Endocrine correlates of gender and throat colouration in the Southern Ground-Hornbill (*Bucorvus leadbeateri*)

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Abstract

The southern ground-hornbill (SGH) is a cooperatively breeding bird endemic to east and southern Africa, but is endangered in its southern distributional range. The national conservation restoration programme harvests redundant chicks for captive breeding and reintroduction; with sexing and social grouping of the species evaluated by the determination of throat-skin colouration, with adult males displaying a completely red colour compared to and a panel of dark blue within the red observed in adult females. However, recent findings indicate that both dominant and subordinate adult males exhibit patches of blue throat-skin. To optimize SGH management practices for reintroductions and captive breeding, it is vital to determine the role of red and blue colouration, as well as the possible drivers thereof. As a prerequisite, an enzyme immunoassay for monitoring faecal androgen metabolite (fAM) concentrations in SGH was established. Following this, fresh faecal samples were collected from 78 SGH, of various demographics and origin, across 12 captive institutions, to determine whether fAM concentrations differ between blue (B), partially blue (sB) and fully red (R) skin-throat coloured male SGH. Furthermore, fAM concentrations were compared between males housed in different social groups of different age and sex classes (M/F, M/M). Individual median fAM concentrations of blue (B), partially blue (sB) and fully red (R) adult males did not differ significantly, but were considerably higher in B and sB males compared to R males. Social dynamics within captivity played no role as a driver of male gonadal activity or throat skin coloration. The results of the study indicate that androgens and apparent social dynamics are not the only determinants of throat colouration in male SGH.

Keywords: southern ground-hornbill; coloration; validation; faecal glucocorticoid metabolites; non-invasive

Introduction

The increase in human populations has led to a considerable decrease in the density and distribution range of some of the most enigmatic avian species globally (McKee et al., 2004; Pimm et al., 2006). In order to conserve many of the most threatened species from extinction, the implementation of intense management protocols is required (Gittleman et al., 2002; Runge et al., 2015). However, with limited information available on the reproductive biology of many bird species, it is impossible to formulate and implement ideal management protocols within both captive and free-ranging environments. As such, there is an urgent need for conservationists to supplement the limited information about threatened avian species by conducting research that will enhance management actions and improve species survivability.

The southern ground-hornbill (*Bucorvus leadbeateri*, hereafter SGH) is currently listed as vulnerable by the IUCN Redlist (Birdlife International, 2016), with a decreasing population trend throughout its natural distribution. Recent findings suggest that there has been a 65% reduction in the natural distribution and population size of the species within southern Africa across three generations (Wilson and Hockey, 2013). In addition to anthropogenic activities leading to population declines, several biological parameters inherent in the species has led to limited recruitment of new individuals into the population (Engelbrecht et al., 2007; Msimanga, 2004). As the largest cooperative avian breeder, only one male-female breeding pair is present within a social group (2-11 individuals), with the subordinate animals assisting in chick rearing (Kemp and Kemp, 1980; Wilson and Hockey, 2013). Furthermore, the species is known to have a low breeding success rate, with a monogamous breeding pair rearing a single chick every 9.3 years on average (Kemp, 1988). Finally, due to their comparatively large body size (2-6 kg), individuals require large cavities in trees and rock faces as nest sites. Such nest sites within their natural environment are, however, an increasingly limited resource (Carstens et al., 2019; Kemp and Joubert, 1989). As a consequence of these compounding factors, several breeding and management programmes have been developed for the species within both captive and free-ranging environments to assist with the breeding success and survival of the species (Hulley and Craig, 2007). As sexual dimorphism is absent in this species, managers regularly use throat-skin colouration as an indication for sexing to pair breeding birds in managed populations, with the hope of increasing reproductive success. The throat skin in adult

males is thought to be completely red, while females present red throat skin with a blue-violet patch and sub-adults a cream colouration (Fig. 1) (Hockey et al., 2005; Kemp and Kemp, 1980). These distinguishable colourations were thought to be inherent in the species, but the underlying physiological mechanisms responsible for colouration are currently unknown. However, there have been several reported incidences from managers at conservation and breeding facilities of male individuals of varying age, origin, and social status displaying female-like throat coloration. That deviation will have considerable implications for managed breeding populations, as well as, the release of new populations into the wild. Therefore, in order to assist conservation efforts of the SGH, it is of the utmost importance to understand the physiological drivers and variations of throat-skin colouration between and among sexes.

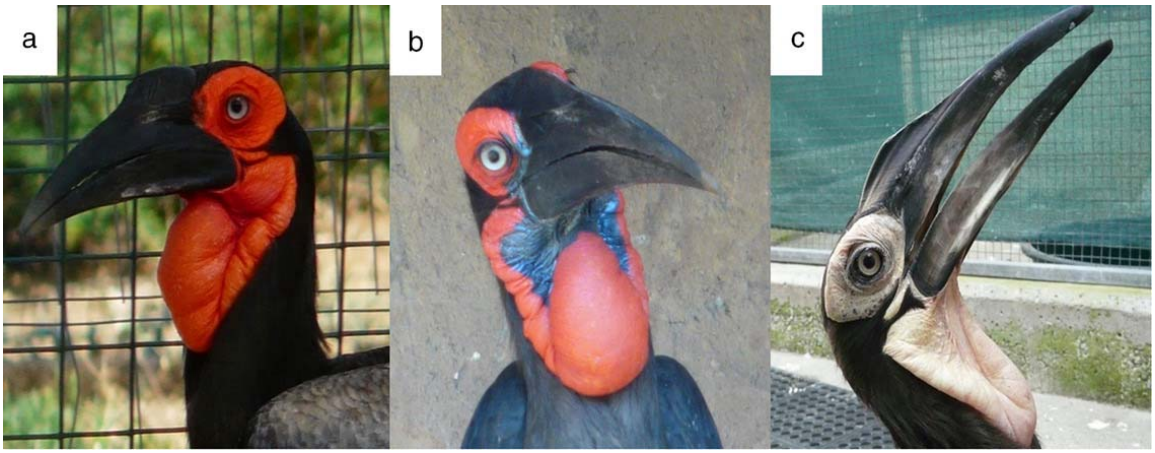

Figure 1. Photographic examples of typical adult male (a), adult female (b), and subadult (c) throat coloration.

Bare-skin and plumage coloration in vertebrate species act as an important biological signal, conveying important information regarding individual health, fitness and defensive abilities (Blount and McGraw, 2008; Cooper and Greenberg, 1992; Noonan and Comeault, 2009; Václav et al., 2007). Research on avian species have shown that plumage and bare-skin coloration indicate aspects of male fitness, age, sex, sexual status, and mate quality (Andersson, 1994; Delhey et al., 2007). Androgens and carotenoids are often indicated as primary factors driving coloration and ornamentation in birds (Ardia et al., 2010; Kimball, 2006; Murphy et al., 2009). Furthermore, although colouration in birds is believed to be a male-based trait, research has shown that androgens are also responsible for throat-skin size and colouration in females of a sex-role reversed species (Muck and Goymann, 2011). As such, the role of androgens in driving colouration in both sexes, especially in a co-operative breeding species should be carefully assessed.

Historically, hormone analyses have predominantly involved the use of serum or plasma, which requires specific skills for sampling and presents several animal welfare considerations (Goymann, 2012). For example, capture and restraint practices in avian species have been shown to lead to an increase in stress-associated glucocorticoid levels (Lynn et al., 2010). As a consequence, a preference for non-invasive determination of steroid hormone metabolite concentrations using alternative matrices have been developed for a number of birds (Goymann, 2005). The use of such matrices has some clear advantages over the traditional use of blood. Firstly, unlike blood samples bird urofaecal samples are less affected by episodic fluctuations or pulsatile secretions of hormones and therefore, short-term alterations in plasma hormone concentrations are not detected (Touma and Palme, 2005). Secondly, urofaecal samples may be collected without direct human-animal contact. This greatly reduces feedback alterations in hormone concentrations, allows for longitudinal sampling periods without any welfare implications, and ensures the safety of both the study subjects and the researchers (Romero and Reed, 2005). However, prior to the use of the specific matrix and chosen assay for monitoring androgen metabolite concentrations in a species for the first time, it is important that the approach be validated to ensure a reliable quantification of respective androgens, or their metabolites. Although this can be attempted via a physiological validation (i.e. the injection of gonadotropin-releasing hormone), the invasive nature of this approach is often not advisable for vulnerable and endangered species (Goymann, 2005). In such cases, researchers can attempt a biological validation by demonstrating the ability of an enzyme immunoassay (EIA) to distinguish between sexes and male maturation stages in terms of immunoreactive faecal androgen metabolite (fAM) concentrations (see eg. Scheun et al., 2017).

The aim of the study was to (1) biologically validate an appropriate EIA for monitoring fAM concentrations in SGH by comparing respective steroid concentrations of adult and sub-adult males and females, respectively, and (2) to examine the relationship of fAM concentrations and the variation in throat-skin colouration of adult male SGHs, specifically pure red and red/blue variations.

Table 1. The social group composition of all 12 southern ground-hornbill facilities within South Africa used during the study. Shown are the aviary ID, along with the number of adult male (AM), adult female (AF), sub-adult male (SM) and sub-adult females (SF) for each location. Key ■ = individuals not sampled

Location	Aviary ID	Group Composition				Group Size
		AM	AF	SM	SF	
Bester Birds and Animal Zoo Park, Willowglen, Pretoria, South Africa	B1	1	1	0	0	2
	B2	1	0	0	1	2
Boscia Birds, Kleinfontein, Brits, South Africa	C1	2	1	1	0	4
	C2	0	1	0	1	2
Hoedspruit Endangered Species Centre, Hoedspruit, South Africa	K1	1	1	0	0	2
Johannesburg Zoo, Johannesburg, South Africa	J1	1 ■	0	0	1	3
	J2	1	0	1	1	3
Lory Park Zoo, Midrand, South Africa	P1	1	1	0	0	2
Loskop Dam Nature Reserve, Mpumalanga, South Africa	L1	1	1	0	0	2
	L2	0	0	0	4	4
	L3	1	1	0	0	2
	L4	1	1	■	0	3
	L5	0	0	2	4	6
	L6	0	0	0	2	2
Mitchell Park Zoo, Morningside, Durban, South Africa	M1	1	1	0	0	2
Mohloholo Wildlife Rehabilitation Centre	O1	1	0	0	0	1
Montecasino Bird Gardens, Johannesburg, South Africa	T1	1	■	0	0	2
	T2	1	1	0	0	2
	T3	0	0	0	1	1
National Zoological Garden, Pretoria, South Africa	N1	■	■	0	4	6
	N2	1	0	0	1	2
	N3	0	0	0	1	1
Umgeni River Bird Park, Durban, South Africa	U1	1	1	0	0	2
	U2	1	1	0	0	2
	U3	0	0	1	0	1
	U4	0	0	1	0	1
Zaagkuilsdrift Bird Sanctuary and Lodge, Pienaarsriver, South Africa	Z1	1	1	0	0	2
	Z2	1	0	0	1	2
	Z3	0	0	2	1	3
	Z4	1	0	0	2	3
Total number of animals						72
Total number of animals sampled						67

Materials and methods

Study site and animals

The study was performed on 67 SGHs from 12 captive institutions within South Africa (Table 1). All individuals were DNA sexed according to the method described by Theron et al. (2013) prior to the start of the study. Focal birds were housed either alone, in pairs or in groups ranging from three to six individuals of varying social combinations, including three instances of all-female groups. Eight of the captive facilities contained more than one aviary housing a SGH group, resulting in all, but four groups of study subjects being in auditory and/or visual proximity to a neighbouring group. There were no changes to existing diet or husbandry routines of the captive study subjects for the duration of the sampling period. Study animals were categorised according to their age at the start of this study: adult male (AM) and female (AF, >5 years); sub-adult male (SM) and female (SF, 2-5 years). Adult male SGHs were categorized according to the quantity of blue coloration perceived on the throat, which was assessed using visual observations and photographic profiles: R, 0% blue throat coloration; sB, minor blue coloration of the throat skin (Fig. 2a–c) and b, major blue coloration of the throat skin (Fig. 2d–f).

Sample collection and steroid extraction

For the purpose of this study, only the faecal component of each urofaecal sample excreted was collected. In this regard, a minimum of at least one fresh sample was collected from each study subject on three consecutive days (n=3) within the breeding season (September – December 2016). Each faecal sample was collected using a new wooden spatula, and placed into a 30-ml screw-top specimen container. To enable individual identification of faecal samples, occupants in each aviary were observed constantly from outside until a fresh sample was produced. Only when individual identification and location of a sample was certain, did the researcher enter the aviary for collection. The faecal material was put on ice immediately and stored at -20 ˚C within one hour of collection.

A total of 256 faecal samples were collected, lyophilized, pulverized, and sieved through a thin mesh to remove fibrous material (Fieß et al., 1999). Subsequently, 0.050 – 0.055 g faecal powder was extracted with 1.5 ml of 80 % ethanol by vortexing for 15 min at room temperature. Following centrifugation for 10 min at 1500 *g*, the supernatant was decanted into microcentrifuge tubes and stored at -20 ˚C until analysis (Ganswindt et al., 2002).

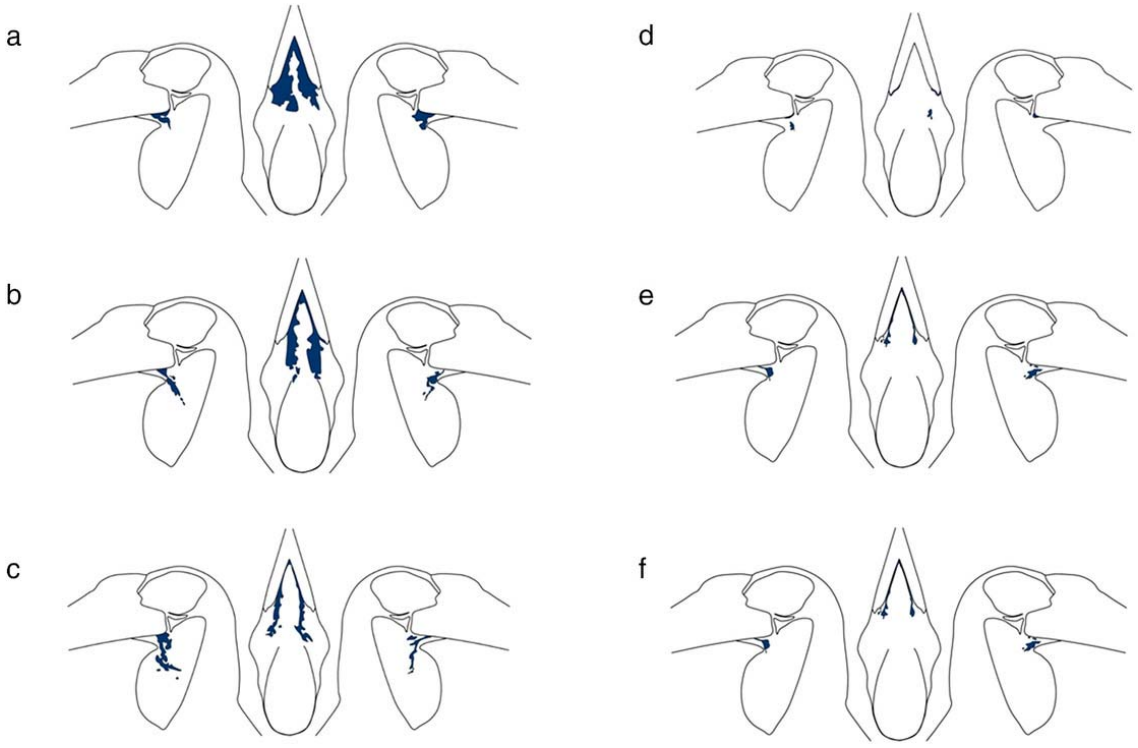

Figure 2. Graphical representation of "Blue" (B: a–c) and "Small blue" (sB: d–f) coloration as used in the current study. All red (R) individuals displayed 0% blue coloration.

fAM quantification

Faecal extracts were measured using an EIA for immunoreactive fAM concentrations utilizing an antibody against testosterone-3-CMO:BSA and 5α-androstane-3β,17β-diol-3-HS:DADOO-biotin as label. Further details of the EIA, including cross-reactivities of the antibody used, are described Palme and Möstl (1993). The sensitivity of the EIA was 2.4 ng/g dry weight (DW). Serial dilutions of extracted samples of male and female SGHs gave displacement curves that were parallel to the respective standard curve (relative variation (%) in the slope of the trend lines <5% for males, <3% for females; Fig. 3). Intra-assay coefficient of variance (CV), determined by repeated measurements of high and low quality controls, were 5.00% and 5.05%, respectively. Intra-assay CV, also determined by repeated measurements of high and low quality controls, were 9.97% and 12.03%, respectively. All analyses were performed at the Endocrine Research Laboratory, University of Pretoria, South Africa, as described by Scheun et al. (2016).

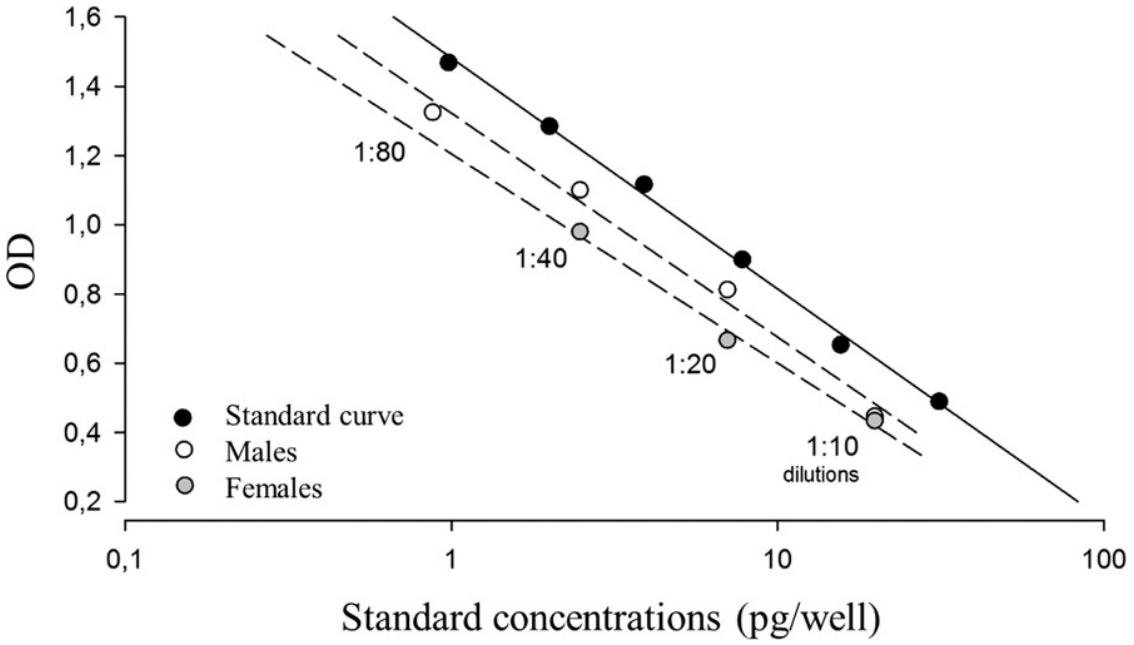

Figure 3. Parallelism test for serial dilutions of male and female sample pools for the selected fAM enzyme-immunoassay utilizing an antibody against testosterone-3-CMO:BSA.

Biological validation of immunoassays

To evaluate if the chosen EIA detects biologically meaningful alterations in fAM concentrations, individual median fAM levels of sexually mature (adult) and sexually immature (sub-adult) birds were compared for both sexes (males: mature n = 21, immature n = 9; females: mature n = 13, immature n = 25). Since full gonadal development typically results in an increased output of reproductive hormones, the contrast of higher androgen levels in sexually mature compared to immature individuals is generally deemed as a suitable biological validation for an androgen assay (eg. Möhle et al., 2002).

Data analysis

Individual median fAM values were calculated for each study subject and these values were used for all further analyses. A Shapiro-Wilks test was used to determine normality of relevant data subsets. For the assay validation, individual median fAM concentrations of adult male and female SGHs were compared to their sub-adult counterparts using Wilcoxon rank sum test.

The relationship of fAM concentrations and variation in throat-skin colouration of adult male SGHs (either R, sB, or B; Fig. 2) were analysed using a one-way analysis of variance (ANOVA). The relationship between fAM concentration of adult males and the presence/absence of sub-adult male(s) within the same group was tested using a Welch's two

sample t-test. Similarly, the relationship between fAM concentration of adult males and the presence/absence of adult females within the same group was tested using the same test. Statistical significance was placed at p = 0.05 for all tests, which were performed using the software R, version 3.4.1 (R Development Core Team 2013).

Results

Biological validation of the fAM EIA

Adult male SGHs (167.43 ± 98.63 ng/g DW; mean ± standard deviation (SD)) had significantly (W = 222, p = 0.002) higher individual mean fAM concentrations than sub-adult males (81.38 ± 69.24 ng/g DW, Fig. 4). Similarly, adult female SGHs (W=266, p=0.03) had significantly higher fAM concentrations (141.20 ± 67.87 ng/g DW) than their sub-adult counterparts (102.34 ± 87.49 ng/g DW, Fig. 4).

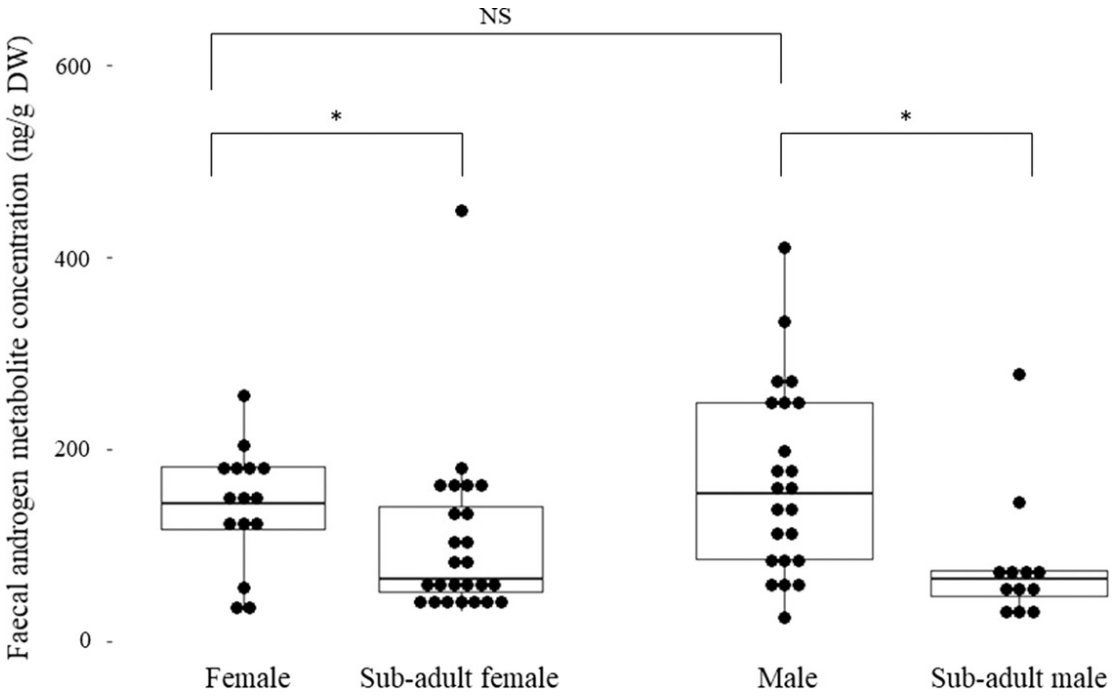

Figure 4. The median fAM concentration of adult male, subadult male, adult female, and subadult female used to biologically validate the androgen EIA. Significance is indicated by *. Non-significance is indicated by NS.

Faecal androgen metabolite concentration and throat colouration

There were no significant differences ($F_{(2,20)} = 3.49$, p = 0.74) in fAM concentrations between males with different throat colourations (R, sB, and B; Fig. 5). Overall individual median fAM concentrations for both B (163.96 ± 84.91 ng/g DW) and sB (165.77 ± 122.07 ng/g DW) birds

were considerably higher than in R birds (100.14 ± 95.47 ng/g DW), but SD for all three groups were high (54% - 95% of respective median values; Fig. 5).

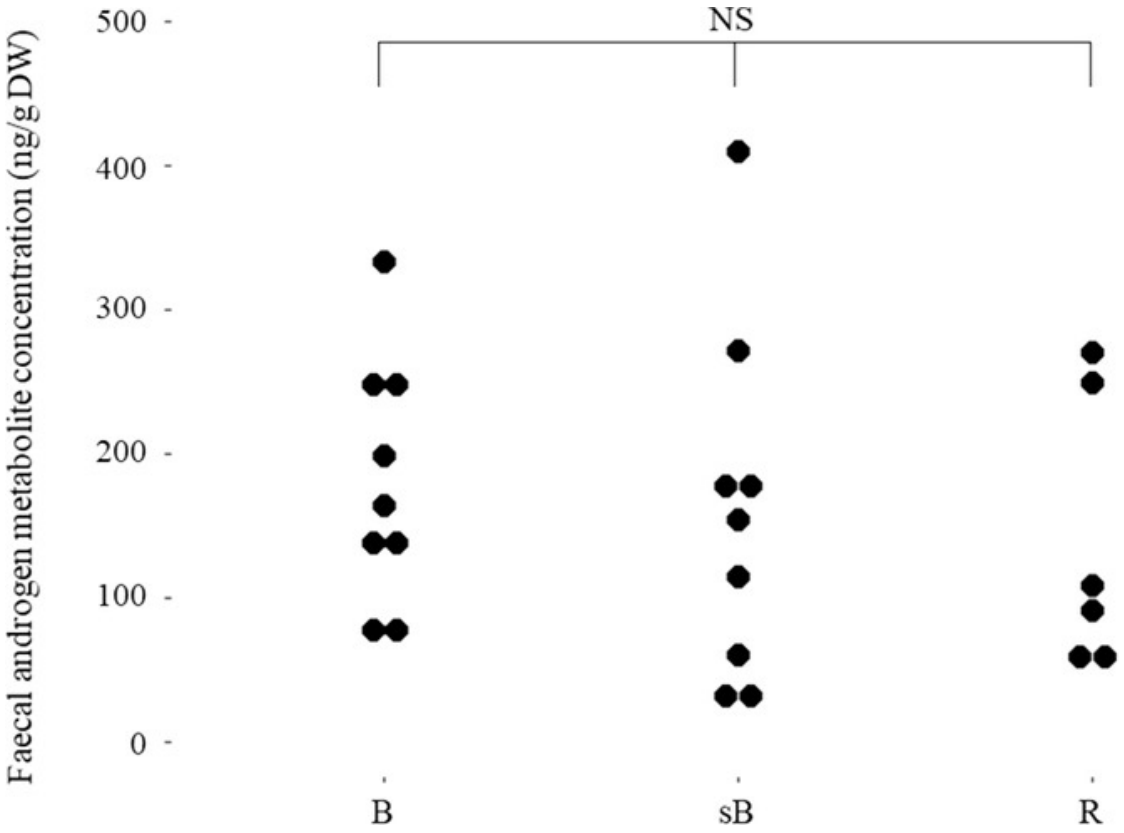

Figure 5. The median fAM concentrations of B, sB, and R categorized male individuals in the study. NS, non-significance.

External influences on fAM concentrations

Although not significant (t = -1.49, p = 0.22), adult males housed with sub-adult males had overall 75 % higher median fAM concentrations (200.51 vs 115.00 ng/g DW) than adult males in enclosures where sub-adults were absent. Similarly, no significant difference in fAM concentrations was found between males housed with or without a female SGH (t = 0.90, p = 0.39). However, where females were present, B and sB (difference of 13 – 45 ng/g DW) males had more comparable median fAM concentrations to their paired females (157.76 ± 49.50 ng/g DW) than R males (difference of 147.87 ng/g DW).

Although not significantly different (auditory: t = 1.00, p = 0.33; visual: t = 1.40, p = 0.18), adult males in close proximity to other SGH aviaries, in terms of auditory/visual interaction, had considerably lower fAM concentrations than their counterparts housed away

from other SGH enclosures (auditory: difference of 56.7%; visual: difference of 86.4 %). This pattern was not repeated in female SGH, with adult animals across all aviaries showing similar fAM concentrations (auditory: Δ 13.6%. $t = 0.06$, $p = 0.95$; visual: Δ 17.71%, $t = 0.61$, $p = 0.55$).

Discussion

The findings of this study showed that androgen concentrations were not the primary driver of throat skin coloration in SGHs. Male and female SGH individuals had significantly higher fAM concentrations than their sub-adult counterparts, indicating that the applied EIA was able to detect biologically meaningful differences in fAM output.

The pattern of elevated androgen concentrations in adults, compared to their sub-adult counterparts, has been shown for a number of avian species such as the bar-headed goose (*Anser indicus*, Dittami, 1981) and magpies *(Gymnorhina tibicen Latham*, Schmidt et al., 1991). Although the use of a biological validation was successful in the current study, a number of avian species do not show the expected age-related androgen difference as described for the SGH. For example, Deviche et al. (2000) found no significant difference in androgen concentrations when comparing different age and breeding classes of the dark-eyed juncos (*Junco hyemalis*), with the exception of a short breeding period (April – mid-May). Similarly, no significant difference in androgen concentrations were found in a study comparing young, middle and old aged Japanese quail *(Coturnix japonica*, Ottinger et al., 1995). As a number of factors driving androgen production and secretion in birds, most notably factors associated with breeding and social mechanisms inherent in a species (Garamszegi et al., 2005; Lynn, 2008; Wingfield et al., 1990), they should be taken into consideration when deciding on the use of a biological validation as performed here. To ensure the assay of choice is sufficient for monitoring the hormone metabolites of interest in a species, both, a biological and physiological (GnRH challenge) validation should be performed if possible (Touma and Palme, 2005).

In this study, no significant difference in fAM concentrations were found between R, sB, and B males. As androgens have been shown to enhance avian ornamentations and plumage in general (Adkins-Regan, 2005; Kimball, 2006), it was assumed that SGH males with red throat-skin would have higher fAM concentrations. Furthermore, it would be expected for the development of colouration associated with females to be an indicator of suppression of a beta-male by a more dominant individual, as found in several other species (Setchell and Dixson,

2001; Slotow et al., 2000). In contrast to the expected outcome, sB and B males showed higher fAM concentrations compared to R males, although not statistically significant. One possible explanation for this could be the species-specific biochemical pathways responsible for colouration (Hill, 2006). For example, androgens determine the deposition of melanins in house Sparrows (*Passer domesticus*) resulting in black bill colouration, while the interactions of androgens and carotenoids are responsible for the orange bill colouration of the American goldfinch (*Spinus tristis*) during the breeding season (Haase, 1975; Murphy et al., 2009; Pham et al., 2014). Furthermore, androgens have been found to induce the expression of structural blue bare-part colouration in female budgerigars *(Melopsittacus undulatus*, Lahaye et al., 2014). Elevated androgens may thus lead to the blue expression observed in the SGH and not the expected red. However, looking closer into the regulatory mechanisms of SGH throat coloration would require additional research, incorporating factors such as melanin and carotenoid concentration monitoring as well as experimental study setups.

When evaluating a dichromatic colouration in a socially complex and cooperatively breeding species like the SHG, a logical conclusion of its cause, based on similar research in other species, would be a visual indication of social standing, dominance or submission(Dey et al., 2017; Gerald, 2001; Renoult et al., 2011; Setchell and Dixson, 2001). For example, short term exposure to elevated androgen concentrations in zebra finches (*Taeniopygia guttata*) led to a change in bill coloration and increased dominance behaviour (Ardia et al., 2010). However, the findings of the current study did not support this, with dominant and submissive animals displaying both red and blue throat skin coloration with varying fAM concentrations. Furthermore, there are several instances of blue colouration in adult males housed with a single female, which eliminates the need to compete. It should be noted that all study animals were held in captivity, thus reaching social inertia, a factor that may explain the fAM patterns observed in this study (Wingfield et al., 1987). Furthermore, the random grouping of birds at the various study sites may results in the variation in fAM concentrations observed. As such, it would appear that throat skin coloration is a poor predictor of social standing in captive SGH. Future research should focus on monitoring free-ranging SGH populations in order to determine whether the results obtained here are universal for the species.

No significant difference in fAM concentrations was found between adult male and female SGHs in this study. This is in stark contrast to the accepted notion that males generally produce higher androgen concentrations than their female counterparts (Feder *et al*. 1977; Silverin & Wingfield 1982; Gill *et al*. 2007). The overlap in fAM concentrations in this and

other avian species, such as the domestic fowl, *Gallus gallus domesticus* (Cockrem & Rounce 1994), Adelie penguins *Pygoscelis adeliae* (Ninnes *et al*. 2009), and the northern bald ibis G*eronticus eremita* (Sorato & Kotrschal 2006), may be explained by the social-sexual parameters present in each species. Polygynous species show significant hormonal asymmetry between sexes, with considerably higher androgen concentrations observed in males. Although elevated androgen concentrations can be beneficial to polygynous males, allowing for the establishment and defense of territories and mating partners (Beletsky *et al*. 1995; Garamszegi *et al*. 2005), chronically elevated androgen levels can have a range of deleterious effects (Wingfield *et al*. 1990). In the absence of competition, monogamous males have lower baseline androgen concentrations during breeding periods (Hirschenhauser & Oliveira 2006). This leads to lower energy expenditure and rates of aggressive behaviors, as well as encouraging parental behavior (Wingfield *et al*. 1990). Furthermore, the morphological and behavioral symmetry between monogamous male and female SGH may be responsible for the similarities in fAM levels between sexes (Kellam *et al*. 2004; Sorato & Kotrschal 2006). Competition for resources, dominance, and mating opportunities, along with the need to defend chicks, are all factors responsible for elevated androgen concentrations in females (Langmore *et al*. 2002; Smith *et al*. 2005; Cantarero *et al*. 2015). Future SGH studies should include a behavioral component to assess the importance of social-sexual factors, in combination with species physiology, in driving male and female fAM levels as well as skin coloration.

It is worth mentioning that the fAM concentrations observed in the current study may reflect plasma androgen concentrations in the SGH. Previous research has shown fAM concentrations to be a reliable indicator of plasma androgen patterns in wildlife species (Bishop & Hall 1991; Hesterman & Jones 2009; Hirschenhauser *et al*. 2000). However, Ninnes *et al*. (2009) indicated that although fAM concentrations were similar between sexes of the Adelie penguin, plasma androgen concentrations were significantly higher in males of the species. This may be as a result of (i) the sex-specific metabolism of hormones inherent in the species (Goymann 2012), (ii) the ratio of free to bound androgens present within the blood of each sex, or (iii) the stability (consistency vs. fluctuation) of plasma androgen concentrations over time in male and female counterparts (Ninnes *et al*. 2009). In order to determine the correlation strength between fAM and plasma androgen concentrations in the SGH, additional research is required which incorporates plasma androgen analysis.

Possible coloration mechanisms and future research on SGHs

This study aimed to determine the importance of androgens as drivers of throat skin coloration in SGHs. The androgen–ornamentation relationship is a commonly accepted mechanism controlling coloration in birds (Adkins-Regan 2005; Leary & Knapp 2014; Schuppe & Fuxjager 2019). However, in the absence of such a relationship in the SGH, several other factors may be responsible for the observed coloration in the species. (i) Carotenoids, which are pigments derived from different food sources, are important in producing yellow and red coloration in bird feathers and bare parts (Hill *et al*. 2002; Nordén & Price 2018). Differences in the diet presented to SGHs at the various sample locations may have played a role in driving bare skin coloration and should be monitored when conducting multi-location studies. (ii) Several large-bodied, dark bird species inhabiting hot environments present red, bare-skin coloration; this highly vascularized tissue found under the epidermis is filled with blood, providing the red coloration, while assisting with thermoregulation (Negro *et al*. 2006). In order to determine whether this is applicable for SGHs, ambient and individual thermoregulation should be monitored under controlled conditions. (iii) Melanin is a naturally produced pigment in all birds and is largely responsible for darker coloration such as black and brown (Siefferman & Hill 2003; Nordén & Price 2018). Although it would not be directly responsible for the red and blue coloration observed in SGH, it would be interesting to see if melanin plays a major role in SGH coloration. (iv) Finally, although often linked to feather coloration (Fox 1976), structural coloration might be responsible for the blue coloration observed in the species. Additional information on the physical structure of the throat skin in SGH would need to be collected in order to determine whether this is applicable.

Conclusion

This study has successfully validated an EIA for monitoring androgen concentrations non-invasively in the SGH. In contrast to several studies, no correlation was found between androgen concentrations and the alteration in male throat skin coloration of captive SGH. Further research is, however, required to determine whether the results of this study also hold true for free-ranging SGHs, where blue coloration in males has also been observed. Finally, future research on the SGH should include additional parameters in order to determine the role of enclosure setup, skin structure, and environmental factors in driving coloration in the species.

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