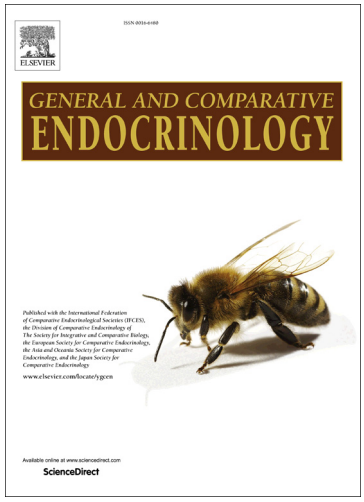

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Baseline and stress-induced blood properties of male and female Darwin's small ground finch (*Geospiza fuliginosa*) of the Galapagos Islands

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Running head: Blood stress response in Darwin's finches.

Abstract

Birds are renowned for exhibiting marked sex-specific differences in activity levels and reproductive investment during the breeding season, potentially impacting circulating blood parameters associated with stress and energetics. Males of many passerines often do not incubate, but they experience direct exposure to intruder threat and exhibit aggressive behaviour during the nesting phase in order to defend territories against competing males and predators. Nesting females often have long bouts of inactivity during incubation, but they must remain vigilant of the risks posed by predators and conspecific intruders approaching the nest. Here, we use 33 free-living male (n = 16) and female (n = 17) Darwin's small ground finches (*Geospiza fuliginosa*) on Floreana Island (Galapagos Archipelago) to better understand how sex-specific roles during the reproductive period impact baseline and stress-induced levels of plasma corticosterone (CORT), blood glucose and haematocrit. Specifically, we hypothesise that males are characterised by higher baseline values given their direct and relatively frequent exposure to intruder threat, but that a standardised stress event (capture and holding) overrides any sex-specific differences. In contrast with expectations, baseline levels of all blood parameters were similar between sexes (13.4±1.9 ng ml^{-1} for CORT, 13.7±0.4 mmol l^{-1} for glucose, 58.3±0.8% for haematocrit). Interestingly, females with higher body condition had lower baseline haematocrit. All blood parameters changed with time since capture (range 1.2 – 41.3 min) in both sexes, whereby CORT increased linearly, haematocrit decreased linearly, and glucose increased to a peak at ~20 min post-capture and declined to baseline levels thereafter. Our results do not support the hypothesis that sex-specific roles during the reproductive period translate to differences in blood parameters associated with stress and energetics, but we found some evidence that blood oxygen transport capacity may decline as finches increase in body condition.

Keywords: glucose; corticosterone; haematocrit; *Geospiza fuliginosa*; Darwin's finches; stress hormones; passerine.

1. Introduction

Many bird species are renowned for displaying sexual dichotomy in behaviour throughout much of the year and especially during the reproductive period (e.g., Silverin et al., 1997; Silverin and Wingfield, 1998), yet comparatively little is known of how this sexual dichotomy may differentially affect physiological processes (e.g., Edwards et al., 2013). The major stress hormone in birds, corticosterone (CORT), is generally maintained within diurnally-cycling baseline levels during normal activities (Breuner et al., 1999; Tarlow et al., 2003) and plays an important role in the activation of social behaviours such as courtship, aggression and vigilance (Ball and Balthazart, 2008; Wingfield et al., 1995; Wingfield and Silverin, 1986), exploratory and foraging behaviours (Crino et al., 2016; Martins et al., 2007; Wingfield, 2003), and may even alter the feeding preferences of mosquitoes that act as vectors of avian disease (Gervasi et al., 2016). In concert, baseline levels of other blood parameters, such as glucose and haematocrit, are maintained to ensure that aerobic, energy-demanding processes can be met. Indeed, haematocrit is often assumed to be an indicator of condition in birds, although a reported lack of correlation between haematocrit and avian body condition has called this assumption into question (Smith and Barber, 2012). Given the distinct roles of males and females during the breeding season of many bird species, it may be expected that sex-specific differences in baseline blood parameters (aside from sex hormones) should exist, yet evidence for this remains scant (Glomski and Pica, 2011; Wingfield et al., 1992).

Beyond sex-specific differences in baseline blood properties, it may be anticipated during the breeding season that sexes differ in the magnitude of the acute stress response following a challenging encounter (e.g., Butler et al., 2009; Scheuerlein et al., 2001). The first reaction to acute stress is generally the activation of the hypothalamus-pituitary-adrenal (HPA) axis and release of epinephrine and norepinephrine as part of the fight-or-flight response. Subsequently, glucocorticoids including CORT are released and act directly on the liver to stimulate glycogenolysis, which results in the mobilisation of glucose in the blood (Kubokawa et al., 1999; Remage-Healey and Romero, 2001; Silverin, 1998). Thus, it may be expected that changes in CORT and glucose are correlated during an acute stress response (Remage-Healey and Romero, 2001 and references within). Increased CORT in birds may negatively affect territorial, reproductive and foraging activities, sometimes leading to nest abandonment (Crino et al., 2013; Wingfield and Silverin, 1986), however it may also favour 'survival-related' behaviours like feeding activity when under nutritional stress (Astheimer et al., 1992; Kitaysky et al., 2001; Wingfield et al., 1995).

Acute stress and/or exercise result in an abrupt increase in oxygen requirements, which often corresponds with an increase in blood oxygen transport capacity via mobilisation of erythrocytes into the circulation (i.e., increased haematocrit). While the increase in circulating erythrocytes stems from splenic contraction in animals such as mammals and fish (Barcroft et al., 1925; Pearson and Stevens, 1991), the bird spleen is thought to lack the trabeculae necessary for contraction and therefore may not serve the same function (or at least not to the same degree; John, 1994; Sturkie, 1943). Notably, a review by Fair et al. (2007) highlighted that most of our understanding of haematocrit dynamics in birds has come from descriptive studies without any manipulative component. Thus, while it is known that birds can regulate circulating haematocrit

in response to chronic challenges (e.g., Borras et al., 2010; Hõrak et al., 1998), it remains unclear to what degree birds are able to modulate circulating haematocrit over short temporal scales (e.g., between flying bouts) via erythrocyte pooling/storage and other mechanisms (Goldstein and Afshan, 1990; Jenni et al., 2006; John, 1994; Khorrami et al., 2008).

Owing to their role in testing Charles Darwin's theory of natural selection (Darwin, 1859; Grant and Grant, 2014b), the iconic Darwin's finches of the Galapagos Islands, Ecuador, have been the subject of many long-term investigations into sex-specific breeding behaviour and its implications for the evolution of species (Chaves et al., 2016; Grant, 1986; Grant and Grant, 2014a; Grant and Grant, 2014b; Kleindorfer, 2007a). Darwin's finches breed annually with peak activity in January-March. Breeding pairs appear to be seasonally monogamous, although partners may be swapped for different nesting attempts and between years. Predation of the nests can be high (up to 60%) and nests are generally used only once, so nesting attempts are often repeated multiple times throughout a season before a successful brood is achieved (Cimadom et al., 2014; Kleindorfer, 2007a, b; Kleindorfer and Dudaniec, 2009; O'Connor et al., 2010). Males establish small (20 m^2) nesting territories, defend the nesting area against intruders, and build several nests while singing to attract a female to select one of the newly built nests (Kleindorfer, 2007b). After egg laying, female Darwin's finches are uniparental incubators (Kleindorfer, 2007a, b). During the ~12 day incubation period, males provide incubation feeding to the female outside the nest and invest effort into defending the nest against frequent intruders or predators. Females spend approximately 60% of their time inside the nest during the incubation period and are exposed to the risk of potential attacks by predators (e.g., rats, hawks) as well as the detrimental effects of blood-sucking larvae from parasitic *Philornis downsi* flies that inhabit the nesting material of nearly every nest (Huber et al., 2010; Kleindorfer and Dudaniec, 2016;

Knutie et al., 2013). Upon egg hatching, both the male and female feed the nestlings until fledging approximately 12-14 days later (O'Connor et al., 2014).

Given the behavioural dichotomy between male and female Darwin's finches during the breeding season (Grant and Grant, 2008), they make interesting model species for testing whether sexes differ in their baseline physiological processes and their responses to stress (e.g., Edwards et al., 2013). Here, we test for the presence of sexual dichotomy in baseline and stress-related blood parameters of Darwin's small ground finch (*Geospiza fuliginosa*) during the breeding season on Floreana Island in the Galapagos Archipelago. We made three predictions *a priori*: (1) the heightened activity levels and social conflicts experienced by males during the breeding season translate to elevated baseline blood stress indices and blood oxygen transport capacity in comparison with females, (2) comparable stress-induced blood responses exist in males and females when faced with a standardised stressor, and (3) baseline haematocrit correlates positively with body condition within sexes. To test these predictions, we analyse CORT, glucose and haematocrit in blood samples from male and female finches immediately after mid-air capture (baseline) and following a defined period of holding (stress-induced).

2. Materials and methods

2.1. Animal capture and sampling

Mature small ground finches (*Geospiza fuliginosa*) were captured in daylight hours (N=24 between 07:10 and 09:45; N=9 between 14:51 and 17:02) during the onset of the breeding phase (late February 2010) using mist-nets in the highlands of Floreana Island (1°17'43S, 90°27'23W), Galapagos Archipelago, Ecuador. Environmental conditions at our field site can vary widely during the January-March breeding period from year to year, and this may affect finch breeding

and related physiological parameters. For example, years that are extremely dry (e.g., 2006: 108 mm) or wet (e.g., the 1998 El Niño: 1,008 mm from Santa Cruz Island) may cause asynchronous breeding and additional stress (Kleindorfer et al., unpublished data; Dudaniec et al., 2007). However, in the year of the present study (2010), rainfall during the January-March breeding period on Floreana Island was quite typical at 572 mm (see Discussion), and finch breeding was synchronous. Birds were removed from the net immediately (<10 s) following capture and a 50 µl blood sample was taken from the jugular vein with a 0.5-ml insulin syringe and 29 G hypodermic needle. Blood sampling was completed within 3.0±1.0 min (mean±SD) of the bird contacting the mist-net (range 1.2-5.6 min; quantified using a stopwatch), which was assumed to provide a representative indication of the bird's baseline blood parameters prior to capture (Clark et al., 2011; Handasyde et al., 2003; Romero and Reed, 2005). The majority of the blood sample was immediately transferred to a heparinised micro-capillary tube for measurement of haematocrit (haematocrit; centrifuged at 7,000 x *g* for 4 min), while a subsample was used for glucose measurements (Accu-chek, Compact Plus, Mannheim, Germany). The plasma was collected from the micro-capillary tube following the haematocrit measurement and stored in Eppendorf tubes on ice for subsequent transfer to a -20^{o}C freezer. The bird was fitted with an aluminium identification leg band and colour bands and measurements were made of tarsus length, wing length and body mass, before placing the bird into a cotton bag for subsequent sampling.

A second blood sample was taken from the jugular vein of each bird within 41.3 min after capture, and time was considered a continuous variable in the statistical analyses. The blood samples were processed as detailed above, and the bird was released near the point of capture. All birds flew away immediately after release, and many individuals were observed again

throughout the 2-week experimental period. Mist-nets were relocated each morning and routinely throughout the day to prevent recapture of the same individuals. A linear sampling transect of about 2 km through *Scalesia* forest was covered over the 2-week experimental period.

Subsequent nest searches as part of our annual three-month nest monitoring program (see Kleindorfer et al., 2014; O'Connor et al., 2010) identified the colour-banded birds at nests, and enabled confirmation of nesting activity in 10 of 17 female birds (59%) and 9 of 16 male birds (56%). In all cases, the birds were at the early stages of nesting: three single males were singing at a display nest to attract a female, six males were at nests with an incubating female, and all 10 females were incubating on first observation. In addition, all females sampled in the present study had a brood patch and were therefore nesting, likely close to or during the incubation period.

2.2. Plasma analyses

Plasma samples were moved into a -80^{o}C freezer upon completion of the study and were subsequently thawed for measurements of CORT. CORT concentration of each sample was measured in duplicate using radioimmunoassay following extraction of steroids from 5 µl plasma samples. Detailed procedures are outlined in Lindsay et al. (2011). The average intra-assay coefficient of variation was 10.84% and the average inter-assay coefficient of variation was 11.62%.

2.3. Data analyses and statistics

Issues with equipment occasionally prevented us from measuring all morphological and blood parameters for each bird; sample sizes and degrees of freedom are provided herein. JMP 11 (SAS

Institute Inc., NC, USA) and SigmaPlot 13.0 (Systat Software Inc., CA, USA) were used for all statistical tests and graphing. Body condition was calculated for each individual from the residuals of a linear regression between body mass and tarsus length (sexes combined). Student t-tests were used to determine whether sex influenced body mass, body condition, morphometrics (e.g., tarsus and wing length; methods in Kleindorfer et al. (2014)), or the baseline levels (i.e., samples taken within 3.0±1.0 min of capture) of any of the measured blood parameters (CORT, glucose, haematocrit). Student t-tests were also used to examine for any influence of time of day on baseline blood parameters. Data were log-transformed where indicated to satisfy tests of normality and equal variance. Restricted maximum likelihood (REML) mixed models were used to test the effects of sex, body mass, body condition, tarsus length, wing length, time of day and time post-capture on each of the measured blood parameters. Individual ID was included as a random factor in all REML models. Minimum adequate models (based on the lowest Akaike Information Criterion scores; AICc) were calculated by removing non-significant parameters from the models using stepwise backward exclusion. Significance was considered at $P<0.05$.

3. Results

Males and females did not differ in their body mass (log-transformed; $t_{(30)}=0.505$, $P=0.617$), yet males had a significantly greater tarsus length ($t_{(29)}=-2.137$, $P=0.041$) and wing length ($t_{(29)}=-2.794$, $P<0.001$) (Table 1). Consequently, females had a higher body condition, calculated from the residuals of the relationship between body mass over tarsus length (both sexes combined; Table 1). Time of day (morning [07:10-09:45] vs. afternoon [14:51-17:02]) did not affect any of the baseline blood parameters (t-tests (CORT and glucose log-transformed): P range = 0.217-

0.770). Moreover, none of the baseline blood parameters were influenced by the time between capture and initial blood sampling (linear regression analyses (CORT and glucose log-transformed): P range = 0.189-0.361), suggesting that the measured baseline values were representative of the blood glucose, CORT and haematocrit of freely-roaming finches.

Sex did not have a significant effect on baseline levels of glucose (log-transformed; $t_{(28)}$=-0.016, P=0.988), CORT ($t_{(30)}$=-1.041, P=0.306) or haematocrit ($t_{(29)}$=-0.741, P=0.465) (Fig. 1, Table 1). Combining both sexes, baseline levels for blood parameters were 13.7±0.4 mmol l^{-1} for glucose, 13.4±1.9 ng ml^{-1} for CORT and 58.3±0.8% for haematocrit. There was a significant negative relationship between body condition and baseline haematocrit for females ($F_{1,12}$=8.097, P=0.016) but not males ($F_{1,13}$=0.740, P=0.406), indicating that higher conditioned females had lower circulating haematocrit (Fig. 2, Table 2). No other significant sex-specific correlations existed between body condition and the measured baseline blood parameters.

Neither sex nor time of day had an effect on any of the measured blood parameters across the duration of sampling (i.e., 1.2-41.3 min post-capture), so both variables were excluded from all REML models (Table 2). Combining both sexes as well as baseline and subsequent blood samples, model estimates revealed a significant effect of time post-capture on all measured blood parameters. The pattern in glucose was best described by a second-order polynomial, whereby glucose increased for ~20 min post-capture and returned to baseline levels by ~38 min post-capture (Fig. 3, Table 2). CORT increased linearly and exhibited more inter-individual variability over time post-capture (Fig. 3, Table 2), perhaps indicative of different individual stress responses. Haematocrit declined linearly over time and was generally lower in birds with higher condition (Fig. 3, Table 2).

4. Discussion

We characterised baseline and stress-related levels of CORT, glucose and haematocrit in free-living island birds during the reproductive season on Floreana Island, Galapagos Archipelago. Contrary to our first prediction, the heightened activity levels and social conflicts experienced by male finches during the breeding season did not translate into elevated baseline levels of blood indices associated with stress (CORT), energetics (glucose) and oxygen transport (haematocrit). Our second prediction was confirmed, as we observed a comparable blood stress response in males and females when faced with capture stress and holding: CORT increased linearly and became more variable across individuals, haematocrit decreased in a manner consistent with erythrocyte pooling/storage within the circulatory system (e.g., spleen) once flight was prevented, and glucose increased by ~20 min post-capture and returned to baseline levels in the subsequent ~18 min. Notably, the time-dependent decrease in haematocrit is more suggestive of erythrocyte pooling/storage rather than haemodilution associated with blood sampling, as the values from the second blood sample were typically lower when the blood was sampled later (>25 min) rather than earlier (<25 min) after capture. Interestingly, our final prediction was the opposite of what we subsequently observed, whereby haematocrit had a negative rather than a positive relationship with body condition (similar to Smith and Barber (2012) but in contrast with Jenni et al. (2006)). We discuss the main findings and their ecological implications below.

4.1. Sex-specific differences and temporal dynamics of blood parameters

Our results indicate that differential investment in reproductive activities observed in breeding male versus female Darwin's small ground finches did not result in sex-specific baseline or stress-related values of CORT, glucose or haematocrit. Our values of plasma CORT corroborate

the only previous study on stress hormones in Darwin's finches, in which Knutie et al. (2013) sampled blood from breeding female medium ground finches (*Geospiza fortis*) within 3 min of capture and again at 15 min post-capture. Mean baseline CORT for *G. fortis* during the breeding season in Knutie et al. (2013) was 12.6±0.9 ng ml^{-1}, and our mean baseline CORT for *G. fuliginosa* was 13.7±0.4 ng ml^{-1}. Mean stress-induced CORT (15 min post-capture) in Knutie et al. (2013) was 57.9±4.9 ng ml^{-1}, which is slightly higher than our values at a similar time point (see 15 min in Fig. 3B) but may be an underestimate of the values that can be attained in some individuals following longer holding periods (see Fig. 3B; mean CORT 15-41 min post-capture = 58.5±6.3 ng ml^{-1}). Indeed, it may be speculated that Darwin's finches, perhaps like other island fauna without endemic mammalian predators, have a delayed initiation of the blood stress response and a prolonged duration until reaching the peak response (see below). It is also possible that differences in body mass of approximately 6 g between *G. fortis* in Knutie et al. (2013) and *G. fuliginosa* in our study (see Grant, 1986) may have contributed to some of the minor variation between studies. We are not aware of any published studies of Darwin's finches that have measured blood glucose, and only one study that has measured adult haematocrit (baseline haematocrit of female *G. fortis* ~47.4% in Knutie et al. (2013), which is lower than the baseline values measured here), but our values for both variables fall within normal ranges reported for other passerines of similar body size (Braun and Sweazea, 2008; Fair et al., 2007; Lill, 2011; Potti, 2007). Our values of glucose in small ground finches corroborate previous observations that birds generally maintain higher levels of glucose than similar-sized mammals (Braun and Sweazea, 2008).

CORT concentrations are known to vary throughout the breeding season or with time of day (Johnstone et al., 2012), although this is not always the case (e.g., Tilgar et al., 2009).

Environmental variation was reported to influence CORT in pied flycatchers (*Ficedula hypoleuca*), whereby concentrations were lower in northern populations where the breeding season was shorter and environmental conditions more harsh and unpredictable, compared with more stable conditions faced by southern populations (Silverin and Wingfield, 1998). Environmental conditions often show annual fluctuations on the Galapagos Islands with annual highland rainfall on Floreana Island oscillating between 'dry' (<200 mm), 'normal' (~500-600 mm) and 'wet' (>800 mm) over the January to March breeding period (Grant, 1986; Grant and Grant, 2014b). Such variability could create stressful environments across years and alter associated blood properties within populations (Wingfield and Sapolsky, 2003). However, in the current study, 2010 was a 'normal' rainfall year (572 mm), indicating that birds were not subject to atypical environmental stress. Furthermore, temporal and environmental influences should not have been particularly influential in our study because we used a relatively short sampling period (2 weeks), we consistently sampled in daylight hours, and we restricted our sampling to a single, synchronous breeding season in which temperatures on the Galapagos Islands typically remain stable with low variability (Grant and Grant, 2008; O'Connor et al., 2010). We acknowledge however, that our study is representative of a single 'normal' rainfall year and blood parameters may be altered in years of extreme environmental conditions, or in other seasons of the year.

Notably, while there was no significant difference in haematocrit between the sexes at any time point, three findings suggest a tendency for lower haematocrit in females: (1) Females had higher body condition than males (Table 1), (2) body condition (combined sexes) was found to have a negative influence on haematocrit in the REML mixed model describing the post-capture data (Table 2), and (3) there was a tendency for lower haematocrit in females during the holding period following capture (Fig. 3C). Given that haematocrit is a determinant of blood

oxygen carrying capacity, these findings may be reflective of the lower flight requirements of females during the reproductive season. It is clear from the baseline haematocrit measured immediately post-capture that flight requires elevated, perhaps maximal, levels of circulating erythrocytes. Thus, it is possible that previous studies have failed to detect sex-specific differences in haematocrit (reviewed in Fair et al., 2007) because birds utilise haematocrit at full capacity during routine flight activities, making sex differences in baseline haematocrit measureable only after birds have remained stationary and inactive for prolonged periods (this has obvious implications for using the term 'baseline' to describe values taken immediately after capture). Moreover, sex differences in haematocrit may be regulated by stress-induced suppression of sex hormones that cause vasodilation and vasoconstriction of renal microvasculature, thus varying the volume of circulating erythrocytes within different parts of the circulatory system independent of changes in erythropoiesis (Fair et al., 2007; Murphy, 2014). Sex-specific differences in this mechanism may have played some role in the tendency for female ground finches to exhibit a greater decrease in haematocrit during the holding period (Fig. 3C). Indeed, it may be postulated that the primary male and female sex hormones (androgens and estrogens, respectively) exhibit different temporal dynamics in response to stress, subsequently impacting physiological systems to differing degrees and revealing sex-specific differences that are not apparent under more benign conditions.

4.2. Parasitism and blood stress indices

Blood stress indices can be predictive of pathogen risk and tolerance, as they may interact with immunity, host behaviour and reproductive success (Gervasi et al., 2016). Glucocorticoids, in particular, may provide information on host susceptibility and attractiveness to ectoparasites, as

found for bird-feeding mosquito vectors by Gervasi et al. (2016). Given the ongoing ecological stressors to Galapagos fauna imposed by human impacts, climate, introduced species and pathogens (Causton et al., 2006; Kleindorfer and Dudaniec, 2016; Romero and Wikelski, 2010), blood stress responses can provide insight into sub-lethal population impacts.

Of particular relevance to Darwin's finches is the introduced fly ectoparasite *Philornis downsi*, which is causing extremely high mortality (i.e., mean of 55% of nestlings) within finch populations (Kleindorfer and Dudaniec, 2016). The use of verified physiological protocols is necessary for making accurate impact assessments of *P. downsi* as well as other avian pathogens (e.g., Kleindorfer and Dudaniec, 2009). Knutie et al. (2013) measured CORT in breeding female medium ground finches (*Geospiza fortis*) to test if within-nest parasitism, by the predominantly nestling-feeding *P. downsi*, affected the adult female blood stress response. In that study, the female stress response did not differ with parasite infestation and could not be related to the lower nestling survival from parasitised nests. Notably, Knutie et al. (2013) removed nest parasites 1-2 days after egg hatching, presumably allowing time for adult females to be parasitised in this time and during incubation (S. Kleindorfer, personal observation). Evidence for this suggestion stems from a study that found a higher concentration of *P. downsi*-specific antibodies in nesting female *G. fortis* compared with nesting males and with females prior to nesting (Huber et al., 2010).

A reduction in blood oxygen carrying capacity can also act as an indicator of poor body condition in birds, such as those that are diseased or infected with parasites (Minias, 2015). In an earlier study on nestling *G. fuliginosa*, Dudaniec et al. (2006) measured blood parameters in *P. downsi*-parasitised nestlings and found that increasing parasite intensity within nests corresponded with a linear decrease in haemoglobin and a corresponding decrease in

reticulocytes (immature red blood cells). Conversely, Dawson and Bortolotti (1997) found that haematocrit increased with the level of infection of a blood parasite. While a disconnect between haemoglobin and haematocrit is intuitively confusing, this may be explained by a disproportionate increase in immature erythrocytes in parasitised individuals (Carleton, 2008). In any event, when combined with our finding of a negative relationship between haematocrit and body condition in female finches, these observations highlight a complex interplay between environmental challenges and blood oxygen carrying capacity in birds (see Dawson and Bortolotti, 1997; Lill et al., 2013).

4.3. Conclusions and implications

This study of male and female *G. fuliginosa* provides the first analysis of baseline and stress-induced levels of CORT, glucose and haematocrit in a Darwin's finch species. In general, we found little evidence for differences in the measured blood parameters between sexes, except for a tendency for lower haematocrit in females with higher body condition. Our time-series data (Fig. 3) highlight the roles of capture and holding in trying to ascertain baseline values; an important consideration when conducting both field and laboratory experiments. Moreover, the dramatic increase in variability of CORT across individuals throughout the post-capture holding period (Fig. 3B) is interesting given previous observations that link inter-individual differences in CORT to variation in 'personality' and performance (see Cockrem, 2007). Notably, Darwin's finches on the Galapagos Archipelago are renowned for showing reduced vigilance in the presence of humans compared with mainland passerines (Darwin, 1859; Grant and Grant, 2008), which is a frequently noted characteristic of island fauna that has been attributed to a lack of endemic mammalian predators (Blumstein and Daniel, 2005). This island-mainland difference in

behaviour may be detectable in blood stress indices (e.g., delayed initiation of blood stress response and prolonged duration until reaching peak response) and would be a fruitful direction for future research (see Müller et al., 2007). Broadly, our results are relevant for illuminating the breadth of factors that drive individual- and population-level responses to environmental perturbations in wild bird populations.

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References

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis. Scand. 23, 355-365.

Ball, G.F., Balthazart, J., 2008. Individual variation and the endocrine regulation of behaviour and physiology in birds: a cellular/molecular perspective. Philos. T. Roy. Soc. B 363, 1699-1710.

Barcroft, J., Harris, H.A., Orahovats, D., Weiss, R., 1925. A contribution to the physiology of the spleen. J . Physiol. 60, 443-456.

Blumstein, D.T., Daniel, J.C., 2005. The loss of anti-predator behaviour following isolation on islands. Proc. Roy. Soc. B 272, 1663-1668.

Borras, A., Cabrera, J., Senar, J.C., 2010. Hematocrit variation in response to altitude changes in wild birds: a repeated-measures design. Am. Ornithol. Soc. 112, 622-626.

Braun, E.J., Sweazea, K.L., 2008. Glucose regulation in birds. Comp. Biochem. Physiol. B 151, 1-9.

Breuner, C.W., Wingfield, J.C., Romero, L.M., 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. The Journal of experimental zoology 284, 334-342.

Butler, L.K., Bisson, I.-A., Hayden, T.J., Wikelski, M., Romero, L.M., 2009. Adrenocortical responses to offspring-directed threats in two open-nesting birds. Gen. Comp. Endocr. 162, 313-318.

Carleton, R.E., 2008. Ectoparasites affect hemoglobin and percentages of immature erythrocytes but not hematocrit in nestling eastern bluebirds. Wilson J. Ornithol. 120, 565-568.

Causton, C.E., Peck, S.B., Sinclair, B.J., Roque-Albelo, L., Hodgson, C.J., Landry, B., 2006. Alien insects: threats and implications for the conservation of the Galápagos Islands. Ann. Entomol. Soc. Am. 99, 121-143.

Chaves, J.A., Cooper, E.A., Hendry, A.P., Podos, J., De León, L.F., Raeymaekers, J.A., MacMillan, W.O., Uy, J.A.C., 2016. Genomic variation at the tips of the adaptive radiation of Darwin's finches. Mol. Ecol. 25, 5282–5295.

Cimadom, A., Ulloa, A., Meidl, P., Zöttl, M., Zöttl, E., Fessl, B., Nemeth, E., Dvorak, M., Cunninghame, F., Tebbich, S., 2014. Invasive parasites, habitat change and heavy rainfall reduce breeding success in Darwin's finches. PLOS ONE 9, e107518.

Clark, T.D., Donaldson, M.R., Drenner, S.M., Hinch, S.G., Patterson, D.A., Hills, J., Ives, V., Carter, J.J., Cooke, S.J., Farrell, A.P., 2011. The efficacy of field techniques for obtaining and storing blood samples from fishes. J. Fish Biol. 79, 1322-1333.

Cockrem, J.F., 2007. Stress, corticosterone responses and avian personalities. J. Ornithol. 148, 169-178.

Crino, O.L., Buchanan, K.L., Trompf, L., Mainwaring, M.C., Griffith, S.C., 2016. Stress reactivity, condition, and foraging behavior in zebra finches: effects on boldness, exploration, and sociality. Gen. Comp. Endocrinol. 244, 101-107.

Crino, O.L., Johnson, E.E., Blickley, J.L., Patricelli, G.L., Breuner, C.W., 2013. Effects of experimentally elevated traffic noise on nestling white-crowned sparrow stress physiology, immune function and life history. J. Exp. Biol. 216, 2055-2062.

Darwin, C.R., 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London.

Dawson, R.D., Bortolotti, G.R., 1997. Are avian hematocrits indicative of condition? American kestrels as a model. J. Wildlife Manage. 61, 1297-1306.

Dudaniec, R.Y., Kleindorfer, S., Fessl, B., 2006. Effects of the introduced ectoparasite *Philornis downsi* on haemoglobin level and nestling survival in Darwin's Small Ground Finch (*Geospiza fuliginosa*). Austral Ecol. 31, 88-94.

Edwards, D.B., Chin, E.H., Burness, G., Gilchrist, H.G., Schulte-Hostedde, A.I., 2013. Linking sex differences in corticosterone with individual reproductive behaviour and hatch success in two species of uniparental shorebirds. Comp. Biochem. Physiol. A 166, 169-176.

Fair, J., Whitaker, S., Pearson, B., 2007. Sources of variation in haematocrit in birds. Ibis 149, 535-552.

Gervasi, S.S., Burkett-Cadena, N., Burgan, S.C., Schrey, A.W., Hassan, H.K., Unnasch, T.R., Martin, L.B., 2016. Host stress hormones alter vector feeding preferences, success, and productivity. Proc. Roy. Soc. B 283, 1278.

Glomski, C.A., Pica, A., 2011. The avian erythrocyte: its phylogenetic odyssey. CRC Press, FL, USA.

Goldstein, D.L., Afshan, Z., 1990. Variation in osmoregulatory parameters of captive and wild house sparrows (*Passer domesticus*). The Auk 107, 533-538.

Grant, P.R., 1986. Ecology and evolution of Darwin's finches. Princeton University Press.

Grant, P.R., Grant, B.R., 2008. How and why species multiply: The radiation of Darwin's finches. Princeton University Press, Princeton.

Grant, P.R., Grant, B.R., 2014a. 40 Years of Evolution: Darwin's Finches on Daphne Major Island. Princeton University Press.

Grant, P.R., Grant, B.R., 2014b. Evolutionary biology: speciation undone. Nature 507, 178-179.

Handasyde, K.A., McDonald, I.R., Evans, B.K., 2003. Plasma glucocorticoid concentrations in free-ranging platypuses (*Ornithorhynchus anatinus*): response to capture and patterns in relation to reproduction. Comp. Biochem. Physiol. A 136, 895-902.

Hõrak, P., Ots, I., Murumägi, A., 1998. Haematological health state indices of reproducing Great Tits: a response to brood size manipulation. Func. Ecol. 12, 750-756.

Huber, S.K., Owen, J.P., Koop, J.A.H., King, M.O., Grant, P.R., Grant, B.R., Clayton, D.H., 2010. Ecoimmunity in Darwin's finches: invasive parasites trigger acquired immunity in the medium ground finch (*Geospiza fortis*). PLOS ONE 5, e8605.

Jenni, L., Müller, S., Spina, F., Kvist, A., Lindström, Å., 2006. Effect of endurance flight on haematocrit in migrating birds. J. Ornithol. 147, 531.

John, J.L., 1994. The avian spleen: a neglected organ. The Quarterly review of biology 69, 327-351.

Johnstone, C.P., Reina, R.D., Lill, A., 2012. Interpreting indices of physiological stress in free-living vertebrates. J. Comp. Physiol. B 182, 861-879.

Khorrami, S., Tazawa, H., Burggren, W., 2008. `Blood-doping' effects on hematocrit regulation and oxygen consumption in late-stage chicken embryos (*Gallus gallus*). J. Exp. Biol. 211, 883-889.

Kitaysky, A.S., Kitaiskaia, E.V., Wingfield, J.C., Piatt, J.F., 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. J. Comp. Physiol. B 171, 701-709.

Kleindorfer, S., 2007a. The ecology of clutch size variation in Darwin's small ground finch *Geospiza fuliginosa*: comparison between lowland and highland habitats. Ibis 149, 730-741.

Kleindorfer, S., 2007b. Nesting success in Darwin's small tree finch (*Camarhynchus parvulus*): Evidence of female preference for older males and more concealed nests. Anim. Behav. 74, 795-804.

Kleindorfer, S., Dudaniec, R.Y., 2009. Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's tree finches. Behav. Ecol. Sociobiol. 63, 731-739.

Kleindorfer, S., Dudaniec, R.Y., 2016. Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. BMC Zoology 1, 1.

Kleindorfer, S., O'Connor, J.A., Dudaniec, R.Y., Myers, S.A., Robertson, J., Sulloway, F.J., 2014. Species collapse via hybridization in Darwin's tree finches. Am. Nat. 183, 325-341.

Knutie, S., Koop, J.A.H., French, S.S., Clayton, D.H., 2013. Experimental test of the effect of introduced hematophagous flies on corticosterone levels of breeding Darwin's finches. Gen. Comp. Endocrinol. 193, 68-71.

Kubokawa, K., Watanabe, T., Yoshioka, M., Iwata, M., 1999. Effects of acute stress on plasma cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture 172, 335.

Lill, A., 2011. Sources of variation in blood glucose concentrations of free-living birds. Avian Biol. Res. 4, 78-86.

Lill, A., Yachou-Wos, L., Johnstone, C.P., 2013. Are haematocrit and haemoglobin concentration reliable indicators of body condition in adult Welcome Swallows? Emu 113, 93-98.

Lindsay, W.R., Webster, M.S., Schwabl, H., 2011. Sexually selected male plumage color is testosterone dependent in a tropical passerine bird, the red-backed fairy-wren (*Malurus melanocephalus*). PLOS ONE 6, e26067.

Martins, T.L.F., M.L., R., I., G., R., H., Evans, M.R., 2007. Speed of exploration and risk-taking behavior are linked to corticosterone titres in zebra finches. Horm. Behav. 52, 445–453.

Minias, P., 2015. The use of haemoglobin concentrations to assess physiological condition in birds: a review. Conserv. Physiol. 3, cov007.

Müller, C., Jenni-Eiermann, S., Blondel, J., Perret, P., Caro, S.P., Lambrechts, M.M., Jenni, L., 2007. Circulating corticosterone levels in breeding blue tits Parus caeruleus differ between island and mainland populations and between habitats. Gen. Comp. Endocrinol. 154, 128-136.

Murphy, W.G., 2014. The sex difference in haemoglobin levels in adults — Mechanisms, causes, and consequences. Blood Rev. 28, 41-47.

O'Connor, J.A., Dudaniec, R.Y., Kleindorfer, S., 2010. Parasite infestation and predation in Darwin's small ground finch: contrasting two elevational habitats between islands. J. Trop. Ecol. 26, 285-292.

O'Connor, J.A., Robertson, J., Kleindorfer, S., 2014. Darwin's finch begging intensity does not honestly signal need in parasitised nests. Ethology 120, 228-237.

Pearson, M.P., Stevens, E.D., 1991. Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 9, 39.

Potti, J., 2007. Variation in the hematocrit of a passerine bird across life stages is mainly of environmental origin. J. Avian Biol. 38, 726-730.

Remage-Healey, L., Romero, L.M., 2001. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. Am. J. Physiol.-Reg. I. 281, R994-R1003.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A 140, 73-79.

Romero, L.M., Wikelski, M., 2010. Stress physiology as a predictor of survival in Galapagos marine iguanas. 277, 3157-3162.

Scheuerlein, A., Van't Hof, T., Gwinner, E., 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). Proc. Roy. Soc. B 268, 1575-1582.

Silverin, B., 1998. Stress responses in birds. Poult. Avian Biol. Rev. 9, 153-168.

Silverin, B., Arvidsson, B., Wingfield, J., 1997. The adrenocortical responses to stress in breeding Willow Warblers Phylloscopus trochilus in Sweden: effects of latitude and gender. Func. Ecol. 11, 376-384.

Silverin, B., Wingfield, J.C., 1998. Adrenocortical responses to stress in breeding pied flycatchers *Ficedula hypoleuca:* Relation to latitude, sex and mating status. J. Avian Biol. 29, 228-234.

Smith, K.D., Barber, C.A., 2012. Hematocrit does not indicate condition in nestling or adult European starlings. Wilson J. Ornithol. 124, 788-792.

Sturkie, P.D., 1943. The reputed reservoir function of the spleen of the domestic fowl. Am. J. Physiol. 138, 599-602.

Tarlow, E.M., Hau, M., Anderson, D.J., Wikelski, M., 2003. Diel changes in plasma melatonin and corticosterone concentrations in tropical Nazca boobies (*Sula granti*) in relation to moon phase and age. 133, 297-304.

Tilgar, V., Saag, P., Moks, K., 2009. Development of stress response in nestling pied flycatchers. J. Comp. Physiol. A 195, 799-803.

Wingfield, J.C., 2003. Control of behavioural strategies for capricious environments. Anim. Behav. 66, 807-815.

Wingfield, J.C., O'Reilly, K.M., Astheimer, L.B., 1995. Ecological bases of the modulation of adrenocortical responses to stress in Arctic birds. Am. Zool. 35, 285-294.

Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. 15, 711–724.

Wingfield, J.C., Silverin, B., 1986. Effects of corticosterone on territorial behaviour of free-living male song sparrows *Melospiza melodia*. Horm. Behav. 20, 405–417.

Wingfield, J.C., Vleck, C.M., Moore, M.C., 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert. J. Exp. Zool. 264, 419-428.

Table 1. Body mass, morphometrics and baseline blood parameters (means±SE) of male and female small ground finches (*Geospiza fuliginosa*) on Floreana Island.

Variable	Sex	N	Value
Body mass (g)	Male	16	14.8±0.2
	Female	16	15.1±0.4
Tarsus length (mm)	Male	16	20.3±0.2*
	Female	15	19.6±0.2
Wing length (mm)	Male	16	64.1±0.4*
	Female	16	61.3±0.5
Body condition (residual)	Male	16	-0.37±0.15*
	Female	15	0.40±0.29
Blood glucose (mmol l^{-1})	Male	13	14.0±0.9
	Female	17	13.6±0.4
Plasma corticosterone (ng ml^{-1})	Male	16	15.4±3.3
	Female	17	11.5±1.8
Haematocrit (%)	Male	14	57.7±1.0
	Female	17	58.9±1.2

Blood samples were obtained within 1.2-5.6 min of birds hitting the mist-net. * significantly different from the corresponding value for females (student t-tests; see text).

Table 2. Model parameters indicating the effects of time post-capture and body condition on blood parameters of male and female small ground finches (*Geospiza fuliginosa*) on Floreana Island. Bird ID was included as a random factor, and only the minimum adequate models are presented. There was no significant effect of sex on any of the variables, so it was excluded from all models.

Response variable	Explanatory variable	Estimate	SE	F Ratio	P
Blood glucose (mmol l^{-1})	Full model: R^2=0.405, N=58				
	Intercept	11.963	0.866		<0.0001
	Time	0.712	0.135	27.72	<0.0001
	$Time^2$	-0.018	0.003	28.28	<0.0001
	Condition	–	–	–	ns
Plasma corticosterone (ng ml^{-1})	Full model: R^2=0.637, N=65				
	Intercept	8.717	4.799		0.0745
	Time	1.729	0.219	62.27	<0.0001
	$Time^2$	–	–	–	ns
	Condition	–	–	–	ns
Haematocrit (%)	Full model: R^2=0.675, N=52				
	Intercept	59.326	0.912		<0.0001
	Time	-0.238	0.042	33.08	<0.0001
	$Time^2$	–	–	–	ns

Condition	-2.582	0.687	14.61	0.0009

Figure legends

Fig. 1 Box-and-whisker plots of baseline (a) blood glucose, (b) plasma corticosterone and (c) haematocrit of male and female small ground finches (*Geospiza fuliginosa*) sampled within 1.2-5.6 min of being caught in a mist-net. Boxes encompass the 25th and 75th percentiles (median indicated by horizontal line), while whiskers indicate the 10th and 90th percentiles. Outliers are indicated by black circles. No significant differences existed between sexes. Inset: small ground finch on Floreana Island (photo credit: Timothy D. Clark).

Fig. 2 Haematocrit as a function of body condition (residuals of body mass over tarsus length) for male (open circles) and female (closed circles) small ground finches (*Geospiza fuliginosa*) sampled within 1.2-5.6 min of being caught in a mist-net. Linear regression represents only female finches, where the relationship was significant (haematocrit = -2.662 • body condition + 61.123; $F_{1,12}$=8.097, P=0.016). Dashed lines indicate 95% confidence intervals.

Fig. 3 Time series data for (a) blood glucose, (b) plasma corticosterone and (c) haematocrit of male (open circles) and female (filled circles) small ground finches (*Geospiza fuliginosa*) sampled within 5.6 min after capture and again within 41.3 min after capture. No significant differences existed between sexes. Combining both sexes, restricted maximum likelihood (REML) mixed models were formulated and are represented as black regression lines (details in Table 2). Body condition was significant in the model for haematocrit, so regressions are presented to depict the individual with the lowest (-1.515; upper line) and highest (2.210; lower line) condition in this study (condition calculated as residuals of body mass over tarsus length;

both sexes combined). Mean baseline values (measured within 5.6 min of capture; see text) are indicated with grey horizontal lines for visual reference.

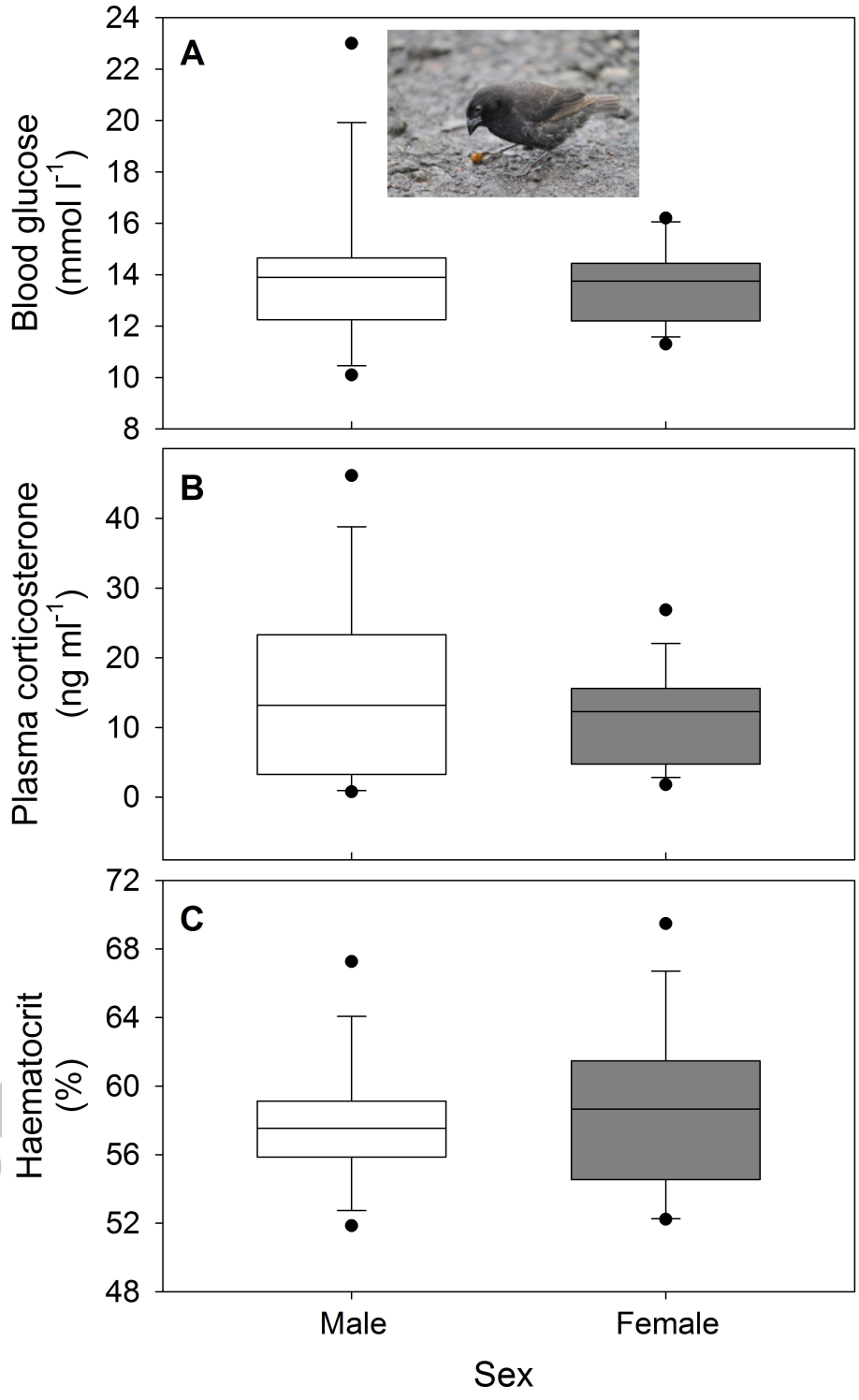

Fig. 1

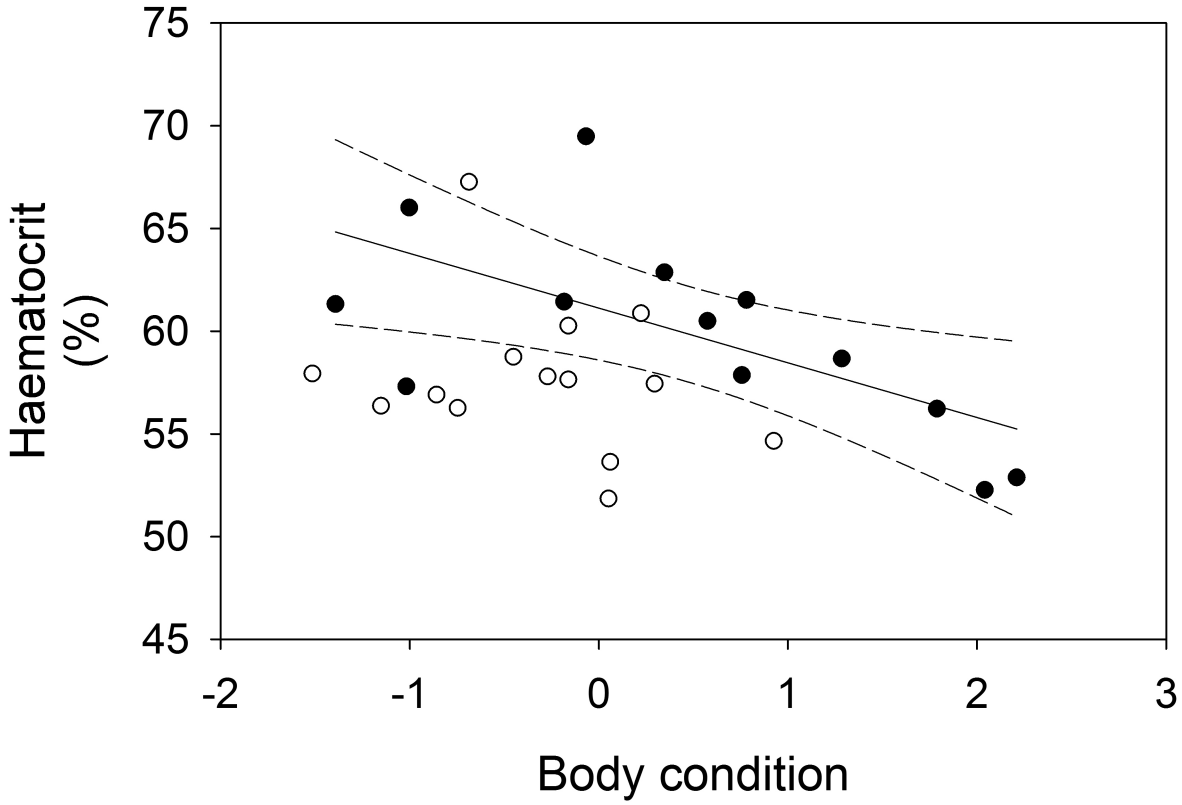

Fig. 2

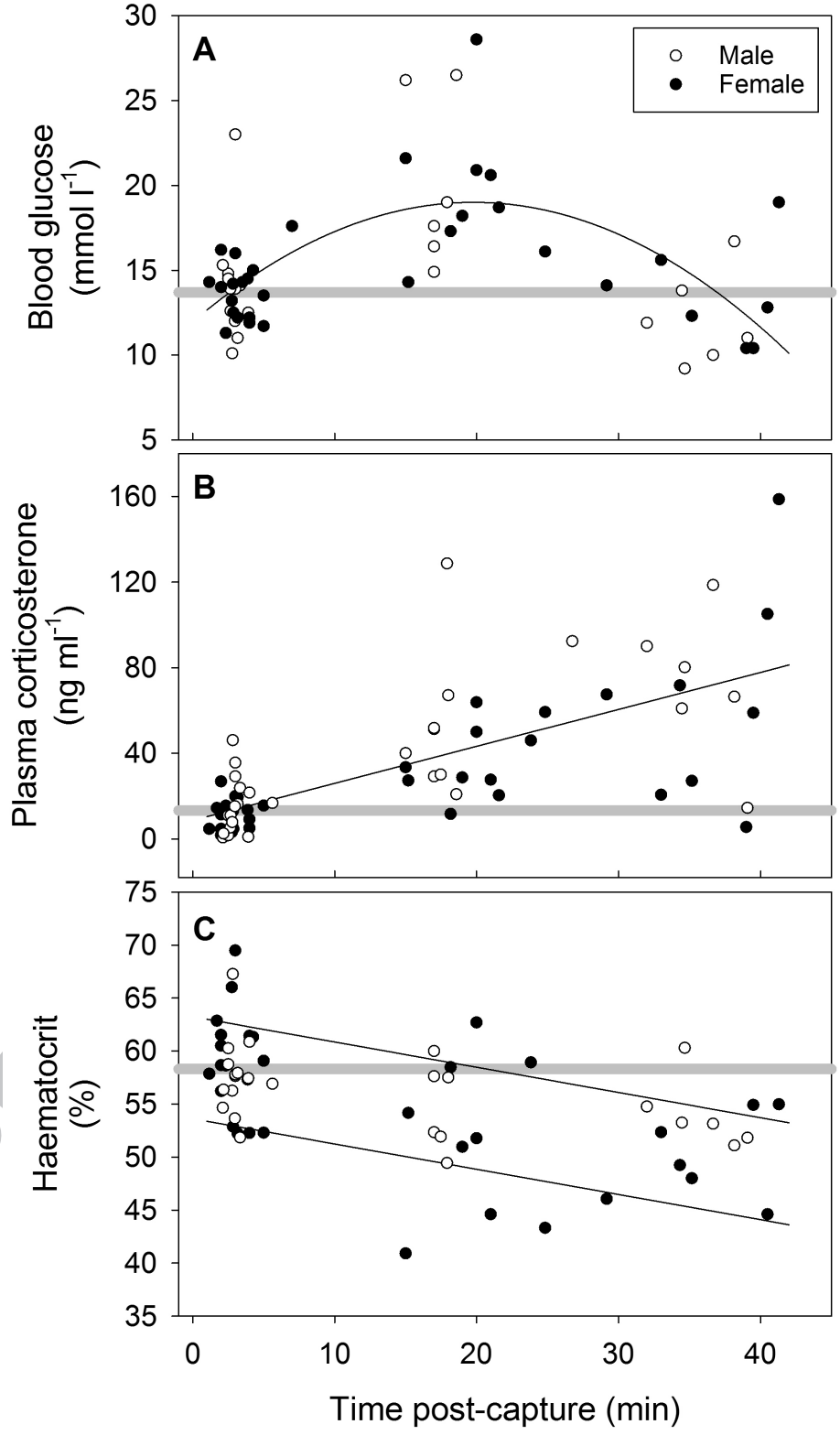

Fig. 3

- Sexes of Darwin's finches have similar blood corticosterone, glucose and haematocrit
- Female finches with high body condition have lower haematocrit
- Corticosterone increases after capture and holding, while haematocrit decreases
- Sex differences in behaviour are independent of the measured blood parameters