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# Sources of variation in baseline and stress-induced blood glucose levels in two free-living tropical passerine species

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**Abstract.** Blood glucose plays an essential role in the body's energy metabolism; however, sources of variation in baseline and stress-induced changes in glucose concentration remain poorly understood in tropical passerines. This study assesses intra-annual variation in baseline and stress-induced glucose concentration changes in two common free-living Afrotropical passerines, the village weaver (*Ploceus cucullatus*) (Müller, 1776) and the black-crowned waxbill (*Estrilda nonnula*) (Hartlaub, 1883), captured around Dschang, western Cameroon, using intrinsic (sex, body mass, moult and breeding status) and environmental (season, temperature and rainfall) variables as predictors. In black-crowned waxbills, we found that baseline glucose was significantly elevated in the dry season, with the same trend observed in the village weaver. Stress-induced elevations in glucose concentration were observed in the black-crowned waxbills in the dry season, but only when the temperature was fitted as a covariate. Village weaver females showed higher stress-induced changes in glucose compared to males. Body mass and moulting were not predictors of baseline or stress-induced changes in glucose concentrations. This study reveals that some fluctuations in glucose levels may be mediated by seasonal changes in temperature (but not rainfall), sex, and breeding status.

**Key words:** passerines, tropical environment, seasonality, breeding, temperature, stress

## Introduction

Adaptation to the periodicity of the annual cycle is an important life-history component in a given species (Varpe 2017). In other words, a species' life history is controlled by seasonality, defined as variation in certain biotic (predation, pathogens and parasites) and abiotic (temperature and rainfall) environmental

variables across defined periods of the year (Lieth 1974) and organisms will exhibit evolutionary adaptations to such seasonal variation in abiotic and biotic factors (Alerstam et al. 2003, Forrest & Miller-Rushing 2010, Williams et al. 2017). For example, seasonal environmental variation, including temperature, day-length dynamics and precipitation, has an undoubted effect on a bird's annual cycle,

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including its timing of reproduction, song activity and related changes in physiological traits such as steroid hormone concentrations, immunity or energy metabolism (Hau 2001). In fact, the annual cycle of birds that inhabit highly seasonal northern temperate zone environments is characterised by seasonal peaks in breeding and moulting activity associated with cyclic high amplitude fluctuations in hormones (Quispe et al. 2018). These seasonal peaks are supported by large seasonal variations in mean daily temperature, day length or food abundance (Gwinner 2003).

In contrast, relatively stable tropical environments appear to select for low seasonal variation in avian physiological status (Stutchbury & Morton 2022); however, such tropical regions have so far remained relatively understudied with regard to research focusing on annual changes in avian physiology. This situation is despite the fact that tropical regions often experience distinct seasonality in humidity and rainfall, with clearly defined rainy and dry seasons (Rao et al. 2016). Tropical birds have been shown to use slight seasonal changes in daylight length for the optimal timing of breeding (Hau 2001). Several studies on tropical bird species also report restricted breeding seasons (Skutch 1950), moulting seasons (Nwaogu & Cresswell 2021), migration in specific periods of the year (Jahn et al. 2020), relatively low long-term repeatability in basal metabolic rates resembling temperate species (Bushuev et al. 2021) and seasonal changes in innate immunity mediated by rainfall (Nwaogu et al. 2019). Heavy rainfall is an important cue for tropical bird phenology (Quispe et al. 2018). Generally speaking, heavy rains are likely to be associated with increased stress and elevated energy metabolism (Vaughn et al. 2020, Tomášek et al. 2022), which may alter the physiological status of a bird. Thus, as stated by Stutchbury & Morton (2008), tropical birds offer unique opportunities to test ecological and evolutionary theory as their life history traits are diverse and differ from temperate zone models upon which most empirical studies are based.

In addition to abiotic environmental variables, intrinsic parameters and their interactions with environmental conditions will also influence the adaptation capacity of birds. As such, life-history variation is underpinned by changes in numerous physiological processes and consequently, energy metabolism is the main physiological system studied in this context (Boyce et al. 2020, Tomášek et al. 2019, 2022). All living organisms require energy for their

growth, maintenance and reproduction. Though lipids may play a more important role in birds than mammals due to their importance for long-distance flight (Jenni & Jenni-Eiermann 1998), carbohydrates (mainly glucose) are still an important source of energy for birds (Braun & Sweazea 2008, Sweazea 2022). Blood glucose concentrations have recently been shown not only to be species-specific but also repeatable across species (Tomášek et al. 2019), being comparatively lower in tropical lowland species than temperate species, and co-evolving intimately with a key avian life history trait, clutch size (Tomášek et al. 2022).

Birds use glucose as a rapid source of energy for intense short-term activities and naturally maintain considerably higher blood glucose levels than mammals of the same size (Braun & Sweazea 2008, Polakof et al. 2011). The maintenance of a high blood glucose concentration in birds usually reflects their high metabolic demands. Importantly, baseline and stress-induced blood glucose levels will be influenced by several environmental factors, including season (Remage-Healey & Romero 2000), temperature (Jimeno et al. 2018) and rainfall (Vaughn et al. 2020). Blood glucose concentration is also known to be influenced by intrinsic factors such as sex (Tomášek et al. 2019, 2022), body mass (Kaliński et al. 2014, 2015), breeding status (Tomášek et al. 2022) and moulting (Podlaszczuk et al. 2017).

The impact of the environment on blood glucose regulation in birds is still poorly understood, especially in the Afrotropical region. Given the general lack of detailed knowledge of factors influencing the physiological status of Afrotropical passerines, and particularly seasonal changes in blood glucose concentration, we evaluated sources of variation in baseline and stress-induced blood glucose levels in two common passerine species of western Cameroon, the village weaver (*Ploceus cucullatus*) and the black-crowned waxbill (*Estrilda nonnula*). Both species were selected as they are abundant year-round in this part of the country (Tamungang et al. 2016) and are classified as of least concern according to the IUCN red list. We hypothesised that 1) these two tropical species would have high baseline blood glucose in the rainy season (Vaughn et al. 2020), a prediction supported previously in an extensive analysis of blood glucose levels across multiple Afrotropical passerine species (Tomášek et al. 2022), 2) stress-induced changes in blood glucose would be low in the rainy season due to elevated baseline glucose concentrations (Tomášek et al. 2022), and 3) body



mass, sex, breeding and moulting parameters will all affect individual energetic demands and influence blood glucose concentrations (Deviche et al. 2016, Podlaszczuk et al. 2017, Tomášek et al. 2019, 2022). While several relevant studies are available for temperate birds (Remage-Healey & Romero 2000, Lill 2011, Sweazea et al. 2020), our study is the first to provide a detailed examination of intraspecific seasonal dynamics in blood glucose concentration for Afrotropical passerines over an entire year.

## Material and Methods

### Study area

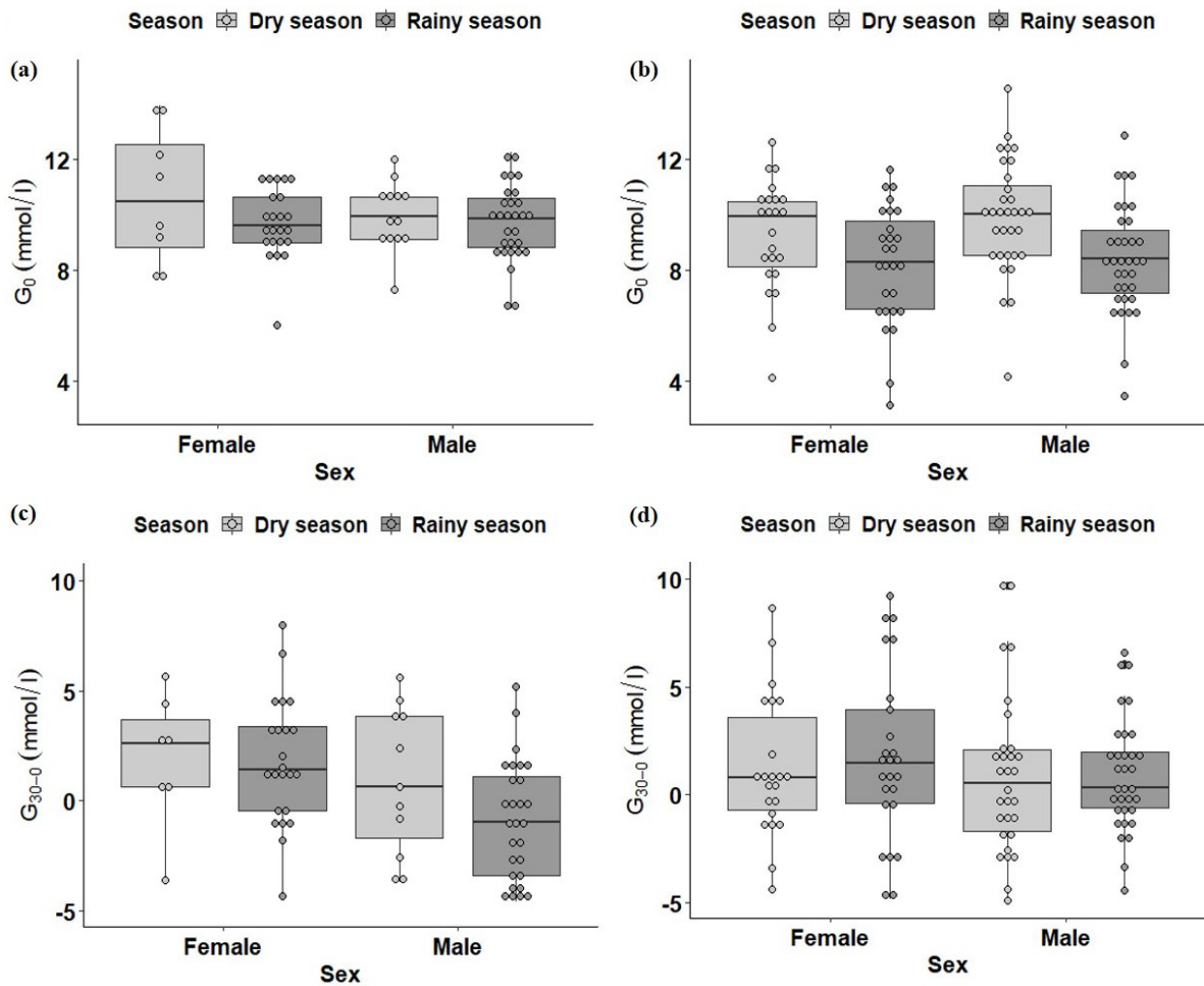
This study was conducted in Dschang (5°26'58" N 10°3'0" E), a city in western Cameroon, Africa. The city is between 1,380 m and 1,615 m in elevation and has an equatorial monsoon climate type, with an average annual rainfall of 1,790 mm (Tazen et al. 2013). Seasonal divisions are represented by the main dry season (MDS: November to mid-March), a short rainy season (SRS: mid-March to May), a short dry season (SDS: June to July) and the main rainy season (MRS: August to October) (Olivry 1986). For the present study, bird captures took place in November, December, January and February during the dry season, and March, April, May, August and September in the rainy season. Birds were captured at three main sites, i.e. around the Dschang Municipal Lake, the University campus and the Government Bilingual High School (Fig. S1). Vegetation at all three main sites consisted of fruit trees, grasses, pine trees and small plantations.

### Study design and sample collection

Village weavers and black-crowned waxbills were captured following an established protocol (Albrecht et al. 2013, Tomášek et al. 2019, 2022). In short, birds were trapped during the dry and rainy seasons over two consecutive years (2019-2020) using a 12 m mist net. The mist net was placed along cleared pathways and opened around sunrise (usually 6:00 am) and closed in the afternoon (usually 3:00 pm). Upon capture, a small amount of blood (max. approx. 0.5% of body mass) was drawn from the ulnar vein using a heparinised syringe within three minutes of the bird hitting the mist net. This blood sample was then used to determine the baseline blood glucose level ( $G_0$ ). Captured birds were enclosed in individual breathable cloth bags for thirty minutes to elicit an acute stress response and kept under shade. Subsequently, a second blood sample was taken to assess the level of stress-induced blood glucose ( $G_{30}$ ). Blood left after glucose measurements was stored

in 96% ethanol in tube vials. Blood glucose level measurements were performed directly in the field using FreeStyle Freedom Lite portable glucometers (Abbott Diabetes Care, USA; linear range: 1.1-27.8 mmol/l), previously shown to be reliable under such circumstances (Breuner et al. 2013, Tomášek et al. 2019). In passerines, measurements taken using a similar human-calibrated glucose meter (Bayer Contour, UK) based on the same principle as the device used in this study (glucose dehydrogenase with flavin adenine dinucleotide coenzyme) have been shown to be highly correlated ( $R^2 = 0.82$ ) with measurements obtained using a reference laboratory method (Morales et al. 2020). However, it is important to note that the blood glucose concentrations measured should be considered relative, as absolute concentrations will differ due to the meter's calibration to human blood (Morales et al. 2020). The measurements were duplicated on two different glucometers of the same brand. A new duplicate measurement was taken if the difference between the two devices was higher than 1.0 mmol/l. Measurement repeatability was assessed using the rptR package (version 0.9.22) and found to be very high ( $R = 0.93$ ). The glycaemic difference between  $G_0$  and  $G_{30}$ , calculated as  $G_{30} - G_0$ , was considered the stress-induced change in blood glucose levels. After glycaemia measurement, the trapped birds were weighed, checked for moult and brood patches and sexed. Village weavers were sexed based on their marked sexual dichromatism, while the sexually monomorphic black-crowned waxbills were sexed later using molecular methods. In short, DNA was extracted using the HotSHOT alkaline lysis method (Truett et al. 2000), and sex was determined via amplification of the sex-linked CHD gene (primers used for amplification and PCR protocol followed the protocol developed by Pérez et al. 2011), the PCR products being inspected on 2% agarose gel. All molecular sexing was performed at the Studenec Outside Research Facility of the Czech Academy of Science's Institute of Vertebrate Biology (Czech Republic). Finally, to avoid resampling of the same individuals, the birds were fitted with an aluminium ring with a unique SAFRING number (South African Bird Ringing Unit, Cape Town, South Africa) before being released.

Brood patches were checked in both species and scored using a six-degree scale, where 0 = absence; 1 = starting; 2 = well-defined, with breast muscle and gut still visible through the skin; 3 = skin of the belly opaque, thickened or engorged, veined and red with broad, undulating wrinkles; 4 = skin of the



**Fig. 1.** Seasonal variation in baseline glucose ( $G_0$ ) and stress-induced change in glucose ( $G_{30-0}$ ) in village weaver and black-crowned waxbills. Panels (a) and (b) compare for each sex, values of glycaemia between seasons, village weaver (Fig. 1a, female = 31, male = 42) and black-crowned female = 51, male = 67). Panel (c) and panel (d) compare stress-induced change variation in blood glucose in each sex between seasons, village weaver (Fig. 1c, female = 31, male = 42) black-crowned waxbill (Fig. 1d, female = 51, male = 67). Individual points show individual observations. The horizontal bar is the median (2<sup>nd</sup> quartile); boxes around the median represent the 1<sup>st</sup> (value above which at least 25% of data are found) and 3<sup>rd</sup> (value below which at least 75% of data are located) quartiles while the vertical lines show the 1.5 times the interquartile range of the data.

belly has thin wrinkles, engorgement of the skin now absent but the skin still stretched; 5 = feathering over (Redfern & Clark 2001). For analysis, “brood patch absent” (levels 0 and 5) was contrasted with the grouped category “brood patch present” (levels 1-4; indicating active incubation/brooding). Likewise, moulting in both sexes was scored using a six-degree scale, where 0 = old feathers; 1 = feathers missing or in pin; 2 = feathers just emerging from the sheath, up to one-third grown; 3 = new feathers between one and two-thirds grown; 4 = new feathers more than two-thirds grown, but waxy sheath still at base; 5 = new feather fully grown with no trace of a sheath (Ginn & Melville 1983), with scores 1-4 considered as moulting and scores 0 and 5 as not moulting.

Seventy-three village weavers were caught in all, 22 in the dry season (14 males and eight females) and 51 in the rainy season (28 males and 23 females), and 118 black-crowned waxbills, 55 in the dry season (32 males and 23 females) and 63 in the rainy season (35 males and 28 females).

### Statistical analysis

All analyses were conducted using the R software package (version 4.0.5; R Core Team 2020) in Rstudio (version 1.4.1106; Rstudio Team 2020). Glucose data were first checked for normality and homoscedasticity, then analysed using a linear model with either  $G_0$  or  $G_{30-0}$  as the dependent variable. We used  $G_{30-0}$  change (together with  $G_0$  fitted as a covariate) rather

**Table 1.** Variability and sample size of baseline glucose ( $G_0$ ), stress-induced glucose ( $G_{30}$ ) and stress-induced change in glucose ( $G_{30-0}$ ) in village weavers and black-crowned waxbills.

Species	Village weaver			Black-crowned waxbill		
	$G_0$	$G_{30}$	$G_{30-0}$	$G_0$	$G_{30}$	$G_{30-0}$
Mean	9.85	9.96	0.10	8.87	10.24	1.36
SD	1.47	3.47	3.54	2.21	4.02	4.27
Min	6.00	1.60	-9.00	2.30	3.35	-6.75
Max	13.95	17.60	8.00	14.60	25.75	18.25
Individuals	73	73	73	118	118	118

than fitting  $G_{30}$  as dependent and  $G_0$  as a covariate as we consider  $G_{30-0}$  to be a more intuitive way to express the stress-induced change in blood glucose, and both models provided the same results (except for the estimate of  $G_0$ ). To verify the regression to mean effect on the  $G_0$  estimate, a correction of  $G_{30-0}$  was made, as previously described by Berry et al. (1984) and Kelly & Price (2005), using the formula  $Y = (G_{30} - \mu_{G_{30}}) - p(G_0 - \mu_{G_0})$ , where  $Y$  is the corrected value of  $G_{30-0}$ ,  $p$  the correlation coefficient of  $G_{30}$  vs.  $G_0$ , and  $\mu$  the mean of  $G_0$  or  $G_{30}$ .  $Y$  was then used instead of  $G_{30-0}$  in a supplementary analysis. Body mass (continuous variable), sex, season (dry or rainy) and moult (present or absent) were used as explanatory variables. Season and sex were also fitted as an interaction to reveal potential sex differences in seasonal blood glucose level patterns. The model was progressively simplified to obtain the minimum adequate model (MAM), where non-significant ( $P$

$> 0.05$ ) independent variables were removed by backward, stepwise selection following Crawley (2014). As the simplification of complex models by stepwise removal of non-significant terms can lead to cryptic multiple hypothesis testing (Forstmeier & Schielzeth 2011), we always performed full model (model including all predictors) tests and interpreted the simplified model results if the full model was significantly different from the null model (model that contains only the overall mean) to reduce bias (Crawley 2014).

The average temperature and total precipitation of the day before each capture day were used to determine the potential contribution of weather variables on seasonal variation in  $G_0$  and  $G_{30-0}$  (Table S1), using hourly temperature and precipitation data purchased from Open Weather Map (London, UK). These parameters were then used to examine

**Table 2.** Variation in baseline glucose ( $G_0$ ) with season, sex, body mass and moult in village weavers ( $n = 73$ ) and black-crowned waxbills ( $n = 118$ ). In the linear model used,  $G_0$  was a dependent variable while season, sex, body mass and moult were predictors. The full model was not significantly different from the null model for village weavers ( $F = 1.07$ ,  $P = 0.381$ ,  $df = 5$  and  $67$ ) but statistically different for black-crowned waxbills ( $F = 3.45$ ,  $P = 0.006$ ,  $df = 5$  and  $112$ ). Significant  $P$ -values are indicated in bold. Abbreviations: SE = standard error;  $F$  = F-statistic;  $df$  = degree of freedom;  $R^2$  = adjusted R-squared.

Species	village weaver			black-crowned waxbill		
	Estimate $\pm$ SE	t-value	$P$ -value	Estimate $\pm$ SE	t-value	$P$ -value
	Full model					
Intercept	10.65 $\pm$ 1.46	7.25	<b>&lt; 0.001</b>	11.39 $\pm$ 2.19	5.18	<b>&lt; 0.001</b>
Season (rainy)	-1.32 $\pm$ 0.64	-2.03	<b>0.045</b>	-1.12 $\pm$ 0.64	-1.74	0.083
Sex (male)	-0.98 $\pm$ 0.80	-1.22	0.22	0.73 $\pm$ 0.58	1.26	0.20
Body mass	0.01 $\pm$ 0.04	0.26	0.79	-0.28 $\pm$ 0.29	-0.95	0.34
Moult (present)	-0.88 $\pm$ 0.59	-1.48	0.14	-0.46 $\pm$ 0.58	-0.78	0.43
Sex: season	0.93 $\pm$ 0.80	1.16	0.24	-0.46 $\pm$ 0.82	-0.56	0.57
	<b><math>R^2 = 0.0052</math></b>			<b><math>R^2 = 0.0947</math></b>		
	Minimal Adequate Model					
Intercept	9.85 $\pm$ 0.17	57.23	<b>&lt; 0.001</b>	9.64 $\pm$ 0.28	34.01	<b>&lt; 0.001</b>
Season (rainy)				-1.44 $\pm$ 0.38	-3.71	<b>&lt; 0.001</b>
				<b><math>R^2 = 0.0987</math></b>		

**Table 3.** Variation in baseline glucose ( $G_0$ ) with body mass, moult, breeding and season in female village weavers ( $n = 31$ ) and black-crowned waxbills ( $n = 43$ ). In the linear model used,  $G_0$  was a dependent variable, while body mass, moult, breeding and season were predictors. The full model was not significantly different from the null model for village weavers ( $F = 2.14$ ,  $P = 0.104$ ,  $df = 4$  and  $26$ ) but statistically different for black-crowned waxbills ( $F = 2.88$ ,  $P = 0.035$ ,  $df = 4$  and  $38$ ). Significant  $P$ -values are indicated in bold. Abbreviations: SE = standard error;  $F$  = F-statistic;  $df$  = degree of freedom;  $R^2$  = adjusted R-squared.

Species	Village weaver			Black-crowned waxbill		
Parameters	Estimate $\pm$ SE	t-value	P-value	Estimate $\pm$ SE	t-value	P-value
	Full model					
Intercept	10.70 $\pm$ 2.85	3.74	< <b>0.001</b>	11.76 $\pm$ 3.82	3.07	<b>0.003</b>
Body mass	0.04 $\pm$ 0.08	0.55	0.58	-0.41 $\pm$ 0.54	-0.76	0.45
Moult (present)	-1.81 $\pm$ 1.00	-1.80	0.082	-1.23 $\pm$ 0.79	-1.55	0.12
Breeding (present)	-1.54 $\pm$ 0.80	-1.91	0.066	1.65 $\pm$ 0.74	2.22	<b>0.032</b>
Season (rainy)	-1.15 $\pm$ 0.76	-1.54	0.14	-1.93 $\pm$ 0.75	-2.58	<b>0.013</b>
	<b><math>R^2 = 0.1321</math></b>			<b><math>R^2 = 0.152</math></b>		
	Minimal Adequate Model					
Intercept	9.94 $\pm$ 0.29	33.21	< <b>0.001</b>	9.10 $\pm$ 0.46	19.38	< <b>0.001</b>
Season (rainy)				-1.35 $\pm$ 0.65	-2.06	<b>0.045</b>
				<b><math>R^2 = 0.0718</math></b>		

seasonal differences between the dry and rainy seasons, using the Student's  $t$ -test. Any correlation between temperature and precipitation was assessed using Pearson's correlation test. As expected, the results showed that ambient temperature was significantly higher in the dry season (Fig. S2a), while precipitation was significantly lower than in the rainy season (Fig. S2b); thus, temperature was negatively correlated with precipitation (Fig. S2c). Consequently, we also ran models with  $G_0$  and  $G_{30-0}$  ( $Y$ ) as dependent variables, where season was replaced by temperature and precipitation. To ascertain the effect of precipitation, we ran a further model where season, temperature and precipitation were all included.

We also undertook a broad check for seasonality in breeding status, finding that, while both species breed in both seasons, the percentage of birds breeding during the rainy season was higher than in the dry season (Figs. S3a and S3b). Analyses were then performed with  $G_0$  and  $G_{30-0}$  set as dependent variables and body mass, moulting status, season and breeding status (females only) as independent variables.

## Results

### Species-specific variation in glucose levels

Mean baseline blood glucose concentration was  $8.87 \pm 2.21$  mmol/l for the black-crowned waxbill and  $9.85 \pm 1.47$  mmol/l for the village weaver, while

the stress-induced change in blood glucose was  $1.36 \pm 4.27$  mmol/l for the black-crowned waxbill and  $0.10 \pm 3.54$  mmol/l for the village weaver. However, high variability in individual stress responses ( $G_{30} - G_0$ ) were observed in both species due to high intraspecific variation in  $G_{30}$  (Table 1).

### Sources of variation in baseline glucose

Seasonal trends in  $G_0$  for each sex of each species are provided in Figs. 1a and 1b. For the black-crowned waxbill,  $G_0$  was significantly lower in the rainy season. However, for the village weaver, though season appeared to be a weakly significant predictor of  $G_0$  in the full model, it was not selected as a predictor in the MAM (effect of season as a sole predictor of  $G_0$  in village weavers:  $F = 1.07$ ,  $P = 0.381$ ,  $df = 5$  and  $67$ ).  $G_0$  was unaffected by moulting status, body mass, sex or season  $\times$  sex interaction in either species (Table 2).

To identify seasonal parameters that may drive change in  $G_0$ , average temperature and total precipitation of the day before the day of capture were fitted as predictors in the models instead of season. As suspected,  $G_0$  did not vary significantly with temperature or precipitation in either species when both predictors were fitted together. However, in black-crowned waxbills, when one of the two parameters was removed along with non-significant predictors, the other significantly affected  $G_0$  (precipitation:  $P = 0.013$ , temperature:  $P = 0.024$ ; Table S2). To confirm whether precipitation or temperature were drivers of seasonal blood glucose variation,



**Table 4.** Variation in stress-induced change in glucose ( $G_{30-0}$ ) with baseline glucose ( $G_0$ ), season, sex, body mass and moult in village weavers ( $n = 73$ ) and black-crowned waxbills ( $n = 118$ ). In the linear model used,  $G_{30-0}$  was a dependent variable while  $G_0$ , season, sex, body mass and moult were predictors. The full model was significantly different from the null model for village weavers ( $F = 2.70$ ,  $P = 0.020$ ,  $df = 6$  and  $66$ ) and black-crowned waxbills ( $F = 3.82$ ,  $P = 0.001$ ,  $df = 6$  and  $111$ ). Significant  $P$ -values are indicated in bold. Abbreviations: SE = standard error;  $F$  = F-statistic;  $df$  = degree of freedom;  $R^2$  = adjusted R-squared.

Species Parameters	Village weaver			Black-crowned waxbill		
	Estimate $\pm$ SE	t-value	$P$ -value	Estimate $\pm$ SE	t-value	$P$ -value
	Full model					
Intercept	6.49 $\pm$ 4.43	1.46	0.14	10.87 $\pm$ 4.63	2.34	0.020
$G_0$	-0.65 $\pm$ 0.27	-2.36	<b>0.021</b>	-0.81 $\pm$ 0.17	-4.53	<b>&lt; 0.001</b>
Season (rainy)	0.34 $\pm$ 1.50	0.22	0.81	-1.14 $\pm$ 1.23	-0.92	0.35
Sex (male)	-1.93 $\pm$ 1.82	-1.05	0.29	-0.15 $\pm$ 1.11	-0.13	0.89
Body mass	0.03 $\pm$ 0.09	0.35	0.72	-0.17 $\pm$ 0.56	-0.30	0.76
Moult (present)	0.15 $\pm$ 1.36	0.11	0.90	-1.53 $\pm$ 1.10	-1.38	0.16
Sex : season	-1.13 $\pm$ 1.82	-0.61	0.53	-0.28 $\pm$ 1.56	-0.17	0.85
	<b><math>R^2 = 0.1243</math></b>			<b><math>R^2 = 0.1263</math></b>		
	Minimum Adequate Model					
Intercept	8.02 $\pm$ 2.64	3.03	<b>0.003</b>	7.71 $\pm$ 1.52	5.06	<b>&lt; 0.001</b>
$G_0$	-0.65 $\pm$ 0.25	-2.53	<b>0.013</b>	-0.71 $\pm$ 0.16	-4.29	<b>&lt; 0.001</b>
Sex (male)	-2.48 $\pm$ 0.76	-3.24	<b>0.001</b>			
	<b><math>R^2 = 0.1639</math></b>			<b><math>R^2 = 0.1296</math></b>		

a model was constructed where season, temperature and precipitation were fitted together.  $G_0$  remained significantly elevated in the dry season ( $P < 0.001$ ), while the effects of temperature and precipitation disappeared (Table S3).

In a separate analysis using only females, breeding and season affected black-crowned waxbill blood glucose levels in the full model ( $F = 2.88$ ,  $P = 0.035$ ,  $df = 4$  and  $38$ ), but season remained the only predictor of  $G_0$  for black-crowned waxbills females in the MAM, with  $G_0$  elevated in the dry season (Table 3).

### Sources of variation in stress-induced changes in blood glucose

Seasonal trends in  $G_{30-0}$  for each sex in each species are presented in Figs. 1c and 1d. We recorded no effect of moulting status, body mass or the interaction of sex with season on  $G_{30-0}$  in either species. Likewise, no seasonal variation in  $G_{30-0}$  was observed in either species. While we observed a negative correlation between  $G_{30-0}$  and  $G_0$  (Table 4), the correlation disappeared when  $G_{30-0}$  was replaced with  $Y$ . Overall, the  $G_{30-0}$  stress response was stronger in village weaver females (Table 4; Table S4).

There was no correlation in either species when average temperature and total precipitation of the day before capture were used instead of season as

predictors for  $G_{30-0}$  (Table S4); however, when season, temperature and precipitation were included in the model, the stress response was significantly lower in the rainy season ( $P = 0.009$ ) and decreased with increasing temperature ( $P = 0.034$ ) in black-crowned waxbills (Table S5), while only sex affected  $G_{30-0}$  in the village weaver (Table S5).

Additionally,  $G_{30-0}$  was not associated with moulting status, season or breeding status in females of either species (Table S6).

### Discussion

Relatively stable tropical environments appear to select for low seasonal variation in avian physiological status; however, tropical avian species remain understudied. To understand whether blood glucose remains stable or displays seasonal fluctuations in tropical environments, we examined seasonal variation in  $G_0$  across two years in selected songbird species. In black-crowned waxbills,  $G_0$  was significantly affected by season, with a higher  $G_0$  observed during the dry season. Although only significant in the full model, a similar trend was observed in village weavers. In contrast, the only study assessing seasonal variation in  $G_0$  that involved tropical passerines (Tomášek et al. 2022) reported a higher  $G_0$  in tropical passerines during the rainy





season. While the above-mentioned comparative study drew its general conclusion based on the analysis of 99 Afrotropical passerine species, this does not exclude the possibility of among-species variation, which could account for the discrepancy with the current study, which analysed intraspecific variation of  $G_0$ . Indeed, our analysis suggests that seasonal variation in baseline blood glucose could be species-specific in Afrotropical passerines.

Dry and wet seasons differ in the amount of precipitation and mean daily temperatures. To better understand the causes of the seasonal variation observed in  $G_0$  in the species studied, we examined the impacts of weather variables on baseline blood glucose. For this purpose, we first identified average temperature and total precipitation for the day preceding capture as variables that differ significantly across seasons at the study site and further examined their contribution to  $G_0$  variation. Temperature and precipitation were not predictors of  $G_0$  in village weavers; however, when season was substituted with temperature and precipitation in the analysis, neither parameter was significant in black-crowned waxbills. As the removal of one of the parameters from the model resulted in the other having a significant effect, this is most likely due to collinearity between precipitation and temperature and their subsequent negative correlation.

Additional tests were performed to ascertain the effect of temperature and precipitation on blood glucose levels where season, temperature and precipitation were fitted into the model. Season appeared to be the only significant predictor, suggesting that, in black-crowned waxbills,  $G_0$  varies with season but that temperature and precipitation are not the main drivers of this seasonal variation. Importantly, when season was included in the model with temperature, the effect of temperature turned negative (though non-significant due to collinearity with season), suggesting that it cannot explain increased  $G_0$  in the dry season, i.e. the warmer season. Moreover, if the effect of season was driven by temperature, we would expect its effect to be stronger than that of season, and thus temperature, rather than season, should be retained in the MAM. Both lines of reasoning suggest that temperature does not drive the effect of season and that the actual effect of temperature on  $G_0$  is negative rather than positive. The positive effect of temperature in the model when the effect of season was not controlled was probably due to the confounding effect of season, which is unrelated to temperature and may include differences in body mass (Kaliński

et al. 2014, 2015, Vágási et al. 2020), diet (Vaughn et al. 2020), moulting (Podlaszczuk et al. 2017) or breeding status (Tomášek et al. 2022), the energy costs of which can further differ between sexes (Hambly et al. 2007, Vézina & Salvante 2010, Podlaszczuk et al. 2017; see also the discussion of these potential drivers of seasonal effects below). Furthermore, the negative effect of temperature is biologically more meaningful as lower temperatures (below the thermoneutral zone of the species) are expected to increase the energy demands of thermogenesis. This hypothesis is in accord with empirical evidence from great tits (*Parus major*; Kaliński et al. 2014), zebra finches (*Taeniopygia guttata*; Jimeno et al. 2018, Montoya et al. 2018) and African striped mice (*Rhabdomys pumilio*; Schradin et al. 2015). Though the thermoneutral zone of the species in this study are unknown, data from other Afrotropical species (Wikelski et al. 2003), including three estrildids (Pacioni et al. 2023), suggest that most of the temperature range in our dataset (approx. 19–23 °C) falls well below this zone. It is worth noting that, in the present study, only immediate (day before capture) temperature and precipitation were used and that, consequently, this may not perfectly describe the seasonal effect, which is a long-term adaptation. As such, the present results should be discussed with caution.

In the present study, no correlation was observed between  $G_0$  and body mass in either species. This result is consistent with a comparative study by Tomášek et al. (2019, 2022), where the relationship between  $G_0$  and individual body mass was also absent. At the intra-specific level, therefore, our results support the idea that blood glucose is independent of individual body mass in Afrotropical passerines and is an unlikely driver of seasonal differences in  $G_0$  detected in this study.

No differences in blood  $G_0$  levels were found between the sexes in the two species studied, suggesting that male and female carbohydrate needs do not differ. This finding contrasts with Tomášek et al.'s (2019, 2022) results, which found higher blood glucose levels in female Afrotropical passerine species. However, the finding is similar to the results of Ramage-Healey & Romero (2000) on starlings (*Sturnus vulgaris*), which also showed no sex difference in glucose levels. As such, we can hypothesise a similar glucose mobilisation in the usual physical and physiological activities of males and females. A further explanation may be that sex differences are species-specific. Examination of the interaction between sex and season was carried out for both birds, though no pattern was observed,



suggesting that males and females of both species do not differ in metabolic glucose across the dry and rainy seasons. It is also possible that sex differences depend on breeding status. The comparative analysis of Tomášek et al. (2022) only reported on sex differences during the breeding season. In the current study, only females were analysed regarding their breeding status and its effect on glucose levels.  $G_0$  did not differ between breeding and non-breeding females in the village weaver and was higher in breeding black-crowned waxbills only when considering the full model analysis. This finding suggests that breeding status is not a predictor of  $G_0$  in the village weaver or a strong predictor in the black-crowned waxbill. This finding is somewhat contradictory to that obtained by Tomášek et al. (2022), who observed a lower  $G_0$  during the breeding season, which could be justified by the fact that they performed an extensive comparative analysis involving many Afrotropical species, though with a limited number of individuals sampled per species. Overall, these findings indicate substantial interspecific variation in the effect of breeding on glucose levels in Afrotropical passerines. Additionally,  $G_0$  was unaffected by moulting status in either species studied, despite Podlaszczuk et al. (2017) observing a high baseline blood glucose level in moulting common snipes (*Gallinago gallinago*). Further investigations will be needed to establish the relationship between moulting and blood glucose in Afrotropical passerines.

The response to acute stress is an energy-demanding biological phenomenon. In stressful situations such as climatic variation, physiological changes occur that disrupt homeostasis, leading to increased activity of the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. This results in the production of catecholamine and glucocorticoids, which act synergistically to stimulate glycogenolysis and gluconeogenesis, and thus increase glucose mobilisation (Malisch et al. 2018) and its availability to the brain, muscle and other tissues (Sapolsky et al. 2000, Sweazea et al. 2020). Our study shows that  $G_0$  could be used to predict  $G_{30-0}$  in two Afrotropical bird species, showing that  $G_{30-0}$  and  $G_0$  are negatively correlated in both species. These findings suggest that birds with an elevated glucose level at the time of the capture may be unable to elevate glucose levels further after exposure to stress. When the  $G_{30-0}$  regression was subsequently corrected, the correlation between  $G_{30-0}$  and  $G_0$  disappeared, suggesting that the correlation between  $G_{30-0}$  and  $G_0$  observed in this study was purely a mathematical rather than a biological effect.

Our results also showed that the blood glucose stress response is stronger in female than male village weavers, which corroborates the findings of Tomášek et al. (2022) and Banerjee & Banerjee (1976) on Indian weavers (*Ploceus philippinus* and *Ploceus benghalensis*). Female village weavers may be more predisposed to stress and mobilise more energy substrates than males in stressful situations to survive. In black-crowned waxbills, no sex differences were observed in response to stress, suggesting a species-dependent response to stress.

As already noted with  $G_{0r}$  black-crowned waxbills displayed a seasonal difference in  $G_{30-0r}$  but only when season, precipitation and temperature were fitted in the same model, when  $G_{30-0}$  was low in the rainy season and the slope of the relationship between  $G_{30-0}$  and temperature was negative, implying a low-stress response at high temperatures. The seasonal variation in stress response observed in black-crowned waxbills was at least partly independent of the effect of temperature.

Neither body mass, moulting, nor breeding status (in females) were predictors of stress response in either species studied. These findings align with the observations on  $G_{0r}$  and the same reasons mentioned above may apply.

Our findings indicate interspecific variation in the physiological response to seasonal changes in environmental conditions, with only one of the two species studied (waxbills) showing a significant drop in baseline blood glucose concentration during the rainy season, along with an increase in stress-induced glucose concentrations during the dry season. This finding suggests that tropical passerines show seasonal flexibility in glucose regulation. Some of these fluctuations may be partly mediated by seasonal changes in temperature (but not rainfall), though they also include temperature-independent components whose effects need to be clarified in further studies. It is worth noting that, in the present study, we used average temperature and total precipitation values from the day before capture and that other time scales may produce different effects on blood glucose. To the best of our knowledge, our study is the first to provide a detailed examination of seasonal variation in blood glucose levels in any free-living tropical passerine species. Nevertheless, we could only assess detailed seasonal changes in two species, though each was represented by dozens of individuals. We encourage more detailed studies on seasonal variation in physiological status in the



presently understudied bird species inhabiting seasonal tropical environments.

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## Author Contributions

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## Supplementary online material

**Fig. S1.** Map of the study area, showing capture points in the city of Dschang.

**Fig. S2.** Seasonal variation in temperature and precipitation in Dschang ( $n = 45$  days). (a) represents the seasonal variation of average temperature one day before capture (Dry season = 18 days, Rainy season = 27 days) while (b) represents the seasonal variation of total precipitation one day before capture (Dry season = 18 days, Rainy season = 27 days). Student' t-test was used to evaluate its. (c) represents the relationship between average temperature and total precipitation one day before capture evaluated by Pearson's correlation test. Blue line depicts predicted values while grey band depicts 95% confidence interval, and black points are original data. \*\*\*:  $p < 0.001$ . The horizontal bar is the median (2<sup>nd</sup> quartile); boxes around the median represent the 1<sup>st</sup> (value above which at least 25% of data are found) and 3<sup>rd</sup> (value below which at least 75% of data are located) quartiles while the vertical bars show the lower and upper whisker.

**Fig. S3.** Distribution of females breeding in the dry and the rainy season of village weaver (a,  $n = 31$  individuals) and black-crowned waxbill (b,  $n = 43$  individuals). The orange colour represents the breeding individuals while the green colour is the non-breeding individuals.

**Table S1.** Average temperature and total precipitation of the day preceding capture during fieldwork.

**Table S2.** Variation of  $G_0$  with sex, body mass, moult, precipitation and temperature in village weavers and black-crowned waxbills ( $n = 118$  individuals).  $G_0$  was a dependent variable while sex, body mass, moult, precipitation and temperature were predictors in the linear model. Temperature become a significant predictor of  $G_0$  only when precipitation (and not temperature) was removed first from the full model for the black-crowned waxbill (estimate =  $0.36 \pm 0.16$ ,  $t$  value = 2.27,  $p = 0.024$ ). This was tested because of the negative correlation between temperature and precipitation. The full model was not significantly different from the null model for the village weaver ( $F = 0.42$ ,  $p = 0.826$ ,  $Df = 5$  and 67) and for the black-crowned waxbill ( $F = 1.96$ ,  $p = 0.089$ ,  $Df = 5$  and 112). Significant  $p$ -values are indicated in bold. Abbreviations: SE, Standard error;  $F$ ,  $F$ -statistic;  $Df$ , Degree of freedom;  $R^2$ , Adjusted R-squared.

**Table S3.** Variation of  $G_0$  with season, sex, body mass, moult, temperature and precipitation in the village weaver ( $n = 73$  individuals) and the black-crowned waxbill ( $n = 118$  individuals).  $G_0$  was a dependent variable while season, sex, body mass, moult and temperature were predictors in the linear model. The full model was not significantly different from the null model for the village weaver ( $F = 0.83$ ,  $p = 0.560$ ,  $Df = 7$  and 65) but statistically different for the black-crowned waxbill ( $F = 2.66$ ,  $p = 0.013$ ,  $Df = 7$  and 110). Significant  $p$ -values are indicated in bold. Abbreviations: SE, Standard error;  $F$ ,  $F$ -statistic;  $Df$ , Degree of freedom;  $R^2$ , Adjusted R-squared.

**Table S4.** Variation of  $G_{30-0}$  with  $G_0$ , sex, body mass, moult, precipitation and temperature in the village weaver ( $n = 73$  individuals) and the Black-crowned Waxbill ( $n = 118$  individuals).  $G_{30-0}$  was a dependent variable while  $G_0$ , sex, body mass, moult, precipitation and temperature were predictors in the linear model. The full model was significantly different from the null model for the village weaver ( $F = 3.02$ ,  $p = 0.011$ ,  $Df = 6$  and 66) and for the black-crowned waxbill ( $F = 3.69$ ,  $p = 0.002$ ,  $Df = 6$  and 111). Significant  $p$ -values are indicated in bold. Abbreviations: SE, Standard error;  $F$ ,  $F$ -statistic;  $Df$ , Degree of freedom;  $R^2$ , Adjusted R-squared.

**Table S5.** Variation of  $G_{30-0}$  with  $G_0$ , season, sex, body mass, moult, temperature and precipitation in the village weaver ( $n = 73$  individuals) and the black-crowned waxbill ( $n = 118$  individuals).  $G_{30-0}$  was a dependent

variable while  $G_0$ , season, sex, body mass, moult and temperature were predictors in the linear model. The full model was significantly different from the null model for the Village Weaver ( $F = 2.27$ ,  $p = 0.032$ ,  $Df = 8$  and 64) and for the black-crowned waxbill ( $F = 3.65$ ,  $p < 0.001$ ,  $Df = 8$  and 109). Significant  $p$ -values are indicated in bold. Abbreviations: *SE*, Standard error; *F*, *F*-statistic; *Df*, Degree of freedom;  $R^2$ , Adjusted R-squared.

**Table S6.** Variation of  $G_{30-0}$  with breeding, body mass, moult and season in female's the village weaver ( $n = 31$  individuals) and the black-crowned waxbill ( $n = 43$  individuals).  $G_{30-0}$  was a dependent variable while  $G_0$ , body mass, moult, breeding and season were predictors in the linear model. The full model was not significantly different from the null model for the village weaver ( $F = 1.39$ ,  $p = 0.261$ ,  $Df = 5$  and 25) but statistically significant for the black-crowned waxbill ( $F = 3.17$ ,  $p = 0.017$ ,  $Df = 5$  and 37). Significant  $p$ -values are indicated in bold. Abbreviations: *SE*, Standard error; *F*, *F*-statistic; *Df*, Degree of freedom;  $R^2$ , Adjusted R-squared.

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