Hot and bothered: alterations in faecal glucocorticoid metabolite concentrations of the sungazer lizard, *Smaug giganteus*, in response to an increase in environmental temperature

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Abstract

Despite the commonly held belief that reptiles are immune to extreme temperatures, global warming is predicted to result in the loss of 40 % of all reptile species by 2080. In order to understand the effects of elevated temperature on African reptile physiology and health, additional research is required. We studied the physiological stress response of sungazer lizards (*Smaug giganteus*) facing elevated temperatures within captivity. Control animals (1M, 2F) were kept at 30 °C throughout the 13-week study period. Test animals (1M, 2F) were exposed to 30 °C for 4 weeks (stage 1), 39 °C for six weeks (stage 2) and 30 °C for 3 weeks (stage 3). Faecal samples were collected from both control and test animals to monitor faecal glucocorticoid metabolite (fGCM) concentrations as a proxy of physiological stress. While the fGCM levels of control females remained constant, test females showed an acute fGCM increase following the increase and subsequent decrease of temperatures. The test male had significantly higher fGCM levels during stage 3 compared to stage 1 and 2. The control male, although displaying constant fGCM levels throughout the study, had higher fGCM levels than the test male. The results indicate that a considerable temporal increase in environmental temperature did not lead to chronically elevated adrenocortical activity in sungazer lizards. It is likely that inherent traits within the species allows the species to adapt to such changes. Furthermore, the endangered status of the species and limited availability of study animals

restricted the sample size in this study to a few, captive-based lizards. Therefore, while our results are an important addition to reptile conservation, they should be extrapolated to free-ranging populations with caution. Future research should consider the effects of chronically elevated temperature in relation to water and food shortages, as well as inter-individual variation in physiological responses.

Introduction

In the early 21st century more than 25% of all known reptile species were defined as vulnerable, endangered or extinct by the International Union for Conservation of Nature (Bohm et al. 2013; Gibbons et al. 2000). Primary drivers of population declines include several complex and interlinked factors such as anthropogenic habitat modification, disease, invasive predators, the wildlife trade, human-animal conflict and climate change (see Fitzgerald et al. 2018 for a full review; Tingley et al. 2016; Todd et al. 2010). Although argued to be less vulnerable to environmental warming, due to their ability to evade thermal stress, tolerate high body temperatures and resist water loss (Huey et al. 2010), other research has suggested that global warming will result in the extinction of 40% of all reptiles by 2080 (Sinervo et al. 2010). In order to avoid the negative effects of climate change, many reptile species are able to migrate to more favourable areas (Barrows 2011; Moreno-Rueda et al. 2012) or implement behavioural plasticity within their current habitat (Caldwell et al. 2017; Czaja et al. 2020; Le Galliard et al. 2012; Refsnider and Janzen 2012). However, due to the limited dispersal ability of certain reptile species (Ceia-Hasse et al. 2014), as well as the inability of behavioural plasticity and genetic evolution to account for rapid environmental change (Catullo et al. 2019; Gunderson et al. 2017; Gunderson and Stillman 2015; Telemeco et al. 2017), many reptile populations likely remain vulnerable to climate change impacts (Griffis-Kyle et al. 2018).

One of the main factors associated with climate change is an increase in ambient temperature (T_a). This is especially important for ectotherms that depend on ambient conditions to drive optimal body temperatures, resulting in healthy biochemical and physiological processes (Carey and Alexander 2003; Gangloff and Telemeco 2018; Seebacher and Franklin 2005). However, an increase in T_a which exceeds the thermal tolerance of an individual can lead to a significant change in those processes (Bickford et al. 2010; Carey and Alexander 2003), reflected in oxidative damage, metabolic change, a reduced immune function and the activation of the physiological stress response (Han et al. 2020; Litzgus and Hopkins 2003;

Sandmeier et al. 2016; Stahlschmidt et al. 2017; Telemeco and Addis 2014). There remains a paucity of information on reptile physiological responses to temperature change - such data being integral to understand the likely ability of reptiles to cope with environmental change, both through evolutionary and phenotypically plastic responses (Clusella-Trullas and Chown 2014).

The physiological stress response is activated in an organism once a noxious stimulus is perceived within its immediate surrounding (Moberg 2000). One of the major components of the physiological stress response is the hyperactivation of the hypothalamic-pituitary-adrenal axis resulting in an increase in secreted glucocorticoid (GC) concentrations. An acute increase in GC concentrations is adaptive in nature, helping an individual to survive a stressful event (predation event, injury etc.) by ensuring energy demands are met, enhancing cardiovascular activity and altering behaviour (Liu et al. 2019; Romero 2002; Romero et al. 2009; Sapolsky et al. 2000). However, a chronic elevation in GC concentrations can lead to several deleterious effects, including immune and reproductive suppression, increased susceptibility to disease and reduced fitness (Cohen et al. 2012; Hing et al. 2016; Nicolaides et al. 2015; Sapolsky et al. 2000; Webster Marketon and Glaser 2008). As such, monitoring GC concentrations of endangered species can be an ideal method for assessing the physiological stress experienced by individuals exposed to anthropogenic and natural stressors. This has previously been done to determine the effect of elevated temperature on GC concentrations of reptile species. For example, an increase in ambient temperature led to elevated GC concentrations in northern and southern alligator lizards (*Elgaria coerulea* and *Elgaria multicarinata*; Telemeco and Addis 2014) and garter snakes (*Thamnophis elegans*; Gangloff et al. 2016); however, this pattern was reversed in Children's pythons (*Antaresia childreni*; Dupoué et al. 2013). Further work on garter snakes suggest that population-level differences in life history, body size and sex explain much variation in plasma GC concentrations, but that local climate variables do not (Holden 2020).

The use of non-invasive endocrine monitoring techniques, through the collection of faeces, urine and hair, hold several advantages above the commonly used, invasive blood collection, including (1) minimal human-animal interaction, (2) the ability to collect repeated samples over prolonged periods with minimal effort (Whitham and Wielebnowski 2013) and (3) hormone metabolite concentrations assessed in faeces are less affected by episodic fluctuations in hormone secretions due to the pooling of metabolites within the gut prior to excretion (Kersey and Dehnhard 2014; Russell et al. 2012). Several studies have used non-invasive faecal glucocorticoid metabolite (fGCM) monitoring to assess the level of

physiological stress experienced by reptiles in response to direct human-animal interaction (Borgmans et al. 2018; Kalliokoski et al. 2012; Rittenhouse et al. 2005) or seasonal changes in social and reproductive parameters (Augustine et al. 2020; West and Klukowski 2018). However, the use of fGCM monitoring to address the physiological response of reptiles to temperature increases has been limited. Megía-Palma et al. (2020) illustrated a positive relationship between extreme temperature events and fGCM levels in adult Gallot's lizards, *Gallotia galloti*. There is thus a need in increase our understanding of the effect of elevated temperature on the physiology of reptile species using non-invasive endocrine monitoring techniques.

Sungazer lizards (*Smaug giganteus*) are endemic to the grasslands of the Free State and Mpumalanga provinces of South Africa (Jacobsen 1989). The species has been listed as vulnerable by the International Union for the Conservation of Nature (Alexander et al. 2018) due to the decline in population size as a result of extensive anthropogenic habitat modification by e.g. mining as well as the medicine and pet trade (McIntyre and Whiting 2012; Mouton 2014; Parusnath et al. 2017; Van Wyk 1992). While the temperate grasslands inhabited by sungazer lizards are potentially less vulnerable to direct climate change effects (Lawal et al. 2019; Li et al. 2018), the region is expected to be subject to longer warm spells (New et al. 2006). Sungazer lizards display substantial plasticity in basking behaviour and may be able to respond to changing climate conditions through behavioural means (Stanton-Jones et al. 2018). However, their likely physiological response to changes in climate is poorly understood. So far, only one study investigated the physiological stress response in sungazer lizards; here, Scheun et al. (2018) validated the most appropriate enzyme immunoassay (EIA) for monitoring glucocorticoid metabolite concentrations in the urine and faeces of sungazer lizards. As such, no information exists on the physiological response of this species to changes in temperature, specifically elevated ambient temperature. Therefore, we aimed to determine the fGCM patterns in the species, as a proxy of physiology stress, in response to an elevation in T_a.

Material and methods

Study Site and Animals

The study was conducted at the National Zoological Garden (NZG), South African National Biodiversity Institute, Pretoria, South Africa (25.73913°N, 28.18918°E) between the 2nd of December 2019 and the 28th of February 2020. Animal availability within the captive environment resulted in the use of two male (164.0g and 184.0g body weight) and four females (185.7 ± 19.9g body weight) sungazer lizards. The lizards were kept at the NZG Reptile and

Amphibian Section in an area that is not on display to the public. The six sungazer lizards were housed in four separate enclosures (1.5 m x 1.5 m; 1M/1M/2F/2F) within a reptile-pit. Enclosures consisted of a substrate of coarse river sand, an artificial burrow constructed from cardboard, and a UV-radiation lamp (Ultra Vitalux Lamp E27, 300Watt, Osram, Munich, Germany). Water bowls with fresh water were available ad libitum for the duration of the project. Each individual was given 3-5 meal worms or cockroaches (depending on availability) every 1-2 days, along with a vegetable mixture as per the feeding regime dictated by the NZG caretakers and veterinarians. All enclosures had two reptile basking/heat-lamps (Daytime Heat Lamp PT2114, 150Watts, Exo Terra, Montreal, Canada) fitted on opposite sides of each enclosure, while a heat-emitting UV-radiation lamp was placed in the middle of each enclosure; the temperature in the enclosure was increased by lowering the height-above-surface distance of the UV-radiation lamp. Thermometer probes were placed on the surface of the river sand directly beneath the UV-radiation lamps in each enclosure. Temperature measurements under the UV-radiation lamps and at the edge of the enclosure prior to the start of the study showed a temperature difference of $1.2\pm0.2^{\circ}$C. Temperatures were recorded at 07h00 and 13h00 every day, and the average of the two recordings were then used to calculate the average daily enclosure temperature. To simulate natural photoperiod, lamps in the enclosures and all lights in the facility were run for 12 hours every day between 06h00 and 18h00.

Experimental Design

The study subjects were randomly divided into two groups, each consisting of one male and two females (n = 3), and randomly assigned as the 'control group' (CG) and 'test group' (TG). Due to a limitation in the number of available enclosures, the two females in each study group were housed together, while the male in each study group was housed individually. Since faecal samples collected in the female enclosures of each study group could not be identified as belonging to a specific individual, all faecal samples were identified by study group (CG or TG) and sex (male or female); i.e. CG male, CG females, TG male, and TG females. During a two-week acclimatization period, all four enclosures were kept at a standard temperature of approximately 30°C. Following this, T_a of the TG were manipulated over three stages as part of a thermal regime. During stage 1, T_a was kept at 30.7 ± 2.2 standard deviation (SD) °C for a period of four weeks. During stage 2, T_a was increased to 39.1 ± 1.6 SD °C for a period of six weeks. In stage 3, the additional basking/heat lamps were removed, and T_a was reduced to 31.2 ± 1.2 SD °C for a period of three weeks. The T_a of the CG remained relatively constant throughout the study period and was maintained at approximately 30°C during all three stages

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of the study (stage 1: 30.7 ± 2.2 SD °C; stage 2: 32.4 ± 0.9 °C; stage 3: 31.2 ± 1.2 SD °C). The study was approved by the SANBI National Zoological Garden Animal Use and Care Committee (Reference: P19/17).

Sample collection and steroid extraction

Although sungazer lizards excrete faeces and urate in unison, the two matrices do not typically mix in the cloaca (Scheun et al. 2018). Generally, the faecal (dark-coloured substance) and the urate components (white-coloured substance) can easily be separated with minimal cross-contamination occurring. As such, the enclosures were checked by the same female researcher between 07h00 and 16h00 for freshly excreted urofaecal samples throughout the study period. Following separation of the two matrices, faecal samples were placed into labelled 1.5 mL microcentrifuge tubes and immediately stored at -20 °C until processed for analysis. Except for TGF which provided only six faecal samples during stage 3, a total of seven faecal samples per group/stage was collected throughout the study, resulting in a total of 83 samples.

All samples were extracted following standard protocols (Scheun, Greeff and Ganswindt, 2018). Briefly, each faecal sample was lyophilized, pulverized and sifted through a thin mesh strainer to remove any undigested material and produce a fine faecal powder for extraction (Fieß et al. 1999). Approximately 0.050-0.055 g of the resultant faecal powder was extracted with 1.5 mL 80% ethanol in water. The suspension was vortexed for 15 minutes and then centrifuged at 1,600 g for 10 minutes. The resultant supernatant was poured into a 1.5 mL microcentrifuge tube and stored at -20 °C until analysis.

Enzyme Immunoassay Analysis

Faecal glucocorticoid metabolite concentrations were determined in extracts using a 5α-pregnane-3β,11β,21-triol-20-one EIA previously established for the species by Scheun et al. (2018). Details about the assay, including cross-reactivities, are provided by (Touma et al. 2003). Serial dilutions of extracted faecal samples gave displacement curves that were parallel to the respective curves (relative variation in the slope of respective trendlines < 5%). Assay sensitivity was 2.4 ng/g dry weight (DW). Coefficients of variance, determined by repeated measurements of high- and low- quality controls, was 5.13% and 5.61% for intra-assay variance, and 6.22% and 9.60% for inter-assay variance. All analyses were performed at the Endocrine Research Laboratory, University of Pretoria, South Africa.

Data Analysis

A Priori Model Building and Selection

All statistical and graphical analyses were performed using R statistical software (R Core Team 2019). To identify predictors of variation in fGCM concentrations, the packages lme4 (Bates et al. 2015) and MuMIn (Barton and Barton 2019) were utilized for mixed-effects model creation, model averaging, and model selection. The full model consisted of the dependent variable (fGCM concentrations), three fixed-effects variables (environmental temperature, sex, and stage of thermal regime), and the random effects variable (1|group) to account for repeated measures from the same groups (CG male, CG females, TG male, TG female). Thus, the starting full model took the form of: fGCM ~ temperature + sex + stage + (1|group). The standardized global models were then averaged and ranked according to Akaike's Information Criterion with small sample size correction (AICc) and Akaike's weight (wi). Only models with an AICc score that differed by less than two (ΔAICc < 2; Burnham and Anderson 2002) from the best model are reported here. To support these findings, two one-way analyses of variance (ANOVA) were conducted to determine whether significant differences in fGCM levels were present across all thermal stages in male and female animals.

Results

Generalized Linear Mixed-Effects Model and ANOVA analyses

Two models were ranked and reported with a ΔAICc < 2 (Table 1). Both 'sex' and 'thermal regime stage' variables best explained the variation in fGCM concentrations among male and female sungazer lizards (ΔAICc = 0.00). 'Thermal regime stage' was the next best predictor (ΔAICc = 0.32) of variation in fGCM concentrations.

Table 1. Ranked results from the generalized linear mixed-effect model analysis of repeated faecal glucocorticoid metabolite measures in male and female sungazer lizards in relation to various predictor variables.

Model fGCM ~ predictor variables	Degrees of Freedom	Akaike's Information Criterion with Small Size Sampling Correction (AICc)	Akaike Distance from the Best Model (ΔAICc)	Akaike Weight (wi)
1. Sex + Stage	6	282.27	0.00	0.38
2. Stage	5	282.58	0.32	0.32
3. Sex	4	284.28	2.01	0.14

Male fGCM levels differed between control and test animals across all thermal stages (ANOVA, $F_{(5,35)}$ = 3.92, p = 0.006). A Tukey HSD test showed that the CG male (2.14 ± 0.75 µg/g DW) had significantly higher fGCM concentrations than the TG male during stage 1 (1.33 ± 0.26 µg/g DW). In addition to this, the TG male had significantly higher fGCM levels in stage 3 (2.42 ± 0.58 µg/g DW) compared to both stage 1 and stage 2 (1.79 ± 0.57 µg/g DW; Figure 1). In contrast to this, no significant difference in fGCM levels were evident between CG females and TG females across all thermal stages (ANOVA, $F_{(5,35)}$ = 0.94, p = 0.47; Fig. 1).
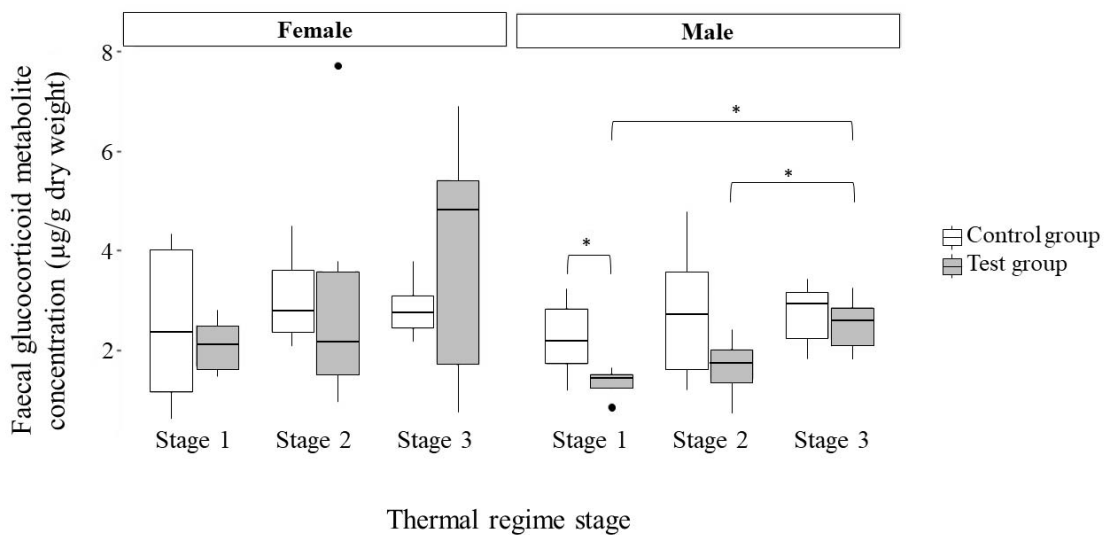

Figure 1. Boxplot (median, 25 % percentile, 75 % percentile) of ufGCM concentrations between sungazer lizard male and female test and control groups. Significant difference is indicated by *.

Post hoc graphical analysis

The fGCM profile of the TG male showed a considerable increase from stage 1 to stage 3 (80% increase in median levels), with peak levels observed in stage 3 (Fig. 2). A gradual increase in fGCM levels were observed in the CG male from stage 1 to stage 3 (35% increase in median levels) with considerable fluctuations in metabolite concentrations (Fig. 2). Median fGCM levels in TG females were ~120% higher in stage 3 (4.72 ± 2.53 µg/g DW) than both stage 1 (2.12 ± 0.53 µg/g DW) and 2 (2.16 ± 2.33 µg/g DW), although a substantial fGCM variation existed within each thermal stage (Fig. 1). However, a considerable peak in fGCM levels were observed following the initial increase (7.712 µg/g DW) and decrease (6.89 µg/g DW) in T_a

(Fig. 3). In contrast to this, fGCM levels of CG females showed no pronounced increase or decrease throughout the study period (Fig. 3).
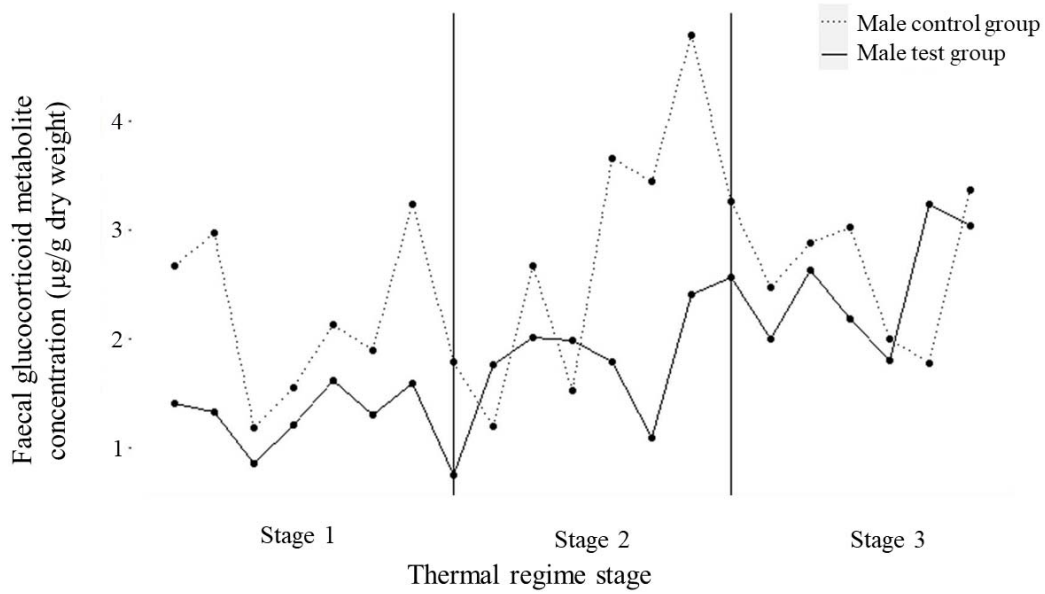

Figure 2. Faecal glucocorticoid metabolite profiles of control and test female groups across the different thermal stages. Solid vertical lines represent points of sudden temperature change.
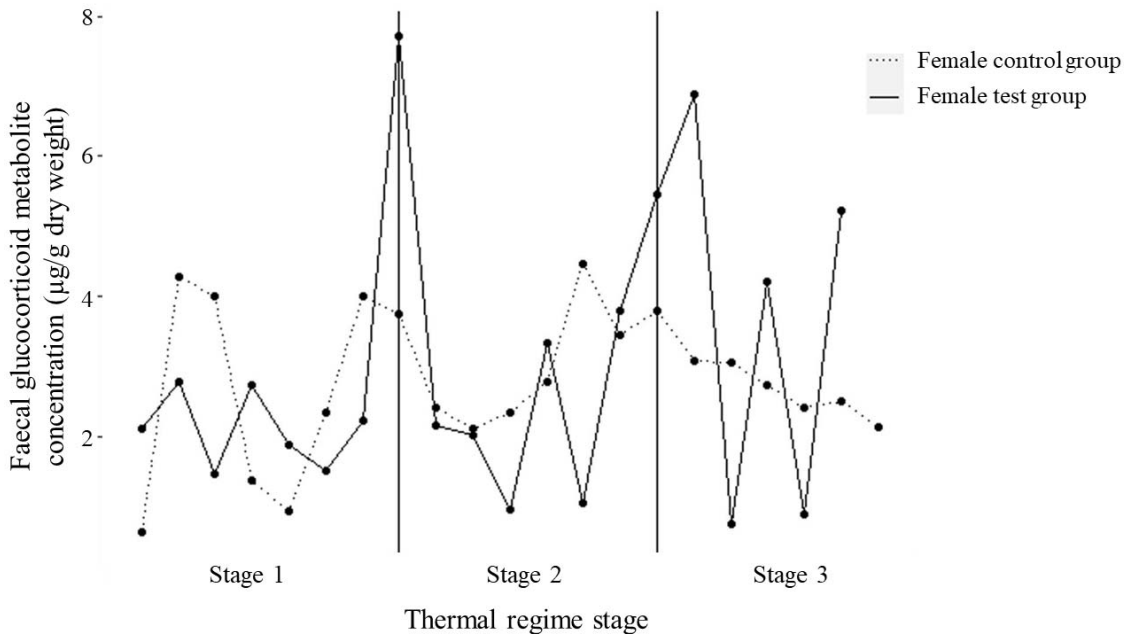

Figure 3. Faecal glucocorticoid metabolite profiles of control and test males across the different thermal stages. Solid vertical lines represent points of sudden temperature change.

Discussion

To our knowledge, this is the first study to report the physiological stress response of an African reptile experiencing temperature fluctuations. An increase in T_a altered the adrenocortical activity in both male and female sungazer lizards. As expected, there was no significant change in fGCM levels observed in control group females kept under constant ambient temperature. Although the control group male showed a fGCM increase between stages, this was not significant. In contrast to both control groups, fGCM levels of treatment male and female sungazer lizards showed varying patterns throughout the thermal stages.

In this study, the sudden increase in T_a correlated with an increase in fGCM levels in TG females, presumably as a means to restore homeostasis (Tasker 2006). A similar response has been observed in both garter snakes, *Thamnophis elegans* (Gangloff et al. 2016) and northern alligator lizards, *Elgaria coerulea*, (Telemeco and Addis 2014). Interestingly, TG female lizards also showed an acute increase in fGCM levels once T_a was returned to control temperatures, indicating a sensitivity of female sungazer lizards to sudden temperature change. As fGCM peaks were only observed in a single sample following temperature increase (stage 1 to stage 2) and decrease (stage 2 to stage 3), it is unlikely that the TG females employed physiological plasticity in response to thermal change. Although the sudden change in temperature implemented here (~9 ^{o}C) is considerably higher than the predicted 4 ^{o}C increase due to climate change by 2100 (Thuiller 2007), the thermal range of this study might well have fallen into the thermal tolerance zone of sungazer lizard females; that is, the thermal minimum and maximum temperatures at which an organism can continue activity required for survival (Kearney and Porter 2004; Kearney et al. 2009). Thermal tolerance is dependent on the physiological and behavioural plasticity, as well as evaporative water loss traits inherent in a species (Caldwell et al. 2015). As all lizards in our study had a constant source of water throughout the study, evaporative water loss due to increased T_a would have been limited. Although behavioural plasticity could have assisted TG females, it is unclear whether it was implemented during stage 2. As such, additional research is required to determine the role of evaporative water loss, in combination with behavioural plasticity in the response of captive and free-ranging female sungazer lizards to elevated temperatures.

Although the TG male of our study showed a similar increase in fGCM levels during an increase in T_a, the significant increase in adrenocortical activity during the return to control temperatures (stage 3) was unexpected. The CG male had considerably higher fGCM levels than his TG counterpart; the latter was only able to match fGCM levels with the CG male during stage 3 of the study. The fGCM patterns observed in the TG and CG males can likely

be explain by two factors. Firstly, individual differences in adrenocortical activity throughout the control and test period may have been present. Such individual fGCM differences in response to an external stressor has been observed in a number of studies including one on the European rabbit (*Oryctolagus cuniculus*; Monclús et al. 2019) and barn swallow (*Hirundo rustica erythrogaster*; Vitousek et al. 2014). As such, the role of individual differences in the physiological stress response should be considered when designing a study and, where possible, the number of study animals increased to reflect population-level response rather than that of the individual (Cockrem 2013; Koolhaas et al. 2010). In addition to this, although the physiological stress response aims to restore the homeostasis of an individual facing an external stressor, short term physiological plasticity, as was needed in the current study, is not always sufficient to ensure optimal fitness or survival (Gangloff et al. 2019). In order to determine which of these two factors were responsible for the fGCM levels observed, additional research on larger study groups is required. However, given the limited number of animals within captivity, as well as the strict conservation protocols currently in place for the species, this might be difficult to implement. Finally, although the results gathered here are of crucial importance to the conservation of this and other reptile species globally, caution should be taken when extrapolating results from habituated/captive animals to free-ranging populations. While no information exists on the possible change in adrenocortical function in sungazer lizards due to captivity, several studies have shown a significant alteration in this regard for other species within captivity (Dickens et al. 2009; Dickens and Romero 2009; Fairhurst et al. 2011). This is especially true since captive animals have access to a constant source of food, water and shelter, which is often absent or highly seasonal in free-ranging populations.

Conclusion

Our results provide the first data on the physiological response of an endemic African reptile to elevated environmental temperatures. The number of lizards monitored in our study was low due to animal availability and the conservation status of the species restricting access to study individuals. As a result, the adrenocortical activity and response to ambient temperatures observed here is likely driven by individual variation in the stress response (Koolhaas et al. 2010; Koolhaas et al. 2007) and thus specific to the study animals. Nonetheless, our results indicate a physiological sensitivity in sungazer lizards to periods of altered environmental temperature. The long-term consequences of elevated GCM concentrations in response to temperature variability is unknown. However, other sub-lethal stressors such as pollution potentially contribute to fitness loss and demographic effects (i.e. skewed sex ratios) in this

species (McIntyre and Whiting, 2012). The potential therefore exists for interactive or cumulative effects of stressors such as environmental temperature changes, changes in rainfall and industry-related pollution to further threaten the fitness and continued survival of sungazer lizard populations. Future research should aim to not only increase the number of study animals used when monitoring the physiological response to environmental change, but include monitoring of parameters such as body condition, evaporative water loss and behavioural change. Furthermore, monitoring of *in situ* sungazer lizard population demographics, stress and behaviour would facilitate conservation planning efforts for this species.

Funding

References

Alexander GJ, Tolley, K, Mouton, PFN. 2018. Giant dragon lizard, *Smaug giganteus*. p. e.T5336A115650269

Augustine L, Miller, K, Peters, A, Franklin, AD, Steinbeiser, CM, Brown, JL, Prado, NA. 2020. Impacts of the season and reproductive status on fecal reproductive and adrenocortical steroid metabolites in zoo Cuban crocodiles (*Crocodylus rhombifer*). *Zoo Biology* 39(6): 411-421.

Barrows CW. 2011. Sensitivity to climate change for two reptiles at the Mojave–Sonoran Desert interface. *Journal of Arid Environments* 75(7): 629-635.

Barton K, Barton, KM. 2019. Package "MuMIn". *Version 1-6*.

Bates DM, Maechler, M, Bolker, B, Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67(1): 1-48.

Bickford D, Howard, SD, Ng, DJJ, Sheridan, JA. 2010. Impacts of climate change on the amphibians and reptiles of southeast Asia. *Biodiversity and Conservation* 19(4): 1043-1062.

Böhm M, Collen, B, Baillie, JE, Bowles, P, Chanson, J, Cox, N, Hammerson, G, Hoffmann, M, Livingstone, SR, Ram, M. 2013. The conservation status of the world's reptiles. *Biological Conservation* 157: 372-385.

Borgmans G, Palme, R, Sannen, A, Vervaecke, H, Van Damme, R. 2018. The effect of environmental provisioning on stress levels in captive green anole (*Anolis carolinensis*). *Animal Welfare* 27(1): 35-46.

Burnham KP, Anderson, DR, 2002. Model selection and multimodel inference: a practical information-theoretic approach.New York: Springer Science.

Caldwell AJ, While, GM, Beeton, NJ, Wapstra, E. 2015. Potential for thermal tolerance to mediate climate change effects on three members of a cool temperate lizard genus, Niveoscincus. *Journal of Thermal Biology* 52: 14-23.

Caldwell AJ, While, GM, Wapstra, E. 2017. Plasticity of thermoregulatory behaviour in response to the thermal environment by widespread and alpine reptile species. *Animal Behaviour* 132: 217-227.

Carey C, Alexander, MA. 2003. Climate change and amphibian declines: is there a link? *Diversity and Distributions* 9(2): 111-121.

Catullo RA, Llewelyn, J, Phillips, BL, Moritz, CC. 2019. The potential for rapid evolution under anthropogenic climate change. *Current Biology* 29(19): R996-R1007.

Ceia-Hasse A, Sinervo, B, Vicente, L, Pereira, HM. 2014. Integrating ecophysiological models into species distribution projections of European reptile range shifts in response to climate change. *Ecography* 37(7): 679-688.

Clusella-Trullas S, Chown, SL. 2014. Lizard thermal trait variation at multiple scales: a review. *Journal of Comparative Physiology B* 184(1): 5-21.

Cockrem JF. 2013. Individual variation in glucocorticoid stress responses in animals. *General and Comparative Endocrinology* 181: 45-58.

Cohen S, Janicki-Deverts, D, Doyle, WJ, Miller, GE, Frank, E, Rabin, BS, Turner, RB. 2012. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proceedings of the National Academy of Sciences* 109(16): 5995-5999.

Czaja RA, Scholz, AL, Figueras, MP, Burke, RL. 2020. The role of nest depth and site choice in mitigating the effects of climate change on an oviparous reptile. *Diversity* 12(4): 151.

Dickens MJ, Earle, KA, Romero, LM. 2009. Initial transference of wild birds to captivity alters stress physiology. *General and Comparative Endocrinology* 160(1): 76-83.

Dickens MJ, Romero, LM. 2009. Wild European starlings (*Sturnus vulgaris*) adjust to captivity with sustained sympathetic nervous system drive and a reduced fight-or-flight response. *Physiological and Biochemical Zoology* 82(5): 603-610.

Dupoué A, Brischoux, F, Lourdais, O, Angelier, F. 2013. Influence of temperature on the corticosterone stress–response: an experiment in the Children's python (*Antaresia childreni). General and Comparative Endocrinology* 193: 178-184.

Fairhurst GD, Frey, MD, Reichert, JF, Szelest, I, Kelly, DM, Bortolotti, GR. 2011. Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. *PLoS ONE* 6(3): e17663.

Fieß M, Heistermann, M, Hodges, JK. 1999. Patterns of urinary and fecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). *General and Comparative Endocrinology* 115(1): 76-89.

Fitzgerald L, Walkup, D, Chyn, K, Buchholtz, E, Angeli, N, Parker, M. 2018. The future for reptiles: advances and challenges in the Anthropocene. *Encycl. Anthropocene* 3: 163-174.

Gangloff EJ, Holden, KG, Telemeco, RS, Baumgard, LH, Bronikowski, AM. 2016. Hormonal and metabolic responses to upper temperature extremes in divergent life-history ecotypes of a garter snake. *The Journal of Experimental Biology* 219(18): 2944-2954.

Gangloff EJ, Sorlin, M, Cordero, GA, Souchet, J, Aubret, F. 2019. Lizards at the peak: physiological plasticity does not maintain performance in lizards transplanted to high altitude. *Physiological and Biochemical Zoology* 92(2): 189-200.

Gangloff EJ, Telemeco, RS. 2018. High temperature, oxygen, and performance: insights from reptiles and amphibians. *Integrative and Comparative Biology* 58(1): 9-24.

Gibbons JW, Scott, DE, Ryan, TJ, Buhlmann, KA, Tuberville, TD, Metts, BS, Greene, JL, Mills, T, Leiden, Y, Poppy, S, Winne, CT. 2000. The global decline of reptiles, déjà vu amphibians. *BioScience* 50(8): 653-666.

Griffis-Kyle KL, Mougey, K, Vanlandeghem, M, Swain, S, Drake, JC. 2018. Comparison of climate vulnerability among desert herpetofauna. *Biological Conservation* 225: 164-175.

Gunderson AR, Dillon, ME, Stillman, JH. 2017. Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. *Functional Ecology* 31(8): 1529-1539.

Gunderson AR, Stillman, JH. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences* 282(1808): 20150401.

Han X, Hao, X, Wang, Y, Wang, X, Teng, L, Liu, Z, Zhang, F, Zhang, Q. 2020. Experimental warming induces oxidative stress and immunosuppression in a viviparous lizard, *Eremias multiocellata*. *Journal of Thermal Biology* 90: 102595.

Hing S, Narayan, EJ, Thompson, RA, Godfrey, SS. 2016. The relationship between physiological stress and wildlife disease: consequences for health and conservation. *Wildlife Research* 43(1): 51-60.

Holden KG. 2020. Physiological ecology of stress in a terrestrial ectothermic vertebrate (garter snakes, Thamnophis spp.). Doctor of Philosophy. Iowa State University. pp 133.

Huey RB, Losos, JB, Moritz, C. 2010. Are lizards toast? *Science* 328(5980): 832-833.

Jacobsen NHG. 1989. A herpetological survey of the Transvaal. PhD Dissertation. University of Natal. Pietermaritzburg, Kwazulu-Natal, South Africa. pp

Kalliokoski O, Timm, JA, Ibsen, IB, Hau, J, Frederiksen, A-MB, Bertelsen, MF. 2012. Fecal glucocorticoid response to environmental stressors in green iguanas (*Iguana iguana*). *General and Comparative Endocrinology* 177(1): 93-97.

Kearney M, Porter, WP. 2004. Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology* 85(11): 3119-3131.

Kearney M, Shine, R, Porter, WP. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences* 106(10): 3835-3840.

Kersey DC, Dehnhard, M. 2014. The use of noninvasive and minimally invasive methods in endocrinology for threatened mammalian species conservation. *General and Comparative Endocrinology* 203(0): 296-306.

Koolhaas J, De Boer, S, Coppens, C, Buwalda, B. 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology* 31(3): 307-321.

Koolhaas JM, de Boer, SF, Buwalda, B, van Reenen, K. 2007. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain, Behavior and Evolution* 70(4): 218-226.

Lawal S, Lennard, C, Hewitson, B. 2019. Response of southern African vegetation to climate change at 1.5 and 2.0° global warming above the pre-industrial level. *Climate Services* 16: 100134.

Le Galliard JF, Massot, M, Baron, J-P, Clobert, J. 2012. Ecological effects of climate change on European reptiles. Wildlife conservation in a changing climate. Chicago: University of Chicago Press. pp 179-203.

Li D, Wu, S, Liu, L, Zhang, Y, Li, S. 2018. Vulnerability of the global terrestrial ecosystems to climate change. *Global Change Biology* 24(9): 4095-4106.

Litzgus JD, Hopkins, WA. 2003. Effect of temperature on metabolic rate of the mud turtle (*Kinosternon subrubrum*). *Journal of Thermal Biology* 28(8): 595-600.

Liu B, Zhang, T-N, Knight, JK, Goodwin, JE. 2019. The glucocorticoid receptor in cardiovascular health and disease. *Cells* 8(10): 1227.

McIntyre T, Whiting, MJ. 2012. Increased metal concentrations in Giant Sungazer Lizards (*Smaug giganteus*) from mining areas in South Africa. *Archives of Environmental Contamination and Toxicology* 63(4): 574-585.

Megía-Palma R, Arregui, L, Pozo, I, Žagar, A, Serén, N, Carretero, MA, Merino, S. 2020. Geographic patterns of stress in insular lizards reveal anthropogenic and climatic signatures. *Science of The Total Environment* 749: 141655.

Moberg G. 2000. Biological response to stress: implications for animal welfare. in: Moberg G, Mench J (eds), The Biology of Animal Stress: Basic Principles and Implications. Wellingford: CAB International. pp 1-21.

Monclús L, Lopez-Bejar, M, De la Puente, J, Covaci, A, Jaspers, VL. 2019. Can variability in corticosterone levels be related to POPs and OPEs in feathers from nestling cinereous vultures (*Aegypius monachus*)? *Science of The Total Environment* 650: 184-192.

Moreno-Rueda G, Pleguezuelos, JM, Pizarro, M, Montori, A. 2012. Northward shifts of the distributions of Spanish reptiles in association with climate change. *Conservation Biology* 26(2): 278-283.

Mouton PFN. 2014. *Smaug giganteus* in: M. F. Bates, W. R. Branch, A. M. Bauer, M. Burger, J. Marais, G. J. Alexander, Villiers MSD (eds), Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute.

New M, Hewitson, B, Stephenson, DB, Tsiga, A, Kruger, A, Manhique, A, Gomez, B, Coelho, CA, Masisi, DN, Kululanga, E. 2006. Evidence of trends in daily climate extremes over southern and west Africa. *Journal of Geophysical Research: Atmospheres* 111(D14).

Nicolaides NC, Kyratzi, E, Lamprokostopoulou, A, Chrousos, GP, Charmandari, E. 2015. Stress, the stress system and the role of glucocorticoids. *Neuroimmunomodulation* 22(1-2): 6-19.

Parusnath S, Little, IT, Cunningham, MJ, Jansen, R, Alexander, GJ. 2017. The desolation of Smaug: The human-driven decline of the Sungazer lizard (*Smaug giganteus*). *Journal for Nature Conservation* 36: 48-57.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Refsnider JM, Janzen, FJ. 2012. Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination. *Biological Conservation* 152: 90-95.

Rittenhouse CD, Millspaugh, JJ, Washburn, BE, Hubbard, MW. 2005. Effects of radiotransmitters on fecal glucocorticoid metabolite levels of three-toed box turtles in captivity. *Wildlife Society Bulletin* 33(2): 706-713.

Romero LM. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology* 128(1): 1-24.

Romero LM, Dickens, MJ, Cyr, NE. 2009. The reactive scope model — a new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior* 55(3): 375-389.

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(5): 589-601.

Sandmeier FC, Horn, KR, Tracy, CR. 2016. Temperature-independent, seasonal fluctuations in immune function of the Mojave Desert Tortoise (*Gopherus agassizii*). *Canadian Journal of Zoology* 94(8): 583-590.

Sapolsky RM, Romero, LM, Munck, AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21(1): 55-89.

Scheun J, Greeff, D, Ganswindt, A. 2018. Non-invasive monitoring of glucocorticoid metabolite concentrations in urine and faeces of the Sungazer (*Smaug giganteus*). *PeerJ* 6: e6132.

Seebacher F, Franklin, CE. 2005. Physiological mechanisms of thermoregulation in reptiles: a review. *Journal of Comparative Physiology B* 175(8): 533-541.

Sinervo B, Méndez-de-la-Cruz, F, Miles, DB, Heulin, B, Bastiaans, E, Villagrán-Santa Cruz, M, Lara-Resendiz, R, Martínez-Méndez, N, Calderón-Espinosa, ML, Meza-Lázaro, RN, Gadsden, H, Avila, LJ, Morando, M, De la Riva, IJ, Sepulveda, PV, Rocha, CFD, Ibargüengoytía, N, Puntriano, CA, Massot, M, Lepetz, V, Oksanen, TA, Chapple, DG, Bauer, AM, Branch, WR, Clobert, J, Sites, JW. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328(5980): 894-899.

Stahlschmidt ZR, French, SS, Ahn, A, Webb, A, Butler, MW. 2017. A simulated heat wave has diverse effects on immune function and oxidative physiology in the corn snake (*Pantherophis guttatus*). *Physiological and Biochemical Zoology* 90(4): 434-444.

Stanton-Jones WK, Parusnath, S, Alexander, GJ. 2018. The impact of posture and basking orientation on thermoregulation in the sungazer (*Smaug giganteus*). *Journal of Thermal Biology* 75: 45-53.

Tasker JG. 2006. Rapid glucocorticoid actions in the hypothalamus as a mechanism of homeostatic integration. *Obesity* 14(S8): 259S-265S.

Telemeco RS, Addis, EA. 2014. Temperature has species-specific effects on corticosterone in alligator lizards. *General and Comparative Endocrinology* 206: 184-192.

Telemeco RS, Fletcher, B, Levy, O, Riley, A, Rodriguez-Sanchez, Y, Smith, C, Teague, C, Waters, A, Angilletta Jr, MJ, Buckley, LB. 2017. Lizards fail to plastically adjust nesting behavior or thermal tolerance as needed to buffer populations from climate warming. *Global Change Biology* 23(3): 1075-1084.

Thuiller W. 2007. Climate change and the ecologist. *Nature* 448(7153): 550-552.

Tingley R, Meiri, S, Chapple, DG. 2016. Addressing knowledge gaps in reptile conservation. *Biological Conservation* 204: 1-5.

Todd B, Wilson, J, Whitfield, J. 2010. The global status of reptiles and causes of their decline. in: Spalding D, Linder G, Bishop C, Krest S (eds), Ecotoxicology of Amphibians and Reptiles. Florida: CRC Press. pp 47-67.

Touma C, Sachser, N, Möstl, E, Palme, R. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *General and Comparative Endocrinology* 130(3): 267-278.

Van Wyk JH. 1992. Life history and physiological ecology of the lizard, *Cordylus giganteus*. PhD Dissertation. University of Cape Town. Capte Town, South Africa. pp 294.

Vitousek MN, Jenkins, BR, Safran, RJ. 2014. Stress and success: individual differences in the glucocorticoid stress response predict behavior and reproductive success under high predation risk. *Hormones and Behavior* 66(5): 812-819.

Webster Marketon JI, Glaser, R. 2008. Stress hormones and immune function. *Cellular Immunology* 252(1): 16-26.

West JM, Klukowski, M. 2018. Seasonal changes in baseline corticosterone, association with innate immunity, and effects of confinement in free-ranging Eastern Box Turtles, Terrapene carolina carolina. *General and Comparative Endocrinology* 262: 71-80.

Whitham JC, Wielebnowski, N. 2013. New directions for zoo animal welfare science. *Applied Animal Behaviour Science* 147(3-4): 247-260.