






ORIGINAL RESEARCH

Hematocrit, age, and survival in a wild vertebrate population

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Abstract

Understanding trade-offs in wild populations is difficult, but important if we are to understand the evolution of life histories and the impact of ecological variables upon them. Markers that reflect physiological state and predict future survival would be of considerable benefit to unraveling such trade-offs and could provide insight into individual variation in senescence. However, currently used markers often yield inconsistent results. One underutilized measure is hematocrit, the proportion of blood comprising erythrocytes, which relates to the blood's oxygen-carrying capacity and viscosity, and to individual endurance. Hematocrit has been shown to decline with age in cross-sectional studies (which may be confounded by selective appearance/disappearance). However, few studies have tested whether hematocrit declines within individuals or whether low hematocrit impacts survival in wild taxa. Using longitudinal data from the Seychelles warbler (*Acrocephalus sechellensis*), we demonstrated that hematocrit increases with age in young individuals (<1.5 years) but decreases with age in older individuals (1.5–13 years). In breeders, hematocrit was higher in males than females and varied relative to breeding stage. High hematocrit was associated with lower survival in young individuals, but not older individuals. Thus, while we did not find support for hematocrit as a marker of senescence, high hematocrit is indicative of poor condition in younger individuals. Possible explanations are that these individuals were experiencing dehydration and/or high endurance demands prior to capture, which warrants further investigation. Our study demonstrates that hematocrit can be an informative metric for life-history studies investigating trade-offs between survival, longevity, and reproduction.

KEYWORDS

aging, biomarkers, birds, condition markers, hematocrit, life history, senescence, survival, trade-offs, wild populations

1 | INTRODUCTION

An organism's fitness is the product of many integrated physiological systems, and their interaction with the environment. Activity in

one physiological system can limit resource availability and generate negative consequences (e.g., by-products) for another (Harshman & Zera, 2007; Leroi, 2001; Ricklefs & Wikelski, 2002). These trade-offs form the basis of life history. Physiological markers provide valuable

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insights into life-history trade-offs, condition, and senescence—particularly in wild populations, where complex environmental factors can weaken associations between life-history traits and observed fitness (Nussey et al., 2008). Many physiological markers (oxidative stress, hormone regulation, etc.) yield complex and/or inconsistent associations with life history, survival, and reproductive success (e.g., Johnstone et al., 2017; Norris & Evans, 2000; Speakman & Selman, 2011; Wilder et al., 2016); thus, there is a continued need to identify and validate such markers.

Aerobic capacity, which contributes to endurance and performance, is a vital physiological trait for organismal health and fitness. Aerobic capacity depends on the oxygen-carrying capacity of blood, which is determined by the concentration of hemoglobin and the rate of blood flow, which is inversely proportional to blood viscosity (Birchard, 1997; Calbet et al., 2006; Wagner, 1996). These properties are reflected by hematocrit or packed cell volume (PCV); the proportion of whole-blood volume is comprised of erythrocytes. Hemoglobin and blood viscosity increase linearly and exponentially, respectively, with hematocrit (Hedrick et al., 1986). Blood becomes harder to circulate with increasing viscosity (i.e., requiring greater cardiovascular effort), but less viscous blood contains less hemoglobin. Therefore, intermediate hematocrit levels (ca. 40%) are optimal for maximum oxygen-carrying capacity and endurance (Birchard, 1997; Jensen et al., 2013; Schuler et al., 2010).

Hematocrit levels observed in nature are variable (range ca. 30%–60%) within and between endothermic species (Stark & Schuster, 2012). For example, hematocrit is higher in species/individuals requiring greater blood oxygen storage and endurance (Lourdais et al., 2014; Minias, 2015; Yap et al., 2019). Similarly, hematocrit tends to increase within individuals in response to elevated oxygen demands, such as during altitudinal migration (Borras et al., 2010) and exercise regimes (reviewed in Yap et al., 2017). Elevated hematocrit occurs via the production of new erythrocytes (erythropoiesis) and/or the release of reticulocytes (immature erythrocytes) from the bone marrow, which is triggered by hypothalamus–pituitary–adrenal-mediated stress (see Voorhees et al., 2013). More rapid (<1 hr) increases in hematocrit can occur due to a reduction in blood plasma volume (hemoconcentration), which happens during exercise and dehydration (Bury et al., 2019; Kaltreider & Meneely, 1940). In some mammal species, splenic reservoirs of erythrocytes can also increase hematocrit at the onset of stress and exercise (Böning et al., 2011).

Anemia—characterized by chronically low hematocrit and hemoglobin—occurs when an individual's rate of erythrocyte loss exceeds that of erythropoiesis, for example, during blood parasitism (O'Brien et al., 2001). However, anemia can occur without affecting hematocrit, since the release of reticulocytes, which are larger than mature erythrocytes, can rapidly complement hematocrit despite them having lower hemoglobin content (Fair et al., 2007). Anemia can also arise as a secondary outcome of competing physiological systems. For example, egg production in birds causes a reduction in hematocrit via an estrogen-mediated suppression of erythropoiesis and hemodilution—an increase in blood plasma volume (Wagner, Prevorsek, et al., 2008; Wagner, Stables, et al., 2008; Williams et al., 2004).

Therefore, both within-individual increases and decreases in oxygen-carrying capacity and associated factors (hematocrit and hemoglobin) have the potential to reflect a multitude of life-history events and trade-offs (for reviews, see Fair et al., 2007; Johnstone et al., 2017; Minias, 2015).

Uncertainty remains regarding associations between hematocrit, age, and senescence in wild animals. From birth to maturity, hematocrit increases with age (e.g., Cornell & Williams, 2017; Eklom & Lill, 2006; Trillmich et al., 2008), but few studies have determined the age dependence of hematocrit in adult life. This likely stems from the difficulty of obtaining samples of known-age adults in many wild systems. In captive mice and humans, low hematocrit in extreme old age reflects senescence in erythrocyte renewal mechanisms (Boggs & Patrene, 1985; Gaskell et al., 2008). Similarly, cross-sectional studies of other captive and wild vertebrates have observed lower hematocrit in old age, suggestive of senescence (Elliott et al., 2015; Jégo et al., 2014; Prinzinger & Misovic, 2010; Smucny et al., 2004). However, such observations may also arise from compositional changes in successive age classes of a population, for example, due to selective disappearance of individuals with high hematocrit. Longitudinal studies are needed to explicitly investigate within-individual change with age (Elliott et al., 2015; Nussey et al., 2008).

Factors that cause hematocrit to deviate from the theoretical optimum (for oxygen-carrying capacity and general health) could have long-term impacts on the fitness of wild taxa. For example, experimental reductions in hematocrit in birds can result in reduced reproductive success (Fronstin et al., 2016) and flight performance (Yap et al., 2018). However, few studies have investigated associations between hematocrit levels observed under natural conditions in the wild and subsequent survival. Anemia results in lethargy and fatigue, but even minor decreases in oxygen-carrying capacity could represent an energetic disadvantage that reduces survival prospects in wild settings. Conversely, more viscous blood, and the cardiovascular loading this creates, is linked to negative health impacts in humans (Brækkan et al., 2010; Coglianese et al., 2012; Stack & Berger, 2009; Walton et al., 2017). Extreme high or low hematocrit can also be a noncausal indicator of factors detrimental to self-maintenance, such as stress, parasitism, and nutrient deficiencies (see Johnstone et al., 2017). Therefore, intermediate hematocrit levels are expected to be optimal for survival (e.g., Boffetta et al., 2013; Bowers et al., 2014).

The isolated Seychelles warbler (*Acrocephalus sechellensis*) population on Cousin Island provides an excellent model system for studying associations between hematocrit, age, and survival in a wild population. This system benefits from over 30 years of continuous monitoring and extremely accurate survival estimates of known-age individuals that are not confounded by dispersal (Hammers et al., 2019; Komdeur, 1992; Richardson et al., 2007). Individuals have been captured and blood sampled repeatedly across their lifetime, providing a wealth of longitudinal physiological data (Hammers et al., 2015), including hematocrit. Here, we first assess the relationship between hematocrit and age. Based on previous findings across vertebrate taxa, we predict that

hematocrit increases during early life up to maturity, followed by an age-related decline. Crucially, we determine the relative contribution of longitudinal (i.e., within-individual) and cross-sectional (i.e., between-individual) effects to any age patterns observed. Hematocrit is also likely to vary between and within individuals independently of age. We determine whether this variation is explained by other factors, namely sex and social status and breeding stage, and assess within-individual repeatability of hematocrit. Lastly, we determine the relationship between hematocrit and annual survival probability. Given the potentially negative effects of both low and high hematocrit, we predicted that individuals with intermediate hematocrit values would have higher survival. Our study will therefore assess the validity of hematocrit as a marker of condition within wild animal populations, and explore its usefulness in terms of providing insights into the costs and trade-offs that individual animals face during life.

2 | METHODS

2.1 | Study species and data collection

The Seychelles warbler is a small insectivorous passerine endemic to the Seychelles. Seychelles warblers can (exceptionally) reach ages of up to 19 years (Hammers & Brouwer, 2017), though the average life span is 5.5 years for individuals that reach fledgling age (Komdeur, 1991). The population of *ca.* 320 adult individuals on Cousin Island (29 ha, 4°209 S, 55°409 E) has been extensively monitored since 1986. Monitoring is carried out for *ca.* 6 months of each year (January–March, June–September) during the minor and major breeding seasons, respectively (Komdeur & Daan, 2005). Since 1997, nearly all individuals (>96%) have been ringed with a unique combination of a British Trust for Ornithology (BTO) metal ring and three color rings for identification (Richardson et al., 2001). Individuals are usually first caught and ringed as nestlings or dependent fledglings, before sexual maturity (<8 months old). Juveniles are assigned to age categories (fledgling 1–3 months, old fledgling 3–5 months, or sub-adult 5–8 months), based on behavior and eye color, which transitions from gray in fledglings to red-brown in adults (Komdeur, 1992).

The population is structured into clearly defined territories that are defended year round. Breeding groups is comprised of one socially monogamous dominant pair (hereafter dominant breeders), but may also include 1–5 sexually mature subordinates (Richardson et al., 2002) which sometimes engage in helping behavior and co-breeding (Hammers et al., 2019). An Individual's social status in a given field season is determined through observations of behavior (see Komdeur, 2001).

During the breeding season, each territory is visited at least every 2 weeks and checked for the presence–absence of individuals identified by their color ring combination. Dominant females are followed for 15 min to determine whether an active nest is present. Once a nest is found, it is visited every 3 days for 15–60 min (to determine breeding stage) until completion or failure. For nests

that were discovered during or after the start of incubation, the egg-laying date is estimated from the timing of hatching (determined from provisioning observations) and/or fledging. Given that interisland dispersal is exceptionally rare (Komdeur et al., 2004) and resighting probabilities are close to one (Brouwer et al., 2009), birds that are not seen during a field season can be assumed dead (Hammers et al., 2013). The last day of a field season for which an individual is observed as present is taken as the date of death.

Individuals were captured using mist nets and conspecific playback (see Kingma et al., 2016 for details). *Ca.* 70 μ l of blood was drawn with a microcapillary tube from the brachial vein. A small amount (*ca.* 10 μ l) of blood sample was also stored in absolute ethanol at 4°C for future DNA extraction. This procedure is the routine, nonlethal way to sample blood from passerine birds and has been shown to have no measurable effect on condition or survival (Sheldon et al., 2008). Within *ca.* 3 hr of bleeding, microcapillary tubes were centrifuged for 8 min at 6,000 g to separate erythrocytes from plasma, white blood cells, and platelets. Hematocrit was measured (using sliding calipers \pm 0.01 mm) as the proportion of erythrocytes relative to whole-blood volume. Between the years of 2003 and 2017, 1,383 hematocrit measurements were obtained from 733 individuals. DNA was extracted using a salt extraction technique following Richardson et al. (2001), and sex of the individual was confirmed using the PCR-based method outlined by Griffiths et al. (1998).

2.2 | Statistical analyses

All statistical analyses were performed with RStudio (v1.2.5033, RStudio Team, 2020). Firstly, we investigated the relationship between hematocrit and age across all samples with a generalized additive mixed model (GAMM) using the *gamm4* package (v0.2–6). In this model, we fitted a nonparametric smoothing parameter for age to evaluate expected nonlinear relationships between hematocrit and age. Compared with linear mixed models (LMMs), which require prespecified functions between dependent and continuous predictor variables, GAMMs are more appropriate when the shape of age-dependent patterns are unknown (Hammers et al., 2016). In addition to age, the model included factors known to influence hematocrit in avian taxa (see Fair et al., 2007): sex, social status (dominant breeders vs. subordinates + juveniles), and time of day of sampling. Sex differences are likely to depend on social status; thus, a two-way interaction between sex and status was included. To control for non-independent samples, individual identity, breed group identity, and catch year were included as random intercepts.

Age-related patterns across populations can arise from selective disappearance, whereby certain phenotypes are associated with shorter life spans (Nussey et al., 2008). To control for selective disappearance effects, we repeated the model using only individuals that were dead at the time of analysis and included age at death as an additional factor (de Pol & Verhulst, 2006; van Hammers et al., 2019).

Our GAMM analysis revealed that dominant females had significantly lower hematocrit than dominant males and subordinates

(male or female). This suggested an effect of reproductive anemia on hematocrit levels of dominant females, since they produce the majority of offspring and sampling coincided with the breeding seasons. To determine whether sex-by-status differences were maintained in individuals not engaged in reproduction, we repeated the model including only sexually mature individuals (>8 months old) sampled outside of known breeding attempts; either no egg was laid for that breed group or the individual was sampled >50 days from the breed groups lay date. For a given individuals breed group, we calculated the number of days between the estimated lay date and the date of sampling. For breed groups with two or more broods (which occurs if, e.g., the first brood was predated), the closest lay date from the sample date was selected. For individuals sampled during breeding attempts (<50 days from breed groups lay date), we expected hematocrit to be lowest nearer the lay date, and only in dominant females. Since hematocrit was expected to fluctuate nonlinearly across breeding stages, nonparametric smoothing parameters were fitted for days from lay date for males and females. Separate models were created for dominant breeders and subordinates to avoid the need for complex three-way interactions between sex, status, and days from lay date. Individuals were rarely caught multiple times within the same breeding attempt; thus, this section of our analysis is cross-sectional in nature.

To separate the role of between- versus within-individual variation with age (i.e., cross-sectional from longitudinal effects), we used the within-subject centering method described by van de Pol & Wright, 2009. Briefly, age at sampling is split into two predictors, (a) mean age across all sampling events for a given individual (mean age), and (b) within-individual deviation from mean age (Δ age). Our GAMM indicated a peak in hematocrit at ca. 1.5 years of age (see Results, Figure 1). To investigate the initial increase and subsequent decrease in hematocrit in more detail, we performed within-individual centering for individuals <1.5 months and for individuals ≥ 1.5 months in separate analyses (following Hammers et al., 2016).

This allowed us to compare the drivers of age-related hematocrit patterns in early life versus later adulthood.

We created linear mixed models using the lme4 package (v1.1-21, Bates et al., 2014) with hematocrit as the response and mean age, Δ age, sex, social status, and time of day of sampling as predictors. Age terms were entered as both linear and quadratic terms to test for possible nonlinear patterns. Two-way interactions between Δ age, sex, and status were included to determine whether within-individual changes in hematocrit were dependent on these factors. Consistent with the GAMMs outlined above, individual identity, breed group identity, and catch year were included as random intercepts. Due to the relationship observed between hematocrit and breeding stage in dominant breeders (see Figure 2), we repeated the analysis excluding samples from breeding stages where hematocrit deviates from typical levels; 20 days before to 5 days after laying for dominant males, and 30–50 days after laying in dominant females. Using the rptR package (v0.9.22; Nakagawa & Schielzeth, 2010), we also calculated repeatability estimates for hematocrit within individuals to determine how consistent individual hematocrit levels are across repeated samples at different times.

Lastly, we investigated whether hematocrit predicts short-term survival. We used a binomial generalized linear mixed models (GLMMs) to test the probability of surviving 1 year beyond the date of sampling (Y/N) in relation to hematocrit. Since hematocrit exhibited different age-specific patterns in early life (<1.5 years) and adulthood (1.5–13 years; see Results), we investigated the relationship between hematocrit and survival for these two age groups in separate models. Where multiple hematocrit samples were taken per individual, only the last sample was selected, which allowed us to identify whether individuals facing imminent mortality have different hematocrit levels compared with those that survive. Additional fixed effects included age, sex, status, and quadratic functions of hematocrit and age. We also included an interaction term between age and hematocrit to see whether

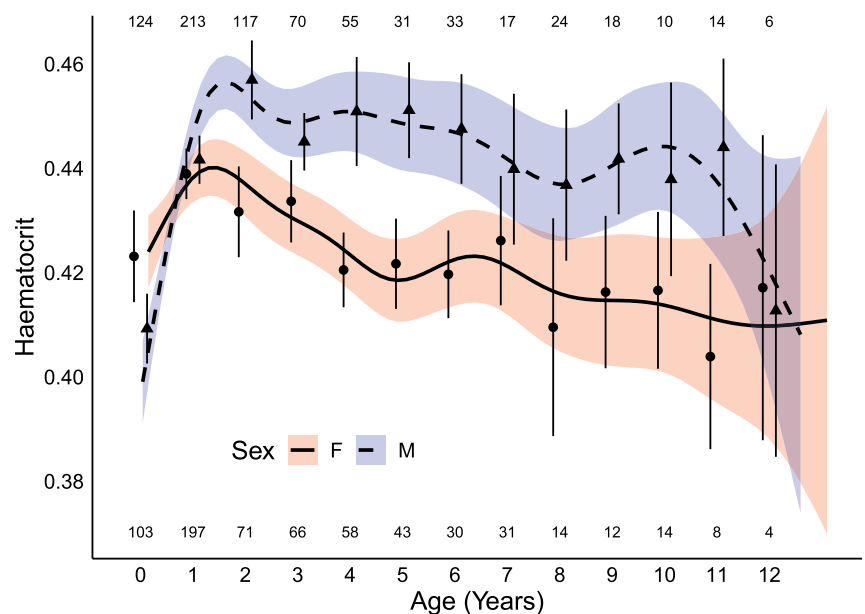


FIGURE 1 Hematocrit in Seychelles warblers in relation to age and sex. The fit lines (solid = female, dashed = male) show nonparametric smoothing functions for age with 95% confidence intervals. Points (round = female, triangles = male) are means and 95% confidence intervals for each age (rounded years). Ages 12 and 13 are grouped for graphical purposes (denoted as 12 here). Within-graph numbers represent sample sizes per age for females (lower) and males (upper)

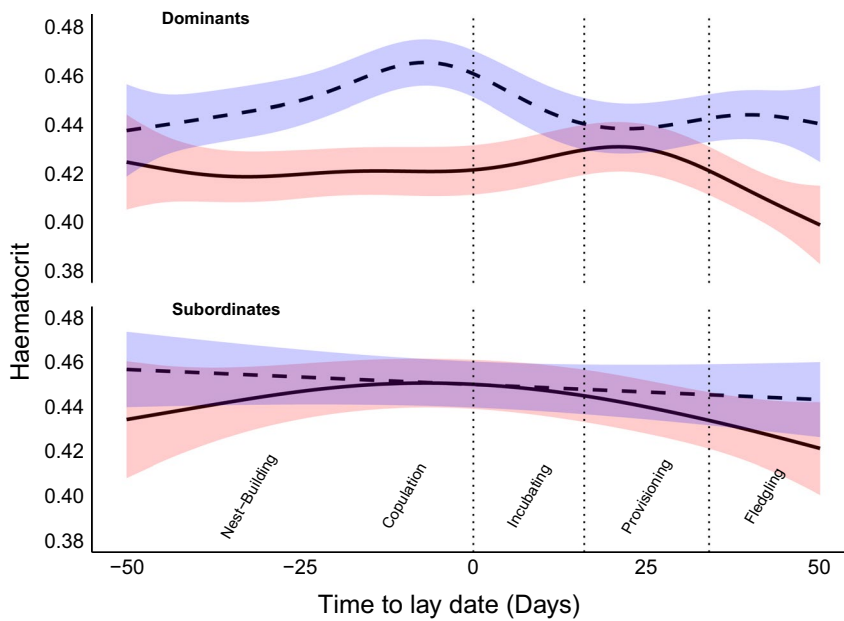


FIGURE 2 Haematocrit in Seychelles warblers in relation to days from lay date for dominant breeders (left) and subordinates (right). The fit lines (solid = female, dashed = male) show non-parametric smoothing functions for days from lay date, and the shaded area is the 95% confidence interval for the smoothing functions. The annotations and dotted lines denote theoretical nest stages relative to lay date

TABLE 1 Haematocrit in relation to cross-sectional age and other factors in Seychelles warblers

Predictor	β	SE	t	p
Intercept	0.460	0.005	94.701	<.001
Sex (male)	-0.003	0.003	-1.202	.230
Status (dominant)	-0.021	0.003	-6.440	<.001
Sample time	-0.002	0.000	-5.981	<.001
Sex \times Status	0.028	0.004	7.612	<.001
Smoothed terms	df	F	p	
Age	7.805	16.73	<.001	
Random factors	1,379 observations	Variance		
Individual identity	730 individuals	<0.000		
Breed group	747 breed groups	<0.000		
Catch year	14 years	<0.000		

Note: Results are from a GAMM analysis with a nonparametric smoothing parameter for age. Significant effects are in bold.

the effect of haematocrit on survival changed with age. Survival probability can vary between territories and years, for example, due to varying food availability (Brouwer et al., 2006; Spurgin et al., 2017); thus, breed group and catch year were also entered as random factors. Breed group was subsequently dropped as a random factor in the 1.5- to 13-year group due to model convergence issues. As with the LMM analysis, we repeated models excluding samples from breeding stages where haematocrit deviates from typical levels in dominant breeders.

In all models, nonsignificant interaction terms were removed sequentially (in order of least significance) and only reported if of specific interest. All fixed effects remained in final models (regardless of significance) except for quadratic functions of continuous variables, which were removed when nonsignificant (see Whittingham et al., 2006). Parameter estimates and significance of removed effects were determined by re-entering them into final models.

3 | RESULTS

3.1 | Cross-sectional age

Haematocrit had a distinctive pattern with cross-sectional age (Figure 1). Initially, haematocrit increased rapidly before reaching a peak at ca. 1.5 years of age. From 1.5 years onward (maximum age in this analysis is 13 years), haematocrit showed a consistent downward trajectory (Figure 1). This age-dependent pattern was similar for both sexes (Figure 1) and fitting smoothed age terms for males and females separately resulted in poorer model fit ($\Delta AIC > 4$). Sex differences in haematocrit were dependent on social status (Table 1). For dominant breeders, which are the vast majority of individuals sampled ≥ 2 years of age, females had lower average haematocrit levels than males. Dominant females also had lower haematocrit than subordinates (male or female; Table 1, Figure S1). Individuals sampled in the early

TABLE 2 Hematocrit in relation to cross-sectional age (mean age) and longitudinal age (Δ age) in Seychelles warbler <1.5 years old and 1.5–13 years old

Predictor	β	SE	t	p
<1.5 years old				
Intercept	0.393	0.010	40.92	<.001
Mean age	0.159	0.022	7.327	<.001
Mean age ²	-0.085	0.016	-5.436	<.001
Δ Age	0.036	0.008	4.437	<.001
Δ Age ²	-0.054	0.018	-3.09	.002
Sex (male)	-0.005	0.003	-1.615	.107
Status (dominant)	-0.014	0.007	-2.061	.040
Sample time	-0.002	0.000	-3.276	.001
Sex \times Status	0.017	0.008	2.143	.033
Random factors	637 observations		Variance	
Individual identity	506 individuals		<0.000	
Breed group	452 breed groups		<0.000	
Catch year	14 years		<0.000	
Predictor	β	SE	t	p
1.5–13 years old				
Intercept	0.465	0.006	74.692	<0.001
Mean age	-0.002	0.001	-3.608	.000
Δ Age	-0.002	0.001	-2.715	.007
Sex (male)	0.026	0.002	10.677	<.001
Status (dominant)	-0.013	0.003	-3.619	<.001
Sample time	-0.002	0.000	-4.714	<.001
Random factors	742 observations		Variance	
Individual identity	405 individuals		<0.000	
Breed group	529 breed groups		<0.000	
Catch year	14 years		<0.000	

Note: Parameters shown are from LMM analysis. Significant effects are in bold.

morning had higher hematocrit than those sampled in the late afternoon (Table 1). When the model was run on a subset of individuals known to be dead, including age at death as a predictor, we found that shorter-lived individuals had significantly higher hematocrit (Table S1). Therefore, selective disappearance of individuals with high hematocrit contributed to the age-specific pattern. Crucially, the effect of age was still significant when controlling for age at death (Table S1), indicating that within-individual effects were also present.

3.2 | Reproductive stage

We compared hematocrit of sexually mature (>8 months old) subordinates and dominant breeders. Outside of breeding attempts, dominant females had lower hematocrit than both dominant males and subordinate males and females (Table S2, Figure S1). During breeding

attempts, the effects were more complex (Table S3). There was no evidence of reproductive anemia (i.e., a marked decrease in hematocrit) in dominant females sampled near their lay date, although hematocrit was lower at ca. 35–50 days after laying (Figure 2). The hematocrit of males exhibited a complex relationship with breed group lay date; hematocrit was highest at ca. 7 days prior to laying and was lowest 15–30 days postlaying (Figure 2). In contrast to dominant breeders, there was no significant difference between male and female subordinates sampled during breeding attempts (Tables S3 and S4, Figure 2). The hematocrit of subordinate males did not vary in relation to breed group lay date, but subordinate females exhibited a weak quadratic relationship with days from breed group lay date; peaking at the laying date (Figure 2). Importantly, the decline of hematocrit with increasing age persisted when the analysis was split between nonbreeding and breeding individuals, and (for the latter) when controlling for days from lay date (Figure S2).

3.3 | Longitudinal age

Our within-subject centering analysis, which separates within- and between-individual contributions to age patterns, was consistent with the GAMM analysis. Below 1.5 years of age, hematocrit increased both within and between individuals with age in a quadratic pattern; a strong initial increase (i.e. that plateaued at ca. 1 year of age (Table 2, Figure 3). From 1.5 years of age onward, hematocrit declined linearly with increasing age both within and between individuals (Table 2, Figure 3). All interactions with Δ age were nonsignificant; thus, within-individual increases (<1.5 years) and decreases (1.5–13 years) did not vary between individuals of differing sex or status. Consistent with the GAMM analysis, hematocrit was lower in dominant females and individuals caught later in the day (Table 2). All results were qualitatively identical when samples from dominant breeders caught during key breeding stages (where hematocrit deviated from typical levels; see Figure 2) were excluded from the analysis (Table S5).

For final LMMs (excluding all nonsignificant interactions and nonsignificant quadratic effects), we calculated repeatability of hematocrit within individuals. For individuals below 1.5 years of age, only 1.6% ($p = .434$) of variance was due to within-individual consistency. From 1.5 years of age, within-individual consistency was higher at 7.8% and approaching significance ($p = .063$).

3.4 | Survival

A total of 263 out of the 1,383 samples taken were from individuals that died within the subsequent year. For young (<1.5 years of age), individuals with higher hematocrit were less likely to survive to the next year (Table 3, Figure 4). In contrast, hematocrit did not predict survival over the subsequent year for individuals 1.5–13 years of age (Table 3). Contrary to expectations, there was no quadratic effect of hematocrit on survival; only high hematocrit was associated with lower survival in

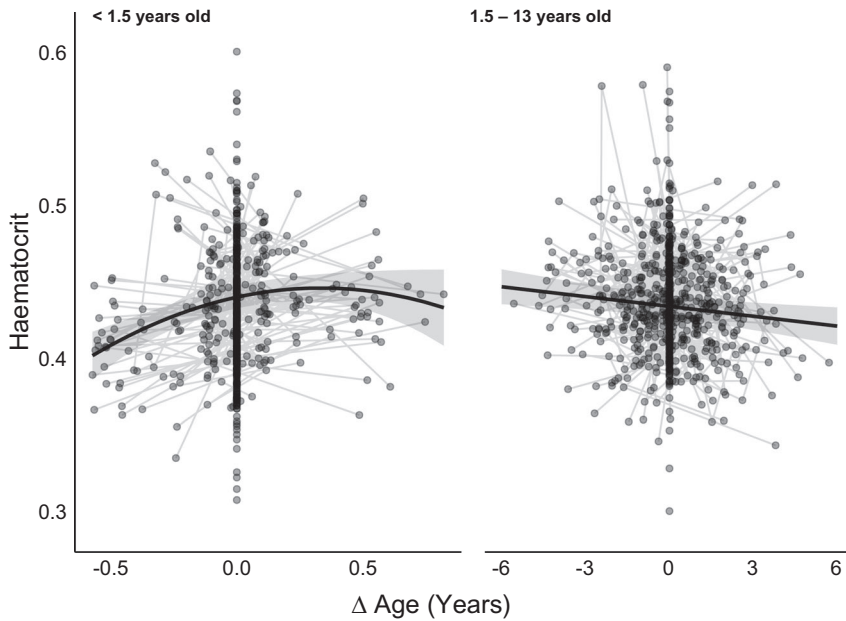


FIGURE 3 Haematocrit in Seychelles warblers in relation to within-individual differences in age (Δ Age) for individuals <1.5 years old and 1.5–13 years old. Thick black lines are the LMM (Table 2) predicted haematocrit \pm 95% CI relative to age. Raw data are haematocrit samples with thin gray lines connecting multiple samples from the same individual

young individuals. The effect of haematocrit on survival was not influenced by age in either age category. Survival probability was lower for males and subordinates from 1.5–13 years of age (Table 3). Repeating the analysis while excluding samples from dominant breeders caught at key breeding stages (where haematocrit deviated from typical levels; Figure 2) did not qualitatively change results (Table S6).

4 | DISCUSSION

Haematocrit increased in juveniles up to 1.5 years of age, beyond which haematocrit declined with increasing age. Both longitudinal change and the selective disappearance of individuals with high haematocrit contributed to this age-specific pattern. In early life (<1.5 years), haematocrit increased within individuals, but individuals with higher haematocrit were less likely to survive to the following year. After 1.5 years of age, haematocrit declined within individuals with advancing age. However, haematocrit did not predict survival in this older age range. In addition to age, haematocrit was lower in females compared with males, but only in dominant breeders (haematocrit did not significantly differ between subordinates and dominant males). The haematocrit of dominant breeders also varied with breeding stage—most notably, male haematocrit peaked in the days prior to the pair-bonded females lay date. However, the relationship between haematocrit with both age and survival persisted when samples from dominant individuals caught during key breeding stages were excluded from analyses.

4.1 | Age

Increases in haematocrit and oxygen-carrying capacity during development have been observed in a range of vertebrates (Fair et al., 2007; Petschow et al., 1978; Trillmich et al., 2008). In birds,

adult levels of haematocrit are usually achieved at late-nestling age, presumably in preparation for fledging (Eklom & Lill, 2006a, 2006b). However, in the Seychelles warbler haematocrit continues to increase postfledging (up to 1.5 years). Our longitudinal and survival analyses confirmed that increases in haematocrit occurred within juveniles and were not due to juveniles with low haematocrit having lower

TABLE 3 Survival in the Seychelles warbler in relation to haematocrit for individuals 1.5 years old and 1.5–13 years old

Predictor	β	SE	z	p
<1.5 years old				
Intercept	5.016	1.283	3.909	<.001
Haematocrit	-9.293	3.004	-3.093	.002
Sex (male)	-0.224	0.224	-1.001	.317
Status (dominant)	0.565	0.369	1.533	.125
Age	0.459	0.395	1.161	.246
Random factors		506 observations	Variance	
Breed group	418 breed groups		<0.000	
Catch year	14 years		0.340	
Predictor	β	SE	z	p
1.5–13 years old				
Intercept	1.485	1.668	0.89	.3733
Haematocrit	-1.398	3.684	-0.379	.7044
Sex (male)	-0.557	0.254	-2.196	.0281
Status (dominant)	0.724	0.345	2.099	.0358
Age	-0.009	0.043	-0.202	.8403
Random factors		408 observations	Variance	
Catch year	13 years		0.370	

Note: Results are from binomial GLMMs with survival to the following year (Y/N) as the response variable. Significant effects are in bold.

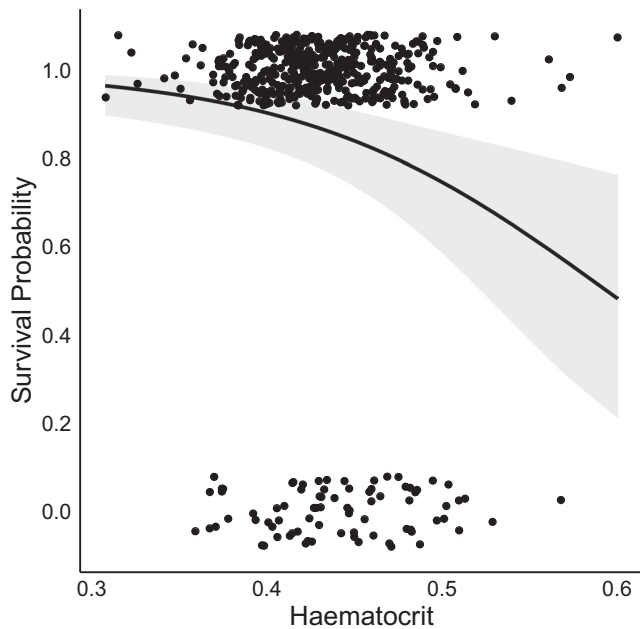


FIGURE 4 The probability of young (<1.5 years of age) Seychelles warblers surviving one year after sampling relative to haematocrit. The fit-line is a binomial regression between survival (Y/N) and haematocrit with 95% confidence limits. Raw data depicts the distribution of observed survival counts (1 = survived, 0 = deceased)

annual survival, that is, selective disappearance. In this species, fledglings receive parental care (provisioning) for up to 3 months (Komdeur, 1996) and may delay dispersal from the natal territory (i.e., become subordinates) for 1–3 years (Hammers et al., 2013). Socially dominant individuals are expected to have higher aerobic demands (for territory defense and reproduction) than juveniles, which might explain why haematocrit increases up to 1.5 years of age. Interestingly, haematocrit levels at this age (1–3 years) were higher than levels across prime reproductive ages (ca. 4–7 years; Komdeur, 1996; Hammers et al., 2012), which could reflect elevated oxygen demands and/or stress in subordinates competing for dominant social positions at this age (Creel, 2001; Kingma et al., 2016).

We observed a gradual decrease in haematocrit within individuals with advancing age during most of adulthood. This relationship is similar to that observed in thick-billed murres (*Uria lomvia*) by Elliott et al. (2015), the only other longitudinal study of haematocrit in a wild system to date, and concurs with results from cross-sectional studies. For example, Jégo et al. (2014) found a decrease in haematocrit in roe deer (*Capreolus capreolus*) from the onset of actuarial senescence (8 years old). Thus, declining haematocrit could be indicative of physiological senescence (i.e., diminished ability to maintain circulating erythrocyte mass) in adult Seychelles warblers. By restricting endurance, such declines could contribute to lower survival in elderly life; onset of actuarial senescence in the Seychelles warbler is ca. 7 years old (Hammers et al., 2015). However, we did not find evidence of this in our study since only high haematocrit (not low haematocrit) was associated with lower survival, and only in young individuals. Alternatively, decreases in haematocrit could reflect other behavioral

and/or physiological changes with age observed in wild vertebrates. For instance, gains in experience could relax demands for oxygen-carrying capacity during foraging (Daunt et al., 2007; Zimmer et al., 2011). Furthermore, the intensity of stress responses—which can elevate haematocrit (Johnstone et al., 2012)—often decline with age (Lendvai et al., 2015; Wilcoxon et al., 2011). Such changes may be expected of older Seychelles warblers living in long-established territories.

4.2 | Sex and reproduction

We found that haematocrit was lower in female, compared with male, Seychelles warblers, but only for dominant breeders. This suggests an effect of reproduction on haematocrit, given that dominant breeders produce the vast majority of offspring in the population (Raj Pant et al., 2019; Richardson et al., 2001) and sampling coincided with peaks in breeding activity (Komdeur & Daan, 2005). A well-documented phenomenon (see Fair et al., 2007) in female birds is reproductive anemia—a reduction in haematocrit during egg laying due to the pleiotropic effects of elevated estrogen (Wagner, Prevorsek, et al., 2008; Wagner, Stables, et al., 2008; Williams et al., 2004). Haematocrit declines observed in other species range from 5% to 10% (Davey et al., 2000; Morton, 1994) and can persist for several weeks, through incubation and chick-rearing (Williams et al., 2004). In contrast to females, males can have elevated haematocrit prior to and during reproduction as a consequence of elevated testosterone, which stimulates erythropoiesis (Mirand et al., 1965). Therefore, sex differences may only occur during reproduction (e.g., Morton, 1994). This was not the case in our study since sex-by-status differences were similar both during and outside of breeding attempts. Additionally, there was no evidence of reproductive anemia (low haematocrit at egg laying) in dominant females. Taken together, these findings indicate that dominant females maintain haematocrit levels at a constant low level (relative to dominant males and subordinates of either sex). In other species, estrogen is positively related to territorial behaviors (e.g., singing and aggression) in females (Pärn et al., 2008; Woodley & Moore, 1999). This suggests that female dominance in Seychelles warblers might be accompanied by an upregulation of estrogen, which subsequently lowers haematocrit. However, we do not currently have data on estrogen dynamics in this species.

Sex differences in haematocrit were greatest prior to egg laying due to increased haematocrit in dominant males. Peak dominant male haematocrit coincided with his female partners fertile period (6 days prior to egg laying), during which testosterone levels of (pair-bonded) dominant males is also highest (Van De Crommenacker et al., 2004). Therefore, elevated haematocrit in males may be a consequence of elevated testosterone (e.g., Buttemer & Astheimer, 2000; Ezenwa et al., 2012). A lack of elevated haematocrit in subordinate males supports this explanation, since subordinate males do not elevate testosterone levels during the female fertile period (Van De Crommenacker et al., 2004). Elevated haematocrit might also reflect broader behavioral and physiological changes during this period. For example, dominant—but not subordinate—males invest in energetically costly guarding of

mates during their fertile period to prevent extra-pair copulations (Komdeur, 2001). Thus, elevated hematocrit could reflect increased activity levels—and therefore higher oxygen demands—during this critical period for dominant males (see Hammond et al., 2000).

4.3 | Survival

Intermediate hematocrit levels are predicted to be advantageous for survival, given that both high and low hematocrit are associated with increased mortality in humans and mice (Boffetta et al., 2013; Heller et al., 1998; Wagner et al., 2001). However, we found young Seychelles warblers with low hematocrit had the highest survival. This finding contradicts a study by Bowers et al. (2014), which found that house wren (*Troglodytes aedon*) nestlings with intermediate hematocrit had higher recruitment. However, in this study a more extreme lower range of hematocrit values (i.e., <30%) was apparent, likely due to age; neonates having lower hematocrit compared with juveniles and adults. Extreme-low hematocrit in neonates likely reflects developmental immaturity, which would reduce the probability of successful fledging (Cornell et al., 2017).

In adulthood, low hematocrit can be indicative of anemia (Campbell, 1994), which in wild populations may increase mortality risk via lethargy and fatigue. However, these symptoms are also likely to preclude anemic individuals from being captured using mist nets. In our sample, only 11 individuals were caught with what are considered anemic hematocrit levels in captive avifauna (<35%; Campbell, 1994). However, the threshold of anemic hematocrit might be higher in wild populations, given that overall hematocrit can be higher in wild compared with captive populations (Sepp et al., 2010). Nevertheless, the ability to detect a negative survival effect of extreme-low hematocrit in wild populations may be limited by an under-representation of anemic individuals. Furthermore, hematocrit has been criticized as indicator of ongoing/recent anemia due to disproportionate effect of reticulocytes (Fair et al., 2007; O'Brien et al., 2001). These immature erythrocytes are larger and contain less hemoglobin, meaning hematocrit can recover more rapidly than oxygen-carrying capacity following anemic episodes. Therefore, anemia could impact survival in wild populations without a detectable change in hematocrit values.

Higher hematocrit was associated with reduced survival probabilities in young individuals, despite nearly all hematocrit values falling within what is considered to be a healthy reference range for captive avifauna, 35%–55% (Campbell, 1994). Short-term increases in hematocrit can result from dehydration/hemoconcentration, which in turn limit oxygen-carrying capacity, or increase the cardiovascular effort required to maintain optimal oxygen-carrying capacity, due to negative relationship between blood viscosity and flow rate. Several studies have observed a lowering of hematocrit (by ca. 2%–5%) in birds during endurance activities via an increase in blood plasma (hemodilution). In line with optimal hematocrit theory (Birchard, 1997), these authors suggest that hemodilution is an adaptive response to prolonged exercise, facilitating faster blood flow for less cardiovascular effort (Bury

et al., 2019; Jenni et al., 2006; Yap et al., 2018). Thus, high hematocrit in young Seychelles warblers may reflect a failure to maintain optimal hematocrit, for example, due to dehydration. Alternatively, hematocrit could reflect physiological traits and/or life histories with potential costs to survival. For example, hematocrit has been positively associated with reproductive effort (e.g., Hörak et al., 1998), male ornamentation (Saino et al., 1997), metabolic rate (Yap et al., 2019) and stress (Johnstone et al., 2012). However, further—ideally experiment—studies are needed to confirm the link between hematocrit and pace of life in the Seychelles warbler.

Our study provides novel insights into the dynamics of hematocrit, and its impact on survival, in a wild population. Hematocrit was highly variable within individuals and varied in relation to time of day and (in dominant breeders) breeding stage. This variation limits the utility of hematocrit as a marker of age or senescence. However, the overarching relationship observed with advancing age supports the concept of changing oxygen demands with age. Interestingly, we show that hematocrit can be an indicator of survival prospects in wild populations. Whether survival is directly impacted by (suboptimal) oxygen-carrying capacity, or factors that increase hematocrit (dehydration, stress, etc.) remains to be tested. Since changes in erythrocyte mass occur over longer timescales than, for example, stress hormones and oxidative stress (Bonier et al., 2009; van de Crommenacker et al., 2017), hematocrit may be a better indicator an individual's baseline stress levels (Johnstone et al., 2012). However, short-term changes in blood plasma volume can affect hematocrit levels independently of erythrocyte mass, which makes unraveling the drivers of elevated hematocrit difficult without data on additional blood metrics, such as plasma protein and hemoglobin concentrations (see Johnstone et al., 2017). Nevertheless, hematocrit can aid in quantifying physiological state or condition in wild vertebrates, which is a fundamental concept in the study of life-history trade-offs.

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CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTION

Thomas James Brown: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Investigation (equal); Methodology (equal); Writing-original draft (lead); Writing-review & editing (lead). **Martijn Hammers:** Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Writing-review & editing (equal). **Martin Taylor:** Investigation (equal); Methodology (equal); Supervision (supporting); Writing-review & editing (equal). **Hannah Dugdale:** Funding acquisition (equal); Investigation (supporting); Methodology (supporting); Supervision (supporting); Validation (equal); Writing-review & editing (equal). **Jan Komdeur:** Funding acquisition (equal); Writing-review & editing (equal). **David Richardson:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Supervision (lead); Writing-review & editing (equal).

ETHICS APPROVAL

All fieldwork was conducted in accordance with local ethical regulations and agreements. The Seychelles Bureau of Standards and Department of Environment gave permission for sampling and fieldwork. Nature Seychelles gave permission to carry out research on Cousin Island.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.7wm37pvrp>

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REFERENCES

- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Birchard, G. F. (1997). Optimal hematocrit: Theory, regulation and implications. *Integrative and Comparative Biology*, 37(1), 65–72.
- Boffetta, P., Islami, F., Vedanthan, R., Pourshams, A., Kamangar, F., Khademi, H., Etemadi, A., Salahi, R., Semnani, S., Emadi, A., Abnet, C. C., Brennan, P., Pharoah, P. D., Dawsey, S. M., & Malekzadeh, R. (2013). A U-shaped relationship between haematocrit and mortality in a large prospective cohort study. *International Journal of Epidemiology*, 42(2), 601–615. <https://doi.org/10.1093/ije/dyt013>
- Boggs, D. R., & Patrene, K. D. (1985). Hematopoiesis and aging III: Anemia and a blunted erythropoietic response to hemorrhage in aged mice. *American Journal of Hematology*, 19(4), 327–338. <https://doi.org/10.1002/ajh.2830190403>
- Bonier, F., Martin, P. R., Moore, I. T., & Wingfield, J. C. (2009). Do baseline glucocorticoids predict fitness? *Trends in Ecology and Evolution*, 24(11), 634–642. <https://doi.org/10.1016/j.tree.2009.04.013>
- Böning, D., Maassen, N., & Pries, A. (2011). The hematocrit paradox – How does blood doping really work? *International Journal of Sports Medicine*, 32(4), 242–246. <https://doi.org/10.1055/s-0030-1255063>
- Borras, A., Cabrera, J., & Senar, J. C. (2010). Hematocrit variation in response to altitude changes in wild birds: A repeated-measures design. *The Condor*, 112(3), 622–626. <https://doi.org/10.1525/cond.2010.090113>
- Bowers, E. K., Hodges, C. J., Forsman, A. M., Vogel, A. L., Masters, B. S., Johnson, B. G. P., Johnson, L. S., Thompson, C. F., & Sakaluk, S. K. (2014). Neonatal body condition, immune responsiveness, and hematocrit predict longevity in a wild bird population. *Ecology*, 95(11), 3027–3034. <https://doi.org/10.1890/14-0418.1.Neonatal>
- Brækkan, S. K., Mathiesen, E. B., Njølstad, I., Wilsgaard, T., & Hansen, J.-B. (2010). Hematocrit and risk of venous thromboembolism in a general population. The Tromsø study. *Haematologica*, 95(2), 270–275. <https://doi.org/10.3324/haematol.2009.008417>
- Brouwer, L., Richardson, D. S., Eikenaar, C., & Komdeur, J. (2006). The role of group size and environmental factors on survival in a cooperatively breeding tropical passerine. *Journal of Animal Ecology*, 75(6), 1321–1329. <https://doi.org/10.1111/j.1365-2656.2006.01155.x>
- Brouwer, L., Tinbergen, J. M., Both, C., Bristol, R., Richardson, D. S., & Komdeur, J. (2009). Experimental evidence for density-dependent reproduction in a cooperatively breeding passerine. *Ecology*, 90(3), 729–741. <https://doi.org/10.1890/07-1437.1>
- Bury, A., Niedojadlo, J., Sadowska, E. T., Bauchinger, U., & Cichoń, M. (2019). Contrasting response of haematological variables between long-term training and short exercise bouts in zebra finches (*Taeniopygia guttata*). *The Journal of Experimental Biology*, 222(4), jeb193227. <https://doi.org/10.1242/jeb.193227>
- Buttemer, W. A., & Astheimer, L. B. (2000). Testosterone does not affect basal metabolic rate or blood parasite load in captive male White-plumed Honeyeaters *Lichenostomus penicillatus*. *Journal of Avian Biology*, 31(4), 479–488. <https://doi.org/10.1034/j.1600-048X.2000.310407.x>
- Calbet, J. A. L., Lundby, C., Koskolou, M., & Boushel, R. (2006). Importance of hemoglobin concentration to exercise: acute manipulations. *Respiratory Physiology and Neurobiology*, 151(2–3), 132–140. <https://doi.org/10.1016/j.resp.2006.01.014>
- Campbell, T. W. (1994). Cytology. In B. W. Ritchie, G. J. Harrison, & L. R. Harrison (Eds.), *Avian medicine: Principles and applications* (pp. 199–221). Winger.
- Coglianesi, E. E., Qureshi, M. M., Vasan, R. S., Wang, T. J., & Moore, L. L. (2012). Usefulness of the blood hematocrit level to predict development of heart failure in a community. *American Journal of Cardiology*, 109(2), 241–245. <https://doi.org/10.1016/j.amjcard.2011.08.037>
- Cornell, A., Gibson, K. F., & Williams, T. D. (2017). Physiological maturity at a critical life-history transition and flight ability at fledging. *Functional Ecology*, 31(3), 662–670. <https://doi.org/10.1111/1365-2435.12777>
- Cornell, A., & Williams, T. D. (2017). Variation in developmental trajectories of physiological and somatic traits in a common songbird approaching fledging. *Journal of Experimental Biology*, 220(21), 4060–4067. <https://doi.org/10.1242/jeb.162248>
- Creel, S. (2001). Social dominance and stress hormones. *Trends in Ecology and Evolution*, 16(9), 491–497. [https://doi.org/10.1016/S0169-5347\(01\)02227-3](https://doi.org/10.1016/S0169-5347(01)02227-3)
- Daunt, F., Wanless, S., Harris, M. P., Money, L., & Monaghan, P. (2007). Older and wiser: Improvements in breeding success are linked to better foraging performance in European shags. *Functional Ecology*, 21(3), 561–567. <https://doi.org/10.1111/j.1365-2435.2007.01260.x>
- Davey, C., Lill, A., & Baldwin, J. (2000). Variation during breeding in parameters that influence blood oxygen carrying capacity in shearwaters. *Australian Journal of Zoology*, 48(4), 347–356. <https://doi.org/10.1071/ZO00047>
- Eklom, K., & Lill, A. (2006a). Development of parameters influencing blood oxygen-carrying capacity in nestling doves. *Emu*, 106(4), 283–288. <https://doi.org/10.1071/MU05061>

- Eklom, K., & Lill, A. (2006b). Development pattern of blood oxygen carrying capacity in rainbow bee-eater nestlings. *Australian Journal of Zoology*, 54(1), 1–7. <https://doi.org/10.1071/ZO05063>
- Elliott, K. H., Hare, J. F., Le Vaillant, M., Gaston, A. J., Robert-Coudert, Y., & Anderson, W. G. (2015). Ageing gracefully: Physiology but not behaviour declines with age in a diving seabird. *Functional Ecology*, 29(2), 219–228. <https://doi.org/10.1111/1365-2435.12316>
- Ezenwa, V. O., Stefan Ekernas, L., & Creel, S. (2012). Unravelling complex associations between testosterone and parasite infection in the wild. *Functional Ecology Ltd*, 26(1), 123–133. <https://doi.org/10.1111/j.1365-2435.2011.01919.x>
- Fair, J., Whitaker, S., & Pearson, B. (2007). Sources of variation in haematocrit in birds Sources of variation in haematocrit in birds. *Ibis*, July, 535–552. <https://doi.org/10.1111/j.1474-919X.2007.00680.x>
- Fronstin, R. B., Christians, J. K., & Williams, T. D. (2016). Experimental reduction of haematocrit affects reproductive performance in European starlings. *Functional Ecology*, 30(3), 398–409. <https://doi.org/10.1111/1365-2435.12511>
- Gaskell, H., Derry, S., Andrew Moore, R., & McQuay, H. J. (2008). Prevalence of anaemia in older persons: Systematic review. *BMC Geriatrics*, 8(1), 1. <https://doi.org/10.1186/1471-2318-8-1>
- Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998). A DNA test to sex most birds. *Molecular Ecology*, 7(8), 1071–1075. <https://doi.org/10.1046/j.1365-294x.1998.00389.x>
- Hammers, M., & Brouwer, L. (2017). Rescue behaviour in a social bird: Removal of sticky “bird-catcher tree” seeds by group members. *Behaviour*, 154(4), 403–411. <https://doi.org/10.1163/1568539X-00003428>
- Hammers, M., Kingma, S. A., Bebbington, K., van de Crommenacker, J., Spurgin, L. G., Richardson, D. S., Burke, T., Dugdale, H. L., & Komdeur, J. (2015). Senescence in the wild: Insights from a long-term study on Seychelles warblers. *Experimental Gerontology*, 71, 69–79. <https://doi.org/10.1016/j.exger.2015.08.019>
- Hammers, M., Kingma, S. A., Spurgin, L. G., Bebbington, K., Dugdale, H. L., Burke, T., Komdeur, J., & Richardson, D. S. (2019). Breeders that receive help age more slowly in a cooperatively breeding bird. *Nature Communications*, 10(1), 1301. <https://doi.org/10.1038/s41467-019-09229-3>
- Hammers, M., Komdeur, J., Kingma, S. A., Hutchings, K., Fairfield, E. A., Gilroy, D. L., & Richardson, D. S. (2016). Age-specific haemosporidian infection dynamics and survival in Seychelles warblers. *Scientific Reports*, 6(1), 29720. <https://doi.org/10.1038/srep29720>
- Hammers, M., Richardson, D. S., Burke, T., & Komdeur, J. (2012). Age-dependent terminal declines in reproductive output in a wild bird. *PLoS One*, 7(7), e40413. <https://doi.org/10.1371/journal.pone.0040413>
- Hammers, M., Richardson, D. S., Burke, T., & Komdeur, J. (2013). The impact of reproductive investment and early-life environmental conditions on senescence: Support for the disposable soma hypothesis. *Journal of Evolutionary Biology*, 26(9), 1999–2007. <https://doi.org/10.1111/jeb.12204>
- Hammond, K. A., Chappell, M. A., Cardullo, R. A., Lin, R., & Johnsen, T. S. (2000). The mechanistic basis of aerobic performance variation in red junglefowl. *Journal of Experimental Biology*, 203(13), 2053–2064.
- Harshman, L. G., & Zera, A. J. (2007). The cost of reproduction: The devil in the details. *Trends in Ecology and Evolution*, 22(2), 80–86. <https://doi.org/10.1016/j.tree.2006.10.008>
- Hedrick, M. S., Duffield, D. A., & Cornell, L. H. (1986). Blood viscosity and optimal hematocrit in a deep-diving mammal, the northern elephant seal (*Mirounga angustirostris*). *Canadian Journal of Zoology*, 64(10), 2081–2085. <https://doi.org/10.1139/z86-317>
- Heller, D. A., Ahem, F. M., Stout, J. T., & McClearn, G. E. (1998). Mortality and biomarkers of aging in heterogeneous stock (HS) mice. *Journals of Gerontology – Series A Biological Sciences and Medical Sciences*, 53(3), B217–B230. <https://doi.org/10.1093/gerona/53A.3.B217>
- Hörak, P., Ots, I., & Murumägi, A. (1998). Haematological health state indices of reproducing Great Tits: A response to brood size manipulation. *Functional Ecology*, 12(5), 750–756. <https://doi.org/10.1046/j.1365-2435.1998.00244.x>
- Jégo, M., Lemaître, J.-F., Bourgoin, G., Capron, G., Warnant, C., Klein, F., Gilot-Fromont, E., & Gaillard, J.-M. (2014). Haematological parameters do senesce in the wild: Evidence from different populations of a long-lived mammal. *Journal of Evolutionary Biology*, 27(12), 2745–2752. <https://doi.org/10.1111/jeb.12535>
- Jenni, L., Müller, S., Spina, F., Kvist, A., & Lindström, Å. (2006). Effect of endurance flight on haematocrit in migrating birds. *Journal of Ornithology*, 147(4), 531–542. <https://doi.org/10.1007/s10336-006-0076-2>
- Jensen, K. H., Kim, W., Holbrook, N. M., & Bush, J. W. M. (2013). Optimal concentrations in transport systems. *Journal of the Royal Society Interface*, 10(83), 20130138. <https://doi.org/10.1098/rsif.2013.0138>
- Johnstone, C. P., Lill, A., & Reina, R. D. (2017). Use of erythrocyte indicators of health and condition in vertebrate ecophysiology: A review and appraisal. *Biological Reviews*, 92(1), 150–168. <https://doi.org/10.1111/brv.12219>
- Johnstone, C. P., Reina, R. D., & Lill, A. (2012). Interpreting indices of physiological stress in free-living vertebrates. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 182(7), 861–879. <https://doi.org/10.1007/s00360-012-0656-9>
- Kaltreider, N. L., & Meneely, G. R. (1940). The effect of exercise on the volume of blood. *Journal of Clinical Investigation*, 19(4), 627–634. <https://doi.org/10.1172/jci101165>
- Kingma, S. A., Komdeur, J., Hammers, M., & Richardson, D. S. (2016). The cost of prospecting for dispersal opportunities in a social bird. *Biology Letters*, 12(6), 20160316. <https://doi.org/10.1098/rsbl.2016.0316>
- Komdeur, J. (1991). Cooperative breeding in the Seychelles warbler. *Trends in Ecology and Evolution*, 7(10), 330–331.
- Komdeur, J. (1992). Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature*, 358(6386), 493–495. <https://doi.org/10.1038/358493a0>
- Komdeur, J. (1996). Influence of age on reproductive performance in the Seychelles warbler. *Behavioral Ecology*, 7(4), 417–425. <https://doi.org/10.1093/beheco/7.4.417>
- Komdeur, J. (2001). Mate guarding in the Seychelles warbler is energetically costly and adjusted to paternity risk. *Proceedings of the Royal Society B: Biological Sciences*, 268(1481), 2103–2111. <https://doi.org/10.1098/rspb.2001.1750>
- Komdeur, J., & Daan, S. (2005). Breeding in the monsoon: Semi-annual reproduction in the Seychelles warbler (*Acrocephalus sechellensis*). *Journal of Ornithology*, 146(4), 305–313. <https://doi.org/10.1007/s10336-005-0008-6>
- Komdeur, J., Piersma, T., Kraaijeveld, K., Kraaijeveld-Smit, F., & Richardson, D. S. (2004). Why Seychelles Warblers fail to recolonize nearby islands: Unwilling or unable to fly there? *Ibis*, 146(2), 298–302. <https://doi.org/10.1046/j.1474-919X.2004.00255.x>
- Lendvai, Á. Z., Giraudeau, M., Bókony, V., Angelier, F., & Chastel, O. (2015). Within-individual plasticity explains age-related decrease in stress response in a short-lived bird. *Biology Letters*, 11(7), 20150272. <https://doi.org/10.1098/rsbl.2015.0272>
- Leroi, A. M. (2001). Molecular signals versus the Loi de Balancement. *Trends in Ecology and Evolution*, 16(1), 24–29. [https://doi.org/10.1016/S0169-5347\(00\)02032-2](https://doi.org/10.1016/S0169-5347(00)02032-2)
- Lourdais, O., Gartner, G. E. A., & Brischox, F. (2014). Ambush or active life: Foraging mode influences haematocrit levels in snakes. *Biological Journal of the Linnean Society*, 111(3), 636–645. <https://doi.org/10.1111/bij.12223>
- Minias, P. (2015). The use of haemoglobin concentrations to assess physiological condition in birds: A review. *Conservation Physiology*, 3(1), cov007. <https://doi.org/10.1093/conphys/cov007>

- Mirand, E. A., Gordon, A. S., & Wenig, J. (1965). Mechanism of testosterone action in erythropoiesis. *Nature*, 206(4981), 270–272. <https://doi.org/10.1038/206270a0>
- Morton, M. L. (1994). Hematocrits in montane sparrows in relation to reproductive schedule. *The Condor*, 96(1), 119–126. <https://doi.org/10.2307/1369069>
- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, 85, 935–956. <https://doi.org/10.1111/j.1469-185X.2010.00141.x>
- Norris, K., & Evans, M. R. (2000). Ecological immunology: Life history trade-offs and immune defense in birds. *Behavioral Ecology*, 11(1), 19–26. <https://doi.org/10.1093/beheco/11.1.19>
- Nussey, D. H., Coulson, T., Festa-Bianchet, M., & Gaillard, J.-M. (2008). Measuring senescence in wild animal populations: Towards a longitudinal approach. *Functional Ecology*, 22(3), 393–406. <https://doi.org/10.1111/j.1365-2435.2008.01408.x>
- O'Brien, E. L., Morrison, B. L., & Johnson, L. S. (2001). Assessing the effects of haematophagous ectoparasites on the health of nestling birds: Haematocrit vs haemoglobin levels in House Wrens parasitized by blow fly larvae. *Journal of Avian Biology*, 32(1), 73–76. <https://doi.org/10.1034/j.1600-048X.2001.320110.x>
- Pärn, H., Lindström, K. M., Sandell, M., & Amundsen, T. (2008). Female aggressive response and hormonal correlates – An intrusion experiment in a free-living passerine. *Behavioral Ecology and Sociobiology*, 62(10), 1665–1677. <https://doi.org/10.1007/s00265-008-0595-3>
- Petschow, R., Petschow, D., Bartels, R., Baumann, R., & Bartels, H. (1978). Regulation of oxygen affinity in blood of fetal, newborn and adult mouse. *Respiration Physiology*, 35(3), 271–282. [https://doi.org/10.1016/0034-5687\(78\)90003-8](https://doi.org/10.1016/0034-5687(78)90003-8)
- Prinzinger, R., & Misovic, A. (2010). Age-correlation of blood values in the Rock Pigeon (*Columba livia*). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 156(3), 351–356. <https://doi.org/10.1016/j.cbpa.2009.07.028>
- Raj Pant, S., Komdeur, J., Burke, T. A., Dugdale, H. L., & Richardson, D. S. (2019). Socio-ecological conditions and female infidelity in the Seychelles warbler. *Behavioral Ecology*, 30(5), 1254–1264. <https://doi.org/10.1093/beheco/arz072>
- Richardson, D. S., Burke, T., & Komdeur, J. (2002). Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. *Evolution*, 56(11), 2313–2321. <https://doi.org/10.1111/j.0014-3820.2002.tb00154.x>
- Richardson, D. S., Burke, T., & Komdeur, J. (2007). Grandparent helpers: The adaptive significance of older, postdominant helpers in the Seychelles warbler. *Evolution*, 61(12), 2790–2800. <https://doi.org/10.1111/j.1558-5646.2007.00222.x>
- Richardson, D. S., Jury, F. L., Blaakmeer, K., Komdeur, J., & Burke, T. (2001). Parentage assignment and extra-group paternity in a cooperative breeder: The Seychelles warbler (*Acrocephalus sechellensis*). *Molecular Ecology*, 10(9), 2263–2273. <https://doi.org/10.1046/j.0962-1083.2001.01355.x>
- Ricklefs, R. E., & Wikelski, M. (2002). The physiology/life-history nexus. *Trends in Ecology and Evolution*, 17(10), 462–468. [https://doi.org/10.1016/S0169-5347\(02\)02578-8](https://doi.org/10.1016/S0169-5347(02)02578-8)
- RStudio Team (2020). *RStudio: Integrated development for R*. RStudio, PBC. <http://www.rstudio.com/>
- Saino, N., Cuervo, J. J., Ninni, P., De lope, F., & Moller, A. P. (1997). Haematocrit correlates with tail ornament size in three populations of the Barn Swallow (*Hirundo rustica*). *Functional Ecology*, 11(5), 604–610. <https://doi.org/10.1046/j.1365-2435.1997.00131.x>
- Schuler, B., Arras, M., Keller, S., Rettich, A., Lundby, C., Vogel, J., & Gassmann, M. (2010). Optimal hematocrit for maximal exercise performance in acute and chronic erythropoietin-treated mice. *Proceedings of the National Academy of Sciences of the United States of America*, 107(1), 419–423. <https://doi.org/10.1073/pnas.0912924107>
- Sepp, T., Sild, E., & Hörak, P. (2010). Condition indexes in greenfinches: Effects of captivity and diurnal variation. *Physiological and Biochemical Zoology*, 83(2), 276–282. <https://doi.org/10.1086/648580>
- Sheldon, L. D., Chin, E. H., Gill, S. A., Schmaltz, G., Newman, A. E. M., & Soma, K. K. (2008). Effects of blood collection on wild birds: an update. *Journal of Avian Biology*, 39(4), 369–378. <https://doi.org/10.1111/j.0908-8857.2008.04295.x>
- Smucny, D. A., Allison, D. B., Ingram, D. K., Roth, G. S., Kemnitz, J. W., Kohama, S. G., Lane, M. A., & Black, A. (2004). Changes in blood chemistry and hematology variables during aging in captive rhesus macaques (*Macaca mulatta*). *J Med Primatol* 30:161–173, 2001. *Journal of Medical Primatology*, 33(1), 48–54. <https://doi.org/10.1111/j.1600-0684.2003.00052.x>
- Speakman, J. R., & Selman, C. (2011). The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *BioEssays*, 33(4), 255–259. <https://doi.org/10.1002/bies.201000132>
- Spurgin, L. G., Bebbington, K., Fairfield, E. A., Hammers, M., Komdeur, J., Burke, T., Dugdale, H. L., & Richardson, D. S. (2017). Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. *Journal of Animal Ecology*, 87(1), 187–198. <https://doi.org/10.1111/1365-2656.12741>
- Stack, S. W., & Berger, S. A. (2009). The effects of high hematocrit on arterial flow—A phenomenological study of the health risk implications. *Chemical Engineering Science*, 64(22), 4701–4706. <https://doi.org/10.1016/j.ces.2009.07.017>
- Stark, H., & Schuster, S. (2012). Comparison of various approaches to calculating the optimal hematocrit in vertebrates. *Journal of Applied Physiology*, 113(3), 355–367. <https://doi.org/10.1152/jappphysiol.00369.2012>
- Trillmich, F., Rea, L., Castellini, M., & Wolf, J. B. W. (2008). Age-related changes in hematocrit in the Galápagos sea lion (*Zalophus wollebaeki*) and the Weddell seal (*Leptonychotes weddellii*). *Marine Mammal Science*, 24(2), 303–314. <https://doi.org/10.1111/j.1748-7692.2007.00177.x>
- van de Crommenacker, J., Hammers, M., Woude, J., Louter, M., Santema, P., Richardson, D. S., & Komdeur, J. (2017). Oxidative status and fitness components in the Seychelles warbler. *Functional Ecology*, 31(6), 1210–1219. <https://doi.org/10.1111/1365-2435.12861>
- van de Crommenacker, J., Komdeur, J., & Richardson, D. S. (2011). Assessing the cost of helping: The roles of body condition and oxidative balance in the Seychelles warbler (*Acrocephalus sechellensis*). *PLoS One*, 6(10), e26423. <https://doi.org/10.1371/journal.pone.0026423>
- van de Crommenacker, J., Richardson, D. S., Groothuis, T. G. G., Eising, C. M., Dekker, A. L., & Komdeur, J. (2004). Testosterone, cuckoldry risk and extra-pair opportunities in the Seychelles warbler. *Proceedings of the Royal Society B: Biological Sciences*, 271(1543), 1023–1031. <https://doi.org/10.1098/rspb.2004.2697>
- van de Pol, M., & Verhulst, S. (2006). Age-dependent traits: A new statistical model to separate within- and between-individual effects. *The American Naturalist*, 167(5), 766–773. <https://doi.org/10.1086/503331>
- van de Pol, M., & Wright, J. (2009). A simple method for distinguishing within- versus between-subject effects using mixed models. *Animal Behaviour*, 77(3), 753–758. <https://doi.org/10.1016/j.anbehav.2008.11.006>
- Voorhees, J. L., Powell, N. D., Moldovan, L., Mo, X., Eubank, T. D., & Marsh, C. B. (2013). Chronic restraint stress upregulates erythropoiesis through glucocorticoid stimulation. *PLoS One*, 8(10), e77935. <https://doi.org/10.1371/journal.pone.0077935>
- Wagner, E. C., Prevorsek, J. S., Wynne-Edwards, K. E., & Williams, T. D. (2008). Hematological changes associated with egg production: Estrogen dependence and repeatability. *The Journal of Experimental Biology*, 211(Pt 3), 400–408. <https://doi.org/10.1242/jeb.011205>

- Wagner, E. C., Stables, C. A., & Williams, T. D. (2008). Hematological changes associated with egg production: Direct evidence for changes in erythropoiesis but a lack of resource dependence? *Journal of Experimental Biology*, 211(18), 2960–2968. <https://doi.org/10.1242/jeb.017897>
- Wagner, K. F., Katschinski, D. M., Hasegawa, J. O., Schumacher, D., Meller, B., Gembruch, U., Schramm, U., Jelkmann, W., Gassmann, M., & Fandrey, J. (2001). Chronic inborn erythrocytosis leads to cardiac dysfunction and premature death in mice overexpressing erythropoietin. *Blood*, 97(2), 536–542. <https://doi.org/10.1182/blood.V97.2.536>
- Wagner, P. D. (1996). Determinants of maximal oxygen transport and utilization. *Annual Review of Physiology*, 58(1), 21–50. <https://doi.org/10.1146/annurev.physiol.58.1.21>
- Walton, B. L., Lehmann, M., Skorczewski, T., Holle, L. A., Beckman, J. D., Cribb, J. A., Mooberry, M. J., Wufsus, A. R., Cooley, B. C., Homeister, J. W., Pawlinski, R., Falvo, M. R., Key, N. S., Fogelson, A. L., Neeves, K. B., & Wolberg, A. S. (2017). Elevated hematocrit enhances platelet accumulation following vascular injury. *Blood*, 129(18), 2537–2546. <https://doi.org/10.1182/blood-2016-10-746479>
- Whittingham, M. J., Stephens, P. A., Bradbury, R. B., & Freckleton, R. P. (2006). Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*, 75(5), 1182–1189. <https://doi.org/10.1111/j.1365-2656.2006.01141.x>
- Wilcoxon, T. E., Boughton, R. K., Bridge, E. S., Rensel, M. A., & Schoech, S. J. (2011). Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. *General and Comparative Endocrinology*, 173(3), 461–466. <https://doi.org/10.1016/j.ygcen.2011.07.007>
- Wilder, S. M., Raubenheimer, D., & Simpson, S. J. (2016). Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. *Functional Ecology*, 30(1), 108–115. <https://doi.org/10.1111/1365-2435.12460>
- Williams, T. D., Challenger, W. O., Christians, J. K., Evanson, M., Love, O., & Vezina, F. (2004). What causes the decrease in haematocrit during egg production? *Functional Ecology*, 18(3), 330–336. <https://doi.org/10.1111/j.0269-8463.2004.00829.x>
- Woodley, S. K., & Moore, M. C. (1999). Ovarian hormones influence territorial aggression in free-living female mountain spiny lizards. *Hormones and Behavior*, 35(3), 205–214. <https://doi.org/10.1006/hbeh.1999.1514>
- Yap, K. N., Dick, M. F., Guglielmo, C. G., & Williams, T. D. (2018). Effects of experimental manipulation of hematocrit on avian flight performance in high- and low-altitude conditions. *Journal of Experimental Biology*, 221(22), jeb191056. <https://doi.org/10.1242/jeb.191056>
- Yap, K. N., Serota, M. W., & Williams, T. D. (2017). The physiology of exercise in free-living vertebrates: What can we learn from current model systems? *Integrative and Comparative Biology*, 57, 195–206. <https://doi.org/10.1093/icb/ix016>
- Yap, K. N., Tsai, O. H. I., & Williams, T. D. (2019). Haematological traits covary with migratory status, altitude and energy expenditure: A phylogenetic, comparative analysis. *Scientific Reports*, 9(1), 6351. <https://doi.org/10.1038/s41598-019-42921-4>
- Zimmer, I., Ropert-Coudert, Y., Kato, A., Ancel, A., & Chiaradia, A. (2011). Does foraging performance change with age in female little penguins (*Eudyptula minor*)?. *PLoS One*, 6(1), e16098. <https://doi.org/10.1371/journal.pone.0016098>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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