TECHNICAL COMMENT

Experimental Manipulation of Air Temperature in Captivity Appears Unsuitable for Evaluating Fecal Glucocorticoid Metabolite Responses of Wild-Caught Birds to Heat Exposure

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ABSTRACT

Noninvasive measurement of stress-related alterations in fecal glucocorticoid metabolite (fGCM) concentrations has considerable potential for quantifying physiological responses to very hot weather in free-ranging birds, but practical considerations related to sampling will often make this method feasible only for habituated study populations. Here we evaluate an alternate approach, the use of experimentally manipulated thermal environments for evaluating stress responses to high environmental temperatures in wild-caught birds housed in captivity. Using an enzyme immunoassay utilizing antibodies against 5ß-pregnane-3α,11ß,21-triol-20-one-CMO:BSA (tetrahydrocorticosterone), we quantified fGCMs in captive individuals of three southern African arid-zone species (southern pied babblers [*Turdoides bicolor*], white-browed sparrow-weavers [*Plocepasser mahali*], and southern yellow-billed hornbills [*Tockus leucomelas*]) experiencing daily air temperature maxima (T_{max}) ranging from 30°–32°C to 42°–44°C. For none of the three species did T_{max} emerge as a significant predictor of elevated fGCM concentrations, and no stress response to simulated hot weather was evident. The apparent lack of a stress response to $T_{max} = 42$°C in captive southern pied babblers contrasts with linear increases in fGCMs at $T_{max} >$ 38°C in free-ranging conspecifics. The lack of an effect of T_{max} on fGCM levels may potentially be explained by several factors, including differences in operative temperatures and the availability of water and food between free-ranging and captive settings or the stress effect of captivity itself. Our results suggest that experimental manipulations of thermal environments experienced by wild-caught captive birds have limited usefulness for testing hypotheses concerning the effects of hot weather events on fGCM (and, by extension, glucocorticoid) concentrations.

Keywords: captive birds, fecal corticosterone metabolites, heat stress, noninvasive hormone monitoring, stress.

Introduction

Wild birds often encounter unpredictable environmental perturbations such as extreme weather events with little or no warning (Wingfield and Kitaysky 2002; Breuner and Hahn 2003). To survive these events, individuals must respond rapidly and appropriately via responses that usually include an activation of the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) axes (Harvey et al. 1984; Wingfield et al. 1998; Romero 2002; de Bruijn and Romero 2011). Those neuroendocrine responses culminate, inter alia, in increased secretion of glucocorticoids (GCs; i.e., steroid hormones involved in a wide range of physiological activities, including the vertebrate stress response). Increased GC concentrations facilitate the redirection of resources toward physiological and behavioral changes that promote survival, often at the expense of regular life-history stages such as reproduction (Wingfield et al. 1998; Sapolsky et al. 2000). If sustained over long periods because of chronic stressors, however, high GC concentrations can negatively impact fitness at both individual and population levels through immune suppression and reproductive dysfunction (Sapolsky et al. 2000; Romero and Butler 2007).

Currently, literature regarding avian stress responses to extreme weather is mostly restricted to high-latitude species in north-temperate regions, with snowstorms and blizzards often associated with increased GC concentrations and reduced concentrations of reproductive steroid hormones (Wingfield et al. 1982, 1983;

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Astheimer et al. 1995; Frigerio et al. 2004). However, stress-related adrenocortical responses are also thought to be triggered by air temperature (T_{air}) approaching or exceeding normothermic body temperature on extremely hot days (Siegel 1980; Xie et al. 2017). The direct effects of high T_{air} during heat waves include lethal hyperthermia or dehydration (Finlayson 1932; Saunders et al. 2011; McKechnie et al. 2021) as well as sublethal fitness costs arising from chronic exposure to sustained hot weather (du Plessis et al. 2012; Cunningham et al. 2013; Conradie et al. 2019; van de Ven et al. 2019, 2020). The sublethal fitness costs reported in these studies occur because of behavioral trade-offs between foraging and heat dissipation behaviors, such as panting and resting in shaded microsites, and involve loss of body mass or reduced breeding success during hot weather.

Recent evidence from a southern African arid-zone passerine, the southern pied babbler (*Turdoides bicolor*), suggests that birds perceive hot days associated with sublethal fitness costs related to body mass or breeding success as acute stressors (Moagi et al. 2021). In the habituated population of babblers examined by those authors, levels of fecal GC metabolites (fGCMs) were unrelated to daily maximum air temperature (T_{max}) at $T_{max} < 38$°C but increased linearly at $T_{max} > 38$°C. Taken together with previous evidence of activation of the HPA axis by heat exposure (Siegel 1980; Xie et al. 2017), these findings suggest that stress physiology is likely to prove essential for understanding the physiological responses of birds to anthropogenic climate change. Global heating is anticipated to substantially increase the risks of both acute, lethal effects and chronic, sublethal effects for arid-zone birds during the course of the twenty-first century (McKechnie and Wolf 2010; Albright et al. 2017; Conradie et al. 2019, 2020).

Quantifying avian stress responses to hot weather by directly measuring circulating GCs (corticosterone in birds) is challenging for several reasons. Although GCs can be measured in blood samples collected from free-ranging birds immediately after capture and before the rapid increase in GCs associated with capture and handling (Wingfield et al. 1982; Romero and Reed 2005), birds in hot climates typically curtail activity during the hottest part of the day and remain in shaded microsites, making capture during the heat of day difficult. The use of fGCMs as a proxy for circulating GCs (Millspaugh and Washburn 2004; Touma and Palme 2005; Sheriff et al. 2011) is a suitable alternative approach, as demonstrated by Jepsen et al. (2019) and Moagi et al. (2021), but stress-free serial sample collection from free-ranging birds in natural habitats will often be feasible only for habituated study populations, particularly if fecal samples need to be linked to the individuals that produced them.

The limitations associated with sample collection from wild birds mean that, for many species, experimental manipulations of thermal conditions experienced by birds in a captive setting may be a more feasible alternative approach to examining stress responses to heat exposure. In addition to ease of sample collection, captive environments permit experimental manipulation of multiple potential predictor variables (Calisi and Bentley 2009). Measuring stress-related biomarkers in captive settings where thermal environments can be manipulated is a potentially useful technique for testing hypotheses about the role of stress-related physiological pathways in avian stress responses to heat exposure. In light of evidence that very hot days are experienced as stressors by an arid-zone passerine, here we tested the hypothesis that high T_{max} is experienced as a stressor in captive conditions. We predicted that high T_{max} triggers increases in fGCM levels in captive populations of three southern African arid-zone species, southern pied babblers (*T. bicolor*), white-browed sparrow-weavers (*Plocepasser mahali*), and southern yellow-billed hornbills (*Tockus leucomelas*). We selected these species as they have been the focus of several recent studies exploring physiological or behavioral determinants of vulnerability to rising T_{air} (du Plessis et al. 2012; Smit et al. 2013; Noakes et al. 2016; van de Ven et al. 2019, 2020; Bourne et al. 2020). We used wild-caught individuals temporarily housed indoors in temperature-controlled rooms to examine whether fGCM concentrations vary with T_{max} values between 30° and 44°C. These values range from below to considerably above threshold T_{max} values corresponding with the onset of sublethal fitness costs among wild populations of babblers (du Plessis et al. 2012; Bourne et al. 2020) and hornbills (van de Ven et al. 2019, 2020).

Material and Methods

Capture and Maintenance

Southern pied babblers (*Turdoides bicolor*; $n = 25$; June 2018) and southern yellow-billed hornbills (*Tockus leucomelas*; $n = 10$; May 2019) were caught using spring traps (both species) or walk-in traps (babblers) baited with superworms (*Zophobas morio*) at Radnor Farm (26°11′E, 22°88′S; 1,014 m asl), approximately 40 km from the village of Vorstershoop in South Africa's North West Province. On capture, babblers were individually marked using plastic color rings, while hornbills were fitted with uniquely numbered aluminum rings to allow for individual identification. At the capture site, babblers were kept as social groups, while hornbills were kept as breeding pairs in outdoor aviaries. Each group/pair occupied an aviary (3.48 m long × 1.74 m wide × 1.74 m high) constructed from aluminum square tubing and wire mesh, with a layer of shade cloth inside each mesh panel to prevent injuries and to provide shade for the birds. During the day, babbler groups occupied their respective aviaries, while overnight, each group was transferred to a smaller cage (61 cm long × 43 cm high × 51 cm wide) and kept indoors to prevent harassment by nocturnal predators (following Jepsen et al. 2019). White-browed sparrow-weavers (*Plocepasser mahali*; $n = 28$) were caught at night using two small nets mounted on aluminum poles placed over the entrances of roost nests in February 2019 at the Barberspan Bird Sanctuary (26°33′S, 25°36′E), approximately 17 km northwest of Delareyville, North West Province. At the capture site, sparrow-weavers were individually marked with plastic color rings and kept in pairs (a male and a female) in cages (61 cm long × 43 cm high × 51 cm wide) placed in well-ventilated rooms.

Birds were kept at the various capture sites until sufficient numbers were caught (14 d for babblers and 3 d for hornbills and sparrow-weavers). The birds were then transported by road in modified pet carriers (babblers and sparrow-weavers) or individual cloth bags (hornbills) to the University of Pretoria (UP) Small Animal Physiological Research Facility (25°45′S, 28°15′E).

On arrival, the babblers and sparrow-weavers were transferred to individual cages (61 cm long × 43 cm high × 51 cm wide) at 25°C in temperature-controlled rooms with a photoperiod of 12L : 12D (light period, 0600–1800 hours), while the hornbills were transferred to separate outdoor aviaries (one pair each). Four aviaries were 5.5 m long × 1.8 m high × 1.8 m wide, and one was 5.0 m long × 2.5 m high × 2.0 m wide. Food (babblers: only superworms; hornbills: superworms and hard-boiled eggs; sparrow-weavers: superworms and birdseed) and water were provided ad lib. throughout the study.

The babblers and sparrow-weavers were allowed 2–3 wk to habituate to the new environment, after which experiments commenced. Hornbills remained in the outdoor aviaries for a 6-wk habituation period. Thereafter, the hornbills were transferred into two temperature-controlled rooms within the Small Animal Physiological Research Facility. One room housed three pairs, and the other housed two pairs. Each pair was placed in a cage constructed from aluminum square tubing and nylon bird netting (1.8 m long × 1 m wide × 1 m high) outfitted with perches and water bowls, with each cage divided in half by a separator made of plastic mesh. This functioned to separate paired individuals (to ensure that samples were assigned to the correct individuals) in such a way that they were still in proximity to and in view of their mate in an attempt to limit the perceived stress of separation. The birds were kept at a constant T_{air} of 20°C and a photoperiod of 12L : 12D (light period, 0600–1800 hours) for 14 d to acclimate to the indoor conditions, after which experiments commenced.

Temperature Treatment Protocol

Southern Pied Babblers and White-Browed Sparrow-Weavers. During experiments (babblers: July–September 2018; sparrow-weavers: February–May 2019), all individuals experienced circadian cycles of T_{air}, with night T_{air} = 20°C and diurnal T_{air} following a profile intended to approximate the shape of natural daily T_{air} cycles. Babblers and sparrow-weavers were housed in three temperature-controlled rooms corresponding with three treatments: cool (T_{max} = 32°C), moderate (T_{max} = 37°C), and hot (T_{max} = 42°C). Each group experienced the same T_{air} regime for the entirety of the experimental period (10 wk). In each room, T_{air} started increasing at 0700 hours, and T_{max} occurred between 1200 and 1400 hours, whereafter T_{air} declined to 20°C by 1900 hours. Fecal samples were collected on a weekly basis (between 1200 and 1400 hours) from the week of arrival at the facility to week 10 of the experimental period. Fecal samples were collected with forceps from wax paper lining the floor of each cage within 1 h of defecation to prevent degradation at room temperature. Samples were then transferred to 2-mL Eppendorf tubes and immediately frozen at −20°C. At the end of the 10-wk experimental period, air temperatures were set to a constant 25°C for 1 wk, after which the birds were returned to the site of capture and released.

Southern Yellow-Billed Hornbills. During experiments on the hornbills (July–August 2019), we used a protocol different from that used for the babblers and sparrow-weavers, with hornbills in each of the rooms experiencing each of three T_{max} values (cool: T_{max} = 30°C; moderate: T_{max} = 37°C; hot: T_{max} = 44°C) in random order. Daily T_{air} profiles commenced at 0600 hours and consisted of three 4-h segments. During the first segment, T_{air} increased from the overnight 20°C to the treatment T_{max}, the second segment consisted of 4 h at T_{max}, and during the third segment, T_{air} decreased from T_{max} to 20°C (the T_{air} was maintained overnight). The experiment lasted for 30 d, with each treatment temperature randomly allocated to 10 nonconsecutive days of the experimental period to ensure that exposure to the different light phase maxima remained unpredictable. Each day at 1400 hours (i.e., at the end of the 4-h constant T_{max} segment to accommodate the 3-h fGCM lag time in this species; Bouwer et al. 2021), fecal samples were collected from a removable tray made of a plastic waterproof material, which facilitated the cleaning process after sample collection. After the 30-d experimental period, the birds were transferred back to the outdoor aviaries, where they remained for a 3-wk rest period before being returned to the site of capture, with pairs released close to their original territories.

Fecal Steroid Extraction and Analysis

All fecal samples were frozen at −20°C within 20 min of collection and kept frozen until steroid extraction at the Endocrine Research Laboratory, UP. The frozen samples were lyophilized and pulverized before the addition of 1.5 mL of 80% ethanol in distilled water to 0.050–0.055 g of the fecal powder. The mixture was then vortexed for 15 min to facilitate steroid extraction following Ganswindt et al. (2002). After centrifuging for 10 min at 1,500 *g*, the supernatant was transferred into microcentrifuge tubes and stored at −20°C. Immunoreactive fGCMs were quantified using an enzyme immunoassay (EIA) utilizing antibodies against 5ß-pregnane-3α,11ß,21-triol-20-one-CMO : BSA (tetrahydrocorticosterone). Assay characteristics, including cross-reactivities, are given by Quillfeldt and Möstl (2003). In previous studies, this EIA was validated for the reliable quantification of fGCMs in southern pied babblers (Jepsen et al. 2019), white-browed sparrow-weavers (C. A. Ngcamphalala, S. W. Nicolson, A. Ganswindt, and A. E. McKechnie, unpublished data), and southern yellow-billed hornbills (Bouwer et al. 2021).

The coefficients of variation (CVs) for intra-assay variance determined by repeated measurements of high- and low-quality controls were 6.33% and 6.64% for babblers and hornbills and 5.30% and 7.80% for sparrow-weavers. The interassay CVs, also determined by repeated measurements of high- and low-quality controls, were 9.90% and 14.66% for babblers, 13.6% and 14.4% for sparrow-weavers, and 10.80% and 14.43% for hornbills. The sensitivity of the assay was 9.0 ng/g of fecal dry weight (DW) for babblers and hornbills and 12.0 ng/g DW for sparrow-weavers. Serial dilutions of fecal extracts gave displacement curves that were parallel to the respective standard curve, with the relative variation of the slope of the trend lines being <2% for the hornbills and <4% for the sparrow-weavers and babblers.

Statistical Analyses

All statistical analyses were run in R version 4.0.4 (R Core Team 2021). fGCM data were $\log_{10}$ transformed and normality confirmed

by visual inspection of quantile-quantile plots. For each species, variation in fGCM concentrations in response to daily T_{max} was modeled by fitting a linear mixed model that included T_{max} and Julian day (hornbills) or weeks in captivity (babblers and sparrow-weavers) as predictors and individual identity as a random effect using the R package nlme (Pinheiro et al. 2013).

Results

Treatment T_{max} did not emerge as a significant predictor of fGCM concentrations in any of the three study species (table 1; figs. 1, 2). The time spent in indoor housing did not influence fGCM concentrations in babblers ($F_{1, 247} = 0.233$; $P = 0.630$) or sparrow-weavers ($F_{1, 360} = 2.170$; $P = 0.142$). In contrast, Julian day emerged as a significant ($F_{1, 249} = 4.660$; $P = 0.032$) predictor of fGCM concentration in hornbills, with fGCM levels declining during the period in captivity. The hornbills' fGCM levels during the last 3 d of the experiment were 0.568 ± 0.207 μg/g DW, equivalent to 80.1% of the values during their first 3 d in captivity. For the babblers, fGCM levels in captive individuals in this study were approximately ninefold higher than those previously measured in free-ranging conspecifics in the Kalahari Desert (fig. 1).

Discussion

We found no evidence for an increase in fGCM concentrations in response to hot conditions in any of our three study species. This was a somewhat unexpected finding in light of the significant short-term increases in fGCM concentrations in free-ranging southern pied babblers (*Turdoides bicolor*) at $T_{max} > 38$°C (Moagi et al. 2021) and increases in circulating GC concentrations at high T_{max} in at least one species investigated in captivity, diamond doves (*Geopelia cuneata*; Xie et al. 2017). Additionally, elevations in circulating avian GC concentrations are typically observed in response to temperature increases (de Bruijn and Romero 2018). We expected a pronounced adrenocortical response, particularly at the higher T_{max} values (42° and 44°C) because these are above T_{max} values that represent ecologically important thresholds for body mass maintenance and breeding success in species including the babblers and hornbills (du Plessis et al. 2012; van de Ven et al. 2019, 2020; Bourne et al. 2020). Our findings are, however, consistent with those of Xie et al. (2017) for zebra finches (*Taeniopygia guttata*) and budgerigars (*Melopsittacus undulatus*), in which no significant changes in plasma GC concentrations occurred after exposure to $T_{air} = 45$°C. These observations led Xie et al. (2017) to argue that GC responses to fluctuations in environmental temperatures may be species specific. However, increases in fGCMs occur on very hot days in free-ranging populations of at least one of our study species (babblers; Moagi et al. 2021), confirming that responses may differ between free-ranging and captive conspecifics.

There are several potential explanations for the lack of an effect of high T_{max} on fGCM concentrations in the birds in our study. First, T_{max} experienced by birds housed indoors may not be equivalent to the thermal conditions experienced by free-ranging conspecifics in natural habitats. Operative temperatures (a measure that integrates the effects of T_{air} and radiative, conductive, and convective heat flux; Robinson et al. 1976; Bakken 1992) experienced by wild birds in natural landscapes can be as much as 10°–15°C above T_{air} for small birds in sunlit microsites (Wolf and Walsberg 1996*b*; van de Ven et al. 2019). Thus, the physiological challenges posed by daily $T_{max} = 44$°C in captivity may not be equivalent to those posed by $T_{max} = 44$°C on a hot day in natural settings.

Another potential explanation for the lack of a relationship between T_{max} and fGCM concentrations is that the birds did not perceive the experimental temperatures as stressors because they had unrestricted access to water (Xie et al. 2017). Those authors argued that constant access to water permits birds to replenish body water and offset any increased water loss rates, removing a potential source of stress associated with high temperatures. Brischoux et al. (2020) found higher GC concentrations in water-deprived house sparrows (*Passer domesticus*) than in conspecifics

Table 1: Relationship between maximum daily air temperature (T_{max}) and fecal glucocorticoid metabolite (fGCM) concentrations in three southern African bird species experiencing experimentally manipulated thermal environments while housed indoors

	fGCM concentration (μg/g DW)		Effect of T_{max} on fGCM		
Species, T_{max} (°C)	Mean ± SE	Range	df	*F*	*P*
Southern pied babbler (*Turdoides bicolor*)			1, 247	.216	.643
32	1.32 ± .07	.36–3.51			
37	1.20 ± .05	.28–2.64			
42	1.07 ± .05	.36–2.81			
White-browed sparrow-weaver (*Plocepasser mahali*)			1, 360	.337	.562
32	.93 ± .04	.13–2.13			
37	.94 ± .06	.06–5.85			
42	.95 ± .09	.08–11.46			
Southern yellow-billed hornbill (*Tockus leucomelas*)			1, 249	.097	.756
30	.64 ± .03	.17–1.52			
37	.72 ± .03	.22–1.81			
44	.63 ± .03	.18–1.47			

Note. DW = dry weight.

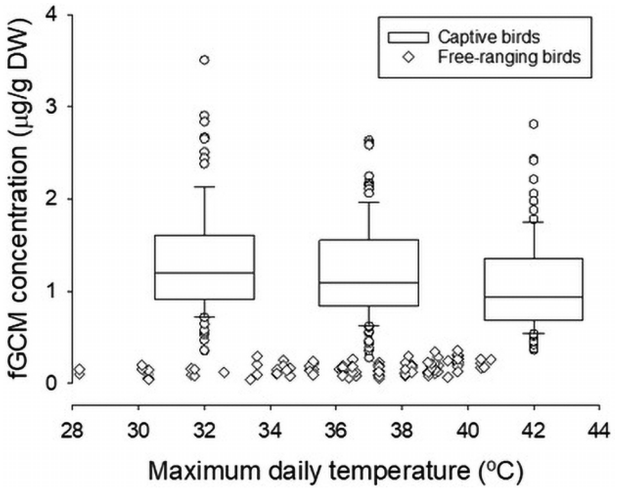

Figure 1. Fecal glucocorticoid metabolite (fGCM) concentrations in wild-caught southern pied babblers (*Turdoides bicolor*) experiencing experimentally manipulated thermal environments while housed indoors were not significantly affected by daily air temperature maxima (T_{max}). Captive birds (boxplots, circles) had higher fGCM concentrations than free-ranging individuals (diamonds; data from Moagi et al. 2021) at similar T_{max} under natural conditions. DW = dry weight.

with access to water, suggesting that restricted access to water is a significant stressor for birds. One of the major challenges posed by high temperatures is elevated water requirements for evaporative cooling (Wolf and Walsberg 1996*a*, 1996*b*). In their natural habitats at high T_{air}, our study species would have had to employ thermoregulatory behaviors such as panting and shade seeking, which are known to reduce foraging success and, by extension, water intake in a wide range of arid-zone species (Smit et al. 2019), including southern pied babblers (du Plessis et al. 2012) and southern yellow-billed hornbills (van de Ven et al. 2019).

It is also possible that increased GC levels in wild birds at high temperatures may be a response to the sublethal effects of high T_{air} rather than a direct response to T_{air} per se. For instance, the elevated fGCM concentrations of the free-ranging babblers in the study by Moagi et al. (2021) may have resulted from constraints on foraging associated with high T_{air} as a stressor, rather than from T_{max} (>38°C) per se. Inclement weather, often associated with low food availability, has been shown to elicit an increase in GC levels in wild birds in cold environments (Schwabl et al. 1985; Wingfield 1988; Frigerio et al. 2004), and there is often a negative correlation between avian body condition and GC concentrations (both baseline and stress induced; Kitaysky et al. 1999; Breuner and Hahn 2003; Fodikis et al. 2011). Additionally, the high mass-specific metabolic rates of small birds make them highly susceptible to short-term food restrictions (Fodikis et al. 2011).

Another possibility is that birds did perceive high T_{max} as a stressor in our study but that the effect on fGCM concentrations was swamped by stress associated with confinement in captivity. Captivity can be a significant stressor for wild birds (Morgan and Tromborg 2007; Fischer et al. 2018; Jepsen et al. 2019). There is almost no overlap in fGCM levels between the captive babblers and free-ranging conspecifics (fig. 1). In a previous study, wild-caught babblers held captive for a few days for an adrenocorticotropic hormone challenge validating fGCMs as a proxy for circulating GCs had 50-fold higher fGCM concentrations compared with free-ranging conspecifics (Jepsen et al. 2019). Captivity-induced stress is typically a response to factors including the capture event itself, transport and handling (Nilsson et al. 2008; Dickens et al. 2009), and exposure to an unnatural environment (e.g., artificial light and noise and a general lack of stimulation or appropriate social housing; Young 2003; Morgan and Tromborg 2007; Calisi and Bentley 2009; Emmerson and Spencer 2018).

The birds in our study showed little evidence of habituation to captive conditions over the 10-wk experimental period. Time in captivity did not affect fGCM concentrations in babblers and sparrow-weavers, although the significance of Julian day as a predictor of fGCM in hornbills raises the possibility that some degree

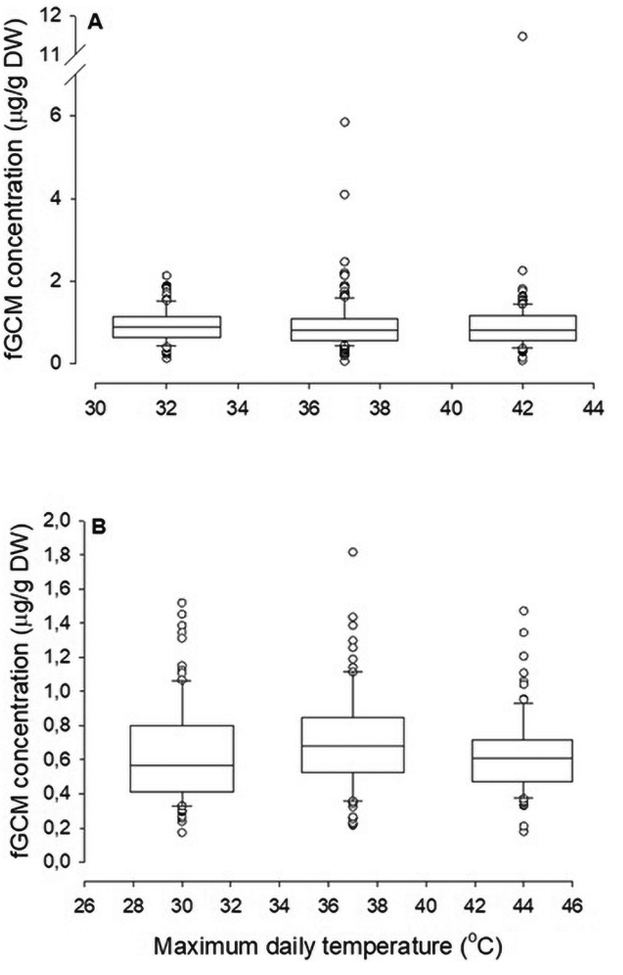

Figure 2. Fecal glucocorticoid metabolite (fGCM) concentrations in white-browed sparrow-weavers (*Plocepasser mahali*; *A*) and southern yellow-billed hornbills (*Tockus leucomelas*; *B*) experiencing experimentally manipulated thermal environments while housed indoors were not significantly affected by daily maximum air temperatures. DW = dry weight.

of habituation was in fact taking place (Cyr and Romero 2009). Although it is often assumed that animals habituate to confinement in captivity, some species appear not to do so (Mason 2010; Love et al. 2017; Fischer and Romero 2018). In their review of GC responses to captivity in vertebrates, Fischer and Romero (2018) found that 45% of the wild-caught species in their study maintained elevated GC concentrations after 3 mo or more of captivity. Being a long-term (chronic) stressor, captivity may alter GC regulation (Dickens and Romero 2013; Fischer and Romero 2018).

Whereas experimental manipulations of thermal conditions in captivity are potentially suitable for testing hypotheses about the role of physiological stress pathways in the modulation of avian responses to heat exposure, our results here reveal that wild-caught birds housed temporarily in captivity over timescales of weeks to months may not be suitable study systems. The obvious alternative is to use captive-bred birds that do not perceive confinement in captivity as a stressor, and stress-related alterations in GC concentrations in response to environmental conditions, including T_{air}, have been demonstrated in captive-bred zebra finches (*T. guttata*; Spencer et al. 2010; Jimeno et al. 2017). However, captive-bred populations are available for only a small subset of species, and the physiological traits of captive-bred and free-ranging conspecifics can differ in consequential ways (Geiser et al. 2000; McKechnie et al. 2006; Homberger et al. 2013, 2014). Given the current rapid pace of anthropogenic global heating, there is an urgent need to develop practical and noninvasive experimental protocols to elucidate the effects of hot weather events on avian stress physiology and to better understand the role of the HPA and SAM axes in modulating avian responses to increasing heat exposure in natural habitats.

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