



Research article

Assessment of biometrics and stress indicators of the adult Village Weaver birds (*Ploceus cucullatus*) during breeding and post-breeding seasons in Jos, Nigeria

Felix A. Andong^{a,b,*}, Vincent C. Ejere^a, Onyinyechukwu A. Agina^c,
Samuel O. Ekere^{d,**}, Ezekiel S. Mayowa^b

^a Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

^b AP Leventis Ornithological Research Institute, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria

^c Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria

^d Department of Veterinary Theriogenology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria

ARTICLE INFO

Keywords:

Seasonal studies
Adult Village Weaver birds
Biometrics and stress indicators
General linear mixed model
APLORI Amurum Forest Reserve

ABSTRACT

In this era of climate change, some biological conservationists' concerns are based on seasonal studies that highlight how wild birds' physiological fitness are interconnected with the immediate environment to avoid population decline. We investigated how seasonal biometrics correlated to stress parameters of the adult Village Weavers (*Ploceus cucullatus*) during breeding and post-breeding seasons of the Weaver birds in Amurum Forest Reserve. Specifically, we explored the following objectives: (i) the seasonal number of birds captured; (ii) whether seasonal baseline corticosterone (CORT), packed cell volume (PCV), and heterophil to lymphocytes ratio (H:L) were sex-dependent; (iii) whether H:L ratio varied with baseline (CORT); (iv) whether phenotypic condition (post-breeding moult) and brood patch varied with baseline (CORT) and H:L ratio; and (v) how body biometrics co-varied birds' seasonal baseline (CORT), (PCV) and (H:L) ratio. Trapping of birds (May–November) coincided with breeding and post-breeding seasons. The birds ($n = 53$ males, 39 females) were ringed, morphologically assessed (body mass, wing length, moult, brood patch) and blood collected from their brachial vein was used to assess CORT, PCV and H:L ratio. Although our results indicated more male birds trapped during breeding, the multiple analyses of variance (MANOVA) indicated that the seasonal temperature of the trapping sites correlated ($P < 0.05$) significantly to baseline (CORT). The general linear mixed model analyses (GLMMs) indicated that the baseline (CORT) also correlated significantly to H:L ratio of the male and female birds. However, PCV correlated significantly to body size of the birds (wing length) and not body mass. Haematological parameters such as the baseline CORT and the H:L ratio as indicators of stress in wild birds. Hence, there is the possibility that the Village Weaver birds suffered from seasonally induced stress under the constrained effect of environmental temperature. Hence, future studies should investigate whether the effect observed is also attributable to other passerine species.

* Corresponding author. Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

** Corresponding author.

E-mail addresses: andongfelix@gmail.com (F.A. Andong), samuel.ekere@unn.edu.ng (S.O. Ekere).

<https://doi.org/10.1016/j.heliyon.2024.e31196>

Received 17 September 2023; Received in revised form 11 May 2024; Accepted 13 May 2024

Available online 14 May 2024

2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Often, quantifying direct fitness parameters (such as reproductive failure and success or survival rate) might be labor intensive, very difficult or nearly impossible in birds [1]. However, as a surrogate, it is suggested that assessing the hematological or physiological indices can give valuable insight into the performance of animals in their environment [2]. To a greater extent also, relating hematological or physiological indices to biometrics or phenotypic conditions of animals such as birds of different sizes is very important [3]. Birds evolved with seasonality; hence, undergo seasonal activities such as migration, wintering, reproduction, post-breeding, recovery, moult and preparation for the next breeding season to survive [4,5]. However, these seasonal activities are demanding [6,7]; they are energetically costly [8,9] and lead to seasonal variation in the body condition of male and female birds [10]. As a result, leading to morphological, hematological or physiological fitness differences [1,5,11,12].

Indeed, most seasonal studies could highlight how wild birds are interconnected with their immediate environment, and could also indicate birds' resilience in the era of climate change [7]. In matured birds such as the Village Weavers (*Ploceus cucullatus*), the males are larger than their female counterparts, and studies have found that the seasonal body condition and size of the birds are sex-dependent [13]. Thus, there could be the possibility that these smaller (females) or larger (males) birds get subjected to contrasting pressures at a certain period [7]. Thus, exploring how the seasonal body conditions of the Village Weaver birds impacts their hematological or physiological fitness is necessary.

This study explored how biometric and phenotypic conditions correlated to stress parameters of the male and female Village Weaver birds during breeding and post-breeding seasons. Specifically, we investigated: (i) the seasonal number of Village Weaver birds trapped during sampling; (ii) whether the seasonal variation in baseline corticosterone (CORT), packed cell volume (PCV), and heterophil to lymphocytes ratio (H:L) were sex-dependent; (iii) whether H:L ratio varied with baseline (CORT) of the birds; (iv) whether phenotypic condition such as post-breeding moult on males, and brood patch on females varied with baseline (CORT) and H:L ratio during breeding and post-breeding seasons; and (v) how birds body biometrics co-varied with male and female seasonal baseline (CORT), packed cell volume (PCV) and heterophil to lymphocyte (H:L) ratio. Packed cell volume was used as the measure of the oxygen demand of the birds, while body mass and wing length were used as the birds' biometrics and body condition. Hematological parameters, i.e. heterophil to lymphocyte ratio (H:L) and baseline corticosterone (CORT) were used as the major stress indicators. Restraint duration, time of the day, and temperature (which is a major environmental factor that usually varies with seasons) of Amurum Forest Reserve was also recorded as factors that could influence the birds' body condition.

Baseline (CORT) measured within 3 min of capture [3,14–16] has made it possible to prevent any change in haematological and physiological parameters [17]. Hence, before the study, we expected variation in birds' baseline hormone [18], and that (CORT) should be higher during breeding [19,20] to maintain allostasis [21,22]. Assuming that (CORT) is very sensitive [23] and influenced by the higher energetic state of the birds during breeding and parental care [24–26].

Second, we expected that the male birds are naturally larger and heavier; therefore, should be more stressed, owing to the much force and energy expended in lifting the body against gravity [27], which in turn, results in elevating (CORT) levels (CORT) [28]. Assuming that females are smaller in size and have less gravitational influence (larger surface area relative to size), and are deemed to have an evolutionary advantage over the larger ones [29–31]; hence, their baseline (CORT) should be lower.

Third, we expected that the higher level of baseline (CORT) should cause a higher level of H:L ratio [1,32]. Assuming that (CORT) is

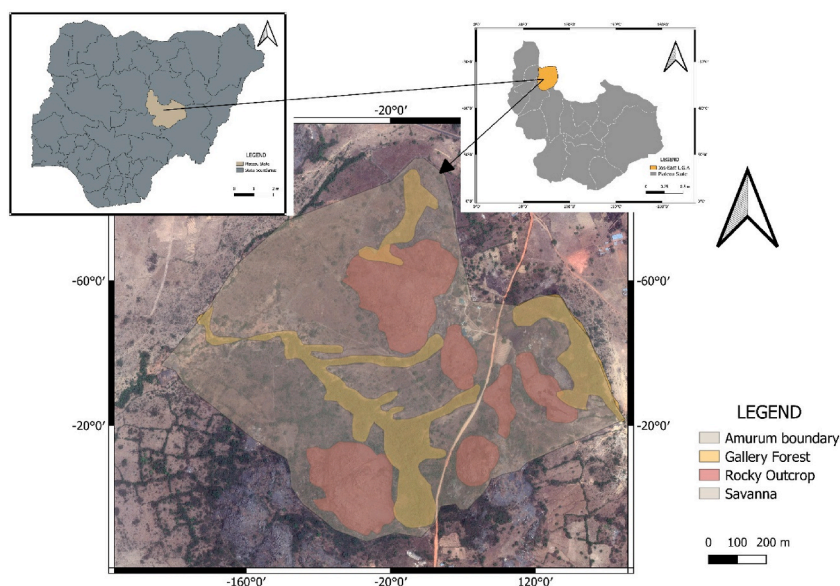


Fig. 1. The QGIS map of the study area. Map of Nigeria (Inset Plateau State upper far left). Map of Plateau State (Inset Jos-East upper far right). Map of Amurum Forest Reserve (Jos-East).

capable for displacing circulating lymphocytes of the blood, while favoring a higher concentration of the blood heterophils [33,34]. In this study, the baseline (CORT) was within the range of 0.7–57 µg/dL for both seasons [35,36], while the normal PCV range was measured at 35 %, any value below this indicated anaemia in birds [37].

2. Materials and methods

2.1. Study area

The following study coincided with the Village Weavers breeding and post-breeding (September–November) in the year 2023. Sample collection in the field was conducted in Amurum Forest Reserve of A.P Leventis Ornithological Research Institute (APLORI) (Fig. 1), while hematological assay of the blood samples was conducted at national veterinary research institute (NVRI) laboratory in Jos, Plateau State [38,39]. Amurum Forest Reserve is ca 300ha area located 15 km North-East of Jos-Plateau in North-Central Nigeria (9° 52' 30" N and 8° 58' 32" E) at an altitude of 1280 m a.s.l [38,40].

Amurum Forest Reserve is among the important bird areas (IBA) of Nigeria [40]. The reserve comprises a remarkably floristic composition of a gallery forest, woodland Savannah and a Rocky outcrop [41]. Within the reserve, trees such as *Daniella oliveri*, *Khaya senegalensis*, *Parkia biglobeasa*, *Lophura lauceolata* and *Ficus* species create the forest habitat [42].

The climatic conditions of the reserve include a temperature range of 8–38 °C, and the mean annual rainfall of 1375–1750 mm [40, 41,43,44]. The rainy and dry seasons usually last for about 6 months each. Usually, the rainy season starts in May and ends in October, while the dry season is between November and April [45]. Village Weavers usually breed when food and nesting materials are available around the rainy season [46,47].

2.2. Description of target species

Adult Village Weavers were targeted during breeding (May–August) and post-breeding periods (September–November). The birds peak of laying is around August [46]. Hence, plumage quality and brood patch were used to confirm adulthood. Generally, Village Weavers are residents and local nomadic African migrants; widely spread across sub-Saharan Africa [48]. These birds flock in numbers, form large nesting colonies, and have a stable population status [49,50]. Hence, before the breeding season, both males and females possess a partial moult (prenuptial moult) of the head and body feather tracts. After breeding, the Village Weavers undergo a complete moult of head, body and flight or wing feathers – males typically then take on a distinctive non-breeding (or eclipse) plumage (since they are sexually dimorphic, males put on a different phenotypical plumage display during the breeding and non-breeding period) [13, 48]. The males are largely polygamous and territorial [48]; offering very little parental care [51], except during nest production [52].

The young Village Weavers are nearly like adult females but more brownish on the back [53]. Over years of ringing in APLORI, the male and female Village Weavers of Amurum Forest Reserve during the non-breeding period (post-breeding) have been distinguished by comparing the wing length. The wing length of the matured non-breeding male Village Weavers is within 91–94 m, while for the females, between 81 and 86 (www.aplori.org).

2.3. Trapping of birds

The birds were trapped away from the constant effort sites (CES) of APLORI by using an understory mist net [45] around 5:30 a. m.-10:00 a.m. Upon capture, each bird was fitted with a unique numbered aluminium ring, following the SAFRING system to avoid re-sampling of individuals [54].

2.4. Morphological and phenotypical assessment

Trapped birds were aged and sexed (based on plumage) using a field assistant and the APLORI ringing guide. Briefly, the resident bird was aged two: when a juvenile bird is not fully matured for the sex to be known; three: having juvenile plumage; four: having all feathers of adult type; five: have a mixture of juvenile and adult feathers; adult: an adult of at least two years and above (www.aplori.org).

Moult is also measured for head, body and flight feathers. Body and head moult were scored based on the percentage of growing feathers. That is, score one: no head or body in moult, while score two: 10–30 % of growing feathers on the head or body region, i.e. at least one growing feather, and score three is: 40–60 % of growing feathers on the head or body region, i.e. approximately half of the feathers growing, while score four: 60 % of growing feathers on the head or body region, i.e. over half of the head and body feather growing. Meanwhile, in scoring the flight or wing feathers the guidelines of Ginn and Melvin [55] was usually adopted. That is, moulting on any part of the body was scored as 1 (yes) or 0 (no-moult).

Brood patch which is largely found on female birds was assessed using guidelines and features adopted from Redfern [56]. Hence, the absence of a brood patch was scored as 0, while its presence was scored as 1.

The native daily resolution temperature of Amurum Forest Reserve supplied by APLORI for the year was recorded at 2 m in degrees Celsius. The data was supplied in collaboration with the NASA Langley Research Center (LaRC) POWER Project through the NASA Earth Science/Applied Science Program.

2.5. Blood collection

To prevent any physiologic change and to obtain a baseline concentration of CORT using blood serum, blood samples were collected from the brachial vein after pricking with a sterilized lancet (into a heparinized and un-heparinized micro hematocrit capillary tubes). Blood was collected within 3 min of capture [3,14,15,57,58]. Subsequently, birds were enclosed in individual cloth bags for extended minutes to undertake another collection, since CORT attains maximum concentration after 30 min [15,59]. Thin smears were made using a drop of blood from the heparinized capillary tube and fixed using 95 % ethanol for 10 min [60]. Afterwards, the films were stained using Giemsa to access the differential blood cell count. The remnant of blood in the heparinized capillary tube was used to access packed cell volume, while the serum obtained from the un-heparinized capillary tube was stored in a non-frost-free freezer (at -20C) in order to quantify the baseline (CORT).

2.6. Hematological assay

The differential leukocytes were sorted based on morphology per slide [61,62]. Hence, heterophils and lymphocytes were assessed by totaling 100 cells on the slide under $1000\times$ magnification using oil immersion [63].

The blood at 75 % mark of the heparinized capillary tube was sealed (using plasticine), then spun (to obtain the proportion of red blood cells in the blood at 10,000 rpm) for 5 min using a micro-haematocrit centrifuge before reading the packed cell volume on a hematocrit reader [64,65]. In birds, the packed cell volume ranges between 35 % and 55 %; however, values below this range suggest anaemia [37].

Owing to the ease of use, low cost and quick results, serum (CORT) was assessed by immunoassay [66]. The process involved the use of Accu-Bind® enzyme-linked immunosorbent assay (ELISA) kits from Monobind Inc (#3625-300, California, USA). The quality control ensured that each assay was controlled at low, normal and high range monitoring assay performance. While controls were treated as unknown and values were determined in every test procedure performed. Furthermore, quality charts were followed, before a pertinent statistical method was employed to assess trends under acceptable assay performance limit for the maximum absorbance. Reagents attained room temperature before use; hence, we thawed the samples and kept them on ice to enhance binding of (CORT) to corticosteroid-binding globulin (CBG) before use [67]. In triplicates, CORT assay was conducted based on the recommendation from the kits manufacturers. The assay dilution factor was fully optimized (25-fold for Accu-Bind). In brief, microplates were formatted for use and about 0.025 ml (25 μL) for each reference, control and specimen (serum) (in which the microplates were formatted) were pipetted into the designated wells. Afterwards, 0.050 ml (50 μL) corticosterone enzyme was added and swirled for a few seconds, before 0.050 ml (50 μL) corticosterone biotin reagent was added and swirled further. Afterwards, the microplate was incubated for 1 h at room temperature, before aspirating contents and adding 0.350 (350 μL) of wash buffer. After aspirating the wash buffer, 0.100 (10 μL) of the working solution was added to the wells and incubated for 15 min before the addition of a stop solution. Finally, the plates were read on a Thermo Fisher Scientific® microplate reader (Fisher Scientific, Porto Salvado, Portugal) at 450 nm wavelength. Within and between plate repeatability were 0.94 and 0.96. The values obtained were used for plotting the standard curve and data extrapolation using the *R* statistical package of version 3.2.5 [68].

2.7. Statistical design and data analyses

Data were analyzed in *R* 3.2.5 [68], a descriptive statistical analysis was used to sum up the mean biometrics and stress parameters of the birds during breeding and post-breeding.

To test whether the seasonal variation in baseline corticosterone (CORT), packed cell volume (PCV), and heterophil to lymphocytes ratio (H:L) was sex-dependent. A multiple analysis of variance (MANOVA). The baseline CORT, PCV and H:L ratio were the response variables. The explanatory axis consisted of sex, body mass, wing length, restraint duration, time of the day, temperature and seasons (breeding and post-breeding). The PCV added to the model as a response variable was to test whether restraint could induce an increase in oxygen demand leading to a higher level of red blood cells [69]. In addition to the model above, there were interactions between: (i) temperature and sex to test if the male or female birds responded differently to the reserves' temperature (assuming that the cold produced during the wet season is not advantageous to the female birds). For instance, we assumed that the female birds' smaller size should enhance the rapid exchange of cold with the environment, in contrast to the males [70]; (ii) the interaction between seasons and temperature was to test whether the seasonal variation in environmental temperature condition of Amurum Forest Reserve impacted the stress parameters (assuming during peak of the breeding period around July and August of the year, there may be a fall in temperature induced by the wet season, leading to cold stress in birds).

To investigate how H:L ratio varied with the baseline (CORT) of the birds, a general linear mixed model (GLMMs) analysis were conducted. The response variable was the H:L ratio, while the explanatory axis consisted of the baseline (CORT), PCV, sex, body mass, wing length, restraint duration, temperature and seasons. The interactions consisted of the (i) temperature and baseline (CORT) (assuming that the variation in environmental environmental condition has a joint interactive effect on (CORT) level; thereby, impacting H:L ratio); (ii) seasons and PCV (assuming seasonal variation in energetic activities impacts the PCV during breeding and post-breeding); (iii) restraint duration and baseline (CORT) (assuming that handling impacts the CORT levels). The random effect consisted of the birds' and ringers' IDs fixed in a nested random effect (i.e. 1|ringers/birds) (assuming that the birds trapped in the reserved were measured by two observers; hence, variation in handling and measurements needs to be controlled). The Akaike Information Criterion (AIC) was used to get the best model through a backward removal of non-significant terms [71]. The goodness of fit, i.e. marginal R^2 (R^2_m), as a result of fixed effects only) and conditional R^2 (R^2_c), as a result of fixed and random effects) for the best

model was assessed using the *MuMIn* package to ascertain how much of the variation is explained by the fixed and random effects respectively [72].

To test how moulting conditions in males, and brooding on females varied with baseline (CORT) and H:L ratio during breeding and post-breeding seasons, the data were sub-set. In MANOVA, the response variables for the male birds was baseline (CORT) and the H:L ratio. The explanatory axis consisted of the moulting (yes/no), temperature, seasons, restraint duration, body mass and wing length of the male birds. The interactions consisted of seasons and temperature, seasons and moulting, and moulting and restraint duration (assuming that the male birds were bound to be more stressed during moulting and restraint). Meanwhile, the response variable for the female birds was the stress parameters, the explanatory axis variables consisted of brooding status (yes/no), temperature, seasons, body mass and wing length. The interaction consisted of the seasons and temperature, moulting and brooding, and brooding and restraint (assuming that the female birds were bound to be more stressed during moulting and restraint).

To test how birds body biometrics co-varied with male and female seasonal baseline (CORT), packed cell volume (PCV) and heterophil to lymphocyte (H:L) ratio, a principle component analyses (PCA) was used for the analyses. There is the possibility that the stress parameters such as baseline CORT should co-vary with lymphocytes to influence the H:L ratio or the body mass of the birds should increase as wing length increases. Assuming that the larger birds are heavier. Hence, the direction of the relationship between male and female birds' seasonal biometric condition and stress parameters was explored on the PCA. Our level of significance was $P < 0.05$.

3. Results

A total of ($n = 92$) Village Weavers were captured. Of the total number, the male birds were 53 individuals and occurred more during breeding than post-breeding season (i.e. 33 and 20 individuals). Conversely, a total of 39 female birds were captured more during post-breeding than the breeding season (25 and 14 individuals). Seasonally, the mean temperature of the reserve was higher, and it was observed that both male and female birds were in better body condition (body mass index = BMI) during post-breeding. During breeding, the mean baseline (CORT) and PCV of the male birds was higher, while their H:L ratio was lower during this period. For female birds, mean PCV and H:L ratio were higher during breeding, while CORT level was lower during this period (Table 1).

In MANOVA, the environmental temperature condition of Amurum Forest Reserve significantly correlated to baseline CORT of the birds, while wing length correlated with PCV levels (Table 2). In GLMMs, baseline (CORT) strongly correlated significantly to H:L ratio (Table 3). The males' CORT level was significantly influenced by seasons and the environmental temperature condition of the reserve (Table 4). Whereas, the females' CORT level was significantly influenced by the joint interaction of environmental temperature condition and seasons, and between restraint duration and brooding status of the females (Table 5).

Loading of the PCA for seasonal variation in biometrics and haematological parameters of the male birds indicated that H:L ratio, body mass, wing length and heterophils contributed more to the first (variance = 22.6 %) and second (variance = 18.5 %) PC (Table 6 and Fig. 2). The lymphocytes contributed more to the first PC of the female birds; and similar to the male birds, the female birds' body mass, wing length, heterophils and H:L ratio contributed more to both the first (variance = 23.4 %) and second (variance = 20.7 %) PC (Table 6 and Fig. 3).

4. Discussion

Conservation biology is usually interested in the mechanisms that result in population declines [73]. As such, changes in haematological or physiological parameters even at suboptimal levels interfere with birds' fitness. Thus, assessing whole blood or its serum is one way to measure the conditions wild animals face in their immediate environment [73]. In this study, more male Village Weavers were trapped during the breeding season. This higher number of male birds might be attributable to either the conducive and

Table 1

The seasonal environmental temperature, biometrics and hematological parameters of the male and female Village Weaver Birds ($n = 53$ males, 39 females).

Year	Variables	Breeding	Post- breeding
	Temperature (°C)	25.5 ± 0.7	28.8 ± 0.2
Males	Body mass index	0.47 ± 0.2	0.55 ± 0.1
	Packed cell volume (%)	57.9 ± 1.5	55.9 ± 0.3
	Heterophil (μL)	53.5 ± 0.9	58.0 ± 0.4
	Lymphocytes (μL)	51.6 ± 0.2	50.9 ± 0.8
	H:L Ratio	1.09 ± 0.9	1.14 ± 0.2
	Baseline (CORT) (μg/dL)	0.87 ± 0.7	0.75 ± 0.0
Females	Body mass index	0.41 ± 0.1	0.47 ± 0.2
	Packed cell volume (%)	58.0 ± 0.2	56.2 ± 0.4
	Heterophil (μL)	60.1 ± 0.7	60.3 ± 0.6
	Lymphocytes (μL)	40.5 ± 0.8	41.0 ± 0.2
	H:L Ratio	1.17 ± 0.2	1.15 ± 0.3
	Baseline (CORT) (μg/dL)	0.59 ± 0.3	0.67 ± 0.9

Table 2

Packed cell volume, corticosterone and H: L ratio of Village Weaver birds in relation to restraint duration, time of the day, sex, environmental temperature of Amurum Forest Reserve. Asterisk indicate significance (n = 53 males, 39 females).

Variables	Sum Sq	Mean Sq	F	P
Response Corticosterone				
Sex	0.03	0.03	0.40	0.53
Body mass	0.09	0.09	1.36	0.25
Wing length	0.01	0.01	0.18	0.67
Restraint duration	0.02	0.02	0.37	0.55
Time of day	0.09	0.09	1.44	0.23
Temperature	0.27	0.27	4.29	0.04*
Seasons	0.15	0.07	1.15	0.32
Sex: Temperature	0.04	0.04	0.66	0.42
Temperature: Seasons	0.25	0.12	1.96	0.15
Response H:L Ratio				
Sex	0.01	0.01	0.03	0.99
Body mass	0.01	0.01	0.02	0.98
Wing length	0.03	0.03	0.18	0.67
Restraint duration	0.02	0.02	0.09	0.76
Time of day	0.03	0.03	0.19	0.66
Temperature	0.11	0.11	0.69	0.41
Seasons	0.24	0.12	0.72	0.49
Sex: Temperature	0.09	0.09	0.56	0.45
Temperature: Seasons	0.47	0.23	1.43	0.24
Response PCV				
Sex	6.87	6.87	0.38	0.54
Body mass	5.10	5.10	0.29	0.59
Wing length	148.8	148.8	8.35	0.01*
Restraint duration	15.53	15.5	0.87	0.35
Time of day	0.49	0.49	0.03	0.87
Temperature	6.95	6.95	0.37	0.53
Seasons	41.2	20.6	1.15	0.32
Sex: Temperature	0.03	0.03	0.01	0.96
Temperature: Seasons	6.91	3.46	0.19	0.82

Table 3

Heterophil and lymphocyte ratio in relation to corticosterone and sex of the birds. Sex was not significant but AIC indicated that the final model with sex as a predictor was the best (i.e. having the least AIC strength). The marginal (R^2M) and condition (R^2C) values are given for the final significant parameters of the models. Significant terms are in asterisk. (n = 53 males, 39 females).

Variables	Estimate	SE	t	P	R^2M	R^2C
					0.52	0.74
Response H:L Ratio						
Intercept	0.89	0.12	7.22	0.01*		
Sex (male)	-0.01	0.08	-0.08	0.94		
Baseline (CORT)	0.36	0.16	2.29	0.02*		

Table 4

Males' body mass, wing length, restraint duration, seasons and moult status in relation to corticosterone and H:L ratio of the Village Weaver birds. Asterisk indicate significance (n = 53 males).

Variables	Sum Sq	Mean Sq	F	P	Sum Sq	Mean Sq	F	P
Males								
Response Corticosterone				Response H:L Ratio				
Body mass	0.04	0.04	0.68	0.41	0.01	0.01	0.02	0.89
Wing length	0.01	0.01	0.03	0.86	0.12	0.12	0.74	0.39
Restraint duration	0.12	0.12	2.04	0.16	0.01	0.01	0.03	0.86
Temperature	0.30	0.30	5.08	0.03*	0.14	0.14	0.87	0.36
Moult	0.21	0.21	3.53	0.07	0.32	0.32	1.94	0.17
Seasons	0.27	0.27	4.48	0.04*	0.20	0.20	1.21	0.28
Temperature × Seasons	0.02	0.02	0.28	0.59	0.51	0.51	3.14	0.08
Moult × Seasons	0.01	0.01	0.01	0.95	0.16	0.16	0.98	0.33
Restraint duration × Moult	0.11	0.11	1.93	0.17	0.31	0.31	1.87	0.18

Table 5

Females' body mass, wing length, restraint, seasons and brooding status in relation to corticosterone and H:L ratio of the Village Weaver birds. Asterisk indicate significance (39 females).

Variables	Sum Sq	Mean Sq	F	P	Sum Sq	Mean Sq	F	P
Females					Response H:L Ratio			
Response Corticosterone								
Body mass	0.05	0.05	0.96	0.33	0.01	0.01	0.01	0.89
Wing length	0.03	0.03	0.55	0.46	0.01	0.01	0.03	0.39
Restraint duration	0.03	0.03	0.60	0.44	0.05	0.05	0.31	0.86
Temperature	0.05	0.05	1.10	0.30	0.01	0.01	0.07	0.36
Brooding	0.09	0.09	1.77	0.19	0.01	0.01	0.01	0.17
Seasons	0.02	0.02	0.41	0.53	0.01	0.01	0.01	0.28
Temperature × Seasons	0.27	0.27	5.54	0.02 ^a	0.01	0.01	0.01	0.08
Brooding × Seasons	0.07	0.07	1.39	0.25	0.06	0.01	0.33	0.33
Restraint duration × Brooding	0.36	0.36	7.21	0.01 ^a	0.13	0.13	0.77	0.18

^a = Emphasizing significance.

Table 6

Loadings of PCA for environmental temperature, time of the day, restraint duration, biometrics and hematological parameters of male and female Village Weaver birds during breeding and post-breeding (n = 53 males, 39 females).

Variables	Males		Females	
	PC1	PC2	PC1	PC2
Packed cell volume	0.13	0.39	0.15	-0.19
Baseline (CORT)	-0.48	-0.03	-0.17	0.43
Heterophils	-0.55	0.55	-0.56	0.57
Lymphocytes	0.45	-0.37	0.62	-0.27
H: L Ratio	-0.72	0.63	-0.62	0.67
Temperature	-0.45	-0.23	0.22	0.33
Wing length	-0.63	-0.59	0.60	0.60
Body mass	-0.54	-0.64	0.64	0.59
Time of day (TOD)	-0.24	0.02	-0.47	-0.43
Restraint duration	0.19	0.20	-0.37	0.02

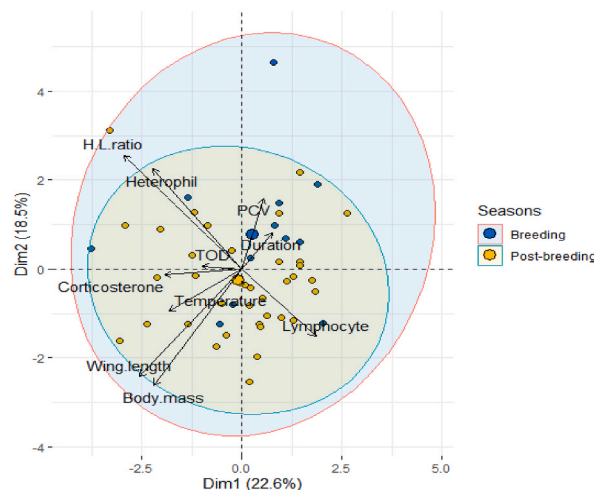


Fig. 2. Principal component analyses (PCA) indicating the score plot for the first and second components. The large oval blue shape indicates the direction of the relationship between the biometrics of the male Village Weaver birds' stress parameters during breeding; the oval yellow shape indicates the direction of the relationship between biometrics and stress parameters of the male birds during post-breeding. The arrows length is approximation of variance of the variables, while angles approximate the correlations. Points closer to each other indicate they have similar score on the PCA components. The biplot suggests that body mass and wing length negatively correlated to packed cell volume (PCV) and restraint duration. On the other hand, heterophil and the heterophil to lymphocyte ratio (H:L) were negatively correlated to males birds' lymphocytes. Time of day (TOD) and temperature moved in the same direction as corticosterone; hence, suggesting a positive correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

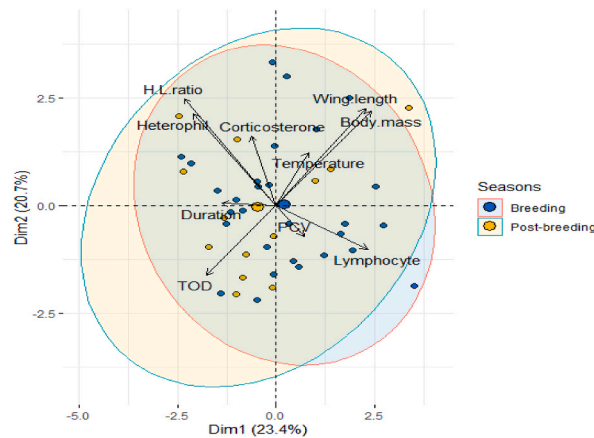


Fig. 3. Principal component analyses (PCA) indicating the score plot for the first and second components. The larger yellow oval shape indicates the direction of the relationship between the biometrics of the female Village Weaver birds' stress parameters during post-breeding; the oval blue shape indicates the direction of the relationship between biometrics and stress parameters of the female birds during breeding. The arrows length is approximation of variance of the variables, while angles approximate the correlations. Points closer to each other indicate they have similar score on the PCA components. The biplot suggests that temperature, body mass and wing length negatively correlated to time of day (TOD). Heterophil, corticosterone and the heterophil to lymphocyte ratio (H:L) correlated positively to each other. However, the increase of these three parameters had a negative effect on the packed cell volume (PCV) and lymphocytes of the female birds. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cooler temperature condition of Amurum Forest Reserve during the breeding season, or the male birds were encountered more during this period because the birds were sexually active and the need to search for mates during the breeding. Conversely, the lower number of female birds encountered in this study was attributed to the fact that some female birds were brooding and incubating the eggs as part of their parental effort during breeding.

The temperature condition of Amurum Forest Reserve was higher during post-breeding; however, during this period, both male and female Village Weavers experienced a higher mean BMI (Table 1). Indeed, relatively short-term seasonal variations in weather indices are crucial extrinsic drivers of avian body condition [26], and at higher mean levels, temperature usually correlates with the body condition of passerine birds [74–76]. In this regard, female Village Weavers' high mean BMI under higher mean temperature (during post-breeding) is attributable to the weight gained to make up for the expended energy during breeding (owing to the draining of the birds' body nutrients during laying and incubation of eggs) [26]. On the other hand, the high male BMI during post-breeding (under higher mean thermal condition) is attributable to the less energetic activities during this period, when compared with the more energy expended to acquire more mates and guard territories during breeding (Table 1).

Sex was not a determining factor influencing the baseline (CORT), PCV and H:L ratio of the Village Weavers; however, contrary to our expectation that the birds' baseline (CORT) should be higher during breeding, we found that the mean baseline (CORT) for both male and female birds during breeding and post-breeding exceeded set baseline value of 0.7–57 $\mu\text{g}/\text{dL}$ (of other studies e.g. [35,36]). These higher mean value in baseline (CORT) is usually attributed to the short-term changes in temperature condition during breeding and post-breeding seasons. Basically, environmental temperature is a major determinant of (CORT) release in most vertebrates [77–80]. As such, the current study found that the baseline (CORT) of both male and female birds significantly correlated to mean temperature of Amurum Forest Reserve (Table 2).

To respond to stimuli, there is a fast transient release of (CORT) far above the normal baseline (CORT) [81,82]. The acute elevation of baseline (CORT) support immune responses and mobilize energy reserves for animals to escape a potentially dangerous situation [83]. However, under chronic situations, prolonged release of (CORT) could lead to disrupted cognition, immune function and poorer breeding success [84–86]. In line with our prediction, and owing to the impact on immune function [84], this study found that the H:L ratio correlated to baseline (CORT) levels of the birds (Table 3).

In this study, one of our objectives was to investigate whether post-breeding moult on male birds (an energetically demanding period when sexually dimorphic male birds replace their breeding plumage feathers) [87], and whether brooding status in female birds correlated to stress indicators (i.e. baseline CORT and H:L ratio). However, we found that seasons and environmental temperature mainly correlated to males' baseline (CORT) (Table 4). Conversely, we observed that the joint interactive effect between restraint and brooding, and between environmental temperature and seasons correlated to females' baseline (CORT) (Table 5). The possible reason for female passerine birds such as the Village Weavers to secrete more (CORT) during restraint could be linked to the female birds' smaller size [88]. Even though, in this study, we expected that the female birds' body size should have a less gravitational influence (larger surface area relative to size) that would give them an evolutionary advantage over their male counterparts [29,30,70]; hence, restraint and brooding should not have correlated to female birds' baseline (CORT).

The wing length (which was used as a measure for both male and female birds' body size) correlated to packed cell volume of the birds (Table 2). More so, the direction of the relationship on a PCA biplot suggests that restraint caused an increase in packed cell volume of the male birds (Fig. 2), when compared with the females (Fig. 3). Indeed, packed cell volume is important in the

transportation of oxygen and glucose to aid the production and regulation of energy in body tissues [89]. However, restraining the birds even within 3 min of capture might have had a serious consequence on the female birds' oxygen supply.

In male birds, environmental temperature increased with an increase in baseline (CORT), and there is the possibility that the increase in (CORT) impacted the H:L ratio. As such, there was a negative relationship between heterophils and lymphocytes (Table 6 and Fig. 2). In a previous study, the H:L levels changed due to (CORT)-induced redistribution of the blood cells from the various parts of the body. The process led to an increase in heterophils, while causing the opposite to occur for the lymphocytes [90]. The stress-induced reductions in the number of circulating lymphocyte is not due to the killing or destruction of the cells, but rather it is suggested that (CORT)-induced stress usually causes the redistribution of lymphocytes from the blood to other body compartments [33]. Thus, in response to (CORT), the circulating lymphocytes become redistributed to the endothelial cells that line the walls of blood vessels, before undergoing transmigration and get sequestered into other tissues such as the lymph nodes, spleen, bone marrow and skin [33,91,92]. More so, from the first PC of this study, there is the possibility that larger or heavier male birds experienced a higher release of (CORT) which impacted the male birds' lymphocytes (Table 6 and Fig. 2). As such, there is the possibility of the disruption of the male birds' immune function during breeding and post-breeding seasons.

Conversely, the female birds' second PC suggests a positive relationship between baseline (CORT), body mass, body size, H:L ratio, baseline (CORT) and the environmental temperature (Table 6 and Fig. 3). Despite this, there is the possibility that the size or weight of the female birds may associate negatively with the birds' heterophil level as shown by the negative relationship between body mass and wing length of the female birds in relation to the heterophils of the females' on the first PC (Table 6 and Fig. 3). Suggesting that the smaller the female birds are, the lower their chances of fighting infection or performing their role in innate immune function, and mediating the acute inflammation response [93].

5. Conclusion

The breeding and post-breeding biometrics in relation to stress indicators of the Village Weavers has been documented. Suggesting that seasonal condition of the environment impacts wild animals' abundance, morphology and physiology. As such, activities under constrained environmental temperature condition and restraint during breeding and post-breeding affect wild birds' haematological and physiological indices. Basically, the seasonal effect of thermal condition impacted the baseline (CORT) of the birds, which in turn, affected other hematological processes of the birds. For instance, the elevated level of heterophils and the opposite observed for the lymphocytes suggest the effect of (CORT) on stress indicators. In spite that this study confirmed the seasonal physiological condition of the Village Weavers, and suggested factors that could possibly impact them, there is still a need for future studies to confirm whether the variation in morphological and stress indicators is possible in birds of different species.

Data availability statement

Data will be made available on request.

CRedit authorship contribution statement

Felix A. Andong: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vincent C. Ejere:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Onyinyechukwu A. Agina:** Writing – review & editing, Visualization, Validation, Investigation. **Samuel O. Ekere:** Validation, Methodology, Formal analysis. **Ezekiel S. Mayowa:** Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported with materials from the following institutions: (i) The National Veterinary Research Institute, Vom in Plateau State; (ii) The Department of Zoology and Environmental Biology, the University of Nigeria, Nsukka in Enugu State, and (iii) The Department of Zoology, the University of Jos in Plateau State. Specifically, we wish to thank the Head and Director of APLORI (Adams Chaskda; Ph.D.), including the Director of Scientific Research (Talatu Tende; Ph.D.). We also thank the following people for their help with various aspects of this project: Prof. Joseph Eyo, Martha Izang and Arin Izang.

References

- [1] C. Müller, S. Jenni-Eiermann, L. Jenni, Heterophils/Lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings, *Funct. Ecol.* 25 (2011) 566–576, 2011.
- [2] M. Wikelski, S.J. Cooke, Conservation physiology, *Trends Ecol. Evol.* 21 (2006) 38–46.

- [3] M. Bejaei, K.M. Cheng, Effects of pretransport handling stress on physiological and behavioral response of ostriches, *Poultry Sci.* 93 (2014) 1137–1148.
- [4] P.P. Marra, E.B. Cohen, S.R. Loss, J.E. Rutter, C.M.A. Tonra, A call for full annual cycle research in animal ecology, *Biol. Lett.* 11 (2015) 1–4.
- [5] Z. Renthlei, S. Yatung, R. Lalpekhlu, A.K. Trivedi, Seasonality in tropical birds, *J. Exp. Zool.: Ecol. Integr. Physiol.* 337 (2022) 952–966, 2022.
- [6] L.B. Buckley, A.H. Hurlbert, W. Jetz, Broad-scale ecological implications of ectothermy and endothermy in changing environments, *Global Ecol. Biogeogr.* 21 (2012) 873–885.
- [7] J. Goldenberg, K. Bisschop, L. D'Alba, M.D. Shawkey, The link between body size, colouration and thermoregulation and their integration into ecogeographical rules: a critical appraisal in light of climate change, *Oikos* 2022 (2022) 1–15.
- [8] D.M. Bryant, Energy expenditure in wild birds, *Proc. Nutr. Soc.* 56 (1997) 1025–1039.
- [9] N.M. Thometz, T.L. Kendall, B.P. Richter, T.M. Williams, The high cost of reproduction in sea otters necessitates unique physiological adaptations, *J. Exp. Biol.* 219 (2016) 2260–2264.
- [10] R.E. Dunn, S. Wanless, F. Daunt, M.P. Harris, J.A. Green, A year in the life of a North Atlantic seabird: behavioural and energetic adjustments during the annual cycle, *Sci. Rep.* 10 (2020) 1–11.
- [11] P. Minias, The use of haemoglobin concentrations to assess physiological condition in birds: a review, *Conserv. Physiol.* 3 (2015) 1–15.
- [12] L. Li, J. Ge, S. Zheng, L. Hong, X. Zhang, M. Li, J. Liu, Thermogenic responses in Eurasian Tree Sparrow (*Passer montanus*) to seasonal acclimatization and temperature-photoperiod acclimation, *Avian Res* 11 (2020) 1–13.
- [13] A.J. Ishong, T.C. Omotoriogun, Intra- and inter-annual variation in body mass of two species of weavers in an Afrotropical environment, *Hous. Theor. Soc.* 69 (2022) 83–89.
- [14] L.M. Romero, J.M. Reed, Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 140 (2005) 73–79.
- [15] L.M. Romero, C.J. Meister, N.E. Cyr, G.J. Kenagy, J.C. Wingfield, Seasonal glucocorticoid responses to capture in wild free-living mammals, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294 (2008) 614–622.
- [16] E. Beaugeard, F. Brischox, P.Y. Henry, C. Parenteau, C. Trouvé, F. Angelier, Does urbanization cause stress in wild birds during development? Insights from feather corticosterone levels in juvenile house sparrows (*Passer domesticus*), *Ecol. Evol.* 9 (2019) 640–652.
- [17] N. Elabarany, A comparative study of some haematological and biochemical parameters between two species from the Anatidae family within migration season, *J. Basic Appl. Zool.* 79 (2018) 1–9.
- [18] B. Dolka, R. Włodarczyk, A. Zbikowski, I. Dolka, P. Szeleszczuk, W. Kluciński, Hematological parameters in relation to age, sex and biochemical values for mute swans (*Cygnus olor*), *Vet. Res. Commun.* 38 (2014) 93–100.
- [19] L. Romero, Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates, *Gen. Comp. Endocrinol.* 128 (2002) 1–24.
- [20] M.J. Dickens, G.E. Bentley, Stress, captivity, and reproduction in a wild bird species, *Horm. Beyond Behav.* 66 (2014) 685–693.
- [21] C.W. Breuner, B. Delehanty, R. Boonstra, Evaluating stress in natural populations of vertebrates: total CORT is not good enough, *Funct. Ecol.* 27 (2012) 24–36.
- [22] B.S. McEwen, J.C. Wingfield, Allostasis and allostatic load, *Encyclopedia of Stress* (2007) 135–141.
- [23] L.M. Romero, Physiological stress in ecology: lessons from biomedical research, *Trends Ecol. Evol.* 19 (2004) 249–255.
- [24] M. Hau, R.E. Ricklefs, M. Wikelski, K.A. Lee, J.D. Brawn, Corticosterone, testosterone and life-history strategies of birds, *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 277 (2010) 3203–3212.
- [25] D. Mota-Rojas, M. Marcet-Rius, A. Domínguez-Oliva, J. Buenhombre, E.A. Daza-Cardona, K. Lezama-García, A. Olmos-Hernández, A. Verduzco-Mendoza, C. Bienboire-Frosini, Parental behavior and newborn attachment in birds: life history traits and endocrine responses, *Front. Psychol.* 14 (2023) 1–13.
- [26] M.W. McCloy, J.K. Grace, K. J. Short-term weather patterns influence avian body condition during the breeding season, *Front. Ecol. Evol.* 11 (2023) 1–15, 2023.
- [27] C.A. Schmidt-Wellenburg, S. Engel, G.H. Visser, Energy expenditure during flight in relation to body mass: effects of natural increases in mass and artificial load in Rose Coloured Starlings, *J. Comp. Physiol. B.* 178 (2008) 767–777.
- [28] S. Yalçın, H.C. Güler, Interaction of transport distance and body weight on preslaughter stress and breast meat quality of broilers, *Br. Poult. Sci.* 53 (2012) 175–182.
- [29] E. Edeline, G. Lacroix, C. Delire, N. Poulet, S. Legendre, Ecological emergence of thermal clines in body size, *Global Change Biol.* 19 (2013) 3062–3068.
- [30] M.A. Olalla-Tárraga, M.A. Rodríguez, Energy and interspecific body size patterns of amphibian faunas in Europe and North America: anurans follow Bergmann's rule, urodeles its converse, *Global Ecol. Biogeogr.* 16 (2007) 606–617.
- [31] D.L. Moreno-Azócar, A.A. Nayan, M.G. Perotti, F.B. Cruz, How and when melanistic coloration is an advantage for lizards: the case of three closely-related species of *Liolaemus*, *Zool.* 141 (2020) 1–38.
- [32] A.K. Davis, D. L. Maney, J.C. Maerz, The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists, *Funct. Ecol.* 22 (2008) 760–772.
- [33] F.S. Dhabhar, A hassle a day may keep the doctor away: stress and the augmentation of immune function, *Integr. Comp. Biol.* 42 (2002) 556–564.
- [34] L.M. Romero, R.C. Romero, Corticosterone responses in wild birds: the importance of rapid initial sampling, *Condor* 104 (2002) 129–135.
- [35] L.Z. Garamszegi, K. Hirschenhauser, V. Bokony, M. Eens, S. Hurtrez-Bousses, A.P. Moller, R.F. Oliveira, J.C. Wingfield, Latitudinal distribution, migration, and testosterone levels in birds, *Am. Nat.* 172 (2008) 533–546.
- [36] V. Bo'kony, A.Z. Lendvai, A. Liker, F. Angelier, J.C. Wingfield, O. Chastel, Stress response and the value of reproduction: are birds prudent parents? *Am. Nat.* 173 (2009) 589–598.
- [37] T.W. Campbell, Hematology of birds, in: M.A. Thrall, D.C. Baker, T.W. Campbell, M.J. Fettman, E.D. Lassen, A. Rebar, G. Weiser (Eds.), *Veterinary Hematology and Clinical Chemistry*, Wiley-Blackwell Publishing, Oxford, 2004, pp. 225–258.
- [38] J. Vickery, P.J. Jones, A new ornithological institute in Nigeria, *Bull. Afr. Bird Club* 9 (2002) 61–62.
- [39] G.S. Mwanasat, Y.N. Lohdip, F.D. Dami, Activities of the AP Leventis, the West African foremost ornithological research center, *Sci. World J.* 6 (2011) (2011) 9–12.
- [40] N. Janfa, M.J. Francis, C. Kambai, M.S. Chomini, S.A. Popoola, M. Ukwadi, C.J. Ukanyirioha, T.A. Erhabor, Y.E. Sadiku, J.A. Imoh, Structure of plants across habitat types in Amurum Forest Reserve, Plateau State Nigeria, *J. Appl. Sci. Environ. Manag.* 25 (2021) 847–850.
- [41] K. Yessoufou, B. Michelle VanDer, J. Abalaka, B.H. Daru, Evolution of fig-frugivore interactions in West Africa, *Isr. J. Ecol. Evol.* 58 (2012) 39–51, 2012.
- [42] A.U. Ezealor, In, L.D.C. Fishpool, M.I. Evans, Important Bird Areas in Africa and Associated Island: Priority Site for Conservation, Pisces publication and Bird Life International (Bird Life conservation series NO.11), Newbury And Cambridge, UK, 2001, pp. 2–3.
- [43] R.B. Payne, A new species of firefinch *Lagonosticta* from northern Nigeria and its association with the Jos Plateau Indigobird (*Vidua maryae*), *Ibis* 140 (1998) 368–381.
- [44] N.S. Dawang, A. Abdulhameed, G.A. Ezra, Phytodiversity of three habitat types in Amurum forest: an important bird area in Jos, Nigeria, *Afr. J. Nat. Sci.* 13 (2010) 85–94.
- [45] C.J. Nwaogu, W. Cresswell, Body reserves in intra-African migrants, *J. Ornithol.* 157 (2016) 125–135.
- [46] P.A.R. Hockey, W.R.J. Dean, P.G. Ryan, Roberts - Birds of Southern Africa. The Trustees of the John Voelcker Bird Book Fund, Cape Town, 2005.
- [47] D.T.C. Cox, M.J. Brandt, R. McGregor, U. Otosson, M.C. Stevens, W. Cresswell, The seasonality of breeding in savannah birds of West Africa assessed from brood patch and juvenile occurrence, *J. Ornithol.* 154 (2013) 671–683.
- [48] C.H. Fry, S. Keith, *The Birds of Africa*, Christopher Helm, London, 2004, pp. 138–202.
- [49] D.C. Lahti, A case study of species assessment in invasion biology: the Village Weaverbird *Ploceus cucullatus*, *Anim. Biodivers. Conserv.* 26 (2003) 1–11, 2003.
- [50] BirdLife International. Species factsheet: *Ploceus cucullatus*, 2023. Downloaded.
- [51] N.E. Collias, E.C. Collias, The behaviour of the West African village weaverbird, *Ibis* 112 (1970) 457–480.
- [52] K. Khan, B. Habig, D.C. Lahti, Behavioural analysis of Village Weavers *Ploceus cucullatus* in an Ethiopian breeding colony during early incubation: 2. Males, *Ostrich* 90 (2019) 233–239.
- [53] N. Borrow, R. Demey, *Birds of Western Africa*, London WID 3QY, 2004, pp. 1–436.

- [54] A.F. Atawal, A.C. Mgbeahuruike, M. Hammers, Microfilarial infections associated with body mass loss of Village Weavers (*Ploceus cucullatus*), *Ostrich* 90 (2019) 41–44.
- [55] H.B. Ginn, D.S. Melville, Moulting in birds, *Bri. Trust Ornithol.* 1983.
- [56] C.P. Redfern, Brood-patch development and female body mass in passerines, *Ring. Migr.* 25 (2010) 33–41.
- [57] N. Elabarany, A comparative study of some haematological and biochemical parameters between two species from the Anatidae family within migration season, *J. Basic Appl. Zool.* 79 (2018) 1–9.
- [58] J.M. Pouadjeu, O. Tomášek, O. Kauzál, T.B. Nguielefack, T. Albrecht, Sources of variation in baseline and stress-induced blood glucose levels in two free-living tropical passerine species, *J. Vertebr. Biol.* 72 (2023) 1–15.
- [59] J. Wingfield, Modulation of the adrenocortical response to stress in birds, *Pers. Comp. Endocrinol.* (1994) 520–528.
- [60] P.F. Cotter, An examination of the utility of heterophil-lymphocyte ratios in assessing stress of caged hens, *Poultry Sci.* 94 (2015) 512–517.
- [61] A.M. Lucas, C. Jamroz, Atlas of Avian Hematology, Monograph 25, USDA, Washington, DC, 1961, 1961.
- [62] P.F.D. Cotter, D.M. Karcher, C. Robison, Coccinocyte: A Precursor to NK Cells of Commercial Pullets? Abstract No. M10, International Poultry Scientific Forum Georgia World Congress Center, Atlanta, Georgia, 2013.
- [63] J. Skwarska, Variation of heterophil-to-lymphocyte ratio in the Great Tit *Parus major*—a review, *Acta. Ornithol.* 53 (2019) 103–114.
- [64] B.S. Bull, J.A. Koepke, E. Simon, O.W. van Assendelft, Clinical and Laboratory Standards Institute Procedure for Determining Packed Cell Volume by the Microhematocrit Method, Approved standard third edition, USA, 2000, p. 20.
- [65] A.I. Stephen, S.T. Ubwa, O.G. Igbum, S.S. Hati, N. Alex, Analytical comparison between microhematocrit and automated methods for packed cell volume (PCV) determination, *Int. J. Hematol. Blo. Dis.* 2 (2017) 1–4.
- [66] M.G. Burt, B.L. Mangelsdorf, A. Rogers, J.T. Ho, J.G. Lewis, et al., Free and total plasma cortisol measured by immunoassay and mass spectrometry following ACTH 1–24 stimulation in the assessment of pituitary patients, *J. Clin. Endocrinol. Metab.* 98 (2013) 1883–1890.
- [67] A. Cameron, D. Henley, R. Carrell, A. Zhou, A. Clarke, S. Lightman, Temperature-responsive release of cortisol from its binding globulin: a protein thermocouple, *J. Clin. Endocrinol. Metab.* 95 (2010) 4689–4695.
- [68] R Development Core Team, R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2018.
- [69] P. Kilgas, R. Mänd, R. Magi, V. Tilgar, Hematological parameters in brood rearing great tits in relation to habitat, multiple breeding and sex, *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 114 (2006) 224–231.
- [70] D.L. Moreno-Azócar, M.F. Bonino, M.G. Perotti, J.A. Schulte, C.S. Abdala, F.B. Cruz, Effect of body mass and melanism on heat balance in *Liolaemus* lizards of the goetschi clade, *J. Exp. Biol.* 219 (2016) 1162–1171.
- [71] T.C. Munyai, S.H. Foord, Temporal patterns of ant diversity across a mountain with climatically contrasting aspects in the tropics of Africa, *PLoS One* 10 (2015) 1–16.
- [72] S. Nakagawa, H. Schielzeth, A general and simple method for obtaining R^2 from generalized linear mixed-effects models, *Methods Ecol. Evol.* 4 (2013) 133–142.
- [73] C. Müller, S. Jenni-Eiermann, L. Jenni, Heterophils/Lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings, *Funct. Ecol.* 25 (2011) 566–576.
- [74] L. Gardner, T. Amano, W.J. Sutherland, M. Clayton, Individual and demographic consequences of reduced body condition following repeated exposure to high temperatures, *Ecol.* 97 (2016) 786–795, 2016.
- [75] N. McLean, H.P. van der Jeugd, M. van de Pol, High intra-specific variation in avian body condition responses to climate limits generalization across species, *PLoS One* 13 (2018) 1–15.
- [76] M.E. Akresh, D.I. King, P.P. Marra, Hatching date influences winter habitat occupancy: examining seasonal interactions across the full annual cycle in a migratory songbird, *Ecol. Evol.* 11 (2021) 9241–9253.
- [77] J.S. Krause, J.H. Pérez, H.E. Chmura, S.K. Sweet, S.L. Meddle, K.E. Hunt, L. Gough, N. Boelman, J.C. Wingfield, The effect of extreme spring weather on body condition and stress physiology in Lapland longspurs and white-crowned sparrows breeding in the Arctic, *Gen. Comp. Endocrinol.* 237 (2016) 10–18.
- [78] T.S. Jessop, M.L. Lane, L. Teasdale, D. Stuart-Fox, R.S. Wilson, V. Careau, I.T. Moore, Multiscale evaluation of thermal dependence in the glucocorticoid response of vertebrates, *Am. Nat.* 188 (2016) 342–356.
- [79] S. Xie, L.M. Romero, Z.W. Htut, T.J. McWhorter, Stress responses to heat exposure in three species of Australian desert birds, *Physiol. Biochem. Zool.* 90 (2017) 348–358.
- [80] S. Ruuskanen, B.Y. Hsu, A. Nord, Endocrinology of thermoregulation in birds in a changing climate, *Mol. Cell. Endocrinol.* 519 (2021) 1–12.
- [81] J. Beiko, R. Lander, E. Hampson, F. Boon, D.P. Cain, Contribution of sex differences in the acute stress response to sex differences in water maze performance in the rat, *Behav. Brain Res.* 151 (2004) 239–253.
- [82] L.L. Moagi, A.R. Bourne, S.J. Cunningham, R. Jansen, C.A. Ngcamphalala, A. Ganswindt, R.A. Ridley, A.E. McKechnie, Hot days are associated with short-term adrenocortical responses in a southern African arid-zone passerine bird, *J. Exp. Biol.* 224 (2021) 1–23.
- [83] K.L. Buchanan, Reply from K.L. Buchanan, *Trends Ecol. Evol.* 15 (2000) 419–420.
- [84] B.S. McEwen, Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders, *Ann. NY. Acad. Sci.* 1032 (2004) 1–7.
- [85] F.S. Dhabhar, Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology, *Neuroimmunomodulation* 16 (2009) 300–317.
- [86] S. Lupien, B. McEwen, M. Gunnar, et al., Effects of stress throughout the lifespan on the brain, behaviour and cognition, *Nat. Rev. Neurosci.* 10 (2009) 434–445.
- [87] L.M. Romero, J.M. Reed, J.C. Wingfield, Effects of weather on corticosterone responses in wild free-living passerine birds, *Gen. Comp. Endocrinol.* 118 (2000) 113–122.
- [88] K.R. Oddie, Size matters: competition between male and female great tit offspring, *J. Anim. Ecol.* 69 (2000) 903–912.
- [89] V. Vatsalya, K.L. Arora, Association between body weight growth and selected physiological parameters in male Japanese Quail (*Coturnix japonica*), *Int. J. Poultry Sci.* 10 (2011) 680–684.
- [90] F.S. Dhabhar, A.H. Miller, B.S. McEwen, R.L. Spencer, Stress induced changes in blood leukocyte distribution—role of adrenal steroid hormones, *J. Immunol.* 157 (1996) 1638–1644.
- [91] A.S. Fauci, Mechanisms of corticosteroid action on lymphocyte subpopulations. I. Redistribution of circulating T and B lymphocytes to the bone marrow, *Immunol.* 28 (1975) 669–680.
- [92] J.J. Cohen, Thymus-derived lymphocytes sequestered in the bone marrow of hydrocortisone-treated mice, *J. Immunol.* 108 (1972) 841–844.
- [93] C.G. Scanes, Blood, *Sturkie's Avian Physiology* (2015) 167–191.