Haematocrit, 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 unravelling such trade-offs and could provide insight into individual variation in senescence. However, currently used markers often yield inconsistent results. One underutilised measure is haematocrit, the proportional of blood comprising of erythrocytes, which relates to the blood's oxygen-carrying capacity and viscosity, and to individual endurance. Haematocrit 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 haematocrit declines within-individuals or whether low haematocrit impacts survival in wild taxa. Using longitudinal data from the Seychelles warbler (*Acrocephalus sechellensis*), we demonstrated that haematocrit increases with age in young individuals (<1.5 years) but decreases with age in older individuals (1.5–13 years). In breeders, haematocrit was higher in males than females and varied relative to breeding stage. High haematocrit was associated with lower survival in young individuals, but not older individuals. Thus, while we did not find support for haematocrit as a marker of senescence, high haematocrit 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 haematocrit can be an informative metric for life-history studies investigating trade-offs between survival, longevity and reproduction.

Keywords

Ageing, condition markers, biomarkers, haematocrit, life-history, trade-offs, senescence, survival, wild populations, birds.

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 (Leroi, 2001; Ricklefs and Wikelski, 2002; Harshman and Zera, 2007). These trade-offs form the basis of life-history. Physiological markers provide valuable 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. Norris and Evans, 2000; Speakman and Selman, 2011; Wilder, Raubenheimer and Simpson, 2016; Johnstone, Lill and Reina, 2017), thus there is a continued need to identity 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 haemoglobin and the rate of blood flow, which is inversely proportional to blood viscosity (Wagner, 1996; Birchard, 1997; Calbet *et al.*, 2006). These properties are reflected by haematocrit or Packed Cell Volume (PCV); the proportion of whole blood volume comprised of erythrocytes. Haemoglobin and blood viscosity increases linearly and exponentially, respectively, with haematocrit (Hedrick et. al., 1986). Blood becomes harder to circulate with increasing viscosity (i.e. requiring greater cardiovascular effort), but less viscous blood contains less haemoglobin. Therefore, intermediate haematocrit levels (ca. 40%) are optimal for maximum oxygen carrying capacity and endurance (Birchard, 1997; Schuler *et al.*, 2010; Jensen *et al.*, 2013).

Haematocrit levels observed in nature are variable (range ca. 30–60%) within and between endothermic species (Stark and Schuster, 2012). For example, haematocrit is higher in species/individuals requiring greater blood oxygen storage and endurance (Lourdais et al., 2014; Minias, 2015; Yap, et al., 2019). Similarly, haematocrit 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 haematocrit 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 hour) increases in haematocrit can occur due to a reduction in blood plasma volume (haemoconcentration), which happens during exercise and dehydration (Kaltreider and Meneely, 1940; Bury *et al.*, 2019). In some mammal species, splenic reservoirs of erythrocytes can also increase haematocrit at the onset of stress and exercise (Böning, et al., 2011).

Anaemia – characterised by chronically low haematocrit and haemoglobin – occurs when an individual's rate of erythrocyte loss exceeds that of erythropoiesis, for example, during blood parasitism (O'Brien et al., 2001). However, anaemia can occur without affecting haematocrit, since the release of reticulocytes, which are larger than mature erythrocytes, can rapidly complement haematocrit despite them having lower haemoglobin content (Fair et al., 2007). Anaemia can also arise as a secondary outcome of competing physiological systems. For example, egg-production in birds causes a reduction in haematocrit via an oestrogen-mediated suppression of erythropoiesis and haemodilution – an increase in blood plasma volume (Williams et al., 2004; Wagner, et al., 2008). Therefore, both within-individual increases and decreases in oxygen carrying capacity and associated factors (haematocrit and haemoglobin) have the potential to reflect a multitude of life-history events and trade-offs (for reviews see Fair, et al., 2007; Minias, 2015; Johnstone, et al., 2017).

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

Factors that cause haematocrit 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 of haematocrit 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 haematocrit levels observed under natural conditions in the wild and

subsequent survival. Anaemia 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 cardio-vascular loading this creates, is linked to negative health impacts in humans (Stack and Berger, 2009; Brækkan *et al.*, 2010; Coglianese *et al.*, 2012; Walton *et al.*, 2017). Extreme high or low haematocrit can also be a non-causal indicator of factors detrimental to self-maintenance, such as stress, parasitism and nutrient deficiencies (see Johnstone et al., 2017). Therefore, intermediate haematocrit 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 haematocrit, 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 (Komdeur, 1992; Richardson et al., 2007; Hammers et al., 2019). Individuals have been captured and blood sampled repeatedly across their life-time, providing a wealth of longitudinal physiological data (Hammers *et al.*, 2015), including haematocrit. Here, we first assess the relationship between haematocrit and age. Based on previous findings across vertebrate taxa, we predict that haematocrit 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. Haematocrit 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 haematocrit. Lastly, we determine the relationship between haematocrit and annual survival probability. Given the potentially negative effects of both low and high haematocrit, we predicted that individuals with intermediate haematocrit values would have higher survival. Our study will, therefore, assess the validity of haematocrit 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.

Methods

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 old (Hammers and Brouwer, 2017), though the average lifespan 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 and 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 colour 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 behaviour and eye-colour, which transitions from grey in fledglings to red-brown in adults (Komdeur, 1992).

The population is structured into clearly defined territories that are defended year-round. Breeding groups comprise 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 behaviour and co-breeding (Hammers et al., 2019). An Individual's social status in a given field season is determined through observations of behaviour (see Komdeur, 2001; van de Crommenacker et al., 2011).

During the breeding season each territory is visited at least every two weeks and checked for the presence-absence of individuals identified by their colour ring combination. Dominant females are followed for 15 minutes to determine whether an active nest is present. Once a nest is found it is visited every 3 days for 15–60 minutes (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 inter-island 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 µl of blood was drawn with a microcapillary tube from the brachial vein. A small amount (ca. 10 ul) of blood sample was also stored in absolute ethanol at 4°C for future DNA extraction. This procedure is the routine, non-lethal 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. three hours of bleeding, microcapillary tubes were centrifuged for 8 minutes at 8000 rpm to separate erythrocytes from plasma, white blood cells and platelets. Haematocrit was measured (using sliding callipers ± 0.01 mm) as the proportion of erythrocytes relative to whole-blood volume. Between the years of 2003 – 2017, 1383 haematocrit measurements were obtained from 733 individuals. DNA was extracted using

a salt extraction technique following Richardson et al. (2001b) and sex of the individual was confirmed using the PCR-based method outlined by Griffiths et al. (1998).

Statistical analyses

All statistical analyses were performed with RStudio (v1.2.5033, RStudio team, 2020). Firstly, we investigated the relationship between haematocrit 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 non-parametric smoothing parameter for age to evaluate expected non-linear relationships between haematocrit and age. Compared to Linear Mixed models (LMMs), which require pre-specified 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 haematocrit in avian taxa (see Fair et al., 2007); sex, social status (dominant breeders versus 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 (van de Pol and Verhulst, 2006; Hammers *et al.*, 2019).

Our GAMM analysis revealed that dominant females had significantly lower haematocrit than dominant males and subordinates (male or female). This suggested an effect of reproductive anaemia on haematocrit 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, for example, 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 haematocrit to be lowest nearer the lay date, and only in dominant females. Since haematocrit was expected to fluctuate non-linearly across breeding stages, non-parametric smoothing parameters were fitted for

196 days from lay date for males and females. Separate models were created for dominant breeders and
197 subordinates to avoid the need for complex three-way interactions between sex, status and days from
198 lay date. Individuals were rarely caught multiple times within the same breeding attempt; thus this
199 section of our analysis is cross-sectional in nature.

200 To separate the role of between- versus within-individual variation with age (i.e. cross-sectional from
201 longitudinal effects), we used the within-subject centering method described by van de Pol and
202 Wright, 2009. Briefly, age at sampling is split into two predictors, (i) mean age across all sampling
203 events for a given individual (mean age), and (ii) within-individual deviation from mean age (Δ age).
204 Our GAMM model indicated a peak in haematocrit at ca. 1.5 years of age (see results, Fig. 1). To
205 investigate the initial increase and subsequent decrease in haematocrit in more detail, we performed
206 within-individual centering for individuals <1.5 months and for individuals ≥1.5 months in separate
207 analyses (following Hammers et al., 2016). This allowed us to compare the drivers of age-related
208 haematocrit patterns in early life versus later adulthood.

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

221 Lastly, we investigated whether haematocrit predicts short-term survival. We used a binominal
222 Generalised Linear Mixed Models (GLMM) to test the probability of surviving one year beyond the
223 date of sampling (Y/N) in relation to haematocrit. Since haematocrit exhibited different age-specific
224 patterns in early-life (<1.5 years) and adulthood (1.5–13 years; see results), we investigated the
225 relationship between haematocrit and survival for these two age groups in separate models. Where
226 multiple haematocrit samples were taken per individual, only the last sample was selected, which
227 allowed us to identify whether individuals facing imminent mortality have different haematocrit levels
228 compared to those which survive. Additional fixed effects included age, sex, status, and quadratic

functions of haematocrit and age. We also included an interaction term between age and haematocrit to see whether the effect of haematocrit on survival changed with age. Survival probability can vary between territories and years e.g. 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 1.5–13-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, non-significant 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 non-significant (see Whittingham et al., 2006). Parameter estimates and significance of removed effects were determined by re-entering them into final models.

Results

Cross-sectional age

Haematocrit had a distinctive pattern with cross-sectional age (Fig. 1). Initially, haematocrit increased rapidly before reaching a peak at ca. 1.5 years of age. From 1.5 years onwards (maximum age in this analysis is 13 years), haematocrit showed a consistent downward trajectory (Fig. 1). This age-dependent pattern was similar for both sexes (Fig.1) and fitting smoothed age terms for males and females separately resulted in poorer model fit (ΔAIC > 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, Fig S1). Individuals sampled in the early morning had higher heamtocrit than those sampled in the late afternoon (Table 1). When the model was run on a sub-set of individuals known to be dead, including age-at-death as a predictor, we found that shorter-lived individuals had significantly higher haematocrit (Table S1). Therefore, selective disappearance of individuals with high haematocrit 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.

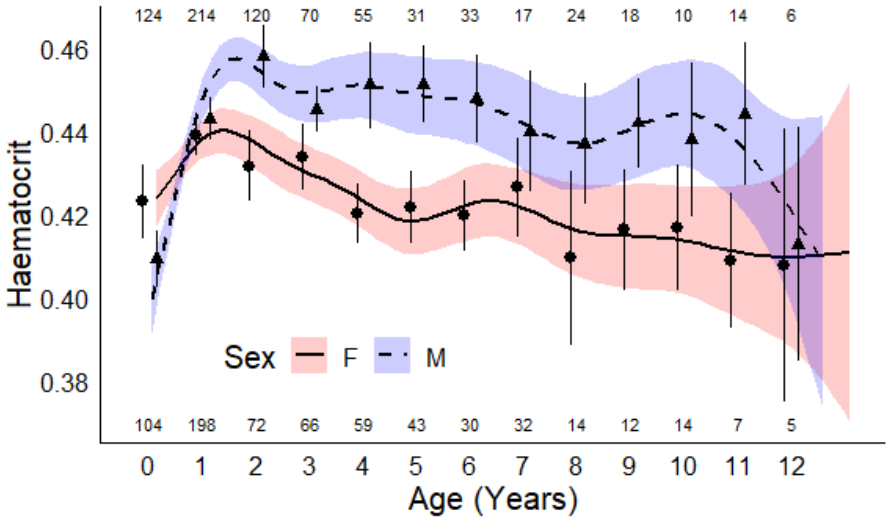

Figure 1: Haematocrit in Seychelles warblers in relation to age and sex. The fit lines (solid = female, dashed = male) show non-parametric smoothing functions for age with 95% confidence intervals. Points (round = female, triangles = male) are means and 95% confidence intervals for each age (rounded 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).

Table 1: Haematocrit in relation to cross-sectional age and other factors in Seychelles warblers. Results are from a GAMM analysis with a non-parametric smoothing parameter for age. Significant effects are in bold.

Predictor	**β**	**SE**	***t***	***P***
(Intercept)	0.460	0.005	94.701	< 0.001
Sex (Male)	-0.003	0.003	-1.202	0.230
Status (Dominant)	**-0.021**	**0.003**	**-6.440**	**< 0.001**
Sample Time	**-0.002**	**0.000**	**-5.981**	**< 0.001**
Sex × Status	**0.028**	**0.004**	**7.612**	**< 0.001**
Smoothed Terms		***df***	**F**	***P***
Age		**7.805**	**16.73**	**< 0.001**
Random factors	**1379 observations**	**Variance**		
Individual identity	730 individuals	< 0.000		
Breed group	747 breed groups	< 0.000		
Catch year	14 years	< 0.000		

Reproductive stage

We compared haematocrit of sexually mature (>8 months-old) subordinates and dominant breeders. Outside of breeding attempts, dominant females had lower haematocrit than both dominant males and subordinate males and females (Table S2, Fig, S1). During breeding attempts, the effects were

more complex (Table S3). There was no evidence of reproductive anaemia (i.e. a marked decrease in haematocrit) in dominate females sampled near their lay date, although haematocrit was lower at ca. 35–50 days after laying (Fig. 2). The haematocrit of males exhibited a complex relationship with breed group lay date; haematocrit was highest at ca. 7 days prior to laying and was lowest 15 – 30 days post laying (Fig. 2). In contrast to dominant breeders, there was no significant difference between male and female subordinates sampled during breeding attempts (Table S3 and S4, Fig. 2). The haematocrit 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 (Fig. 2). Importantly, the decline of haematocrit with increasing age persisted when the analysis was split between non-breeding and breeding individuals, and (for the latter) when controlling for days from lay date (Fig. S2).

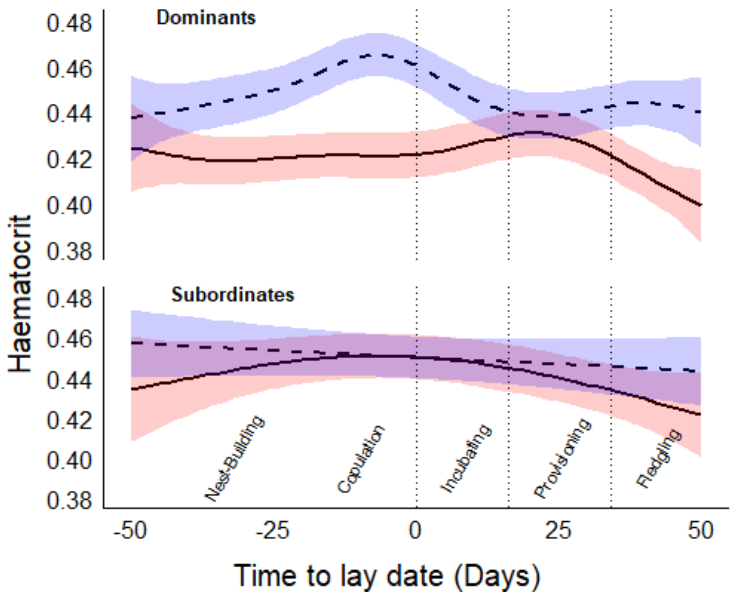

Figure 2: Haematocrit in in Seychelles warblers in relation to days from lay date for dominant breeders (left) and subordinates (right). The fit lines (solid = female, dashed = male) show non-parametric smoothing functions for days from lay date and the shaded area is the 95% confidence interval for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

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, haematocrit increased both within- and between-individuals with age in a quadratic pattern; a strong initial increase which plateaued at ca. 1 year-of-age (Table 2, Fig. 3). From 1.5 years of age onwards,

haematocrit declined linearly with increasing age both within- and between-individuals (Table 2, Fig. 3). All interactions with Δ age were non-significant; 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, haematocrit 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 haematocrit deviated from typical levels; see fig 2) were excluded from the analysis (Table S5).

Table 2: Haematocrit 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. Parameters shown are from LMM analysis. Significant effects are in bold.

< 1.5 YEARS OLD				
Predictor	**β**	**SE**	***t***	***P***
(Intercept)	0.393	0.010	40.92	< 0.001
Mean Age	**0.159**	**0.022**	**7.327**	**< 0.001**
Mean Age^2	**-0.085**	**0.016**	**-5.436**	**< 0.001**
Δ Age	**0.036**	**0.008**	**4.437**	**< 0.001**
Δ Age^2	**-0.054**	**0.018**	**-3.09**	**0.002**
Sex (Male)	-0.005	0.003	-1.615	0.107
Status (Dominant)	**-0.014**	**0.007**	**-2.061**	**0.040**
Sample Time	**-0.002**	**0.000**	**-3.276**	**0.001**
Sex × Status	**0.017**	**0.008**	**2.143**	**0.033**
Random factors	**637 observations**	**Variance**		
Individual identity	506 individuals	< 0.000		
Breed group	452 breed groups	< 0.000		
Catch year	14 years	< 0.000		
1.5 - 13 YEARS OLD				
Predictor	**β**	**SE**	***t***	***P***
(Intercept)	0.465	0.006	74.692	< 0.001
Mean Age	**-0.002**	**0.001**	**-3.608**	**0.000**
Δ Age	**-0.002**	**0.001**	**-2.715**	**0.007**
Sex (Male)	**0.026**	**0.002**	**10.677**	**< 0.001**
Status (Dominant)	**-0.013**	**0.003**	**-3.619**	**< 0.001**
Sample Time	**-0.002**	**0.000**	**-4.714**	**< 0.001**
Random factors	**742 observations**	**Variance**		
Individual identity	405 individuals	< 0.000		
Breed group	529 breed groups	< 0.000		
Catch year	14 years	< 0.000		

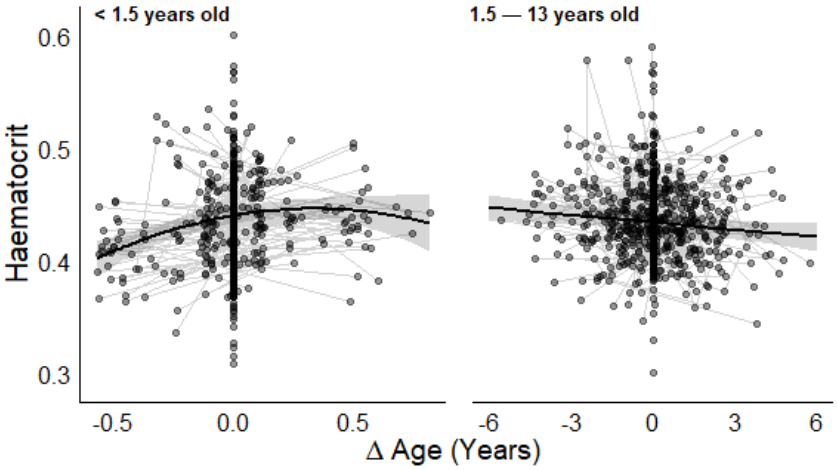

Figure 3: Haematocrit in Seychelles warblers in relation to within-individual differences in age (Δ Age) for individuals <1.5 years old and 1.5–13 years old. Thick black lines are the LMM (Table 2) predicted haematocrit ± 95% CI relative to age. Raw data are haematocrit samples with thin grey lines connecting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

For final LMMs (excluding all non-significant interactions and non-significant quadratic effects), we calculated repeatability of haematocrit within-individuals. For individuals below 1.5 years-of-age, only 1.6% (P = 0.434) 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 = 0.063).

Survival

A total of 263 out of the 1383 samples taken were from individuals that died within the subsequent year. For young (<1.5 years-of-age), individuals with higher haematocrit were less likely to survive to the next year (Table 3, Fig. 3). In contrast, haematocrit 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 haematocrit on survival; only high haematocrit was associated with lower survival in 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. Repeating the analysis while excluding samples from dominant breeders caught at key breeding stages (where haematocrit deviated from typical levels; Fig 2) did not qualitatively change results (Table S6).

Table 3. Survival in the Seychelles warbler in relation to haematocrit for individuals 1.5 years old and 1.5–13 years old. Results are from binominal GLMMs with survival to the following year (Y/N) as the response variable. Significant effects are in bold.

< 1.5 YEARS OLD				
Predictor	**β**	**SE**	***z***	***P***
(Intercept)	5.016	1.283	3.909	< 0.001
Haematocrit	**-9.293**	**3.004**	**-3.093**	**0.002**
Sex (Male)	-0.224	0.224	-1.001	0.317
Status (Dominant)	0.565	0.369	1.533	0.125
Age	0.459	0.395	1.161	0.246
Random factors	**506 observations**	**Variance**		
Breed group	418 breed groups	< 0.000		
Catch year	14 years	0.340		
1.5 - 13 YEARS OLD				
Predictor	**β**	**SE**	***z***	***P***
(Intercept)	1.485	1.668	0.89	0.3733
Haematocrit	-1.398	3.684	-0.379	0.7044
Sex (Male)	**-0.557**	**0.254**	**-2.196**	**0.0281**
Status (Dominant)	**0.724**	**0.345**	**2.099**	**0.0358**
Age	-0.009	0.043	-0.202	0.8403
Random factors	**408 observations**	**Variance**		
Catch year	13 years	0.370		

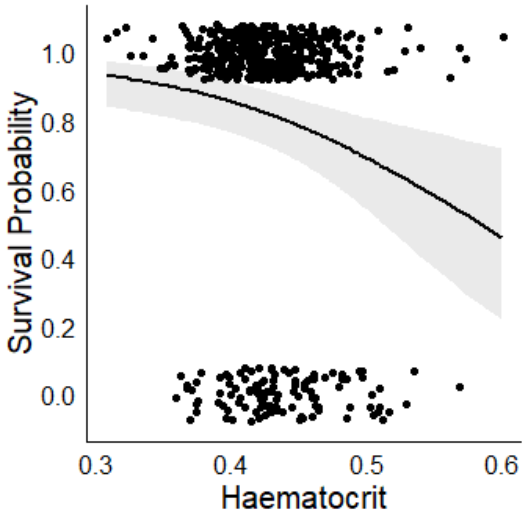

Figure 3: The probability of young (< 1.5 years of age) Seychelles warblers surviving one year after sampling relative to haematocrit. The fit-line is a binomial regression between survival (Y/N) and haematocrit with 95% confidence limits. Raw data depicts the distribution of observed survival counts (1 = survived, 0 = deceased).

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 to 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.

Age

Increases in haematocrit and oxygen carrying capacity during development have been observed in a range of vertebrates (Petschow et al., 1978; Fair et al., 2007; Trillmich et al., 2008). In birds, adult levels of haematocrit are usually achieved at late-nestling age, presumably in preparation for fledging (Eklom and Lill, 2006a, 2006b). However, in the Seychelles warbler haematocrit continues to increase post-fledging (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 annual survival i.e. 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 defence 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 behavioural 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 (Wilcoxen *et al.*, 2011; Lendvai *et al.*, 2015). Such changes may be expected of older Seychelles warblers living in long-established territories.

Sex and reproduction

We found that haematocrit was lower in female, compared to 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 (Richardson *et al.*, 2001; Raj Pant *et al.*, 2019) and sampling coincided with peaks in breeding activity (Komdeur and 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 (Williams *et al.*, 2004; Wagner *et al.*, 2008). Haematocrit declines observed in other species range from 5–10% (Morton, 1994; Davey et al., 2000) 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 anaemia (low haematocrit at egg-laying) in dominant females. Taken together, these findings indicate that dominant females maintain hematocrit 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 (Woodley and Moore, 1999; Pärn *et al.*, 2008). This suggests that female dominance in Seychelles warblers might be accompanied by an upregulation of eostrogen, which subsequently lowers haematocrit. However, we do not currently have data on eostrogen 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 and 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 behavioural 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 haematocrit could reflect increased activity levels – and therefore higher oxygen demands – during this critical period for dominant males (see Hammond et al., 2000)

Survival

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

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

Higher haematocrit was associated with reduced survival probabilities in young individuals, despite nearly all haematocrit values falling within what is considered to be a healthy reference range for captive avifauna; 35–55% (Campbell, 1994). Short-term increases in haematocrit can result from dehydration/haemoconcentration, 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 haematocrit (by ca. 2–5%) in birds during endurance activities via an increase in blood plasma (haemodilution). In line with optimal haematocrit theory (Birchard, 1997), these authors suggest that haemodilution is an adaptive response to prolonged exercise; facilitating faster blood flow for less cardiovascular effort (Jenni *et al.*, 2006; Yap *et al.*, 2018; Bury *et al.*, 2019). Thus, high haematocrit in young Seychelles warblers may reflect a failure to maintain optimal haematocrit, for example, due to dehydration. Alternatively, haematocrit could reflect physiological traits and/or life-histories with potential costs to survival. For example, haematocrit has been positively associated with reproductive effort (e.g. Hõrak, 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 haematocrit and pace-of-life in the Seychelles warbler.

Our study provides novel insights into the dynamics of haematocrit, and its impact on survival, in a wild population. Haematocrit 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 haematocrit 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 haematocrit can be an indicator of survival prospects in wild populations. Whether survival is directly impacted by (suboptimal) oxygen carrying capacity, or factors which increase haematocrit (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.*, 2011, 2017), haematocrit 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 haematocrit levels independently of erythrocyte mass, which makes unravelling the drivers of elevated haematocrit difficult without data on additional blood metrics, such as plasma protein and haemoglobin concentrations (see Johnstone *et al*., 2017). Nevertheless, haematocrit 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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Authors contributions

The study was conceived by T.J.B and D.S.R. Data was collected by T.J.B, D.S.R, H.L.B, M.H and J.K. Statistical analyses were conducted by T.J.B with input from D.S.R and M.H. The paper was written by T.J.B and all authors critiqued the output with important intellectual content. All authors gave final approval for publication.

Competing Interests Statement

We report no competing interests

Ethics statement

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 accessibility statement

All data will be made available upon acceptance in Dryad Digital Repository.

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