (2.29 ± 0.23 kg) and 10 females (1.88 ± 0.09 kg); exact ages unknown) were communally housed in a 42 m^2 outdoor facility at the College

of Agricultural and Veterinary Sciences of São Paulo State University in Jaboticabal, SP, Brazil (21° 14′ 05" S and 48° 17′ 09" W; IBAMA permit no. 02001–000,412/94–28 and SISBIO-ICMBio/n. 26,677–1/ transport and maintenance permit: ICMBio #52,085–1). Throughout the study, animals experienced natural oscillations in ambient temperature, photoperiod, humidity and rainfall. The enclosure is composed of a shared open area, providing options for thermoregulatory, ambulatory, and social behavior in addition to two communal shelters (dimensions: 103 × 72 × 71 cm; lined with wood shavings) that animals retreated to during the night, for protection from direct sunlight and precipitation, and for hibernation during the austral winter. Animals were fed 2–3 times/week with a diet of cooked chicken eggs, dead mice, and fruits. Once per week, animals were provided frozen captive-bred cockroaches (*Nauphoeta cinerea*) supplemented with calcium and vitamin D_3 (Zoo Med Reptivite; San Luis Obispo, CA, USA). Water was provided ad libitum year-round, but food was gradually reduced and completely removed during hibernation when animals voluntarily stop eating. All experimental protocols were approved by a local Animal Care and Use Committee of São Paulo State University (CEUA; # 7.434/16).

Sample collection (blood and skin)

Animals were individually marked with a subcutaneous implanted microchip (transponder ISO FDX-B 134.2 KhZ, AnimallTAG, São Carlos, Brazil). Sheds were collected from individuals from October 2016 to March 2018. Animals were habituated to human presence and handling, enabling opportunistic collection of loosely attached pieces of skin directly from known individuals. Like most lizards, our captive tegus exhibited an erratic body molt, where loose areas of skin occur in a patchwork fashion across the body over days or weeks until the skin of the whole body is shed (see supplementary Fig. S1). Because tegus were communally housed, any sheds found on the ground could not be attributed to any single individual and thus were not analyzed; only sheds pulled directly from the body were quantified for hormone content. The duration of the skin renewal phase in tegus is unknown; that is, a shed collected on a given date contains epidermal tissue that was grown some time prior to that date. Each shed is assumed to contain tissue grown between the date of the previously collected shed and the date of the current shed.

Blood samples were taken monthly from all animals across the year (except November 2016, January, March and July 2017). Briefly, animals were individually handled for ≤ 3 min for blood collection (~ 4 mL) from the ventral coccygeal vein using a heparinized 5 mL syringe and a 21 G needle. Blood samples were placed on ice before centrifugation at 10,000 rpm 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. Plasma methodology and data have been previously published (Zena et al. 2018) and are described again in the present study for comparison with temporal relationships with hormones measured in sheds. Plasma and sheds were shipped to Northern Arizona University (Flagstaff, AZ, USA; CITES permits #17BR023338/DF, #18BR028075/DF and #18BR028073/DF), where all endocrine analyses occurred.

Plasma hormone extraction

Methodology for plasma analyses is fully described elsewhere (Zena et al. 2018). In brief, testosterone and progesterone were extracted from plasma using solid phase extraction, eluted in 90% methanol, dried and stored at −80 °C, and resuspended one day prior to assay in 1 mL assay buffer (1:2 dilution). Plasma corticosterone and T_3 assays were performed using unextracted plasma, as recommended by the assay manufacturer.

Hormone extraction from sheds

Prior to extraction, sheds were washed with distilled water, blotted dry with a paper towel, and freeze-dried for 4 h (Labconco 6L Freeze Dry System with Stoppering Tray Dryer; LabConco, Kansas City, MO, USA). In a small pilot trial comparing hormone content of sheds rinsed with methanol *vs.* unrinsed revealed no apparent effect of a methanol rinse; therefore, samples thereafter were not rinsed with methanol. Owing to the heterogeneity in sheds collected from different body parts, we mixed all sheds obtained during one shedding episode for each individual tegu. Thus, when available, sheds were collected from various places on the body and homogenized before extraction and assay. Dried sheds were cut into 5 mm squares and placed into labeled 13 × 100 mm glass tubes with 2 mL 100% HPLC-grade methanol. We used 25 mg of sheds for assaying corticosterone; however, this amount was not sufficient for consistent detectability of T_3, which required extraction to be done from 50 mg sample of shed material. When assaying for progesterone and testosterone, previous extractions performed on 50 mg sample of shed had to be diluted at 1:16 for consistent detectability. Samples were shaken on a multi-tube vortexer (Glas-Col Large Capacity Mixer; Glas-Col, Terre Haute, IN, USA) at 500 rpm for 2 h, sonicated for 5 min, and then centrifuged at 3000 rpm for 15 min (Fernández Ajó et al. 2020, 2018; Hunt et al. 2018). The supernatant was collected and subsequently dried down in a SpeedVac Evaporator at 35 °C and stored at −20 °C for no longer than two weeks until resuspension. One day prior to assay, samples were resuspended with 1 mL assay buffer (1:2 dilution), shaken for 1 h and then stored at 4 °C overnight. On the day of the assay,

samples were allowed to come to room temperature before assay. Percent recovery was not evaluated in this study, as it is not possible to mimic behavior of native hormone embedded within epidermal sample types via addition of liquid radiolabeled parent hormone (Palme et al. 2013); rather, data analysis focuses on relative patterns and not on absolute concentrations.

Hormone validation from sheds

Immunoassays were validated by using tests of parallelism and accuracy of *S. merianae* shed samples to verify that commercial immunoassay kits can accurately quantify all hormones tested (Grotjan and Keel 1996; Ellsworth et al. 2014; Hunt et al. 2017). To test for parallelism, a pooled skin extract containing our analytes of interest was serially diluted by repeating halving the dilution (1:2–1:128), with all dilutions assayed as unknowns. 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. 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. Assay accuracy (aka "matrix effect test") was determined by spiking each standard with pooled tegu plasma or skin extract, 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 linear relationship with 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.*, skin extract or tegu plasma).

Hormone assays

Commercially available enzyme immunoassay (EIA) kits (Arbor Assays, Ann Arbor, MI, USA) were used to quantify shed and plasma testosterone (kit #K032), progesterone (kit #K025), and corticosterone (kit #K014). T_3 extracted from sheds was quantified by using an enzyme immunoassay kit (kit #K056; Arbor Assays, Ann Arbor, MI, USA), while plasma T_3 was quantified by using a coated-tube ^{125}I radioimmunoassay (RIA) kit (MP Biomedicals, Solon, OH, USA; kit #06B-254215). Additional low-dose standards (created by mixing equal volumes of previous standard + assay buffer) were added to the T_3 standard curve (standard curve range: 78.12–5,000 pg.ml^{-1}) to improve assay precision for low concentrations of hormone in skin and plasma samples. Similarly, one additional low-dose standard was added to the testosterone standard curve for plasma assays (standard curve range: 40.96–10,000 pg.ml^{-1}). Methods for assay validations of plasma were previously published in Zena et al. (2019).

Skin samples from both male and female tegus were assayed for corticosterone and T_3. Skin testosterone was quantified in males only, while skin progesterone was quantified in females only. 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 variations for all skin assays were < 10% (corticosterone: 2.71 ± 0.92%; progesterone: 4.30 ± 1.67%; testosterone: 2.06 ± 0.09%; T_3: 2.32 ± 0.64%) and inter-assay variations were all < 15% (corticosterone: 4.5 ± 0.02%; progesterone: 14.6 ± 0.03%; testosterone: 2.97 ± 0.02%; T_3: 3.00 ± 0.02%). For antibody cross-reactivities, assay sensitivities and other methodological details, see Hunt et al. (2017).

Body temperature

Body temperature was obtained from temperature loggers (iButtons, DS1922L, TX, USA) implanted inside the animals' coelomic cavity as previous described (Zena et al. 2020). Briefly, lizards were initially anesthetized with isoflurane, and a longitudinal incision of less than 3 cm was made on the ventral left side of the body where the logger was inserted and sutured in place. All surgeries were performed under sterile conditions. After recovery from anesthesia, each animal was individually maintained indoors for approximately one week before being released into the communal outdoor facility. Body temperature was collected from April 2017 to March 2018.

Statistical analysis

Statistical analyses were performed using Prism v. 6.0 (graphpad.com) or R software v. 1.1.383 (R Core Team 2018). Parallelism results for skin corticosterone, T_3, progesterone and testosterone were plotted as percentage of antibody bound versus log[concentration], with *F* tests employed to assess differences between slopes of the linear portions of the binding curves for serially diluted skin extract *vs.* the standard curve. Accuracy results were plotted as the concentration of the hormone measured versus the concentration of known standard added. Accuracy results were assessed via linear regression and inspection of slope, with acceptable accuracy defined as $r^2 \geq 0.90$ and slope within the range of 0.7–1.3 (ideal slope = 1.0) (Grotjan and Keel 1996).

We used generalized additive models (package 'mgcv'; Wood, 2017) to account for the nonlinear nature of skin and plasma changes in hormone concentration over time (*i.e.*, day of the year, *doy*), where sex was included as a parametric

term when applicable (*e.g.*, corticosterone and T_3). Individual was included in all models as a random effect (intercept). We used a Gamma distribution (and a log-link function) and added a smoothing function to *doy* (with an automatically chosen number of knots for the model). Finally, we visually examined normality and assessed goodness of fit using histograms and the quantile–quantile plots for all models.

We fitted linear mixed models using the package nlme (Pinheiro et al. 2021) to assess a potential relationship between shed and plasma hormones by accounting for lag time of 0, 1, 2, 3 and 4 months between the time of hormone deposition (when the tissue grew) and collection date (months later). Fitted linear mixed models included each shed's hormone concentration as a response variable, and each correspondent plasma hormone concentration and sex (male/female; when applicable) as independent variables (*i.e.*, global model). Individual identification was included in all models as random effects (intercept) to account for the repeatability of the data throughout the study. Then, we used the MuMIn package (Barton 2018) to create and rank models with all possible combinations of the independent variables (*e.g.*, starting from the null model to the global model) by using Akaike's corrected information criterion (AICc). Since sex did not show a significant effect for any skin hormones analyzed, shed and plasma hormone level relationships were performed with both sexes combined. When the assumption of model residuals normality was not met, the data were log-transformed (*e.g.*, corticosterone skin hormone concentration; see Fig. 6B). Normality of the model residuals was tested by using a Shapiro–Wilk test. Homogeneity of variance for each model was tested by using a Levene's test. Predicted mean and confidence interval values were exported from R software and plots were made in Prism, version 6.0 (www.graphpad.com).

Results

Enzyme immunoassay (EIA) validations

All hormone assays of serially diluted tegu lizard sheds yielded displacement curves parallel to the respective standard curves (Fig. 1). There were no significant differences in slopes between standards and serially diluted tegu shed pools for corticosterone ($F_{(1, 10)} = 0.10$; $P = 0.75$), T_3 ($F_{(1,7)} = 0.59$; $P = 0.46$), progesterone ($F_{(1,10)} = 0.097$; $P = 0.76$) or testosterone ($F_{(1,11)} = 2.15$; $P = 0.17$). Accuracy was acceptable for all hormones tested, as indicated by a linear relationship between observed and expected hormone concentration ($r^2 \geq 0.90$ for all hormones), and a slope within the desired range of 0.7–1.3, as follows: corticosterone (slope = 0.91), T_3 (slope = 0.86), progesterone (slope = 1.06), testosterone (slope = 1.07) (Fig. 2).

Seasonal variation in shed and plasma hormone concentration

The frequency of shedding varied among animals across the year (Fig. 3; individual distribution of sheds contained in Supplementary Table S1 and S2), with some animals shedding as many as six times, and others as few as two times, resulting in an average of 3.6 and 3.2 sheds per year for males and females, respectively. All tegus shed after hibernation, with five of 10 males initiating shedding between August 7th and 26th of 2017, while the remaining males initiated shedding between September 1st and 26th. Most females first shed between September 1st and 20th (8 out of 10), while only one female shed before that in August, and one initiated shedding in October.

We identified a non-linear pattern throughout the year for all hormones in sheds: corticosterone [effective degrees of freedom (e.d.f.) = 3.063, $P = 0.014$]; T_3 (e.d.f. = 3.915, $P = 0.004$); progesterone (e.d.f. = 3.131, $P = 0.007$) and testosterone (e.d.f. = 4.658, $P = 0.00006$), as well as for all plasma hormones: corticosterone (e.d.f. = 7.029, $P < 0.0001$); T_3 (female: e.d.f. = 5.866, $P < 0.0001$; male: e.d.f. = 3.349, $P = 0.01$); progesterone (e.d.f. = 7.752, $P < 0.0001$) and testosterone (e.d.f. = 7.558, $P < 0.0001$) (Figs. 4, 5; Table 1). Models including sex revealed no differences between males and females for either corticosterone (female estimate = 2.6580, male estimate = 2.7555, $P = 0.56$; Fig. 5B) or T_3 (female estimate = 0.9241, male estimate = 1.0136, $P = 0.55$; Fig. 5D) in sheds. However, plasma T_3 concentration differed by sex: female estimate = -0.6358, male estimate = -0.9690, $P = 0.0007$ (Fig. 5C). Female tegus exhibited higher plasma T_3 levels than males from mid-October 2016 to late-March 2017 and from late-August 2017 to mid-February 2018; Fig. 5C). In addition, our analysis predicted relatively high levels of hormones (*i.e.*, peak levels) to fall in the middle of hibernation. This prediction, however, cannot be confirmed with shed data as tegus do not shed during hibernation. It is worth noting that, due to an absence of data points for shed hormones during hibernation, confidence intervals were relatively wide.

Both plasma testosterone and corticosterone were selected as good predictors of skin testosterone and corticosterone for the same month as the shed was collected. Plasma testosterone significantly predicted skin testosterone levels in male tegus ($\chi^2 = 19.028$, d.f. = 1, $P < 0.001$; Table 2 and Fig. 6A) when we paired values for skin and plasma obtained within the same month of sample collection. Conversely, plasma corticosterone negatively correlated with skin corticosterone ($\chi^2 = 7.967$, d.f. = 1, $P = 0.005$; Table 2 and Fig. 6B) also within the same month of collection, while neither skin progesterone nor skin T_3 showed any significant relationship with plasma concentrations. Different time lags (*e.g.*, skin hormones compared to plasma hormones of 1, 2,

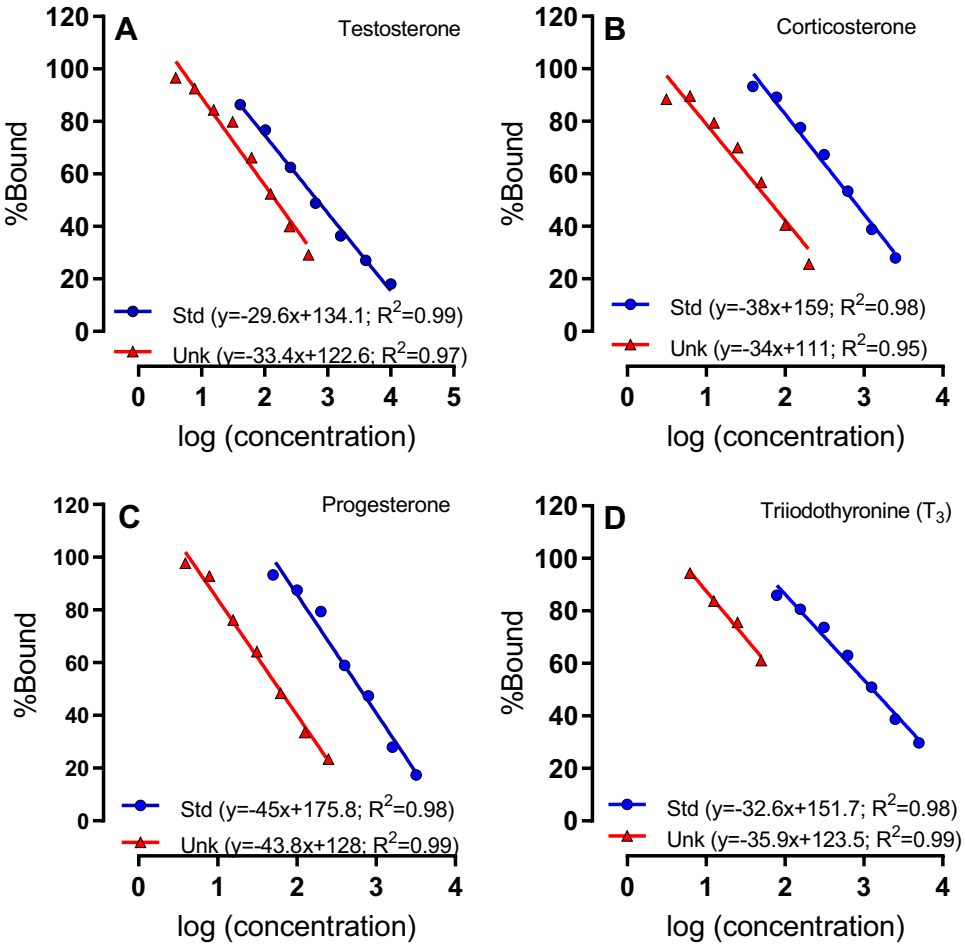

Fig. 1 Parallelism results for shed skin hormones. (**A**) Testosterone, (**B**) corticosterone, (**C**) progesterone, and (**D**) triiodothyronine (T_3) in the shed skin of the tegu lizard *Salvator merianae*; serial dilutions of pooled shed skin (red triangles) are parallel to the standard curve (blue circles). Best-fit regression equations given at bottom. *Std*: standard; *Unk* unknown

3 or 4 months prior) did not improve the fit of the model. For instance, plasma testosterone was also selected as best predictor when the relationship between shed and plasma hormones accounted for lag time of 1, 3 and 4 months; however, a significant inverse relationship was identified for 3 months lag time ($\chi^2 = 33.208$, d.f. = 1, $P < 0.001$) and 4 months lag time ($\chi^2 = 4.951$, d.f. = 1, $P = 0.03$) contrasting with the positive relationship found for the same month of collection. Corticosterone was also selected as best predictor when the relationship between shed and plasma hormones accounted for a lag time of 3 months, but no significant relationship was found between shed and plasma corticosterone ($\chi^2 = 3.185$, d.f. = 1, $P = 0.07$). Progesterone was only selected as a predictor for the relationship between shed and plasma hormones when accounting for a lag time of 4 months, but no significant relationship was found between shed and plasma progesterone ($\chi^2 = 2.856$, d.f. = 1, $P = 0.09$). T_3 was only selected as a predictor for the relationship between shed and plasma hormones when accounting for a lag time of 3 months, with a negative significant relationship found between shed and plasma T_3 ($\chi^2 = 6.075$, d.f. = 1, $P = 0.01$) (see supplementary tables S3-6 for best model subsets when accounting for lag time between 1 and 4 months).

Discussion

We validated assays and quantified concentrations of four different hormones from shed skin opportunistically obtained from tegu lizards held under semi-natural conditions. As demonstrated by the non-linear relationship for skin hormone concentration and day of the year, all four hormones investigated (testosterone, corticosterone, progesterone and T_3) exhibited seasonal variation in shed skins. Shedding frequency was elevated during spring/summer, corresponding to the activity seasons for tegus in southeastern Brazil. Before entering hibernation, shedding frequency decreased until completely ceasing during winter time.

The overall mean shedding frequency of our tegus was 3.6 and 3.2 sheds per year for males and females, respectively. Specifically, frequency of shedding decreased from December 2016 to February 2017 and remained low between April and May 2017 (*i.e.*, occurred in only 3 animals: 2 females and 1 male) until shedding ceased completely during hibernation of 2017 (June and July). Shedding resumed in August 2017 with most males shedding at this time (5 males *vs.* 1 female), marking the protandric emergence pattern observed in our captive tegus (Zena

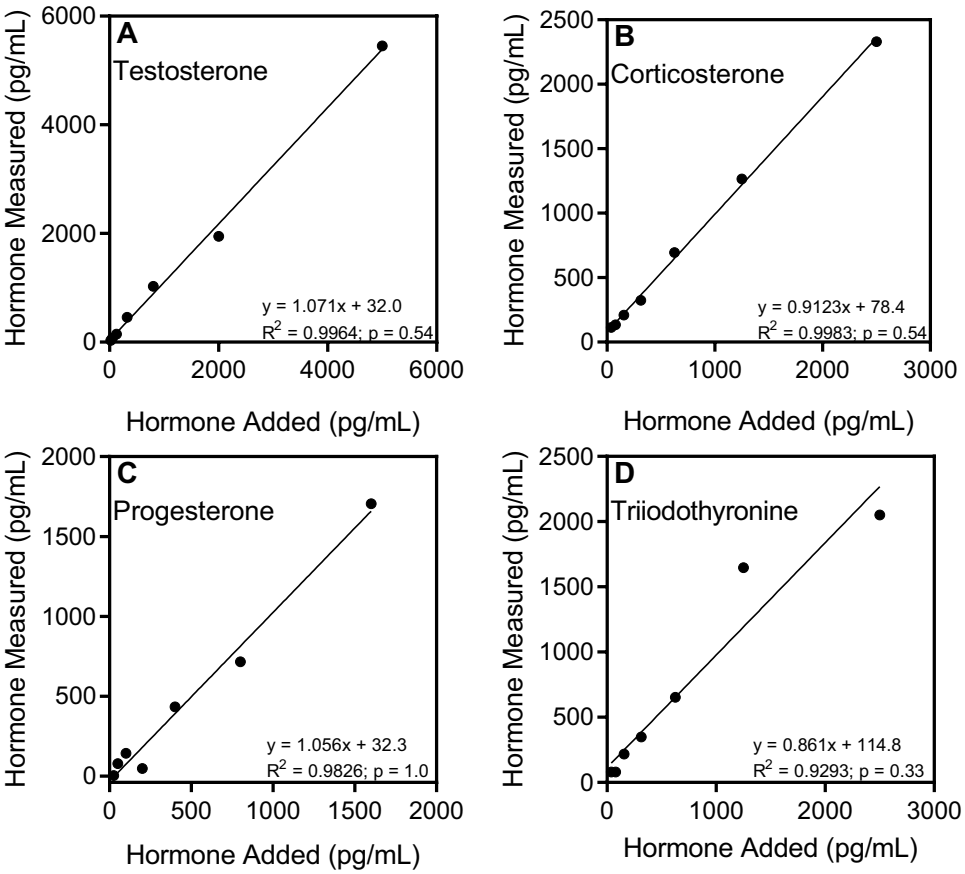

Fig. 2 Accuracy results for shed skin hormones. (**A**) Testosterone, (**B**) corticosterone, (**C**) progesterone, and (**D**) triiodothyronine (T3) in the shed skin of the tegu lizard *Salvator merianae*. Pooled shed skin spiked with known amounts of hormone show good accuracy and little to no matrix effect. Best-fit regression equations given at bottom. $P > 0.05$ refers to absence of deviation from linearity

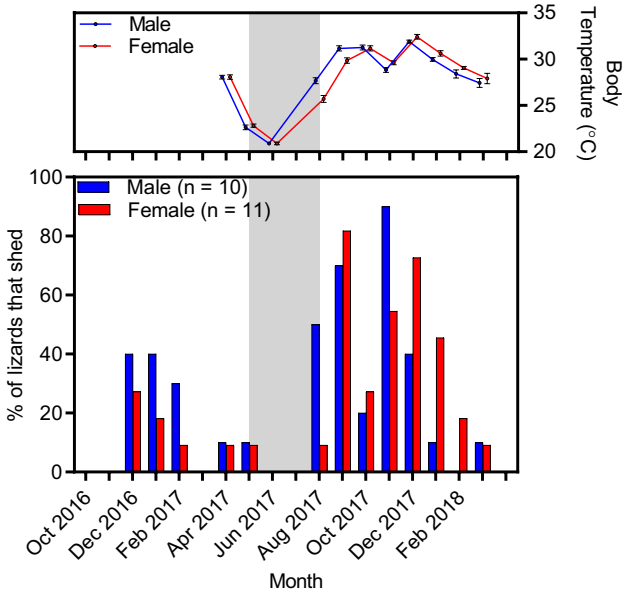

Fig. 3 Frequency distribution histogram for shed skin retrieved from the tegu lizard *Salvator merianae*. Monthly variation in shed skin frequency recorded from December 2016 to March 2018 in male (blue bars) and female (red bars) lizards. Frequency distribution represents the percent of lizards out of total separated by sex that shed in that particular month. Shaded gray area represents hibernation time. Tegu body temperature is included to illustrate the relationship with skin shedding frequency

et al. 2020), also previously shown in wild tegus (Winck and Cechin 2008). Shed frequency then increased between August and December and then slowed until February 2018 when sample collection stopped. These data suggest that shedding may have ceased for the following entrance into hibernation (*i.e.*, May 2018). Annual variation in shedding frequency of another lizard, the Tokay gecko (*Gekko gecko*), depends on the thermal environment (Chiu and Maderson 1980). At ambient temperatures > 32 °C, shedding in the Tokay gecko is more frequent and regular, while below this temperature, shedding is less frequent or even inhibited at temperatures < 15 °C. Thus, monthly variation in shed frequency may be a function of metabolic rate, which is a strongly temperature sensitive process. Elevated frequency of shedding corresponds to the most active time of the year for tegus, that is, spring/summer, while absence of shedding corresponds to the hibernation phase during winter in southeastern Brazil (Abe 1983, 1995; Sanders et al. 2015; Zena et al. 2019, 2020). However, it is important to highlight that although shedding frequency varies throughout the year, it also varied among individuals, with some animals shedding as many as six times, and others as few as two times across the year. Thus, other factors besides ambient/body temperature could have affected the annual shedding frequency in our animals.

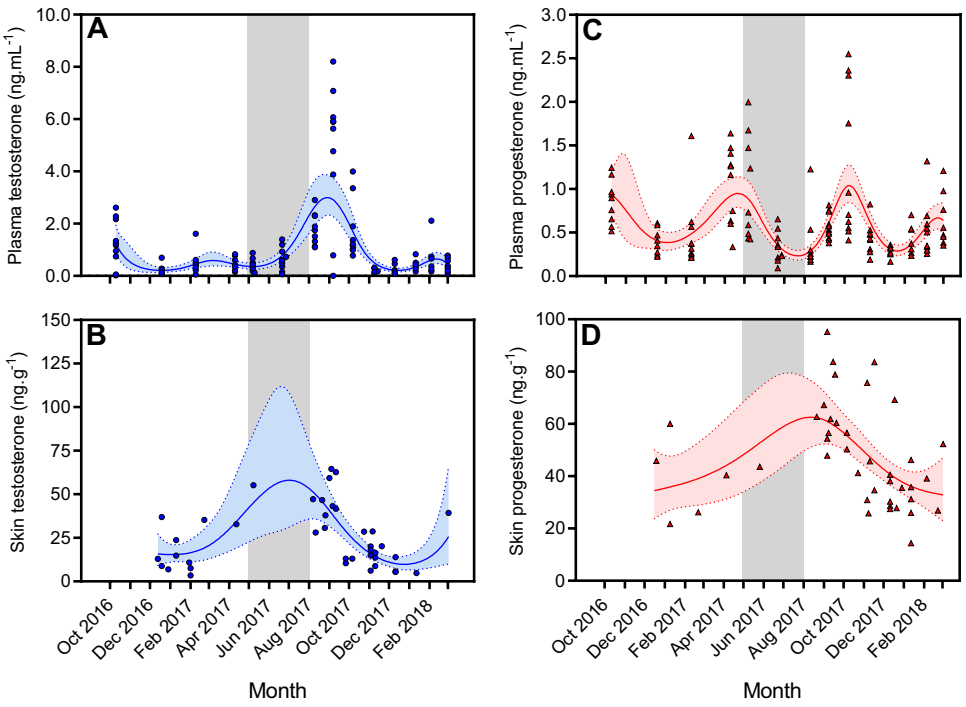

Fig. 4 Monthly changes in plasma and shed skin concentrations of testosterone in adult male and progesterone in adult female tegu lizards *Salvator merianae*. (**A**, **C**) Plasma and (**B**, **D**) shed skin testosterone (blue, left panels) and progesterone (red, right panels) in male and female lizards, respectively, over time with fitted GAMM. The solid line shows the mean prediction; dotted lines indicate 95% confidence intervals. Shaded gray area represents hibernation time. Note this is a rough estimation of hibernation time since it may differ among animals

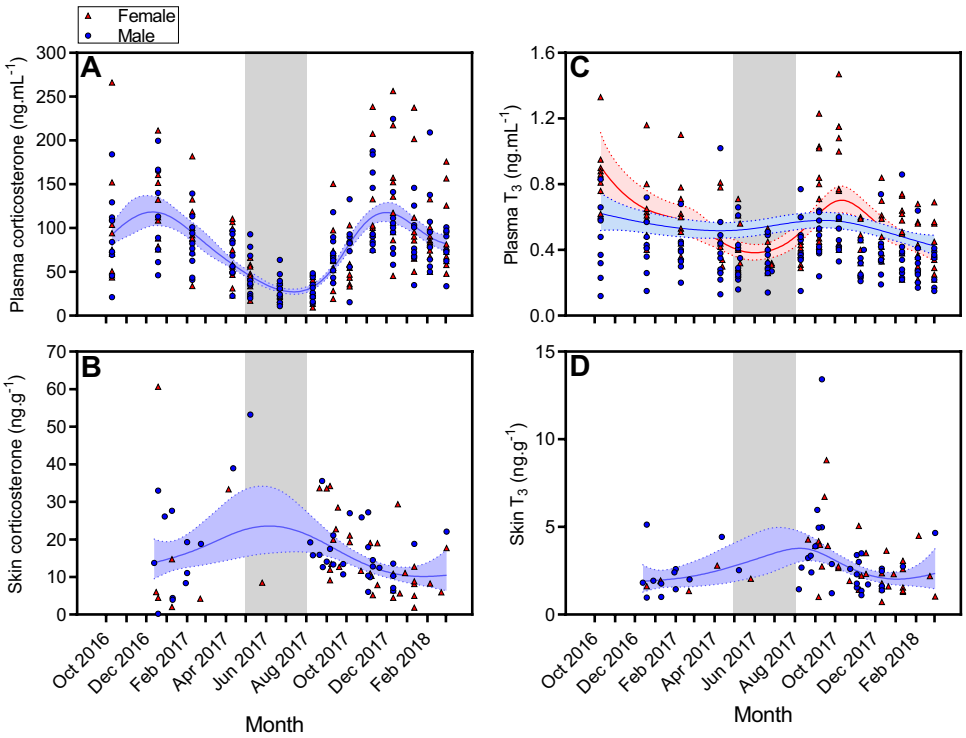

Fig. 5 Monthly changes in plasma and shed skin concentration of corticosterone and triiodothyronine (T_3) in adult male and female tegu lizards *Salvator merianae*. (**A**, **C**) Plasma corticosterone and T_3; and (**B**, **D**) shed skin corticosterone and T_3 in both male (blue circles) and female (red triangles) lizards over time with fitted GAMM. The solid line shows the mean prediction and dotted lines indicate 95% confidence intervals. Due to no significant difference between sexes, data for males and females were combined in A, B and D (A; plasma corticosterone: female estimate = 4.2712 and male estimate = 4.2273, $P = 0.75$; B; skin corticosterone: female estimate = 2.6580 and male estimate = 2.7555, $P = 0.56$; D; skin T_3: female estimate = 0.9241, male estimate = 1.0136, $P = 0.55$). In C the mean prediction and confidence interval were plotted for males and females separately (female estimate = −0.6358, male estimate = -0.9690, $P = 0.0007$). Shaded gray area represents hibernation time. Note this is a rough estimation of hibernation time since it may differ among animals

Table 1 Generalized Additive Mixed Model results showing effects of day of the year (doy) on skin and plasma hormones of tegu lizards

Smooth terms	Effective degrees of Freedom (e.d.f)	F-statistic	*P*	Variation explained (%)
Testosterone				
Plasma; s(doy)	7.558	20.99	< 0.0001	57.7
Skin; s(doy)	4.658	7.112	< 0.0001	52.3
Corticosterone				
Plasma; s(doy)	7.029	65.06	< 0.0001	72.1
Skin; s(doy)	3.063	3.236	0.014	27.7
Progesterone				
Plasma; s(doy)	7.752	13.657	< 0.0001	55.2
Skin; s(doy)	3.13	4.243	0.007	34.8
Triiodothyronine				
Plasma; s(doy × female)	5.866	11.900	< 0.0001	53.2
s(doy × male)	3.349	3.327	0.01	
Skin; s(doy)	3.915	3.875	0.004	41.8

Table 2 Best model subsets for skin hormones in both male and female tegu lizards (*Salvator merianae*)

Response variable	Random effect	Subset model no	Fixed effects	AIC_C	ΔAIC_C	AIC_C wt	Log likelihood
Skin testosterone	Tegu ID	1	Testo plasma	312	0.00	1	− 148.503
Skin corticosterone	Tegu ID	1	Cort plasma	21.0	0.00	0.628	− 14.915
		2	Cort plasma + sex	22.1	1.05	0.372	− 15.847
Skin progesterone	Tegu ID	1	Null model	− 16.6	0.00	0.721	9.011
		2	P4 plasma	− 14.7	1.89	0.279	7.724
Skin T_3	Tegu ID	1	Null model	4.0	0.00	0.673	− 1.363
		2	Sex	5.4	1.44	0.327	− 2.780

AICc refers to Akaike's information criterion corrected for small sample sizes; ΔAICc refers to the difference in AICc between each model and the model with the lowest AICc in the subset (models for which $\Delta AIC_C \leq 2.00$); AIC_C wt refers to the AICc weight; tegu ID refers to individual identity

Tegu identification is included as a random effect (intercept) in the models

T_3: Triiodothyronine; Cort: corticosterone; Testo: testosterone; P4: progesterone

In contrast to snakes, which usually shed a single contiguous piece, most lizards shed in large patches (Ling 1972; Vitt and Caldwell 2008). The shedding pattern of our captive tegus was characterized by release of both small and large pieces of skin, which were retrieved directly from the body of the animals to ensure individual identification. A downside of such an erratic shedding pattern is that comparing sheds from different body parts (*i.e.*, head, back, belly, legs, and tail) was not possible in our animals. Since all tegus were communally housed, when a shed was found on the floor of the facility, we did not use or count that as a sample because individual identification was not possible. Consequently, we mixed sheds from different parts of the individual tegu body before performing the extraction. Previous studies have investigated the potential effect of body location on hormone deposition in sheds of reptiles, more specifically corticosterone. One study performed in Komodo dragons (*Varanus komodoensis*) showed no effect of body region on corticosterone concentration (Carbajal et al. 2018). In another study performed in African House Snake (*Lamprophis fuliginosus*), no difference in shed skin corticosterone along the length of the snake's body was observed, except for the tail section, which showed higher levels than the other parts of the snake's body (Berkvens et al. 2013). The region of the body we sampled could be a source of variation in measured hormone levels and thus may impact results obtained in the present study.

Although our models predict peak levels of hormones in the shed skin at the beginning (*e.g.*, corticosterone) or middle of hibernation (*e.g.*, testosterone, progesterone and T_3), this prediction may not be accurate since we have few or no data points for the time interval of hibernation itself (*i.e.*, no

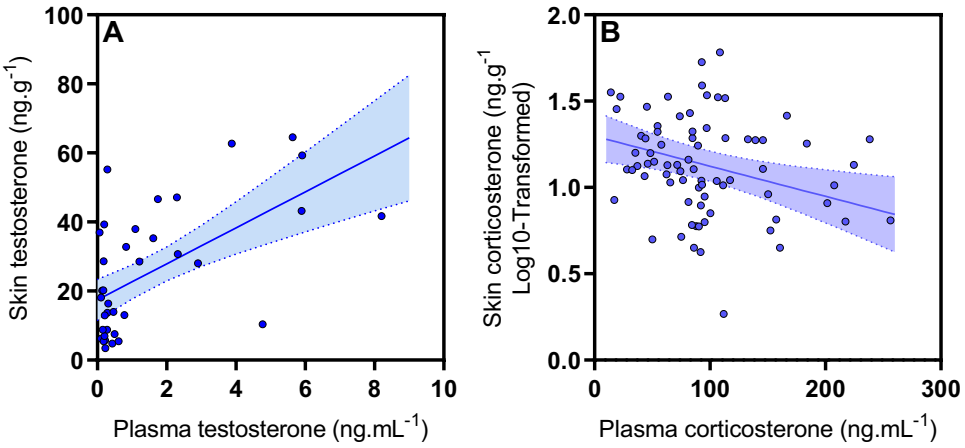

Fig. 6 Relationship between the concentrations of hormone in plasma (ng.mL^{-1}) and in shed skin (ng.g^{-1}) for tegu lizards *Salvator merianae*. (**A**) Plasma testosterone in males and (**B**) corticosterone in males and females combined are estimated from a linear mixed model with lizard individual identification as a random effect. Line depicts estimates and the shaded area represents the 95% confidence interval around the estimated effect of the predictors (plasma hormone) on skin hormone concentration. Skin corticosterone concentration is depicted as log-transformed values to meet assumptions for model residual normality

shedding), and this uncertainty manifests as wide confidence intervals. Therefore, as shown by the large number of data points and relatively high hormonal concentration of sheds around August and September of 2017, we suggest that all hormones peak coincident with the time period during which tegus emerge from hibernation, that is, late-winter and early spring (August–September). These skins are the first sheds to appear after a long resting phase associated with hibernation. Before shedding, tegus resume behavioral thermoregulation (basking) and their daytime body temperatures reach the highest values of the year (Sanders et al. 2015; Zena et al. 2020). When basking, many reptiles, including tegus, rapidly increase heart rate and cutaneous blood perfusion (Sanders et al. 2015). Such a physiological response maximizes heat transfer from the environment to the core of the body (Seebacher and Franklin 2007). A higher heart rate and therefore a faster warming response during basking is observed at the beginning of the reproductive season (August–September; Sanders et al. 2015) as compared to other seasons. Thus, this physiological mechanism of cutaneous and core warming may improve overall circulation of hormones and result in increased rates of incorporation of hormone into the growing skin after hibernation as compared to other times of the year.

It is still unknown when tegu skin accumulates hormone; it might be deposited in skin only during the renewal phase, when a new epidermal layer is laid down underneath the existing layer, a process that may take about 14 days (Vitt and Caldwell 2008). At the end of the renewal phase the new epidermal layer enters the resting phase, which may last for months, as during hibernation, before the epidermal cells come out of "rest" and form the new inner epidermal generation, while the outer epidermal generation is shed (*i.e.*, hormone deposition occurring months prior to shed collection). Alternatively, skin might accumulate hormone during the resting phase when little cell proliferation and differentiation take place (Flaxman and Maderson 1973) or even the very beginning of shedding, *i.e.*, pre-renewal phase (same month as collection), which we think is the case for our tegus since all hormones were elevated in sheds right after emerging from hibernation (August–September). At this time, animals resume behavioral thermoregulation and exhibit high rates of heat transfer with the environment compared to other times of the year (Sanders et al. 2015), which may contribute to hormone transfer from the peripheral circulation to the growing skin. Furthermore, data from bird feather and mammal hair also suggest that in at least some cases, vertebrate epidermal tissues may be able to accumulate hormone even after the tissue is fully grown (Jenni-Eiermann et al. 2015; Colding-Jørgensen et al. 2020). It is unclear at present whether this phenomenon can occur in all vertebrate epidermal tissues or whether it may be specific to thin epidermal tissues (*i.e.*, fine small hairs of rodents, fine barbules of feathers, thin reptile shed skin). Thin tissues may be more easily penetrated by steroid hormones even after growth, and thus might reflect a more recent time period than larger, thicker epidermal structures, for which compelling data exist demonstrating utility of these tissues for retroconstruction of past endocrine events (whale baleen, hair, seal claw) (D'Anna-Hernandez et al. 2011; Mastromonaco et al. 2014; Hunt et al. 2018a; Karpovich et al. 2020). This complexity deserves further investigation, particularly given the increasing use of a variety of epidermal tissues for retrospective analysis of endocrine status.

Although we found a positive relationship between shed skin testosterone and plasma testosterone of the same month,

when accounting for a supposed lag-time of 3 and 4 months between the hormone deposition into the skin and collection date of the shed, a significant inverse relationship was identified contrasting with the positive relationship found for the same month of collection. Therefore, we cannot rule out the fact that such significant relationships were random, since testosterone was the only plasma hormone to exhibit a single and pronounced peak coincident with peak levels of shed testosterone.

An important fact to be considered is that skin itself is an endocrine organ that, at least in mammals, has been shown to produce some glucocorticoids via mechanisms that appear separate from hypothalamus–pituitary–adrenal (HPA) axis. The skin neuroendocrine function thus complicates simple comparisons of plasma levels of corticosterone with levels of hormones taken up by the skin, because melanocytes, fibroblasts, and keratinocytes in the skin express corticotropin-releasing hormone, proopiomelanocortin, corticosterone and cortisol (Slominski 2005; Slominski et al. 2007; Cirillo and Prime 2011; Nikolakis et al. 2016). Therefore, the intriguing inverse relationship found between plasma and skin concentrations of corticosterone in our tegu lizards may reflect two different sources of corticosterone synthesis, one originating from the activation of the HPA axis resulting in elevated levels of corticosterone in the bloodstream, and the other related to the skin as an endocrine organ itself. Although a positive relationship between corticosterone from fecal samples and shed skin samples was found for the African house snake (*Lamprophis fuliginosus*), a physiological validation through adrenocorticotropic hormone stimulation failed to show any increase in skin corticosterone (Berkvens et al. 2013). So far, only the present study and the study by Berkvens and coworkers (2013) have quantified hormone levels of keratinized tissues and tested for correspondence with sample types that reflect relatively short time periods of incorporation, like blood (immediate) and fecal samples (hours to days). Other studies on epidermal tissues (sheds, claws) have not assessed potential correlations with plasma hormones (Baxter-Gilbert et al. 2014; Carbajal et al. 2018).

Previous studies have shown that thyroid hormones affect ecdysis in squamate reptiles (Chiu et al. 1967, 1983; Chiu and Lynn 1970; Kanaho et al. 2006). While thyroid hormones decrease shedding frequency in the shovel-nosed (*Chionactis occipitalis*) and rat snakes (*Ptyas korros*) (Chiu and Lynn 1970; Chiu et al. 1983), they stimulate shedding in the Tokay gecko (*Gekko gecko*) (Chiu et al. 1967). Differential expression of the thyroid hormone receptor (THR) occurs during the shedding process: THRα expresses strongly after shedding (resting phase), while THRβ receptors expresses strongly before (proliferation phase) skin shedding (Kanaho et al. 2006). Collectively, these results are indicative of thyroid hormone involvement in epidermal dynamics, complicating partitioning of influence of thyroid hormone on the shedding process from skin deposition solely as a result of changes in plasma circulating levels.

Conclusion

In the present study, we validated hormone assays to quantify different hormones in shed skin samples of the tegu lizard *S. merianae*. In addition, we found seasonal variation in all hormones investigated in sheds, with highest levels in late winter/early spring (August–September) when tegus shed skins after emerging from almost three months of hibernation. Incorporation of hormones into keratinized tissues is thought to occur via passive diffusion from the surrounding blood vessels when the tissue is growing until shortly before keratinization (Russell et al. 2012); however, we found complex relationships between hormone content of sheds and plasma in tegu lizards. Testosterone, the only hormone showing a single peak in the plasma throughout the seasonal cycle of male tegus may suggest that skin may continue to incorporate testosterone in the same month as it is shed, rather than months prior when the tissue is first grown. Therefore, it seems that hormone incorporation may occur at the end of resting phase (*i.e.*, pre-renewal phase) when the epidermis to be shed is still incomplete, lacking some cell layers that are only incorporated into the epidermis after shedding is completed (Jackson and Sharawy 1978; Alibardi 1995; Abdel-Aal 2018). Furthermore, the pronounced thermoregulatory behavior exhibited by tegus right after emerging from hibernation (Sanders et al. 2015) may contribute to hormone transfer from the peripheral circulation to the newly growing skin.

Inconsistent results regarding correlation between hormone levels in sheds and plasma in our tegus indicate that endocrine content of sheds may be influenced by confounding factors that must be identified before data can be interpreted. We anticipate this will not be an easy task, since molting frequency in squamates is influenced by myriad factors, including nutritional status, growth rate, and season (Semlitsch 1979; Gibson et al. 1989; Maas 2013; Carlson et al. 2014). In addition, adjustments in cutaneous blood flow related to thermoregulatory behavior may directly influence rate of hormone transfer between the blood and the growing skin. These are relevant questions to be addressed in future studies in order to understand the complexity of the phenology of secretion, circulation and deposition of hormones in keratinized tissues in reptiles.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00360-021-01397-3.

Acknowledgements We thank Dr. Augusto S. Abe for providing the animals from Jacarezario, UNESP-Rio Claro and Virginia Tessarine Barbosa for veterinary and technical assistance. We also thank Euclides

Roberto Secatto and Damaris Perecin for technical assistance and Tiago Carabolante and Diogo Caporicci for helping with animal care and blood sample collection.

Author contributions LAZ, CLB, KCB and CAN conceived the study; LAZ, CLB and KCB. designed the study; LAZ. conducted experiments; LAZ, DD, KEH, CLB. and KCB analyzed and interpreted data; CLB, KCB and CAN provided resources for the study; LAZ, DD, KEH, CAN, KCB and CLB wrote and edited the manuscript.

Funding The present study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo–FAPESP (2017/01652-2 and 2018/06192-2, post-doc fellowship to L.A.Z.; 2015/04849-6 grant to K.C.B; 2014/16320-7 grant to C.A.N.), the University of Alaska Anchorage Environment and Natural Resources Institute and Northern Arizona University (both awarded to C.L.B).

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval All experimental protocols were approved by a local Animal Care and Use Committee of São Paulo State University (CEUA; # 7.434/16).

References

Abdel-Aal HA (2018) Surface structure and tribology of legless squamate reptiles. J Mech Behav Biomed Mater 79:354–398. https://doi.org/10.1016/j.jmbbm.2017.11.008

Abe AS (1983) Observations on dormancy in tegu lizard, *Tupinambis teguixin* (Reptilia, Teiidae). Naturalia 8:135–139

Abe AS (1995) Estivation in South American amphibians and reptiles. Br J Med Biol Res 28:1241–1247

Alibardi L (1995) Electron microscopie analysis of the regenerating scales in lizard. Bolletino Di Zool 62:109–120. https://doi.org/10.1080/11250009509356059

Barton K (2018) MuMIn: multi-model inference. R Package Version 1(42):1

Baxter-Gilbert JH, Riley JL, Mastromonaco GF et al (2014) A novel technique to measure chronic levels of corticosterone in turtles living around a major roadway. Conserv Physiol 2:1–9. https://doi.org/10.1093/conphys/cou036

Berkvens CN, Hyatt C, Gilman C et al (2013) Validation of a shed skin corticosterone enzyme immunoassay in the African House Snake (*Lamprophis fuliginosus*) and its evaluation in the Eastern Massasauga Rattlesnake (*Sistrurus catenatus catenatus*). Gen Comp Endocrinol 194:1–9. https://doi.org/10.1016/j.ygcen.2013.08.011

Bortolotti GR, Marchant TA, Blas J, German T (2008) Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct Ecol 22:494–500. https://doi.org/10.1111/j.1365-2435.2008.01387.x

Carbajal A, Tallo-Parra O, Monclús L et al (2018) Corticosterone measurement in Komodo dragon shed skin. Herpetol J 28:110–116

Carlson BE, Williams J, Langshaw J (2014) Is synchronized ecdysis in wild ratsnakes (*Pantherophis alleghaniensis*) linked to humidity? Herpetol Notes 7:471–473

Chang C, Wu P, Baker RE et al (2009) Reptile scale paradigm: Evo-Devo, pattern formation and regeneration. Int J Dev Biol 53:813–826. https://doi.org/10.1387/ijdb.072556cc.Reptile

Chiu KW, Lynn WG (1970) The role of the thyroid in skin-shedding in the Shovel-Nosed Snake, *Chionactis occipitalis*. Gen Comp Endocrinol 14:467–474

Chiu AKW, Maderson PFA (1980) Observations on the interactions between thermal conditions and skin shedding frequency in the Tokay (*Gekko gecko*). J Herpetol 14:245–254

Chiu KW, Phillips JG, Maderson PF (1967) The role of the thyroid in the control of the sloughing cycle in the Tokay (*Gekko gecko*, Lacertilia). J Endocrinol 39:463–472. https://doi.org/10.1677/joe.0.0390463

Chiu KW, Leung MS, Maderson PFA (1983) Thyroid and skin-shedding in the rat snake (*Ptyas korros*). J Exp Zool 225:407–410

Cirillo N, Prime SS (2011) Keratinocytes synthesize and activate cortisol. J Cell Biochem 112:1499–1505. https://doi.org/10.1002/jcb.23081

Colding-Jørgensen P, Hestehave S, Abelson K, Kalliokoski O (2020) Hair glucocorticoids are not a historical marker of stress – exploring the time-scale of corticosterone incorporation into hairs in a rat model. biorXiv. https://doi.org/10.1101/2020.03.27.012377

D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML (2011) Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. Physiol Behav 104:348–353. https://doi.org/10.1016/j.physbeh.2011.02.041

Ellsworth A, Buck CL, Atkinson S, Hollmén T (2014) Longitudinal monitoring of sex steroid hormones in excrement of spectacled eiders (*Somateria fischeri*). Gen Comp Endocrinol 198:59–65. https://doi.org/10.1016/j.ygcen.2013.12.012

Erickson RL, Browne CA, Lucki I (2017) Hair corticosterone measurement in mouse models of type 1 and type 2 diabetes mellitus. Physiol Behav 178:166–171. https://doi.org/10.1016/j.physbeh.2017.01.018

Fernández Ajó AA, Hunt KE, Uhart M et al (2018) Lifetime glucocorticoid profiles in baleen of right whale calves: Potential relationships to chronic stress of repeated wounding by Kelp Gulls. Conserv Physiol 6:1–12. https://doi.org/10.1093/conphys/coy045

Fernández Ajó AA, Hunt KE, Giese AC et al (2020) Retrospective analysis of the lifetime endocrine response of southern right whale calves to gull wounding and harassment: a baleen hormone approach. Gen Comp Endocrinol 296:113536. https://doi.org/10.1016/j.ygcen.2020.113536

Flaxman BA, Maderson PFA (1973) Relationship between pattern of cell migration from the germinal layer and changing patterns of differentiation in the lizard epidermis. J Exp Zool 183:209–216. https://doi.org/10.1002/jez.1401830209

Gardell AM, Dillon DM, Smayda LC et al (2015) Perchlorate exposure does not modulate temporal variation of whole-body thyroid and androgen hormone content in threespine stickleback. Gen Comp Endocrinol 219:45–52. https://doi.org/10.1016/j.ygcen.2015.02.014

Gibson AR, Smucny DA, Kollar J (1989) The effects of feeding and ecdysis on temperature selection by young garter snakes in a simple thermal mosaic. Can J Zool 67:19–23. https://doi.org/10.1139/z89-004

Grotjan HE, Keel BA (1996) Data interpretation and quality control. In: Diamandis EP, Christopoulos TK (eds) Immunoassay. Academic Press, pp 51–95

Hansen WK, Bate LJ, Landry DW et al (2016) Feather and faecal corticosterone concentrations predict future reproductive decisions in harlequin ducks (*Histrionicus histrionicus*). Conserv Physiol 4:1–10. https://doi.org/10.1093/conphys/cow015

Hunt KE, Stimmelmayr R, George C et al (2014) Baleen hormones: a novel tool for retrospective assessment of stress and reproduction

in bowhead whales (*Balaena mysticetus*). Conserv Physiol 2:1–12. https://doi.org/10.1093/conphys/cou030
Hunt KE, Lysiak NS, Moore MJ, Rolland RM (2016) Longitudinal progesterone profiles in baleen from female North Atlantic right whales (*Eubalaena glacialis*) match known calving history. Conserv Physiol 4:1–9. https://doi.org/10.1093/conphys/cow014
Hunt KE, Lysiak NS, Moore MJ et al (2017) Multiple steroid and thyroid hormones detected in baleen from eight whale species. Conserv Physiol 5:1–14. https://doi.org/10.1093/conphys/cox061
Hunt KE, Lysiak NSJ, Matthews CJD et al (2018) Multi-year patterns in testosterone, cortisol and corticosterone in baleen from adult males of three whale species. Conserv Physiol 6:1–16. https://doi.org/10.1093/conphys/coy049
Jackson MK, Sharawy M (1978) Lipids and cholesterol clefts in the lacunar cells of snake skin. Anat Rec 190:41–45. https://doi.org/10.1002/ar.1091900105
Jarnevich CS, Hayes MA, Fitzgerald LA et al (2018) Modeling the distributions of tegu lizards in native and potential invasive ranges. Sci Rep 8:1–12. https://doi.org/10.1038/s41598-018-28468-w
Jenni-Eiermann S, Helfenstein F, Vallat A et al (2015) Corticosterone: effects on feather quality and deposition into feathers. Methods Ecol Evol 6:237–246. https://doi.org/10.1111/2041-210X.12314
Kanaho Y-I, Endo D, Park MK (2006) Molecular characterization of thyroid hormone receptors from the leopard gecko, and their differential expression in the skin. Zoolog Sci 23:549–556. https://doi.org/10.2108/zsj.23.549
Karpovich SA, Horstmann LA, Polasek LK (2020) Validation of a novel method to create temporal records of hormone concentrations from the claws of ringed and bearded seals. Conserv Physiol 8:1–11. https://doi.org/10.1093/conphys/coaa073
Lattin CR, Reed JM, Desrochers DW, Romero LM (2011) Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. J Avian Biol 42:247–252. https://doi.org/10.1111/j.1600-048X.2010.05310.x
Ling JK (1972) Adaptive functions of vertebrate molting cycles. Integr Comp Biol 12:77–93. https://doi.org/10.1093/icb/12.1.77
Lopes HR, Abe AS (1999) Biologia reprodutiva e comportamento do teiú, *Tupinambis merianae*, em cativero (Reptilia, Teiidae). In: Fang TG, Montenegro OL, Bodmer RE (eds) Manejo y conservación de fauna silvestre en América Latina. Editorial Instituto e Ecología, pp 259–272
Maas AK (2013) Vesicular, ulcerative, and necrotic dermatitis of reptiles. Vet Clin Exot Anim 16:737–755. https://doi.org/10.1016/j.cvex.2013.05.007
Mastromonaco GF, Gunn K, McCurdy-Adams H et al (2014) Validation and use of hair cortisol as a measure of chronic stress in eastern chipmunks (*Tamias striatus*). Conserv Physiol 2:1–12. https://doi.org/10.1093/conphys/cou055
Nikolakis G, Stratakis CA, Kanaki T et al (2016) Skin steroidogenesis in health and disease. Rev Endocr Metab Disord 17:247–258. https://doi.org/10.1007/s11154-016-9390-z
Palme R, Touma C, Arias N et al (2013) Steroid extraction: get the best out of faecal samples. Wien Tierarztl Monatsschr 100:238–246
Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2021) nlme: Linear and nonlinear mixed effects models. https://cran.r-project.org/web/packages/nlme/index.html
R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/
Ribeiro-Júnior MA (2015) Catalogue of distribution of lizards (Reptilia: Squamata) from the Brazilian Amazonia. I. dactyloidae, hoplocercidae, iguanidae, leiosauridae, polychrotidae, tropiduridae. Zootaxa 3983:1–110
Russell E, Koren G, Rieder M, Van Uum S (2012) Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. Psychoneuroendocrinology 37:589–601. https://doi.org/10.1016/j.psyneuen.2011.09.009
Sanders CE, Tattersall GJ, Reichert M et al (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. https://doi.org/10.1007/s00360-015-0928-2
Seebacher F, Franklin CE (2007) Redistribution of blood within the body is important for thermoregulation in an ectothermic vertebrate (*Crocodylus porosus*). J Comp Physiol B 177:841–848. https://doi.org/10.1007/s00360-007-0181-4
Semlitsch RD (1979) The influence of temperature on edysis rates in snakes (Genus Natrix) (Reptilia, Serpentes, Colubridae). J Herpetol 13:212–214
Slominski A (2005) Neuroendocrine system of the skin. Dermatology 211:199–208. https://doi.org/10.1002/cyto.a.20594.Use
Slominski A, Wortsman J, Tuckey RC, Paus R (2007) Differential expression of HPA axis homolog in the skin. Mol Cell Endocrinol 265–266:143–149. https://doi.org/10.1016/j.mce.2006.12.012
Vitt LJ, Caldwell JP (2008) Herpetology: an introductory biology of amphibians and reptiles, 3rd edn. Academic Press
Winck GR, Cechin SZ (2008) Hibernation and emergence pattern of *Tupinambis merianae* (Squamata: Teiidae) in the Taim ecological station, southern Brazil. J Nat Hist 42:239–247. https://doi.org/10.1080/00222930701828667
Wood SN (2017) Generalized additive models an introduction with R. Chapman and Hall/CRC, Boca Raton
Zena LA, Dillon D, Hunt KE et al (2019) Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard *Salvator merianae*. Gen Comp Endocrinol. https://doi.org/10.1016/j.ygcen.2018.06.006
Zena LA, Dillon D, Hunt KE et al (2020) Hormonal correlates of the annual cycle of activity and body temperature in the South-American tegu lizard (*Salvator merianae*). Gen Comp Endocrinol 285:113295. https://doi.org/10.1016/j.ygcen.2019.113295

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.