General and Comparative Endocrinology 193 (2013) 178–184

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Influence of temperature on the corticosterone stress–response: An experiment in the Children's python (*Antaresia childreni*)

Andréaz Dupoué [a,b,*], François Brischoux [a], Olivier Lourdais [a,c], Frédéric Angelier [a]

[a] Centre d'Etudes Biologiques de Chizé, CEBC-CNRS UPR 1934, 79360 Villiers en Bois, France
[b] Université de Poitiers, 40 Avenue du recteur Pineau, 86022 Poitiers, France
[c] School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

ARTICLE INFO

Article history:
Received 16 May 2013
Revised 31 July 2013
Accepted 5 August 2013
Available online 12 August 2013

Keywords:
Glucocorticoids
Stress response
Thermal treatments
Temperature
Children's python

ABSTRACT

To cope with environmental challenges, organisms have to adjust their behaviours and their physiology to the environmental conditions they face (i.e. allostasis). In vertebrates, such adjustments are often mediated through the secretion of glucocorticoids (GCs) that are well-known to activate and/or inhibit specific physiological and behavioural traits. In ectothermic species, most processes are temperature-dependent and according to previous studies, low external temperatures should be associated with low GC concentrations (both baseline and stress-induced concentrations). In this study, we experimentally tested this hypothesis by investigating the short term influence of temperature on the GC stress response in a squamate reptile, the Children's python (*Antaresia childreni*). Snakes were maintained in contrasting conditions (warm and cold groups), and their corticosterone (CORT) stress response was measured (baseline and stress-induced CORT concentrations), within 48 h of treatment. Contrary to our prediction, baseline and stress-induced CORT concentrations were higher in the cold *versus* the warm treatment. In addition, we found a strong negative relationship between CORT concentrations (baseline and stress-induced) and temperature within the cold treatment. Although it remains unclear how cold temperatures can mechanistically result in increased CORT concentrations, we suggest that, at suboptimal temperature, high CORT concentrations may help the organism to maintain an alert state.

1. Introduction

Organisms are exposed to predictable (seasonal or diurnal variations) or unpredictable (extreme climatic events) changes of most biotic and abiotic variables (Wingfield, 2003; Wingfield et al., 2011). To cope with such variation and maintain their performances, individuals adjust their behaviour and their physiology to the environmental conditions (the concept of allostasis; (McEwen and Wingfield, 2003, 2010; Romero et al., 2009). These adjustments will organize resource allocation between potentially competing life-history traits and, thus, mediate decisions that aim at optimizing individual's fitness (Ricklefs and Wikelski, 2002). It is therefore crucial to study the central mechanisms that govern behavioural and physiological adjustments adopted when a given environmental change occurs (Hau, 2007; McEwen and Wingfield, 2003, 2010; Ricklefs and Wikelski, 2002; Romero et al., 2009). In vertebrates, one of these central mechanisms is the secretion of glucocorticoids (GCs) that is well-known to be involved in the maintenance of homeostasis (Landys et al., 2006; McEwen and Wingfield, 2003; Romero et al., 2009; Wingfield, 2012, 2013). In response to predictable or unpredictable environmental changes, the Hypothalamic–Pituitary–Adrenal (HPA) axis is activated and this results in a rapid and intense secretion of GCs by the adrenal glands (Romero et al., 2009; Wingfield et al., 1998, 2011; Wingfield, 2003, 2012, 2013; Wingfield and Sapolsky, 2003). In turn, this increase in circulating GCs activates and/or inhibits specific physiological and behavioural traits in order to help the organism to cope with the challenge (Landys et al., 2006; Romero, 2004; Sapolsky et al., 2000).

Environmental temperature is a crucial parameter with important fluctuations at multiple time scales (days, seasons) and unpredictable cold or warm temperature extremes. Most organisms adjust their physiology (metabolic rate, evaporative water loss) and/or behaviour (activity) in order to maintain their body temperature relatively constant (Scholander et al., 1950; Tieleman et al., 2002). Terrestrial ectotherms cannot produce significant amounts of heat and their body temperature is more sensitive to environmental conditions which will influence metabolic rate according to the Q_{10} relationship (Arrhenius equation) (Bennett and Dawson, 1976). Ambient temperature will directly influence major biological processes including digestion, locomotion, and behaviour (Angilletta, 2009; Huey and Stevenson, 1979; Huey and Kingsolver,

* Corresponding author at: Centre d'Etudes Biologiques de Chizé, CEBC-CNRS UPR 1934, 79360 Villiers en Bois, France. Fax: + 33 (0) 5 49 09 65 26.
E-mail address: andreaz.dupoue@gmail.com (A. Dupoué).

0016-6480/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2013.08.004

1989; Stevenson et al., 1985). Ectotherms are subjected to daily and seasonal temperature variations (Huey and Pianka, 1977; Peterson, 1987) and chiefly rely on behavioural means (habitat selection) to regulate their body temperature (Blouin-Demers and Weatherhead, 2001a,b,c, 2002). Because performances are reduced at low (i.e. suboptimal) temperature, one could also expect reduced GCs concentrations at low temperature (Cree et al., 2003; Tyrrell and Cree, 1998). First, low temperature should induce both decreased GCs secretion rates because of a slower activity of the HPA axis; and reduced GCs diffusion because of slower cardiac rhythm and blood circulation. Second, low temperatures are associated with a low metabolism in reptiles (Bennett and Dawson, 1976) and GCs concentrations are known to be positively correlated with metabolic activity in many vertebrate species (reviewed in Landys et al. (2006)).

The positive relation between GCs concentration and temperature has been showed in several species. For example, positive correlations between body temperature and baseline corticosterone (CORT, the primary GC in avian and non avian reptiles, (Greenberg and Wingfield, 1987) have been reported in tuatara, lizards and sea turtles (Cree et al., 2003; Jessop et al., 2000; Jones and Bell, 2004; Romero and Wikelski, 2006; Tyrrell and Cree, 1998; Woodley et al., 2003). However, this relation is not always supported among reptiles (Mathies et al., 2001; Sykes and Klukowski, 2009). While previous studies have focused on the relationship between body temperature and baseline CORT concentrations, much less attention has been paid to the thermal dependence of CORT stress response despite its ecological relevance (Cree et al., 2003; Romero and Wikelski, 2006; Sykes and Klukowski, 2009). Baseline and stress-induced CORT concentrations are known to have different physiological and behavioural actions (Landys et al., 2006; Romero, 2004). Thus, the action of CORT on behaviour and physiology depends on its circulating concentrations because CORT acts through the binding of two different receptors that have very different affinity for CORT. At baseline concentrations, CORT have mainly permissive actions that aim at maintaining energetic balances and deal with seasonal and daily routines (Landys et al., 2006; Romero, 2002; Sapolsky et al., 2000). At stress-induced concentrations, CORT has stimulatory and inhibitory actions that aim at promoting individual's short-term survival (i.e. a life-threatening event; (Angelier et al., 2009; Wingfield et al., 1998, 2011). Therefore it is essential to consider both baseline and stress-induced CORT concentrations.

In this study, we investigated the influence of temperature on both baseline and stress-induced CORT concentrations in a constricting snake, the Children's python (*Antaresia childreni*). Our aim was to test the influence of temperature on CORT stress response in response to a short term ($\leqslant$2 days) change in temperature. We created a thermal contrast between two groups of snakes by either allowing access to preferred body temperature (29 °C, "Warm" treatment) or imposing cold temperature (17 °C, "Cold" treatment). We predicted that (1) baseline CORT concentrations should be higher in individuals accessing preferred temperature because of higher metabolic rate, and (2) stress-induced CORT concentrations – the rate at which CORT is secreted following a stressor – should be higher in warm individuals because of higher CORT secretion and diffusion rates.

2. Materials and methods

2.1. Study individuals

Children's pythons (*A. childreni*) are medium-sized (up to 1.2 m snout vent length, 600 g body mass) constricting snakes that occur in Australian wet-dry tropics (Wilson and Swan, 2003). Preferred temperature has been previously studied in this species and is relatively high in non-reproductive individuals (T_{set}: ~29 °C; Lourdais et al., 2008). Snakes used in this study (16 non-reproductive females) were part of a captive colony of Children's pythons maintained in the Centre d'Etudes Biologiques de Chizé, France. Snakes were housed individually in plastic cages (35.5 × 63 × 15 cm) containing a shelter and providing a thermal gradient (25 to 35 °C). We kept experimental animals fasted before the experiment to avoid any influence of digestive activity on CORT concentrations. Individuals were kept fasted two weeks prior to the experiment and water was provided *ad libitum*.

2.2. Experimental protocol

We manipulated body temperatures applying ecologically relevant conditions. Snakes were exposed to thermal treatment and sampled (baseline and stress-induced CORT) within 48 h. The two thermal treatments were:

"Warm": 8 snakes were housed in individual transparent boxes (35 × 25 × 12.5 cm) with a shelter and equipped with heating cables providing a thermal gradient and therefore access to preferred body temperature.

"Cold": 8 snakes were kept in similar boxes without heating cables and therefore exposed to ambient room temperature (Mean ± SE, 17.03 ± 0.01 °C) well below T_{set}. This temperature is suboptimal but can be seasonally encountered in the field (Madsen and Shine, 1999).

The experiment was conducted in 2012 during two sessions (session 1: July 17–18, session 2: August 29–30). Each snake was randomly assigned to a thermal treatment. The experimental groups were switched during the second session, so that, each individual was sequentially exposed to both treatments. Snake were exposed to the usual housing condition (see above) between sessions. Individuals from both experimental sequence were similar in BM (ANOVA, $F_{1,14} = 0.13$, $P = 0.729$) and SVL (ANOVA, $F_{1,14} = 0.48$, $P = 0.498$).

2.3. Temperature sampling

From 24 to 48 h after the exposure to thermal treatment, each snake was handled to assess CORT concentrations. Skin surface temperature was recorded after capture using an infrared laser thermometer (Raytek Corporation, Santa Cruz, USA). Measurement procedure was similar to (Andrews, 2008) and recommendations from Hare et al. (2007) (emissivity set at 0.95 and the thermometer was oriented in-line with the body axis). We are fully cognizant that this method does not provide an accurate measure of internal temperature. It rather provides a simple (non invasive) measure of the thermal contrast between groups. Previous studies reported that skin surface temperature was strongly related to core temperature and these measures only differed from each other about 1.5 °C in small lizards (Carretero, 2012). In this study, the species is medium size and skin surface temperature is closely related to core temperature (Lourdais *per obs*).

2.4. Blood sampling and CORT assay

All snakes were bled according to a standardized capture/restraint stress protocol (Wingfield, 1994). Immediately after capture, an initial blood sample – 150 µl – was collected through cardiocentesis with a 1 ml heparinized syringe and a 27 gauge needle within approximately 3 min (Mean ± SE, 2.48 ± 0.17 min).

After blood collection, snakes were placed into an empty transparent plastic box without any shelter in their respective thermal conditions. For snake, the combination of being handled, bled and then placed in a new environment is known as a stressful situation

since they typically display increased CORT concentrations (Lourdais unpublished data). Two additional samples – 150 μl each – were collected respectively 30 min (Mean ± SE, 32.34 ± 0.31 min) and 60 min later (Mean ± SE, 61.81 ± 0.16 min). Immediately after sampling, the blood was centrifuged 3 min at 3000*g*. The plasma was separated, collected and stored at −28 °C. Plasma CORT concentrations were then determined at the CEBC by following a well-established radioimmunassay protocol (see Lormée et al., 2003 for details). Samples were run in two assays (intra-assay variation: 7.07%, inter-assay variation: 9.99%). All blood samples were collected between 8:00 and 18:00 and we included this factor (i.e. time of day) in our statistical analyzes since CORT concentrations could be subjected to nycthemeral variations (Cree et al., 2003; Dauphin-Villemant and Xavier, 1987).

2.5. Statistical analyses

All statistical analyses were performed with R software (R Development Core Team, version 2.13.1). First, we built a global model (i.e., model 1) using linear mixed model (lme, package nlme) to assess the effect of the thermal treatment on CORT concentrations. Thermal treatment (warm vs. cold) and sampling time (0, 30 and 60 min post handling) were treated as explanatory factors and the time of day as linear covariate. Female identity was taken in account as a random factor because of repeated samples (two sessions and three blood sampling per session). We used pairwise *post hoc* Tukey tests (lsmeans, package lsmeans) to compare the impact of thermal treatment for each blood sample (0, 30 and 60 min post handling). We did not adjust stress-induced CORT concentrations with baseline CORT concentrations since it is recognized as more relevant to consider absolute values (Romero, 2004).

Second, we built separate linear models (package stat) to test the influence of baseline CORT on stress-induced CORT (30 and 60 min post handling) for each treatment (i.e., model 2: cold treatment; model 3: warm treatment). Sampling time (30 and 60 min post handling) was treated as explanatory factor and baseline CORT as linear covariate.

We used the same design to test the influence of temperature on CORT concentrations. For each thermal treatment (i.e., model 4: cold treatment; model 5: warm treatment), sampling time (0, 30 and 60 min post handling) was treated as explanatory factor and time of day and temperature as linear covariates. We used the residuals of the relation between CORT concentrations and time of the day to graphically present our results. In all analyses, plasma concentration of CORT was Log_{10} transformed to normalized data (Shapiro–Wilk test all, $P > 0.05$).

3. Results

3.1. Effect of the thermal treatment on CORT concentrations

The two thermal treatment resulted in contrasted temperature (ANOVA, session 1: $F_{1,14} = 351.9$, $P < 0.001$, Mean ± SE, cold group: 16.6 ± 0.15 °C, warm group: 29.2 ± 0.65 °C, session 2: $F_{1,14} = 557.2$, $P < 0.001$, cold group: 17.3 ± 0.21 °C, warm group: 29.4 ± 0.47 °C).

CORT concentrations were significantly affected by the thermal treatment (model 1, $F_{1,72} = 29.3$, $P < 0.0001$) and sampling time (model 1, $F_{2,72} = 45.3$, $P < 0.0001$). At baseline, individuals from the cold treatment had higher CORT concentrations than those from the warm treatment (model 1, baseline: Tukey test, $P < 0.001$, Fig. 1). We found similar results with stress-induced concentrations although the difference was significant only at 30 min post handling (model 1, stress-induced, 30 min post handling: Tukey test, $P < 0.01$, 60 min post handling: Tukey test, $P = 0.149$; Fig. 1). There was no interaction between thermal

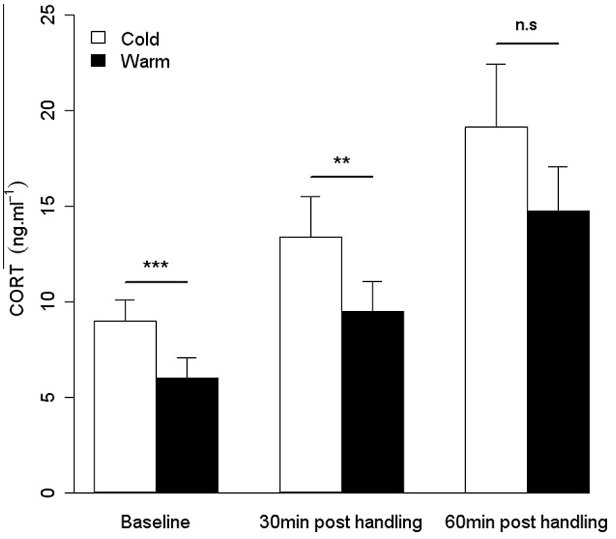

Fig. 1. Comparison of baseline and stress-induced (30 and 60 min post handling) plasma CORT concentrations between snakes maintained at cold (cold treatment: open bars, Mean ± SE, 18.0 ± 0.14 °C) and warm (warm treatment: filled bars, Mean ± SE, 29.6 ± 0.20 °C) temperatures. Bars represent means (±SE) of CORT concentrations (ng ml^{-1}). $^{***}P < 0.001$, $^{**}P < 0.01$, n.s (non-significant).

treatment and sampling time on CORT concentrations (model 1, $F_{2,72} = 0.9$, $P = 0.423$), suggesting a similar kinetic of the CORT stress response for both thermal treatments. Finally, we found a significant influence of time of day on CORT concentrations (model 1, $F_{1,72} = 14.0$, $P < 0.001$), with CORT concentrations increasing during the day.

Stress-induced CORT was significantly influenced by baseline CORT concentration within cold treatment (model 2, 30 min post handling: $t = 4.3$, $P < 0.001$; 60 min post handling: $t = 3.7$, $P < 0.001$; Fig. 2) and warm treatment (model 3, 30 min post handling: $t = 3.9$, $P < 0.001$; 60 min post handling: $t = 2.2$, $P = 0.035$; Fig. 2). Baseline CORT concentration positively influences stress-induced CORT concentrations (30 and 60 min post handling) for all individuals (Fig. 2).

3.2. Influence of temperature on CORT concentrations

Within the cold treatment, baseline and stress-induced CORT concentrations were negatively related to temperature (model 4, baseline: $t = -2.1$, $P = 0.045$; 30 min post handling: $t = -2.8$, $P = 0.008$; 60 min post handling: $t = -2.1$, $P = 0.043$; Fig. 3). As above, CORT concentrations increased during the day (model 4, $t = 3.4$, $P = 0.002$).

Baseline CORT concentrations were marginally positively correlated with temperature for the warm treatment (model 5, $t = 1.7$, $P = 0.089$; Fig. 4). Stress-induced CORT concentrations were not correlated with temperature (model 5, 30 min post handling: $t = 0.3$, $P = 0.747$; 60 min post handling: $t = 0.2$, $P = 0.855$; Fig. 4). As above, CORT concentrations increased during the day (model 5, $t = 2.0$, $P = 0.049$).

4. Discussion

We expected a positive relationship between temperature and CORT concentrations due to positive effect of temperature on metabolism (Cree et al., 2003; Landys et al., 2006; Tyrrell and Cree, 1998). Our study on the Children's python highlights complex effect of temperature on CORT concentrations and contradicts our predictions. First, we found that individuals from the cold treatment had

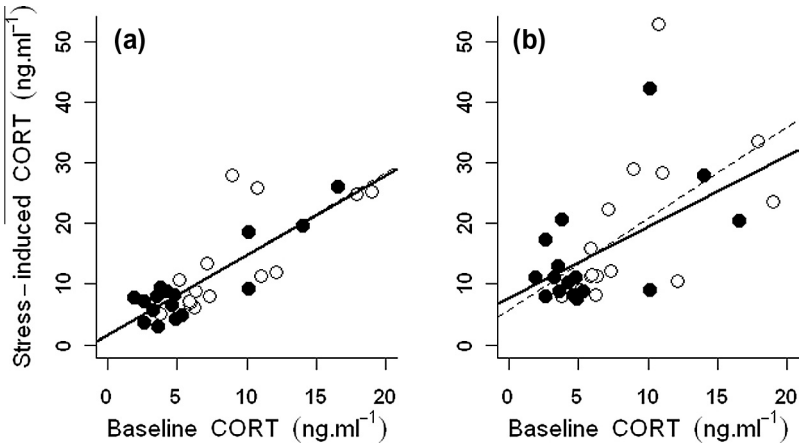

Fig. 2. Influence of baseline CORT concentration on stress-induced CORT concentration in python from cold treatment (open circle, dashed line) and warm treatment (filled circle, solid line) at (a) 30 min post handling and (b) 60 min post handling.

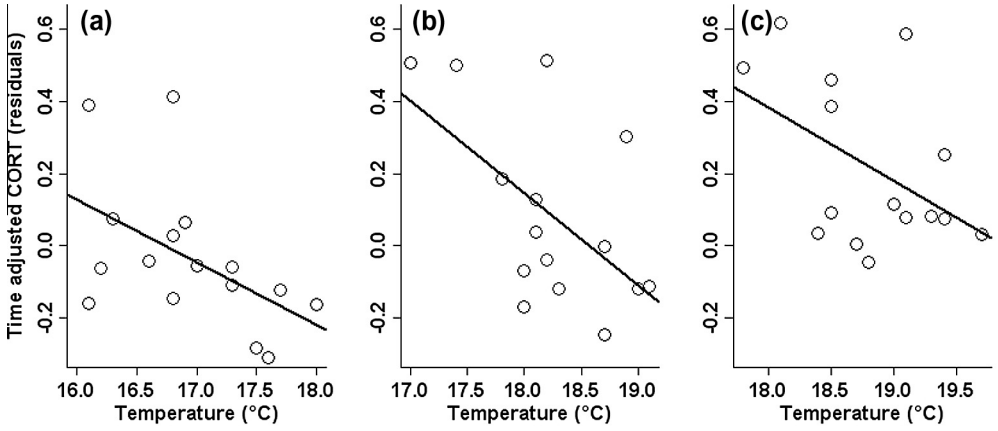

Fig. 3. Influence of temperature on time adjusted CORT concentrations (residuals from the relation between CORT and time of day), in python from cold treatment at (a) baseline concentration, (b) 30 min post handling and (c) 60 min post handling.

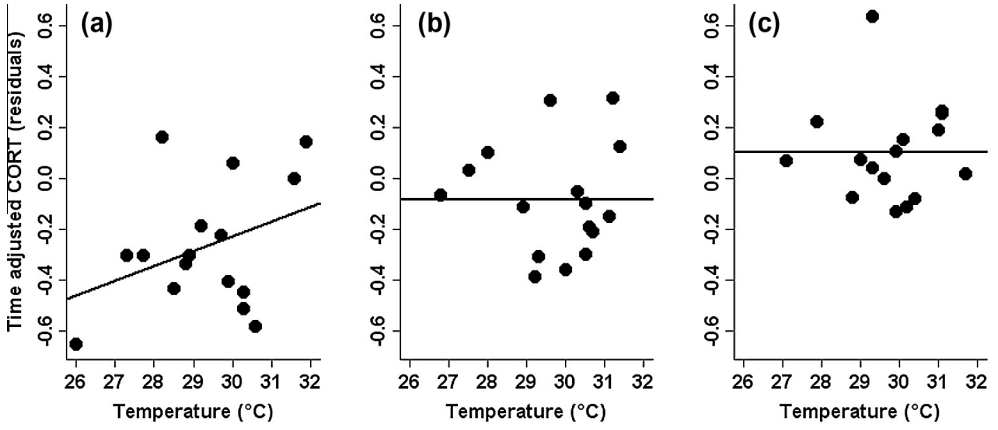

Fig. 4. Influence of temperature on time adjusted CORT concentrations (residuals from the relation between CORT and time of day), in python from warm treatment at (a) baseline concentration, (b) 30 min post handling and (c) 60 min post handling.

higher baseline CORT concentrations than those from the warm treatment. Second, the kinetic of the CORT stress response was not slowered at 17 °C. Such counter-intuitive result could be interpreted as a cold compensation mechanism that stimulates physiological and behavioural response (Landys et al., 2006; McEwen and Wingfield, 2003, 2010; Romero et al., 2009). Our study underlines that short term changes in body temperature affects absolute CORT concentrations (baseline but also stress-induced concentrations) and should therefore be considered when studying CORT concentrations in reptile species (Cree et al., 2003; Jessop et al., 2000; Jones and Bell, 2004; Romero and Wikelski, 2006; Tyrrell and Cree, 1998; Woodley et al., 2003).

How does temperature mechanistically affect the CORT stress response?

In our experiment, snakes were kept in a cold environment (17 °C) from one to two days before sampling. Our predictions were not supported which therefore advocate for alternative hypotheses to the positive relationship between CORT concentration and temperature. For example, cold temperature may have been perceived as a perturbation and, as a result, elicited an activation of the HPA axis. Although such activation might have been progressive because of reduced metabolism and physiological processes, it resulted in increased CORT concentrations within 48 h of continuous exposure. Therefore, the cold treatment may have been stressful for our captive snakes possibly because of altered locomotor performance and inducing increased in CORT concentrations.

In vertebrates, acute environmental stressors influence CORT concentrations and favour the activation of an "emergency" life-history stage (Wingfield et al., 1998) promoting immediate survival (Sapolsky et al., 2000; Wingfield et al., 1998; Wingfield and Sapolsky, 2003). We found that pythons from the cold group had higher absolute stress-induced CORT concentrations than those from the warm group. Remarkably, this suggests that the activation of the HPA axis, the secretion of CORT and its diffusion is not significantly impaired by cold temperature in this species and is less thermally sensitive than other physiological process (Angilletta, 2009; Bennett and Dawson, 1976; Stevenson et al., 1985) and all literature therein]. Accordingly, such high concentrations of CORT in response to short term exposure to low temperature have already been reported in other ectothermic organisms such as fish species (Chen et al., 2002). Temperature compensation has been largely studied in ectotherms after prolonged exposure to a temperature change (Angilletta, 2009; Huey and Berrigan, 1996). For instance, some species display a shift in metabolic rate or a modified thermal sensitivity of performance in response to low temperature (Glanville and Seebacher, 2006; Hare et al, 2010). Such adjustments aim at maintaining functional balance in a new thermal environment. Such integrated acclimation processes usually requires several days to reach a new steady state (Lucassen et al., 2003).

Alternatively, our results may simply reflect more passive effects of temperature on CORT physiology. Indeed plasma CORT concentrations do not only depend on secretion by the adrenal glands and circulation of CORT through the organism (Romero, 2004; Sapolsky et al., 2000). For example, clearance plays a major role in determining circulating CORT concentrations because it determines the rate at which CORT is catabolised. In addition, corticosteroid-binding globulin (CBG) is essential to consider: a large percentage of CORT is bound to CBGs in the blood and it seems that only free CORT can be active and catabolised (Breuner and Orchinik, 2002; Jennings et al., 2000; Mommsen et al., 1999; Siiteri et al., 1982). CORT also binds to receptors directly on tissues and such binding will affect the clearance rate. Blood concentration of CORT also depends on CORT itself through a negative feedback (Romero, 2004). Importantly, these different mechanisms are likely to be temperature-dependent but may show contrasted thermal reaction norms and therefore influence circulating concentrations. For instance, the CORT catabolism in the liver is certainly reduced when body temperature rapidly decreases due to lower enzymatic activity. Similarly, low temperature should affect the affinity of CORT to CBG and receptors and, therefore, the amount of CORT that can be cleared out of the blood. The influence of temperature on these physiological processes has never been investigated in reptiles and further studies are required to better understand the role of temperature.

Non-linear effect of temperature on CORT concentrations

Our results emphasize a complex relationship between temperature and CORT concentrations (baseline and stress-induced). That is, when snakes were imposed cold temperature, a strong negative relationship was detected between CORT concentrations (baseline and stress-induced) and temperature. In turn, this negative relationship was not supported when snakes had access to preferred body temperature (warm treatment). A positive effect of temperature on baseline CORT was found but the relation was marginal. High temperatures (upper limit) are known to be stressful in ectotherms and may therefore elicit an activation of the HPA axis. However, individuals in the warm treatment were at preferred temperature and therefore well below critical thermal maximum (Lourdais et al., 2008). Therefore, our study does not allow assessing the impact of high temperature on CORT. Still, the non-linear relationship suggests complex thermal dependence and the relation between CORT concentrations and temperature should be investigated over species thermal tolerance range.

Elevated CORT concentrations reflect suboptimal temperature in pythons?

Thermal sensitivity of performance attracted considerable interest in ectotherms (Angilletta et al., 2002; Angilletta, 2006; Martin and Huey, 2008; Somero et al., 1996). For instance, relative performance curves are typically asymmetric with a progressive increase toward optimal performance breadth (Angilletta, 2009) and then a rapid decline when body temperature gets closer to critical temperature maximum (Martin and Huey, 2008). Imposed low temperatures are likely to be a constraint resulting in impaired locomotion capacities and lower physiological performances (Angilletta, 2009; Huey and Stevenson, 1979; Huey and Kingsolver, 1989). Defensive response and ability to escape from predators are also dramatically affected by temperature (Cury de Barros et al., 2010; Herrel et al., 2007; Hertz et al., 1982). Hence, the increase of CORT concentrations in response to suboptimal temperature might stimulate compensating mechanisms, either behavioural (increase activity to evade poor thermal conditions) or physiological (increased metabolism) (AlKindi et al., 2003; Belliure et al., 2004; Preest and Cree, 2008). Importantly, this would help the individuals to maintain an alert state. In support with this hypothesis, CORT is known to influence on metabolic rate in reptiles (DuRant et al., 2008; Guillette et al., 1995; Preest and Cree, 2008) and likely stimulates locomotor activity (Belliure and Clobert, 2004; Belthoff and Dufty, 1995, 1998; Dauphin-Villemant et al., 1990; Silverin, 1997). Moreover, some reptile species display small metabolic increase at low temperature (Aleksiuk, 1971; Hare et al., 2010) and therefore at short term, CORT might be one mechanism involved in such compensation. In reptiles, metabolism is primarily driven by body temperature but CORT might allow fine-tuned regulation of metabolic rate independently of temperature.

Overall, our study demonstrates that CORT concentrations respond to short term temperature variation. Elevated baseline and stress-induced CORT concentrations may both promote immediate survival (Wingfield and Sapolsky, 2003) under constraining thermal conditions. Here, we hypothesize that the endocrine response of python could be a short term compensation to suboptimal temperature (Lance and Elsey, 1999; Li et al., 2011; Sykes and Klukowski, 2009). However, CORT concentrations should also depend on the duration, and the intensity of thermal stress. In addition, it is crucial to note that the co-occurrence of multiple stressors (cold temperature, predation risk) may be particularly demanding (very

high allostatic load *sensu* (Landys et al., 2006; McEwen and Wingfield, 2003, 2010; Romero et al., 2009) and CORT secretion may therefore depend on multiple factors. For instance, the availability of a shelter and temperature have recently been shown to interact to affect CORT concentrations in another snake species (Bonnet et al., 2013). Further studies are required to specifically test the effects of multiple stressors on the CORT stress response.

Acknowledgments

We thank Colette Trouvé, Charline Parenteau and Stéphanie Dano for their help in corticosterone assay and Xavier Duchemin for cares to pythons. We also thank Creagh Breuner for helpful comments on the manuscript. Financial support was provided by the Région Poitou-Charentes, the Département des Deux-Sèvres, the Projet BOCAGE and the CNRS.

References

Aleksiuk, M., 1971. Temperature-dependent shifts in the metabolism of a cool temperate reptile, *Thamnophis sirtalis parietalis*. Comp. Biochem. Phys. A 39, 495–503.

AlKindi, A.Y.A., Mahmoud, I.Y., Al-Siyabi, F., 2003. Physiological and behavioral adjustments relative to catecholamine levels during nesting in olive ridley (*Lepidochelys Olivacea*) and hawksbill (*Eretmochelys Imbricata*) sea turtles in Masirah Island, Oman. Sci. Tech. 8, 11–18.

Andrews, R.M., 2008. Effects of incubation temperature on growth and performance of the veiled chameleon (*Chamaeleo calyptratus*). J. Exp. Zool. Part. A 309, 435–446.

Angelier, F., Holberton, R.L., Marra, P.P., 2009. Does stress response predict return rate in a migratory bird species? A study of American redstarts and their non-breeding habitat. Proc. R. Soc. Lond. B Biol. 276, 3545–3551.

Angilletta, M.J., 2006. Estimating and comparing thermal performance curves. J. Therm. Biol. 31, 541–545.

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press, Oxford.

Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249–268.

Belliure, J., Clobert, J., 2004. Behavioral sensitivity to corticosterone in juveniles of the wall lizard, *Podarcis muralis*. Physiol. Behav. 81, 121–127.

Belliure, J., Meylan, S., Clobert, J., 2004. Prenatal and postnatal effects of corticosterone on behavior in juveniles of the common lizard, *Lacerta vivipara*. J. Exp. Zool. 301, 401–410.

Belthoff, J.R., Dufty Jr., A.M., 1995. Locomotor activity levels and the dispersal of western screech-owls, *Otus kennicottii*. Anim. Behav. 50, 558–561.

Belthoff, J.R., Dufty Jr., A.M., 1998. Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. Anim. Behav. 55, 405–415.

Bennett, A.F., Dawson, W.R., 1976. Metabolism. In: Gans, C. (Ed.), Biology of Reptilia, vol. 5. Academic Press, New York, pp. 127–224.

Blouin-Demers, G., Weatherhead, P.J., 2001a. Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. Ecology 82, 3025–3043.

Blouin-Demers, G., Weatherhead, P.J., 2001b. An experimental test of the link between foraging, habitat selection and thermoregulation in black rat snakes *Elaphe obsoleta obsoleta*. J. Anim. Ecol. 70, 1006–1013.

Blouin-Demers, G., Weatherhead, P.J., 2001c. Habitat use by black rat snakes (*Elaphe obsoleta obsoleta*) in fragmented forests. Ecology 82, 2882–2896.

Blouin-Demers, G., Weatherhead, P.J., 2002. Habitatspecific behavioural thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*). Oikos 97, 59–68.

Bonnet, X., Fizesan, A., Michel, C.L., 2013. Shelter availability, stress level and digestive performance in the aspic viper. J. Exp. Biol. 216, 815–822.

Breuner, C.W., Orchinik, M., 2002. Plasma binding proteins as mediators of corticosteroid action in vertebrates. J. Endocrinol. 175, 99–112.

Carretero, M.A., 2012. Measuring body temperatures in small lacertids: infrared vs. contact thermometers, Basic. Appl. Herpetol. 26, 99–105.

Chen, W.H., Sun, L.T., Tsai, C.L., Song, Y.L., Chang, C.F., 2002. Cold-stress induced the modulation of catecholamines, cortisol, immunoglobulin M, and leukocyte phagocytosis in tilapia. Gen. Comp. Endocrinol. 126, 90–100.

Cree, A., Tyrrell, C.L., Preest, M.R., Thorburn, D., Guillette, L.J., 2003. Protecting embryos from stress: corticosterone effects and the corticosterone response to capture and confinement during pregnancy in a live-bearing lizard (*Hoplodactylus maculatus*). Gen. Comp. Endocrinol. 134, 316–329.

Cury de Barros, F., Eduardo de Carvalho, J., Abe, A.S., Kohlsdorf, T., 2010. Fight versus flight: the interaction of temperature and body size determines antipredator behaviour in tegu lizards. Anim. Behav. 79, 83–88.

Dauphin-Villemant, C., Xavier, F., 1987. Nychthemeral variations of plasma corticosteroids in captive female *Lacerta vivipara* Jacquin: influence of stress and reproductive state. Gen. Comp. Endocrinol. 67, 292–302.

Dauphin-Villemant, C., Leboulenger, F., Xavier, F., Vaudry, H., 1990. Adrenal activity in the female lizard *Lacerta vivipara* jacquin associated with breeding activities. Gen. Comp. Endocrinol. 78, 399–413.

DuRant, S.E., Romero, L.M., Talent, L.G., Hopkins, W.A., 2008. Effect of exogenous corticosterone on respiration in a reptile. Gen. Comp. Endocrinol. 156, 126–133.

Glanville, E.J., Seebacher, F., 2006. Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. J. Exp. Biol. 209, 4869–4877.

Greenberg, N., Wingfield, J.C., 1987. Stress and reproduction: reciprocal relationships. In: Norris, D.O., Jones, R.E. (Eds.), Hormones and Reproduction in Fishes, Amphibians, and Reptiles. Plenum Press, New York, pp. 461–502.

Guillette Jr., L.J., Cree, A., Rooney, A.A., 1995. Biology of stress: interactions with reproduction, immunology and intermediary metabolism. In: Warwick, C., Frye, F.L., Murphy, J.B. (Eds.), Health and Welfare of Captive Reptiles. Chapman and Hall, London, pp. 32–81.

Hare, J.R., Whitworth, E., Cree, A., 2007. Correct orientation of a hand-held infrared thermometer is important for accurate measurement of body temperatures in small lizards and tuatara. Herpes Rev. 38, 311–315.

Hare, K.M., Pledger, S., Thompson, M.B., Miller, J.H., Daugherty, C.H., 2010. Nocturnal lizards from a cool-temperate environment have high metabolic rates at low temperatures. J. Comp. Physiol. B 180, 1173–1181.

Hau, M., 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29, 133–144.

Herrel, A., James, R.S., Van Damme, R., 2007. Fight versus flight: physiological basis for temperature-dependent behavioral shifts in lizards. J. Exp. Biol. 210, 1762–1767.

Hertz, P.E., Huey, R.B., Nevo, E., 1982. Fight versus flight: body temperature influences defensive responses of lizards. Anim. Behav. 30, 676–679.

Huey, R.B., Berrigan, D., 1996. Testing evolutionary hypotheses of acclimation. In: Johnston, I.A., Bennett, A.F. (Eds.), Animals and Temperature: Phenotypic and Evolutionary Adaptation. Cambridge University Press, Cambridge, pp. 205–237.

Huey, R.B., Kingsolver, J.G., 1989. Evolution of thermal sensitivity of ectotherm performance. Trends Ecol. Evol. 4, 131–135.

Huey, R.B., Pianka, E.R., 1977. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. Ecology 58, 1066–1075.

Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. Am. Zool. 19, 357–366.

Jennings, D.H., Moore, M.C., Knapp, R., Matthews, L., Orchinik, M., 2000. Plasma steroid-binding globulin mediation of differences in stress reactivity in alternative male phenotypes in tree lizards, *Urosaurus ornatus*. Gen. Comp. Endocrinol. 120, 289–299.

Jessop, T.S., Hamann, M., Read, M.A., Limpus, C.J., 2000. Evidence for a hormonal tactic maximizing green turtle reproduction in response to a pervasive ecological stressor. Gen. Comp. Endocrinol. 118, 407–417.

Jones, S.M., Bell, K., 2004. Plasma corticosterone concentrations in males of the skink *Egernia whitii* during acute and chronic confinement, and over a diel period. Comp. Biochem. Phys. A 137, 105–113.

Lance, V.A., Elsey, R.M., 1999. Hormonal and metabolic responses of juvenile alligators to cold shock. J. Exp. Zool. 283, 566–572.

Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen. Comp. Endocrinol. 148, 132–149.

Li, C., Gu, Y., Tang, S., Fang, H., Jiang, G., Jiang, Z., 2011. Effects of acute low temperature stress on the endocrine reactions of the Qinghai toad-headed lizard. Curr. Zool. 57, 775–780.

Lormée, H., Jouventin, P., Trouve, C., Chastel, O., 2003. Sexspecific patterns in baseline corticosterone and body condition changes in breeding Redfooted Boobies *Sula sula*. Ibis 145, 212–219.

Lourdais, O., Heulin, B., DeNardo, D.F., 2008. Thermoregulation during gravidity in the children's python (*Antaresia childreni*): a test of the preadaptation hypothesis for maternal thermophily in snakes. Biol. J. Linn. Soc. 93, 499–508.

Lucassen, M., Schmidt, A., Eckerle, L.G., Pörtner, H.O., 2003. Mitochondrial proliferation in the permanent vs. temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285, R1410–R1420.

Madsen, T., Shine, R., 1999. Life history consequences of nest-site variation in tropical pythons (*Liasis fuscus*). Ecology 80, 989–997.

Martin, T.L., Huey, R.B., 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. Am. Nat. 171, E102–E118.

Mathies, T., Felix, T.A., Lance, V.A., 2001. Effects of trapping and subsequent short-term confinement stress on plasma corticosterone in the brown treesnake (*Boiga irregularis*) on Guam. Gen. Comp. Endocrinol. 124, 106–114.

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15.

McEwen, B.S., Wingfield, J.C., 2010. What's in a name? Integrating homeostasis, allostasis and stress. Horm. Behav. 57, 105–111.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fisher 9, 211–268.

Peterson, C.R., 1987. Daily variation in the body temperatures of free-ranging garter snakes. Ecology 68, 160–169.

Preest, M.R., Cree, A., 2008. Corticosterone treatment has subtle effects on thermoregulatory behavior and raises metabolic rate in the New Zealand common gecko, *Hoplodactylus maculatus*. Physiol. Biochem. Zool. 81, 641–650.

Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. Trends Ecol. Evol. 17, 462–468.
Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128, 1–24.
Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19, 249–255.
Romero, L.M., Wikelski, M., 2006. Diurnal and nocturnal differences in hypothalamicpituitaryadrenal axis function in Galapagos marine iguanas. Gen. Comp. Endocrinol. 145, 177–181.
Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope modelrama new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55, 375–389.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrinol. Rev. 21, 55–89.
Scholander, P.F., Hock, R., Walters, V., Irving, L., 1950. Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. Biol. Bull. 99, 259–271.
Siiteri, P.K., Murai, J.T., Hammond, G.L., Nisker, J.A., Raymoure, W.J., Kuhn, R.W., 1982. The serum transport of steroid hormones. Recent Prog. Horm. Res. 38, 457–510.
Silverin, B., 1997. The stress response and autumn dispersal behaviour in willow tits. Anim. Behav. 53, 451–459.
Somero, G.N., Dahlhoff, E., Lin, J.J., 1996. Stenotherms and eurytherms: mechanisms establishing thermal optima and tolerance ranges. In: Johnston, I.A., Bennett, A.F. (Eds.), Animal and Temperature. Cambridge University Press, Cambridge, pp. 53–78.
Stevenson, R.D., Peterson, C.R., Tsuji, J.S., 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. Physiol. Zool. 58, 46–57.
Sykes, K.L., Klukowski, M., 2009. Effects of acute temperature change, confinement and housing on plasma corticosterone in water snakes, *Nerodia sipedon* (Colubridae: Natricinae). J. Exp. Zool. 311, 172–181.
Tieleman, B.I., Williams, J.B., Buschur, M.E., 2002. Physiological adjustments to arid and mesic environments in larks (Alaudidae). Physiol. Biochem. Zool. 75, 305–313.
Tyrrell, C.L., Cree, A., 1998. Relationships between corticosterone concentration and season, time of day and confinement in a wild reptile (Tuatara, *Sphenodon punctatus*). Gen. Comp. Endocrinol. 110, 97–108.
Wilson, S., Swan, G., 2003. A Complete Guide to Reptiles of Australia. New Holland, Sydney.
Wingfield, J.C., 1994. Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. Horm. Behav. 28, 1–15.
Wingfield, J.C., 2003. Control of behavioural strategies for capricious environments. Anim. Behav. 66, 807–816.
Wingfield, J.C., 2012. Regulatory mechanisms that underlie phenology, behavior, and coping with environmental perturbations: an alternative look at biodiversity. Auk 129, 1–7.
Wingfield, J.C., 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Funct. Ecol. 27, 37–44.
Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. 15, 711–724.
Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormonebehavior interactions: the "emergency life history stage". Am. Zool. 38, 191–206.
Wingfield, J.C., Kelley, P.J., Angelier, F., 2011. What are extreme environmental conditions and how do organisms cope with them? Curr. Zool. 57, 363–374.
Woodley, S.K., Painter, D.L., Moore, M.C., Wikelski, M., Romero, L.M., 2003. Effect of tidal cycle and food intake on the baseline plasma corticosterone rhythm in intertidally foraging marine iguanas. Gen. Comp. Endocrinol. 132, 216–222.