

**Seasonal stress physiology and body condition differ among co-occurring tropical finch
species**

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Abstract

Seasonal changes in avian hormonal stress responses and condition are well known for common species found at temperate and arctic latitudes, but declining and tropical species are poorly studied. This study compares stress and condition measures of co-occurring declining and non-declining tropical grass-finch species in Australia. We monitored declining Gouldian finches (*Erythrura gouldiae*) and non-declining long-tailed and masked finches (*Poepila acuticauda* and *P. personata*) during two seasons that are potentially stressful: peak breeding (early dry season when food is plentiful) and moult (late dry to early wet season when food may be scarce). We measured body condition (muscle and fat), haematocrit, and stress response to capture using plasma corticosterone and binding globulin concentrations. All species had higher muscle and lower fat indices during breeding than moult. Haematocrit did not consistently differ between seasons. Long-tailed finches had higher stress responses during breeding than moult, similar to other passerines studied. Masked finches showed no seasonal changes in stress response. Gouldian finches had stress response patterns opposite to those of long-tailed finches, with higher stress responses during moult. However, seasonal trends in Gouldian and long-tailed finch stress responses sometimes differed between years or sites. The differences in stress response patterns between species suggest that the declining Gouldian finch is more sensitive to recent environmental changes which are thought to further reduce grass seed food resources during the late dry to early wet season. Retention of stress responsiveness during a protracted moult could increase the survival potential of Gouldian finches. This study highlights the utility of stress and condition indices to determine the sensitivity of co-occurring species to environmental conditions.

Key Words: Body condition, Corticosterone, Gouldian Finch, haematocrit, moult, muscle

Introduction

Studies of variation in avian stress responses and body condition have predominantly described patterns in migratory species in the northern hemisphere (Astheimer et al. 1994; Wingfield et al. 1995; Romero 2002). These birds have highly seasonal lifestyles often characterized by short breeding seasons in resource-rich environments, long non-breeding seasons, and intervening migratory periods (Murton and Westwood 1977). The lifestyles of tropical birds are markedly different, usually characterised by longer breeding seasons, more opportunistic breeding attempts, longer periods of high resource availability, and different moult schedules (Stutchbury and Morton 2001).

In some northern hemisphere birds, baseline and stress-induced plasma concentrations of the key avian stress hormone, corticosterone (CORT), increase during the peak of the breeding season, sometimes accompanied by lower body condition measures than those recorded in non-breeding seasons (Wingfield et al. 1994; Kitaysky et al. 1999; Breuner and Orchinik 2002). Concentrations of circulating corticosterone binding globulin (CBGs), which are thought to limit the availability of plasma stress hormone to tissues by sequestering CORT in plasma, are also higher in breeding birds (Malisch and Breuner 2010). It is hypothesized that only the “free” unbound CORT has biological activity, so increasing CBG concentrations essentially avoids high concentrations of active CORT (Malisch and Breuner 2010). Such containment may facilitate successful breeding, as continually elevated CORT concentrations may interrupt breeding behavior (Wingfield et al. 1983; Silverin 1986; Wingfield and Silverin 1986; Gray et al. 1990; Cyr and Romero 2006; Cyr et al. 2007). CBGs may also help to control CORT concentrations within tissues or movement across cell membranes (Malisch and Breuner 2010). This type of action may be more useful during breeding because the reservoir of CORT bound to CBGs is larger; however, the reason for this is still a matter of conjecture, and the biological significance of ‘free vs bound’ CORT is still under debate (Malisch and Breuner 2010; Breuner et al. 2013).

The typical avian response to a single acute stressor, regardless of season, is characterized by a rapid increase in CORT concentrations (within 1-3 min) (Dawson and Howe 1983), which overwhelm CBG capacity and result in increased circulating free CORT (Fleshner et al. 1995; Lynn et al. 2003; Breuner et al. 2006). Individuals with poor body condition that are exposed to acute stressors often have higher baseline or stress-induced CORT concentrations than birds in good condition. This is particularly apparent among birds exposed to high energetic demands, inclement weather, or food limitations (Rees et al. 1985; Astheimer et al. 1995; Marra and Holberton 1998; Kitaysky et al. 1999; Jenni et al. 2000). Birds exposed to long-term stress (over several days or longer) had reduced baseline and stress-induced CORT concentrations, suggesting that chronically stressed birds suppress the stress response, possibly to avoid corollary effects on breeding and other behaviors (Cyr and Romero 2006).

The pattern of CORT release immediately following stress exposure has been examined in more than 66 species over various stages of their annual cycle, although very few of these were tropical southern hemisphere birds (Astheimer and Buttemer 2002; Malisch and Breuner 2010). Studies contrasting the stress responses of arctic versus temperate breeding bird populations found that species with shorter breeding seasons were more likely to suppress the stress response by reducing CORT secretion (Wingfield 1994; Breuner et al. 2003). The few tropical birds studied showed increases in CORT and CBG capacity during breeding, but sometimes had lower baseline and stress-induced CORT than temperate conspecifics or congeners (Martin et al. 2005; Wada et al. 2006b). However, the focal species in these studies are common birds with relatively stable or increasing population sizes, and may not represent the norm for tropical species or species that are declining in abundance due to environmental stressors.

Seasonality in body condition and haematological state may also differ between birds found at different latitudes. Some tropical birds show less annual variation in body fat concentrations, or even opposite trends in mass variation compared with temperate zone conspecifics (King and

Farner 1966; Blem 1976; Rogers et al. 1993; Breuer et al. 1995). Haematocrit of temperate bird species varies seasonally: lower concentrations are typically found late in the breeding season or during moult, whereas higher values occur in the winter, early breeding season, and during migration, all times when energy requirements are expected to be elevated (Morton 1994; Box et al. 2002; Gayathri and Hegde 2006; Owen and Moore 2006). More research is needed to fully describe this variation, as few studies have looked at possible seasonal differences in haematocrit in tropical species (Fair et al. 2007).

In this study, we characterised the hormonal stress response, body condition, and haematocrit in three closely related and sympatric tropical grass-finches. We compared these indices between one declining finch species, the Gouldian finch (*Erythrura gouldiae*) and two non-declining species, the long-tailed (*Poephila acuticauda*) and masked finches (*P. personata*). To help identify when tropical finches are most stressed, we measured stress and condition indices during two periods of the year that have different environmental and life history pressures.

Methods

Study species

Of the 15 species of grass-finch that inhabit the savannas of northern Australia, four species have suffered large range contractions and decreased abundance within the past 50 years (Franklin 1999). Grazing by introduced herbivores and more frequent, extensive, and intense fires are likely to have decreased the suitability of savanna habitat for grass seed eaters by reducing grass productivity (Franklin et al. 2005).

The declining Gouldian finch has a relatively short life span compared to other Australian finches (usually less than two years), and a long breeding season that lasts up to 8 months (Woinarski and

Tidemann 1992; Tidemann and Woinarski 1994). They have a short and synchronous moult lasting about one month during the late dry to early wet season between October and December (Tidemann and Woinarski 1994). Their seed diet is based almost exclusively on a small number of preferred grass species (Dostine and Franklin 2002). This species is highly mobile with individuals often flying over 17 km a day to visit selected food, water, and breeding sites (S. Legge, unpublished data). This mobile and highly energetic lifestyle, coupled with a narrow diet may make the species vulnerable to environmental changes that result in resources becoming less abundant or more widely dispersed.

Both long-tailed and masked finches have a relatively longer lifespan, more opportunistic moult schedules, lower annual fecundity, and more diverse diets than Gouldian finches (Tidemann and Woinarski 1994; Dostine and Franklin 2002). Recapture data suggest that these species live 50% longer and are less nomadic than the Gouldian finch (K. Maute unpublished data, S. Legge, unpublished data). A smaller proportion of long-tailed and masked finches breed later in the year than Gouldian finches (Tidemann and Woinarski 1994; van Rooij and Griffith 2011). Their diets include a wider variety of grass seed species and are regularly supplemented with other types of plant seeds, insects, lerps, and nectar (Garnett and Crowley 1994; Dostine and Franklin 2002). Moult timing in long-tailed and masked finches is irregular and appears to be extended, with individuals having been recorded in moult during all months of the year (Tidemann and Woinarski 1994). The opportunistic feeding, breeding, and moult strategies of these two species may make them less vulnerable to the environmental effects of increased grazing pressure and fire frequency and extent in northern Australia over the past century (Ash et al. 1997; Russell-Smith et al. 2003).

Study sites and survey seasons

Three study sites were selected where all three study species co-occurred. Each site was sampled twice a year over 2 years. All three properties were in the Northern Territory, Australia: Bradshaw

Field Training Area (latitude $-15^{\circ} 30' 0''\text{S}$, longitude $130^{\circ} 24' 0''\text{E}$, area approximately 4050 km^2), a Department of Defence property; Delamere Station (latitude $-15^{\circ} 41' 59''\text{S}$, longitude $131^{\circ} 35' 59''\text{E}$, area approximately 4000 km^2), a pastoral property; and Yinberrie Hills (latitude $-14^{\circ} 5' 59''\text{S}$, longitude $132^{\circ} 0' 0''\text{E}$, area approximately 200 km^2) aboriginal land. The habitat on all properties was characterized by a predominately grassy understorey and a low density of trees and shrubs.

Populations of all three finch species were located within one area of $5 - 10 \text{ km}^2$ on each property in each sampling trip. The search areas were relatively large due to the mobile behavior of these species. Individuals from each population were captured, measured, and released during the late dry to early wet season (September – early December) when birds are not breeding but most are moulting, and when grass seed availability is low. During this season, spring rains cause annual grass seeds to sprout and finches must switch to feeding on emerging perennial grass seed resources (Dostine and Franklin 2002). Finch populations were also sampled during the early dry season (May-June) which is the late breeding season, and a time when preferred annual grass seeds resources are at their most plentiful (Dostine and Franklin 2002). All properties were visited during the moulting (late dry to early wet) season during 2007 and 2008, and in the breeding (early dry) season in 2008 and 2009. During each visit, 30 - 70 individuals of each species were captured to ensure a range of ages and an equal number of males and females. In total, 453 Gouldian finches, 446 long-tailed finches, and 363 masked finches were included in these analyses. All birds included in analysis were naïve to the capture process.

Finch capture and handling

Birds were captured during fine weather within 4 h after sunrise using mist nets. The length of time between capture (when the bird hit the mist net) and blood sampling was measured to the nearest min to account for the effect of capture stress on CORT. Samples were taken between 5 and 60 min after capture. Blood samples were collected with heparinized microheamatocrit tubes following

venipuncture of the left brachial vein with a 26 - gauge needle. Most adults of all species showed evidence of reproductive status during breeding season based on observations of brood patches in both sexes. Birds were aged as either juvenile (hatching year) or adult (after hatching year). We determined finch age using the presence of juvenile plumage or bill gape. Adult Gouldian finches were sexed by plumage; however, the other two species are not reliably sexed by physical characteristics. Therefore, following Griffiths et al. (1998), a subset of adult long-tailed (149) and masked finches (102) sampled during the first year of the study were sexed using molecular methods from whole blood. Time constraints did not allow for all individuals to be sexed, thus sample sizes for analyses of the effects of sex on stress and condition indices are smaller than the total number of birds captured.

Measurement of finch body and haematological condition

Body condition was measured using two indices. Muscle score was estimated based on the shape and relative volume of the pectoral muscles using a scale of 0 - 3 (Brown 1996; Lindstrom et al. 2000). Fat score was visually estimated from the volume of fat stored in each bird's furculum using a scale of 0 - 5 (Helms and Drury 1960). Finches rarely have observable levels of abdominal fat, therefore our fat scores were based solely on furcular fat. Mass was measured (± 0.5 g) using a Pesola scale. However, mass corrected for body size did not show different seasonal trends from muscle or fat scores, and was confounded by the increased reproductive tissue of egg producing females, and is therefore not shown in this manuscript (see online supplementary content). After microhaematocrit tubes holding whole blood were spun for 7 min at a force of 16 060 G in a Hettich Haematokrit 210 centrifuge, we measured both the length of the tube containing red blood cells and the total length of the tube containing cells and plasma. Haematocrit was calculated using the percentage of the tube length filled with cells and plasma that contained only red blood cells. Haematocrit tubes were kept cool in the field using ice in well insulated transport containers and

Engel brand fridge/freezers. Plasma was drawn out of the tube with a fixed-tip syringe and transferred to a plastic cryovial and frozen at -80°C within 6 h of collection.

Measurement of stress indices from plasma

Total CORT and CBG capacity were determined from plasma. Due to the difficulty of safely bleeding small finches, not all birds had blood samples with a sufficient volume of plasma to test for CORT and CBG. Thus 287 Gouldian, 275 long-tailed, and 224 masked finches were tested for CORT and 212 Gouldian and 136 long-tailed finches were also tested for CBG capacity. The birds tested for CBG capacity represented most Gouldian and long-tailed finches sampled within 5-60 min after capture and those individuals with a sufficient amount of plasma available to test for both CORT and CBG. Masked finch CBG capacity was not measured. Plasma CORT was measured from 5 μL of whole plasma from each bird with an EIA Kit ACETM Competitive Enzyme Immunoassay for corticosterone (Cayman Chemical Co. Ann Arbor, Michigan, USA). Two standard CORT solutions (one high concentration and one low) were tested along with finch plasma on all plates (intra- and an inter-assay coefficient of variation $< 5\%$).

Because each finch in this study could only be bled once, we created a relative measure of CORT response to handling stress over time by performing a linear regression using all CORT results from all seasons against handling time (the time measured between capture in a mist net and blood sampling) for each species (Fig 1). The regression of CORT to handling time for each species did not differ between sampling periods (interaction between CORT, time, and sample period for Gouldian $F_{3, 244} = 0.92$, $P = 0.43$, long-tailed $F_{3, 223} = 0.39$, $P = 0.76$, and masked finches $F_{3, 188} = 1.72$, $P = 0.17$), thus we pooled samples for all seasons and years when setting up the regression between CORT and handling time. Regressions were significant for each species (Gouldian $r^2 = 0.04$ $F_{1, 250} = 10.27$, $P < 0.001$; long-tailed $r^2 = 0.15$ $F_{1, 229} = 41.05$, $P < 0.0001$; masked $r^2 = 0.10$ $F_{1, 194} = 22.55$, $P < 0.0001$). We used the residual values from these regressions as a measure of stress

responses (relative CORT release in response to time since capture). Residual values for each species (residual CORT) represent the release of CORT corrected for handling time and may be either positive or negative. Positive values represent individuals which released more than the mean level of CORT over time, while negative results represent individuals who either suppressed CORT release, returned to baseline within 60 min or simply released less than the mean over time. Residual CORT concentrations were then used in further analyses to compare ages, sexes, seasons, and years.

Gouldian and long-tailed finch plasma CBG capacity was measured using a radioligand-binding assay with tritiated corticosterone (Lynn et al. 2003). Plasma from all finch species was diluted 1:200 and all samples were analysed in triplicate. Pooled plasma from captive Japanese quail (*Coturnix coturnix japonica*) served as a reference standard for all assays of CBG capacity and was tested along with finch plasma samples. This revealed average intra-assay variability to be < 5% and inter-assay variability to be < 10%.

Statistical analysis

All measurements (muscle score, fat score, haematocrit, and stress indices) were normally distributed. Three-factor ANOVA models were used to compare stress and condition indices with year (first year = moult 2007 and breeding 2008, and second year = moult 2008 and breeding 2009), season (breeding and moult), and site (Bradshaw, Delamere and Yinberrie) (SAS institute JMP5.1 software). Separate two-factor ANOVAs were used to examine variation in stress and condition indices between age (adult and juvenile) or sex for each finch species, and whether these differed between seasons. Tukey – Kramer HSD tests (T.K. HSD) were used to determine the direction of significant differences. Correlations between stress and condition indices were analysed separately for each species using correlation analysis (SAS institute JMP 5.1 software).

Results

Residual CORT concentrations and CBG capacity

Gouldian finch

Residual CORT concentrations were higher during moult than breeding seasons (Table 1, Fig. 2).

This effect was dependent on year and site, with all sites having higher residual CORT during moult except Yinberrie in year one (Table 1). Mean CBG capacity was higher during breeding seasons than moult during both years, but different between years (Table 1). While CBG capacity was different between sites, seasonal changes in CBG concentrations were consistent across sites (Table 1).

Gouldian finch juveniles had similar residual CORT to adults during moult (T.K. HSD, $P = 0.11$), but lower concentrations in the breeding season (T.K. HSD, $P = 0.006$, interaction between age and season $F_{1, 247} = 8.23$, $P = 0.005$, Table 2). Recently fledged juveniles had lower CBG concentrations than adults during the breeding season (T.K. HSD, $P = 0.006$), but similar CBG concentrations to adults during moult when they are older (T.K. HSD, $P = 0.38$, interaction between age and season $F_{1, 205} = 5.50$, $P = 0.02$, Table 2). Female Gouldian finches had similar residual CORT ($F_{1, 181} = 1.57$, $P = 0.21$), and CBG capacity to males ($F_{1, 188} = 0.36$, $P = 0.55$).

Long-tailed finch

Unlike Gouldian finches, long-tailed finches had significantly higher residual CORT during both breeding seasons than moult in 2008 (Table 1, Fig. 2). During moult in 2007, birds had high residual CORT concentrations at the Yinberrie site only, which inflated the overall CORT value collapsed across sites and therefore masked the consistently lower residual CORT concentrations

during moult than breeding at other sites (Table 1, Fig. 2). CBG capacity did not vary by season (Table 1).

Juvenile long-tailed finches had lower residual CORT than adults ($F_{1, 275} = 5.77$, $P = 0.02$, Table 2), but CBG capacity was similar to adults ($F_{1, 134} = 0.72$, $P = 0.40$). There was no significant difference in residual CORT between the sexes ($F_{1, 141} = 0.00001$, $P = 0.998$). Female finches had lower CBG capacity than males during breeding in 2008 (T.K. HSD, $P = 0.04$). Females had similar CBG capacity to males during other seasons ($F_{1, 39} = 2.02$, $P = 0.16$).

Masked finch

In masked finches, residual CORT concentrations were stable and moderate across all sampling periods and sites (Table 1, Fig. 2). Juvenile masked finches had similar CORT to adults during the two seasons measured (moult 2008, breeding 2009; $F_{1, 222} = 0.27$, $P = 0.60$), however, because we caught relatively few juveniles in both years ($n < 10$), assigning meaning to this analysis is problematic. Masked finches had similar CORT concentrations between the sexes ($F_{1, 94} = 2.51$, $P = 0.12$).

Body condition

Gouldian finch

Muscle scores were higher during breeding seasons for all Gouldian finches (Table 3, Fig. 3). All finches at Bradshaw and Delamere had higher fat scores during moult than breeding in the first year of the study, but trends were otherwise inconsistent (Table 3). Juvenile Gouldian finches had similar muscle scores to adults ($F_{1, 448} = 3.75$, $P = 0.05$, Table 2). ANOVA results suggested that muscle scores differed between sexes, depending on season (interaction: $F_{1, 309} = 4.47$, $P = 0.04$). However,

post hoc tests could not distinguish where the differences lay (Breeding T.K. HSD, $P = 0.19$; Moulting T.K. HSD, $P = 0.09$). Fat scores showed no significant differences between the ages or sexes (age $F_{1, 446} = 3.17$, $P = 0.08$; sex $F_{1, 311} = 0.05$, $P = 0.82$).

Long-tailed finch

Muscle scores were significantly higher during breeding than moulting during the second year of the study at all sites (Table 3, Fig. 3). Fat scores were much higher during moulting than breeding during the first year of the study at Bradshaw and Delamere, but trends were otherwise inconsistent (Table 3). Juvenile long-tailed finches had lower muscle scores than adults ($F_{1, 443} = 17.45$, $P < 0.0001$, Table 2). Fat scores were similar between ages ($F_{1, 443} = 0.71$, $P = 0.40$). Muscle was similar between sexes ($F_{1, 146} = 1.10$, $P = 0.30$). The effect of sex on fat scores was dependent on season ($F_{1, 144} = 5.66$, $P = 0.02$), with fat scores higher in breeding females than males (female = 1.46 ± 0.11 , male = 0.96 ± 0.10 , T.K. HSD, $P = 0.002$) and similar between the sexes during moulting (female = 1.60 ± 0.11 , male = 1.61 ± 0.09 , T.K. HSD, $P = 0.93$).

Masked finch

Masked finch muscle scores were higher during breeding than moulting (Table 3, Fig. 3). Fat scores were higher during moulting than breeding during the first year but were low during both seasons in the second year (Table 3, Fig. 3). Juvenile masked finches had lower muscle scores than adults ($F_{1, 357} = 22.26$, $P < 0.0001$ Table 2). Fat scores were similar between ages ($F_{1, 357} = 3.37$, $P = 0.07$). Muscle or fat scores were similar between sexes (muscle $F_{1, 100} = 0.21$, $P = 0.65$, fat $F_{1, 100} = 0.73$, $P = 0.39$).

Haematocrit

Gouldian finch

Seasonal differences in haematocrit depended on site (see online supplementary content), with higher haematocrit during breeding at one site only. Gouldian finch adults had higher haematocrit than juvenile birds ($F_{1, 413} = 14.36$, $P = 0.0002$, Table 1). Females had similar haematocrit to males ($F_{1, 292} = 0.004$, $P = 0.95$).

Long-tailed finch

Seasonal changes in long-tailed finch haematocrit differed among sites and years (see online supplementary content), with no consistent trends found. Haematocrit did not differ by age ($F_{1, 423} = 0.64$, $P = 0.42$) nor by sex ($F_{1, 146} = 0.58$, $P = 0.45$).

Masked finch

Haematocrit differed between sites and years (see online supplementary content). However, there was no effect of season (see online content), age ($F_{1, 348} = 2.48$, $P = 0.11$) or sex ($F_{1, 100} = 0.52$, $P = 0.47$).

Correlations between stress and condition indices

Gouldian muscle and fat were positively correlated (Table 4). Muscle and residual CORT were negatively correlated, while residual CORT and haematocrit were positively correlated (Table 4). CBG capacity was not correlated with any indices ($P > 0.05$). Fat was negatively correlated with haematocrit (Table 4). Other Gouldian finch indices were not correlated.

Long-tailed and masked finch muscle and fat scores were positively correlated (Table 4). Though long-tailed finch residual CORT was correlated with fat and haematocrit measures, there was no other correspondence between stress, haematological state, and body condition in these two species.

Discussion

Stress response variation in declining tropical versus common temperate northern hemisphere birds

Results from this study suggest that Gouldian finches have seasonal changes in their stress response that are unique in two ways. Firstly, they differ from seasonal patterns described for other passerines, and secondly, they differ from the patterns of sympatric and closely related, but non-declining finch species.

While many small passerines have their highest stress-induced CORT concentrations during breeding (Romero 2002; Wada et al. 2006b; Malisch and Breuner 2010), Gouldian finches did not follow this pattern. Breeding Gouldian finches sampled during the early dry season had consistently lower residual CORT and higher CBG capacity than non-breeding and moulting individuals sampled during the late dry to early wet season. This combination of CORT and CBG concentrations should translate into lower concentrations of free CORT during breeding (less CORT would be available, being bound to CBGs) and comparatively higher free CORT during moult. The actual calculation of free CORT would involve the determination of CBG affinity for CORT, which can differ by species, season, year and site but was not measured in this study as the biological significance of the relationship is still a point of disagreement (Malisch and Breuner 2010).

Thus during the late breeding season, Gouldian finches had lower stress-induced CORT concentrations because of their lowered CORT release as well as higher CBG concentrations. This could either help to promote the maintenance of breeding behavior later in the season, be a product of lowered stress due to abundant food and good condition, or alternatively, attenuation of the stress response, a symptom of chronic stress during breeding. However, based on current knowledge, chronically stressed birds tend to reduce both their CBG capacity and CORT response (Fleshner et al. 1995; Rich and Romero 2005; Cyr et al. 2007). Thus these breeding Gouldian finches do not fit the profile of chronically stressed birds. Their pattern better fits the strategy used by short-lived birds and those with short breeding seasons in highly productive environments, which are thought to minimize stress responses during nesting because stress-induced interruptions to breeding would severely reduce their lifetime breeding potential (Breuner et al. 2003). The high abundance of grass seed food resources during the Gouldian finch late breeding season (Lewis 2007) is consistent with this hypothesis, as minimized stress responses when food is abundant would be less likely to cause reduced survival due to a reduction in stress hormones that promote feeding behavior.

The increased CORT concentrations of Gouldian finches during moult could be a response to lowered resource availability during the early wet season combined with the energetic challenge of moult. Studies of birds undergoing moult in the northern hemisphere show that most species have lower baseline CORT and stress responses during moult, opposite to our Gouldian finch findings (Astheimer et al. 1995; Romero and Wingfield 1999; Romero and Ramage-Healey 2000; Rich and Romero 2001). Species that undergo an extended molt often retain stress responsiveness (Cornelius et al. 2012); however Gouldian finches undergo a full moult in a matter of weeks, not months (Franklin et al. 1998). Naturally and experimentally fasted birds show high stress responses coupled with lowered CBG capacity, similar to the pattern we have seen in moulting Gouldian finches (Cherel et al. 1988; Astheimer et al. 1995; Vleck et al. 2000; Lynn et al. 2003). Thus the pattern of stress in moulting Gouldian finches may be caused by a poor diet. This hypothesis is consistent with

the lowered availability of grass seed food resources during the late dry/early wet season when Gouldian finches are moulting (Lewis 2007).

Stress response in declining versus non-declining tropical finches

Mean Gouldian finch CBG capacity was lower than that of the common long-tailed finch, suggesting that Gouldian finches retain a smaller reservoir of CORT bound to CBGs with greater variation in free CORT concentrations than long-tailed finches. Long-tailed finches tended to have higher stress responses and CBG capacity during breeding, opposite to Gouldian finches but similar to the pattern reported for the few other passerines for which these data are known (Romero 2002; Wada et al. 2006b; Malisch and Breuner 2010). This suggests that long-tailed finches may maintain low free CORT concentrations all year, but a larger CORT reservoir during breeding than moult. Higher potential CORT concentrations during breeding may promote behaviors to ensure adult survival behavior over breeding during stressful events (Wingfield et al. 1983; Wingfield and Silverin 1986). This hypothesis is consistent with the opportunistic breeding and relatively longer life-span of long-tailed finches, and may also help explain the opposite pattern in Gouldian finches, which tend to have longer uninterrupted breeding seasons and shorter life-spans (Tidemann and Woinarski 1994). Alternatively, long-tailed finches may have an attenuated stress response during moult due to chronic stress or some unknown factor. Studies of wild and captive birds have shown that many species have lower stress responses during moult than other life stages, however, the possible causes of this difference are unknown (Astheimer et al. 1995; Romero and Wingfield 1999; Romero and Ramage-Healey 2000; Rich and Romero 2001).

In the first year of the study, long-tailed finches showed increased stress responses during moult at one site while Gouldian finches did not show increased stress responses during moult across all sites. This suggests that high stress responsiveness during moult may not be a trait specific to

Gouldian finches, and highlights the possibility that habitat condition is likely to be an important factor in determining stress responses of individuals or populations.

Though CBG capacity was not measured in masked finches, the seasonal patterns in stress-induced CORT concentrations highlight a response that is intermediate to those Gouldian and long-tailed finches. Similar to both other species, masked finches had high concentrations of total CORT during moult in 2007. However, masked finches do not have the fluctuating high and low concentrations seen in the other finch species between 2008 and 2009. Further analyses of masked finch stress responses and CBG capacity are needed to determine if any patterns exist.

Seasonal body condition variation

All finches showed seasonal variation in body condition. In particular, birds had higher muscle scores during the breeding season, possibly as a result of improved food resources at that time of year. Though finches rarely carried much fat, all species had significantly higher fat scores during moult in 2007. Since seed resources dwindle by the end of the late dry to early wet season when finches are moulting (Woinarski and Tidemann 1992; Dostine and Franklin 2002), finches may store fat to compensate for periods of food scarcity. It is interesting to note that maximum CORT concentrations for finches were recorded in the same season as maximum fat scores (moult 2007). However, long-tailed finch residual CORT concentrations showed a negative correlation with fat scores, suggesting that long-tailed finches with lower fat stores have increased stress responses. It is possible that poor resource availability during the late dry seasons encourages birds to trade flight agility for increased fat stores as a safeguard against starvation, similar to many other bird species exposed to unpredictability in food access (Ekman and Hake 1990; Hurly 1992; Bednekoff and Krebs 1995; Witter et al. 1995; Smith and Metcalfe 1997; Cuthill et al. 2000). Captive studies have shown that increases in body mass of less than 7% can lower the speed and agility of Zebra finches (*Taeniopygia guttata*) by as much as 30% (Metcalfe and Ure 1995). Compared to 2008, Gouldian

finches displayed 7% higher mean body mass during their moult in 2007, when elevated fat scores and stress responses were also recorded. Telemetry and observational monitoring of Gouldian and long-tailed finch movements suggests they move in response to the seasonal patchiness of their preferred grass-seeds (Dostine et al. 2001; Dostine and Franklin 2002). If finches must travel further and forage longer in order to find and feed on the dwindling sources of their preferred grass seeds during the late dry to early wet season, fat storage may be advantageous by ensuring survival and enough energy resources to moult, despite the cost of lowered agility.

Correlations between stress and condition indices

Compared to the non-declining finches, Gouldian finch stress responses, haematocrit, and condition measures were often correlated. Though weak, the fact that a correlation exists between stress, haematocrit, and condition is evidence that some individuals of this species had low nutrient stores and high energy demands. For example, Gouldian finches with lower muscle measures had higher residual CORT concentrations. This equates to higher plasma CORT in birds with lower condition measures, a state commonly observed in underweight migrating birds (Schwabl et al. 1991; Holberton 1999; Jenni et al. 2000). Small increases in CORT are likely to drive these individuals to forage more and expend more energy, and this could explain their relatively high haematocrit concentrations (mean > 60). High haematocrit concentrations in birds are often attributed to extreme dehydration or increases in energy demands, such as cold stress and increased activity during breeding or migration (Carpenter 1975; Breuer et al. 1995; Horak et al. 1998; Box et al. 2002; Fair et al. 2007). However, all finch species showed no significant seasonal differences in haematocrit, and without obvious changes in haematocrit above or below mean concentrations, interpretation of the reason for relatively high haematocrit values is not possible (Fair et al. 2007).

Variation in stress and condition indices due to age and sex

All finch species displayed some age and sex differences in residual CORT concentrations, body condition and haematocrit, however, these differences were not consistent among species, seasons, and years suggesting that both environmental conditions and individual traits interact to determine individual condition. Juvenile finches occasionally had significantly lower residual CORT concentrations, body condition, and haematocrit concentrations than adults. Studies of other species also show an increase in stress responsiveness with age (Sims and Holberton 2000; Ouillfeldt et al. 2004; Wada et al. 2006a). Stress and condition measures did not vary consistently between the sexes in most finches, though female long-tailed finches had higher fat scores and lower CBG capacity than males during breeding.

Conclusion

The difference in the seasonal stress response patterns of the declining Gouldian finch and common long-tailed and masked finches in this study highlight the usefulness of dynamic stress measures in determining species' interactions with seasonal environments. Combined with parallel changes in body condition, this information also helps narrow down a critical time of year for survival in an endangered species. The results of this study suggest that for Gouldian finches, the transition of the late dry season to the wet season (when food is scarce and birds are moulting) is more physiologically stressful than the early dry (when food is plentiful and birds are breeding). However, similar research in the Kimberley, Western Australia is revealing that the early wet season (when birds are just beginning to breed) is also physiologically stressful for the Gouldian finch (S. Legge, unpublished data). Conservation-based monitoring studies for this endangered species should focus research effort during these seasons in order to better discern the main causes of finch stress.

Comparison of stress responses of tropical finches with northern hemisphere birds is difficult due to the great variety of life histories and environmental conditions experienced by these groups. Further

comparisons within tropical species may be more informative, especially among co-occurring and closely related species.

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References

- Ash A, Mcivor J, Mott J, Andrew M (1997) Building grass castles: Integrating ecology and management of Australia's tropical tall-grass rangelands. *Rangeland J* 19:123-144
- Astheimer LB, Buttemer WA (2002) Changes in latitude, changes in attitude: a perspective on ecophysiological studies of Australian birds. *Emu* 102:19-27
- Astheimer LB, Buttemer WA, Wingfield JC (1994) Gender and seasonal differences in adrenal response to ACTH challenge in an arctic passerine, *Zonotrichia leucophrys gambelii*. *Gen Comp Endocrinol* 94:33-43
- Astheimer LB, Buttemer WA, Wingfield JC (1995) Seasonal and acute changes in adrenocortical responsiveness in an arctic-breeding bird. *Horm Behav* 29:442-457

- Bednekoff PA, Krebs JR (1995) Great tit fat reserves: effects of changing and unpredictable feeding day length. *Funct Ecol* 9:457-462
- Blem CR (1976) Patterns of lipid storage and utilization in birds. *Am Zool* 16:671-684
- Box J, Lill A, Baldwin J (2002) Seasonal variation in body mass and blood oxygen carrying capacity of the superb fairy-wren (*Malurus cyaneus*) *Aust J Zool* 50:313-323
- Breuer K, Lill A, Baldwin J (1995) Hematological and body-mass changes of small passerines overwintering in south-eastern Australia. *Aust J Zool* 43:31-38
- Breuner CW, Delehanty B, Boonstra R (2013) Evaluating stress in natural populations of vertebrates: total CORT is not good enough. *Funct Ecol* 27:24-36
- Breuner CW, Lynn SE, Julian GE, Cornelius JM, Heidinger BJ, Love OP, Sprague RS, Wada H, Whitman BA (2006) Plasma-binding globulins and acute stress response. *Horm Metab Res* 38:260-268
- Breuner CW, Orchinik M (2002) Downstream from corticosterone: seasonality of binding globulins, receptors and behavior in the avian stress response. In: Dawson A (ed) *Avian Endocrinology*. Narosa Publishing, New Delhi and London, pp 385-399
- Breuner CW, Orchinik M, Hahn TP, Meddle SL, Moore IT, Owen-Ashley NT, Sperry TS, Wingfield JC (2003) Differential mechanisms for regulation of the stress response across latitudinal gradients. *Am J Physiol - Reg I* 285:R594-R600
- Brown ME (1996) Assessing body condition in birds. In: Nolan Jr. V, Ketterson ED (eds) *Current Ornithology*, vol 13. Plenum Press, New York, pp 67-135
- Carpenter FL (1975) Bird hematocrits: effects of high altitude and strength of flight. *Comp Biochem Physiol* 50A:415-417
- Cherel Y, Leloup J, LeMaho Y (1988) Fasting in the King Penguin. II. Hormonal and metabolic changes during molt. *Am J Physiol* 23:R178-R184
- Cornelius JM, Perfito N, Zann R, Breuner CW, Hahn TP (2012) Physiological trade-offs in self-maintenance: plumage, molt and stress physiology in birds. *J Exp Biol* 214:2768-2777
- Cuthill IC, Maddocks SA, Weall CV, Jones EKM (2000) Body mass regulation in response to changes in feeding predictability and overnight energy expenditure. *Behav Ecol* 11:189-195
- Cyr NE, Earle K, Tam C, Romero LM (2007) The effect of chronic psychological stress on corticosterone, plasma metabolites, and immune responsiveness in European Starlings *Gen Comp Endocrinol* 154:59-66
- Cyr NE, Romero LM (2006) Chronic stress in free-living European Starlings reduces corticosterone concentrations and reproductive success. *Gen Comp Endocrinol* 151:82-89
- Dawson A, Howe PD (1983) Plasma corticosterone in wild starlings (*Sturnus vulgaris*) immediately following capture and in relation to body weight during the annual cycle. *Gen Comp Endocrinol* 51:303-308
- Dostine PL, Franklin DC (2002) A comparison of the diet of three finch species in the Yinberrie Hills area, Northern Territory. *Emu* 102:159-164

- Dostine PL, Johnson GC, Franklin DC, Zhang Y, Hempel C (2001) Seasonal use of savanna landscapes by the Gouldian Finch, *Erythrura gouldiae*, in the Yinberrie Hills area, Northern Territory. *Wildlife Res* 28:445-458
- Ekman JB, Hake MK (1990) Monitoring starvation risk: adjustments of body reserves in greenfinches (*Carduelis chloris* L.) during periods of unpredictable foraging success. *Behav Ecol* 1:62-67
- Fair J, Whitaker S, Pearson B (2007) Sources of variation in haematocrit. *Ibis* 149:535-552
- Fleshner M, Deak T, Spencer RL, Laudenslager ML, Watkins LR, Maier SF (1995) A long term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. *Endocrinol* 136:5336-5342
- Franklin DC (1999) Evidence of disarray amongst granivorous bird assemblages in the savannas of northern Australia, a region of sparse human settlement. *Biol Conserv* 90:53-68
- Franklin DC, Dostine PL, Tidemann SC (1998) Post-juvenile moult strategies of co-existing Gouldian, Long-tailed and Masked Finches. *Corella* 22:73-79
- Franklin DC, Whitehead PJ, Pardon G, Matthews J, McMahon P, McIntyre D (2005) Geographic patterns and correlates of the decline of granivorous birds in northern Australia. *Wildlife Res* 32:399-408
- Garnett S, Crowley G (1994) Wet season feeding by four species of granivorous birds in the Northern Territory. *Aust Bird Watch* 15:306-309
- Gayathri KL, Hegde SN (2006) Alteration in haematocrit values and plasma protein fractions during the breeding cycle of female pigeons, *Columba livia* *Anim Reprod Sci* 91:133-141
- Gray JM, Yarian D, Ramenofsky M (1990) Corticosterone, foraging behaviour, and metabolism in dark-eyed juncos, *Junco hyemalis*. *Gen Comp Endocrinol* 79:375-384
- Griffiths R, Double MC, Orr K, Dawson RJG (1998) A simple DNA test to sex most birds. *Mol Ecol* 7:1071-1075
- Helms CW, Drury WHJ (1960) Winter and migratory weight and fat field studies on some North American buntings. *Bird Band* 31:1-40
- Holberton RL (1999) Changes in patterns of corticosterone secretion concurrent with migratory fattening in a neotropical migratory bird. *Gen Comp Endocrinol* 116:49-58
- Horak P, Ots I, Murumagi A (1998) Haematological health state indices of reproducing Great Tits: a response to brood size manipulation *Funct Ecol* 12:750-756
- Hurly TA (1992) Energetic reserves of marsh tits (*Parus palustris*): food and fat storage in response to variable food supply. *Behav Ecol* 3:181-188
- Jenni L, Jenni-Eiermann S, Spina F, Schwabl H (2000) Regulation of protein breakdown and adrenocortical response to stress in birds during migratory flight *Am J Physiol - Reg I* 278:R1182-R1189
- King JR, Farner DS (1966) The adaptive role of winter fattening in the white-crowned sparrow with comments on its regulation. *Am Zool* 100:403-418

- Kitaysky AS, Wingfield JC, Piatt JF (1999) Dynamics of food availability, body condition, and physiological stress response in breeding Black-legged Kittiwakes. *Funct Ecol* 13:577-584
- Lewis M (2007) Foraging responses of the endangered Gouldian finch to temporal differences in seed availability in northern Australian savanna grasslands. In: Bissonette JA, Storch I (eds) *Temporal Dimensions of Landscape Ecology: Wildlife Responses to Variable Resources*. Springer, New York, NY,
- Lindstrom A, Kvist A, Piersma T, Dekinga A, Dietz MW (2000) Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. *J Exp Biol* 203:913-919
- Lynn SE, Breuner CW, Wingfield JC (2003) Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Horm Behav* 43:150-157
- Malisch JL, Breuner CW (2010) Steroid-binding proteins and free steroids in birds. *Mol Cell Endocrinol* 316:42-52
- Marra PP, Holberton RL (1998) Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia* 116:284-292
- Martin LB, Gilliam J, Han P, Lee K, Wikelski M (2005) Corticosterone suppresses cutaneous immune function in temperate but not tropical House Sparrows, *Passer domesticus* *Gen Comp Endocrinol* 140:126-135
- Metcalf NB, Ure SE (1995) Diurnal variation in flight performance and hence potential predation risk in small birds. *Proceedings: Biol Sci* 261:395-400
- Morton ML (1994) Hematocrits in montane sparrows in relation to reproductive schedule. *Condor* 96:119-126
- Ouillfeldt P, Masello JF, Mostl E (2004) Blood chemistry in relation to nutrition and ectoparasite load in Wilson's storm-petrels *Oceanites oceanicus* *Polar Biol* 27:168-176
- Owen JC, Moore FR (2006) Seasonal differences in immunological condition of three species of thrushes. *Condor* 108:389-398
- Rees A, Harvey S, Phillips JG (1985) Adrenocortical responses to novel stressors in acutely or repeatedly starved chickens *Gen Comp Endocrinol* 59:105-109
- Rich EL, Romero LM (2001) Daily and photoperiod variations of basal and stress-induced corticosterone concentrations in house sparrows (*Passer domesticus*). *J Comp Physiol B* 171:543-547
- Rich EL, Romero ML (2005) Exposure to chronic stress downregulates corticosterone responses to acute stressors. *Am J Physiol - Reg I* 288:R1628-R1636
- Rogers CM, Ketterson ED, Nolan VJ (1993) Geographic variation in winter fat of dark-eyed juncos: displacement to a common environment. *Ecol* 74:1183-1190
- Romero LM (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen Comp Endocrinol* 128:1-24

- Romero LM, Remage-Healey L (2000) Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): corticosterone. *Gen Comp Endocrinol* 119:52-59
- Romero LM, Wingfield JC (1999) Alterations in hypothalamic-pituitary-adrenal function associated with captivity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Comp Biochem Physiol B* 122:13-20
- Russell-Smith J, Yates C, Edwards A, Allan GE, Cook GD, Cooke P, Craig R, Heath B, Smith R (2003) Contemporary fire regimes of northern Australia, 1997-2001: change since aboriginal occupancy, challenges for sustainable management. *Int J Wildland Fire* 12:283-297
- Schwabl I, Bairlein F, Gwinner E (1991) Basal and stress-induced corticosterone levels of Garden Warbler, *Sylvia borin*, during migration. *J Comp Physiol B* 161:576-580
- Silverin B (1986) Corticosterone-binding proteins and behavioural effects of high plasma levels of corticosterone during the breeding period in the pied flycatcher. *Gen Comp Endocrinol* 64:67-74
- Sims CG, Holberton RL (2000) Development of the corticosterone stress response in young Northern mockingbirds (*Mimus polyglottos*). *Gen Comp Endocrinol* 119:193-201
- Smith RD, Metcalfe NB (1997) Diurnal, seasonal and altitudinal variation in energy reserves of wintering snow buntings. *J Avian Biol* 28:216-222
- Stutchbury BJM, Morton ES (2001) Behavioral ecology of tropical birds. Academic Press San Diego, California, USA,
- Tidemann SC, Woinarski JCZ (1994) Moulting characteristics and breeding seasons of Gouldian, *Erythrura gouldiae*, Masked, *Poepila personata* and Long-tailed finches, *P. acuticauda* in savannah woodland in the northern territory. *Emu* 94:46-52
- van Rooij EP, Griffith SC (2011) Breeding ecology of an Australian estrildid, the long-tailed finch (*Poephila acuticauda*). *Emu* 111:297-303
- Vleck CM, Vertalino N, Vleck D, Bucher TL (2000) Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adlie Penguins. *Condor* 102:392-400
- Wada H, Hahn TP, Breuner CW (2006a) Development of stress reactivity in white-crowned sparrow nestlings: Total corticosterone response increases with age, while free corticosterone response remains low. *Gen Comp Endocrinol* 150:405-413
- Wada H, Moore IT, Breuner CW, Wingfield JC (2006b) Stress responses in tropical sparrows: Comparing tropical and temperate *Zonotrichia*. *Physiol Biochem Zool* 79:784-792
- Wingfield JC (1994) Modulation of the adrenocortical response to stress in birds. In: Davey K, Peter R, Tobe S (eds) *Perspectives in Comparative Endocrinology*. National Research Council of Canada, Ottawa, pp 520-528
- Wingfield JC, Deviche P, Sharbough S, Astheimer LB, Holberton RL, Suydam R, Hunt K (1994) Seasonal changes in the adrenocortical responses to stress in Redpolls, *Acanthis flammea*, in Alaska. *J Exp Zool* 270:372-380

- Wingfield JC, Moore MC, Farner DS (1983) Endocrine responses to inclement weather in naturally breeding populations of White-crowned Sparrows (*Zonotrichia leucophrys pugetensis*). *Auk* 100:56-62
- Wingfield JC, O'Reilly KM, Astheimer LB (1995) Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. *Am Zool* 35:285-294
- Wingfield JC, Silverin B (1986) Effects of corticosterone on territorial behaviour of free-living male song sparrows, *Melospiza melodia*. *Horm Behav* 20:405-417
- Witter MS, Swaddle JP, Cuthill IC (1995) Periodic food availability and strategic regulation of body mass in the European Starling, *Sturnus vulgaris*. *Funct Ecol* 9:568-574
- Woinarski JCZ, Tidemann SC (1992) Survivorship and some population parameters for the endangered Gouldian Finch *Erythrura gouldiae* and two other finch species at two sites in tropical northern Australia. *Emu* 92:33-38

List of Tables

1. Table 1: Three-factor ANOVA models and post hoc tests (Tukey – Kramer HSD) which compare possible differences in finch residual CORT concentrations and CBG capacity between season (moult and breeding), years (1 and 2) and sites (B, D and Y). Table includes include values for Gouldian, long-tailed and masked finches.
2. Table 2: Mean stress and condition indices for different aged finches. Significant differences between adult and juvenile mean values are shown, non-significant results are omitted.
3. Table 3: Three-factor ANOVA models and post hoc tests (Tukey – Kramer HSD) which compare possible differences in finch muscle and fat scores between season (moult and breeding), years (1 and 2) and sites (B, D and Y). Table includes include values for Gouldian, long-tailed and masked finches.
4. Table 4: Key interactions between stress and condition indices determined by correlation analysis. Pearson correlation coefficient (r) is shown and direction of significant correlations is indicated by either a negative or positive r value.

Table 1 Three-factor ANOVA models and post hoc tests (Tukey – Kramer HSD) which compare possible differences in finch residual CORT concentrations and CBG capacity between season (moult and breeding), years (1 and 2) and sites (B, D and Y). Table includes include values for Gouldian, long-tailed and masked finches.

Gouldian finch						
Index	Factor	df	n	F	P	Trend Direction and P value for Tukey Kramer HSD
Residual CORT	Season	1	239	154.61	<.0001*	Moult > Breeding
	Year	1	239	0.78	0.38	
	Site	2	239	5.13	0.007*	
	Season * Year	1	239	1.17	0.28	
	Season * Site	2	239	15.72	<.0001*	
	Year * Site	2	239	2.95	0.05	
	Season * Year * Site	2	239	16.57	<.0001*	Year 1 Moult = Breeding at Y, Year 2 Moult > Breeding at Y, All years Moult > Breeding at sites B and D
CBG Capacity	Season	1	197	37.37	<.0001*	Moult < Breeding
	Year	1	197	18.84	<.0001*	Year 1 > Year 2
	Site	2	197	7.61	0.0007*	B > Y, P = 0.04
	Season * Year	1	197	3.36	0.07	
	Season * Site	2	197	1.27	0.28	
	Year * Site	2	197	0.54	0.58	
	Season * Year * Site	2	197	0.24	0.79	

Long-tailed finch						
Index	Factor	df	n	F	P	Trend Direction and P value for Tukey Kramer HSD
Residual CORT	Season	1	219	8.31	0.004*	Moult > Breeding
	Year	1	219	3.35	0.07	
	Site	2	219	1.62	0.20	
	Season * Year	1	219	22.97	<.0001*	
	Season * Site	2	219	3.96	0.02*	
	Year * Site	2	219	0.69	0.50	
	Season * Year * Site	2	219	6.14	0.003*	Year 1, Moult > Breeding at Y, Year 2 Moult < Breeding at Y, All years Moult < Breeding at sites B and D
<hr/>						
CBG Capacity	Season	1	124	1.94	0.17	
	Year	1	124	0.47	0.49	
	Site	2	124	0.02	0.98	
	Season * Year	1	124	0.02	0.90	
	Season * Site	2	124	1.09	0.34	
	Year * Site	2	124	0.08	0.92	
	Season * Year * Site	2	124	1.09	0.34	
<hr/>						
Masked finch						
Index	Factor	df	n	F	P	Trend Direction and P value for Tukey Kramer HSD
Residual CORT	Season	1	184	3.85	0.05	Moult = Breeding
	Year	1	184	0.32	0.57	
	Site	2	184	0.30	0.74	
	Season * Year	1	184	1.36	0.24	
	Season * Site	2	184	1.41	0.25	

Year * Site	2	184	0.51	0.60
Season * Year * Site	2	184	1.01	0.37

* indicates significant factors

Table 2 Mean stress and condition indices for different aged finches. Significant differences between adult and juvenile mean values are shown, non-significant results are omitted.

Gouldian finch				
Stress or Condition Index	Significant factor(s)	Season	Age	Mean Values \pm SE
Residual CORT	Age*Season	Moult	Adult	7.26 ng/mL \pm 1.50
			Juvenile	11.52 ng/mL \pm 1.31
		Breeding	Adult	-5.38 ng/mL \pm 1.05
			Juvenile	-8.59 ng/mL \pm 1.31
CBG Capacity	Age*Season	Breeding	Adult	98.14 nM \pm 3.58
			Juvenile	67.0 nM \pm 8.15
Muscle Score	Age	All	Adult	2.0 \pm 0.04
			Juvenile	1.9 \pm 0.04
Haematocrit	Age	All	Adult	61 \pm 0.34
			Juvenile	59 \pm 0.36
long-tailed finch				
Residual CORT	Age	All	Adult	0.73 ng/mL \pm 0.87
			Juvenile	-5.82 ng/mL \pm 2.32
Muscle Score	Age	All	Adult	2.0 \pm 0.03
			Juvenile	1.8 \pm 0.06
masked finch				
Muscle Score	Age	All	Adult	2.2 \pm 0.03
			Juvenile	1.9 \pm 0.06

Table 3 Three-factor ANOVA models and post hoc tests (Tukey – Kramer HSD) which compare possible differences in finch muscle and fat scores between season (moult and breeding), years (1 and 2) and sites (B, D and Y). Table includes include values for Gouldian, long-tailed and masked finches.

Gouldian finch						
Index	Factor	df	n	F	P	Trend Direction and P value for Tukey – Kramer HSD
Muscle Score	Season	1	439	7.26	0.007*	Moult < Breeding
	Year	1	439	0.10	0.75	
	Site	2	439	2.40	0.09	
	Season * Year	1	439	2.95	0.09	
	Season * Site	2	439	1.55	0.21	
	Year * Site	2	439	1.56	0.21	
	Season * Year * Site	2	439	1.22	0.30	
Fat Score	Season	1	439	3.09	0.08	
	Year	1	439	8.41	0.004*	
	Site	2	439	6.92	0.001*	
	Season * Year	1	439	26.68	<.0001*	
	Season * Site	2	439	1.38	0.25	
	Year * Site	2	439	1.40	0.25	
	Season * Year * Site	2	439	0.70	0.50	Year 1, Moult > Breeding at B and D Moult = Breeding at Y Year 2, Moult < Breeding at B and Y, Moult = Breeding at D
Long-tailed finch						
Index	Factor	df	n	F	P	Trend Direction and P value for Tukey – Kramer HSD

Muscle Score	Season	1	433	26.06	<.0001*	
	Year	1	433	0.75	0.39	
	Site	2	433	4.87	0.008*	No site differences significant, P > 0.05
	Season * Year	1	433	8.71	0.003*	Year 1, Moulting = Breeding Year 2, Moulting < Breeding
	Season * Site	2	433	1.88	0.15	
	Year * Site	2	433	0.35	0.71	
	Season * Year * Site	2	433	1.45	0.24	
Fat Score	Season	1	433	1.65	0.20	
	Year	1	433	9.70	0.002*	
	Site	2	433	6.08	0.003*	
	Season * Year	1	433	30.24	<.0001*	
	Season * Site	2	433	6.28	0.002*	
	Year * Site	2	433	0.43	0.65	
	Season * Year * Site	2	433	5.86	0.003*	Year 1, Moulting > Breeding at B and D, Moulting = Breeding at Y, Year 2, Moulting < Breeding at B and Y, Moulting = Breeding at D
Masked finch						
Index	Factor	df	n	F	P	Trend Direction and P value for Tukey – Kramer HSD
Muscle Score	Season	1	349	31.52	<.0001*	Moulting < Breeding
	Year	1	349	4.62	0.03*	
	Site	2	349	0.39	0.68	
	Season * Year	1	349	0.17	0.68	
	Season * Site	2	349	1.09	0.34	

	Year * Site	2	349	4.09	0.02*	Both years, B = D = Y, P > 0.05 For B and D, Year 1 = Year 2, For Y, Year 1 > Year 2
	Season * Year * Site	2	349	2.13	0.12	
Fat Score	Season	1	349	5.59	0.02*	
	Year	1	349	7.27	0.007*	
	Site	2	349	0.86	0.42	
	Season * Year	1	349	12.98	0.0004*	Year 1, Moulting > Breeding Year 2, Moulting = Breeding
	Season * Site	2	349	2.65	0.07	
	Year * Site	2	349	4.36	0.01*	Year 1, B < D, P = 0.04 B = Y, D = Y, P = 0.21 Year 2, B > Y, P = 0.03 B = D, D = Y, P = 0.31
	Season * Year * Site	2	349	0.39	0.69	

* indicates significant factors

Table 4 Key interactions between stress and condition indices determined by correlation analysis. Pearson correlation coefficient (*r*) is shown and direction of significant correlations is indicated by either a negative or positive *r* value.

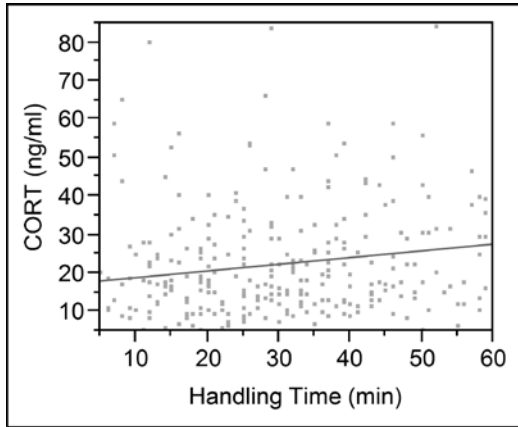
Interaction:	Gouldian Finch			long-tailed Finch			masked Finch		
	r	n	p	r	n	p	r	n	p
Fat and Muscle	0.35	451	<0.0001*	0.18	445	<0.0001*	0.12	361	0.02*
CORT and Muscle	-0.20	251	0.002*	0.08	231	0.26	-0.03	196	0.72
CORT and Fat	0.07	251	0.30	-0.13	231	0.04*	0.01	196	0.86
CORT and Haematocrit	0.16	249	0.01*	0.13	229	0.04*	0.02	196	0.77
CBG and Muscle	0.04	209	0.61	-0.09	136	0.29	Na		
Haematocrit and Fat	-0.13	413	0.009*	-0.05	424	0.33	-0.04	350	0.51
Haematocrit and Muscle	-0.07	413	0.19	0.06	424	0.18	-0.01	350	0.86

* indicates significant correlations

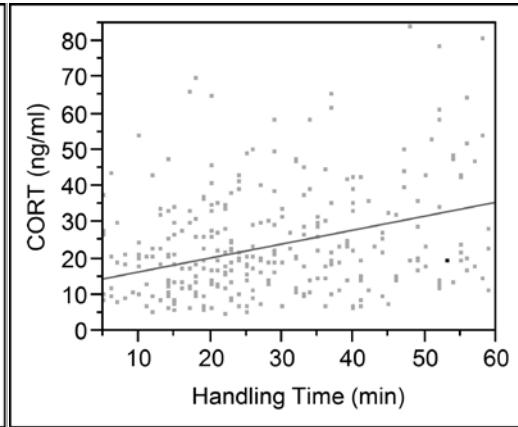
List of Figures

1. Figure 1: Regression of finch plasma CORT concentrations against time handled before blood sampling. Graphs represent regressions for Gouldian (a), long-tailed (b) and masked finches (c).
2. Figure 2: Residual CORT concentrations and CBG capacity for finches during two moult (2007, 2008) and breeding seasons (2008, 2009) and include values for Gouldian, long-tailed and masked finches. Bars represent means plus standard errors.
3. Figure 3: Adult finch muscle scores (a) and fat scores (b). Bars depict means plus errors for two moult (2007 and 2008) and breeding seasons (2008 and 2009) and include values for Gouldian, long-tailed and masked finches.

a)



b)



c)

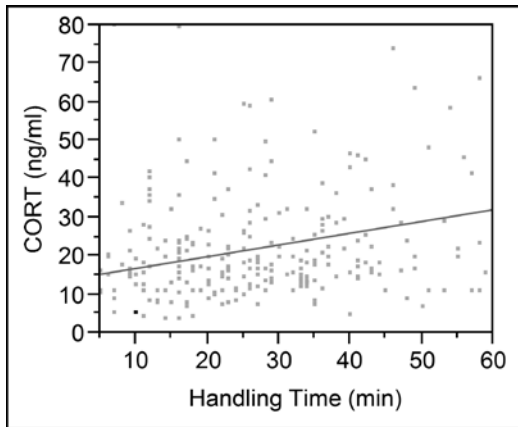


Fig 1 Regression of finch plasma CORT concentrations against time handled before blood sampling. Graphs represent regressions for Gouldian (a), long-tailed (b) and masked finches (c).

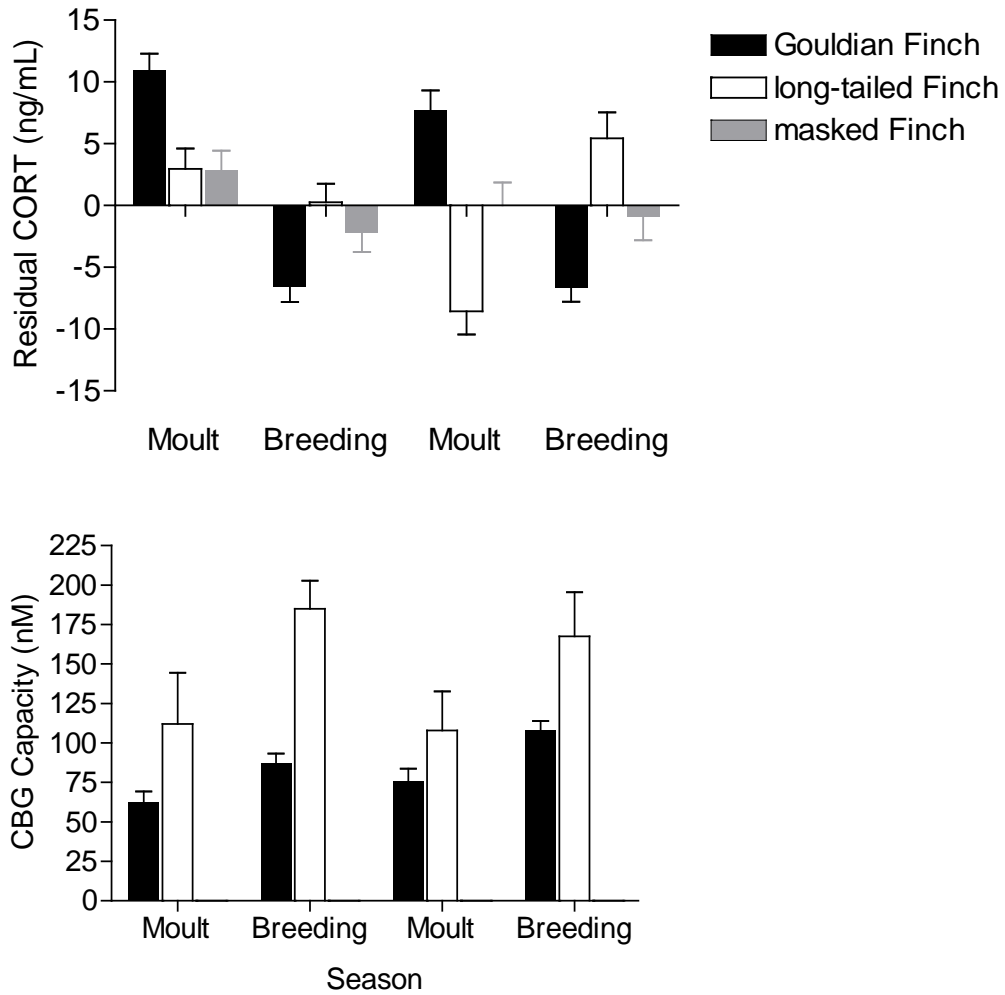


Fig. 2 Residual CORT concentrations and CBG capacity for finches during two moulting (2007, 2008) and breeding seasons (2008, 2009) and include values for Gouldian, long-tailed and masked finches. Bars represent means plus standard errors.

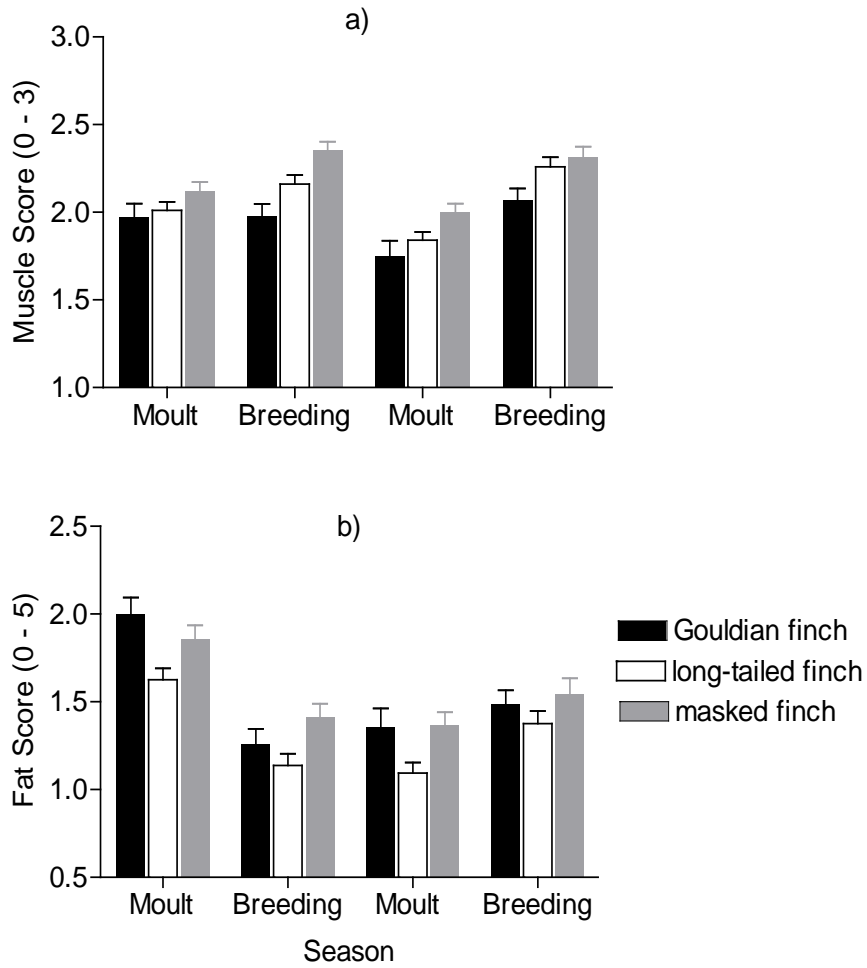


Fig. 3 Adult finch muscle scores (a) and fat scores (b). Bars depict means plus errors for two moult (2007 and 2008) and breeding seasons (2008 and 2009) and include values for Gouldian, long-tailed and masked finches.