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Stress reactivity, condition, and foraging behavior in zebra finches: effects on boldness, exploration, and sociality

O.L. Crino [a,b,*], Katherine L. Buchanan [a], Larissa Trompf [b], Mark C. Mainwaring [b,c], Simon C. Griffith [b]

[a] School of Life and Environmental Sciences, Deakin University, 3216 Victoria, Australia
[b] Department of Biological Sciences, Macquarie University, 2122 New South Wales, Australia
[c] Lancaster Envrionment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom

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ABSTRACT

The arid and semi-arid zones of Australia are characterized by highly variable and unpredictable environmental conditions which affect resources for flora and fauna. Environments which are highly unpredictable in terms of both resource access and distribution are likely to select for a variety of adaptive behavioral strategies, intrinsically linked to the physiological control of behavior. How unpredictable resource distribution has affected the coevolution of behavioral strategies and physiology has rarely been quantified, particularly not in Australian birds. We used a captive population of wild-derived zebra finches to test the relationships between behavioral strategies relating to food access and physiological responses to stress and body condition. We found that individuals that were in poorer body condition and had higher peak corticosterone levels entered baited feeders earlier in the trapping sequence of birds within the colony. We also found that individuals in poorer body condition fed in smaller social groups. Our data show that the foraging decisions which individuals make represent not only a trade-off between food access and risk of exposure, but their underlying physiological response to stress. Our data also suggest fundamental links between social networks and physiological parameters, which largely remain untested. These data demonstrate the fundamental importance of physiological mechanisms in controlling adaptive behavioral strategies and the dynamic interplay between physiological control of behavior and life-history evolution.

1. Introduction

The Australian arid zone is characterized by the unpredictability of rainfall both temporally and spatially (Morton et al., 2011). Many Australian birds, such as the iconic zebra finch (*Taeniopygia guttata*), have responded to the challenges of this environment with a behavioral strategy that combines a high level of mobility and sociality with wariness with respect to the risk of predation or novelty (Robin et al., 2009). The role of hormones in modulating the behavior, ecology, and evolution of Australian species has been well evaluated in marsupials (e.g. Bradley, 2003; McAllan, 2011; Shaw and Renfree, 2006), but much less so in Australian birds (but see Cornelius et al., 2011; Perfito et al., 2007). Here, we use a captive population of wild-derived zebra finches to test the relationships between behavioral strategies relating to food access and stress physiology.

The zebra finch is a socially monogamous estrildid finch that is widely distributed across arid and semi-arid areas of Australia (Zann, 1996). Zebra finches feed on patchily distributed grass seeds and form large foraging flocks that serve both to help individuals locate food and decrease predation risk (Zann, 1996). The relative ease with which zebra finches breed in captivity has contributed to their development as a model avian species for captive-based research in physiology, endocrinology, and the behavioral sciences (Griffith and Buchanan, 2010). Recent zebra finch studies have focused on variation between individuals with respect to behaviors such as foraging, exploratory behavior, risk-taking, activity levels, and neophobia (David et al., 2012a; Mainwaring et al., 2011; Martins et al., 2007; McCowan et al., 2014; Schuett and Dall, 2009). Variation in these consistent behavioral traits ('behavioral syndromes') in captive zebra finches is related to foraging behavior (Beauchamp, 2006; David et al., 2011, 2012b; McCowan and Griffith, 2015) and reproductive success (McCowan et al., 2015;

Abbreviations: GC, glucocorticoid; HPA, hypothalamic–pituitary–adrenal; CORT, corticosterone.

* Corresponding author at: School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, 3216 Victoria, Australia.
E-mail address: ondi.crino@deakin.edu.au (O.L. Crino).

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Schuett et al., 2011). The well-documented variation in individual behavior has important implications, not only for optimal foraging strategies, but also for life-history evolution.

In patchy, unpredictable environments, individual differences in foraging strategy may be important for determining individual fitness. Few studies have investigated the hormonal mechanisms that contribute to such behavioral variation. Glucocorticoids (GCs), known as 'stress' hormones, are a potential modulator of behaviors often studied in relation to 'behavioral syndromes' (e.g. Carere et al., 2003; Crino et al., 2010; Dosmann et al., 2015; Martins et al., 2007; Overli et al., 2002) and have important effects on fitness (Bonier et al., 2009; Breuner et al., 2008; Patterson et al., 2014). GCs are released via activation of the hypothalamic–pituitary–adrenal (HPA) axis and have important roles in metabolism, as well as physiological and behavioral responses to environmental perturbations (i.e. the stress response; Wingfield, 1994).

In birds, differences in the corticosterone (CORT; the dominant avian GC) profile of individuals are likely to affect both exploratory and foraging behavior (Wingfield, 2003). Martins et al. (2007) examined behavioral variation in groups of captive zebra finches that had been selectively bred for five generations with respect to their peak CORT production (Evans et al., 2006). Overall, increased levels of CORT were associated with increased levels of both exploratory and risk-taking behavior (Martins et al., 2007). Although, the selective breeding approach used by this study is an innovative way of addressing genetic control of the links between physiology and behavior, the authors themselves questioned the ecological relevance of assaying behavioral variation in a highly social bird in isolation or small groups in captivity. Subsequent studies of captive zebra finches have made a similar point, that the zebra finch is a highly social bird, and assays conducted on individuals tested alone may be confounded by the stress of isolation (Mainwaring et al., 2011; Schuett and Dall, 2009). In the wild, zebra finches live in social flocks throughout the year and individuals are rarely on their own (McCowan et al., 2015; Zann, 1994). Examining behavioral variation and the hormonal mechanisms that modulate individual differences within an ecologically relevant context, is likely to provide better insights into the selective pressures acting on free-living birds (Dall and Griffith, 2014).

To understand the mechanisms underlying the interactions between sociality, foraging, and stress physiology it is necessary to take an integrative approach within an ecologically relevant social context. We therefore conducted a study of wild-derived female zebra finches to examine associations between foraging behaviors and HPA-axis activity. Although we studied our birds in a social context, we focused on a single sex flock, so that our results were not confounded by hormonal changes associated with reproduction. Specifically, we examined individual variation in sociality, exploratory and foraging behavior (following McCowan and Griffith, 2015), as well as wariness through trappability, condition, and HPA-axis activity. By doing so, we sought to determine the relationships between physiological mechanisms and adaptive behavioral strategies in an Australian passerine.

2. Materials and methods

2.1. Animals and housing

The study focused on 51 female zebra finches that were part of the resident laboratory population at Macquarie University, Sydney, Australia and were wild-caught themselves, or the offspring or grand-offspring of birds taken from the wild (Gilby et al., 2013). All birds carried an individually numbered color band for identification and had a passive integrated transponder (PIT) ID100 tag (11 × 2 mm; Trovan, Hessle, UK) implanted subcutaneously. Prior to the beginning of the experiment the birds had been kept in a single sex group in an outdoor aviary (8 × 8 × 2.5 m) for at least three months. Both the holding and experimental aviaries contained multiple natural branch perches, had a roof that covered about 2/3rd of the ceiling and had at least one side of open mesh admitting sunlight and allowing the circulation of natural air. Commercial finch seed and water were supplied *ad libitum* as well as grit, cuttlefish and a daily provision of green food, which was a mixture of blended frozen peas and spinach.

2.2. Behavioral assays

In December 2013 and early January 2014, all birds ($n = 51$) were assayed over several weeks in replicate trials that were conducted in two large outdoor aviaries measuring 10 × 8 × 2.5 m (Aviary A and B, in that order). Each of these aviaries were differently partitioned using wooden pallets on their side, shade cloth and reed mat garden screens from floor to roof, to make the environment more complex and different from each other so that the second aviary was 'novel'. For the duration of each trial, the only food provided to the birds was given in three cage feeders (70 × 40 × 50 cm), henceforth referred to as feeders, that were present throughout the duration of the trial. These feeders contained a 50 × 30 cm seed tray provisioned with the finch seed and green food, and that could only be entered through a 11 × 11 cm circular opening around which was a powered antenna connected to a PIT-tag detection system (LID-665 decoder, Trovan) that automatically recorded the unique identification of any individual going through the entrance along with date and time information (following McCowan and Griffith, 2015). In total birds spent nine days in Aviary A and were then captured and released into Aviary B for the second trial. During experimental trials, aviaries were only entered once per day (for approximately 30 min) by the experimenter, to download the decoders and replenish food and water. Following the completion of each trial period all birds were caught and PIT tags checked. No birds lost their tags, but two birds died from natural causes during the trial period (Dec 2013–Jan 2014) and they have been excluded from final analysis (although included as companion foragers in the sociality data). During these two trials the following assays were conducted, as described below.

2.2.1. Exploration

Following release into Aviary A in trial 1, the decoder data logged every visit by individuals into the three feeders for the entire duration of the trial. We calculated the time taken for each individual to enter the first, second and third feeder that they entered. Only a small proportion of birds entered a third feeder, so these data are not presented here. Two variables were used to capture individual variation in exploratory behavior; (i) the time taken to enter the first feeder, and (ii) the time taken to enter a second feeder. Both of these measures were standardized relative to the time that the fastest individual in the trial took to enter its first two feeders. Unfortunately, due to human error, the decoder record during the second trial in Aviary B was not complete and therefore we were unable to measure this behavior in both trials. However, in a previous study exploratory behavior measured in this way was repeatable over multiple trials (McCowan and Griffith, 2015).

2.2.2. Sociality

In both trials, birds were allowed a period of five days to settle into the aviary and find at least two feeders that they regularly used (as above). On the fifth day of the trial we assessed sociality by considering how many birds an individual typically foraged with. The 24-h period of the fifth day was broken into 5-min segments (McCowan and Griffith, 2015). During each 5-min period

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that a bird fed, we considered the number of other birds also present during that time, and calculated the average number of birds present over all visits during the 24 h period (variable name – *Companions*). Second, we identified the most popular feeder during the 24 h period in each trial as the distribution of all feeds were split unevenly amongst the feeders (Trial A Feeder 1: 56.1%, Feeder 2: 4.0%;, Feeder 3: 39.9%; Trial B Feeder 1: 57.1%, Feeder 2: 28.5%, Feeder 3: 14.4%). For each individual, we then calculated the proportion of feeds in the day that were in the popular feeder (variable name – *Social feeds*). The social foraging variables were replicated across both trials, and provided a complete record of all feeder visits in that period.

2.2.3. Foraging behavior

Using the data recorded on the fifth day when birds had settled into the new aviaries (in Trial A and B), and again considering the period as 5-min segments, we calculated the total number of periods during the day that an individual was recorded visiting any feeder. There is no reason for an individual to enter the feeder cages other than to forage, and therefore we assume that this variable (*Feeds*) represents the number of feeding bouts that an individual had in a day. During the same 24-h period, we also considered the latency to feed after dawn. For this we measured the time in seconds that it took for each individual to visit a feeder after the first individual fed in the morning. This variable (*Breakfast time*), is likely to represent the individuals need to feed after the fasting over night, but will also be affected by social interactions. Both of these foraging variables were replicated across both trials.

2.3. Trapping and blood collection

We allowed females to habituate to the aviary for 18 days prior to trapping. We trapped for seven consecutive days from 8am to 12 pm using a feeder trap, to measure HPA-axis activity. One of the three cage feeders in the aviary was located flush against a wall of a room adjoining the aviary with a panel of one-way glass. On trap days, we closed the two other traps in the aviaries so food access was restricted to the feeder trap. The door to the feeder trap was held open with a piece of fishing line. After birds entered the feeding trap, we manually dropped the door, and removed the birds from the feeder trap via a hole in the wall that was connected to the trap. To assess HPA-axis activity we subjected birds to a standardized restraint stress protocol (Wingfield, 1994). We obtained an initial blood sample to measure baseline CORT within three minutes of the birds entering the trap. In general, samples collected within three minutes are considered to reflect baseline levels of CORT (Romero and Reed, 2005). After the initial blood sample was obtained, we placed birds in cloth bags for 15 min and then collected a second blood sample to quantify stress-induced CORT before returning the bird to the aviary. To collect blood, we punctured the alar vein with a 27-gauge needle and collected 25–50 μl of blood with heparinized microcapillary tubes. We stored the blood on ice (<4.5 h) until it could be centrifuged to separate plasma from red blood cells (7000 rpm for ten minutes). After separation, the plasma was isolated and stored at −20 °C. We trapped 47 of 51 females (92.2% of the population) after seven days of trapping. Of the 47 females, we trapped almost half (48.9%) within the first two days of trapping. We collected blood to measure baseline CORT for all 47 females and were able to collect a second blood sample after 15 min of restraint stress for 45 females. Two females were accidently released while collecting morphometric data resulting in a sample size of 45 for condition analyses and 43 for analyses involving condition and stress-induced CORT.

2.4. Morphometrics and condition calculations

We measured tarsus, wing chord and mass for females prior to starting the experiment, after collecting blood (see below), and again at the termination of the experiment. We calculated condition for females using the scaled mass index (Peig and Green, 2009). The scaled mass index calculates the expected mass of each individual at a fixed body size using a scaling relationship derived from the population of interest (Peig and Green, 2009). In this way, the scaled mass index accounts for errors associated with residual body mass measurements and is considered a more accurate measure of condition than residual body size to body mass calculations (Peig and Green, 2010). Scaled mass is hereafter referred to as 'condition' and presented in units of grams.

2.5. Corticosterone assays

Baseline and stress-induced corticosterone levels were quantified with Enzyme Immunoassay (EIA) kits (Cat No. ADI 900-097, Enzo Life Sciences). Samples were spiked with 1 pg of tritiated CORT (1,2,6,7-^{3}H; Perkin Elmer, Australia) prior to steroid extraction to determine recovery percentage. We extracted CORT from raw plasma using a double wash of dichloromethane. Samples were then dried under nitrogen gas and reconstituted in buffer solution (1:25 ratio). We adjusted hormone values for the average sample recovery (87.49%). We used an adjusted protocol to assay the reconstituted samples using half the volume of all the reagents supplied with the EIA kits. An external standard of 500 pg/ml was run on every plate and used to calculate inter-plate variation. All samples and standards were run in triplicate. Plates were read on a FLUOstar Omega microplate reader at 405 nm corrected at 595 nm. Levels of CORT were determined from a six point standard curve ranging from 20,000 to 15.53 pg/ml. Intra- and inter-plate variation was 4.44% and 4.24% respectively. Detection limit of the assay was 0.02 ng/ml.

2.6. Statistical analyses

Data for the total amount of feeds were normally distributed (Shapiro–Wilk, $P = 0.87, 0.76$). Data for all other exploratory behaviors were non-normally distributed (Shaprio-Wilk, $P < 0.02$) or trended to non-normality ($P < 0.06$). We used Pearson's correlations to examine the association between the first and second tests for normally distributed variables and Spearman rank correlations to examine associations between time points in non-normally distributed variables. We averaged behavioral data for the first and second tests and used the resulting values in all analyses. The averaged behavioral data were normally distributed ($P > 0.12$), except for the average time to first feed ($P < 0.001$). We used log transformation to normalize the average time to first feed ($P = 0.53$) and used the resulting values in all further analyses. The time to explore the first and second feeder and the time it took to trap females to collect blood for hormone sampling were non-normally distributed ($P < 0.02$) and log transformation failed to normalize data ($P < 0.053$). We accounted for non-normal data in the relevant statistical models (see below). Baseline and stress-induced CORT levels were non-normally distributed ($P < 0.01$ for both). We used log transformation to normalize the CORT data ($P > 0.12$ for both) and used the resulting values in all analyses. To analyze the amount of time it took to trap females, we binned total trap time over the six day period by hours resulting in 32 one-hour bins. We used the resulting variable (trap effort) in statistical analyses.

For variables with normal distributions, we used multiple regression to examine the relationships between HPA-axis activity, condition, and exploratory/social behaviors. For non-normally dis-

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tributed variables we used generalized linear models with poisson distributions for count variables (trap effort) and gamma distributions for continuous variables and log link functions to account for right skewed data.

3. Results

3.1. Body condition, CORT profiles, and trappability

Body condition was highly consistent; females in good condition prior to the start of the experiment were in good condition when trapped to collect blood, and at the end of the experiment ($P < 0.001$, 0.04, $R^2 = 0.56$, 0.34, $n = 47$, 39 respectively; Table 1). Females with high levels of stress-induced CORT were in lower body condition when trapped, compared to females with lower CORT levels ($P = 0.03$, $t = -2.20$, d.f. = 42, Fig. 1b). There was no association between baseline CORT and body condition ($P = 0.71$, $t = 0.38$, d.f. = 43, Fig. 1a). The females trapped early in the trapping effort had higher levels of stress-induced CORT ($P < 0.001$, Wald chi-square = 27.95, d.f. = 1; Fig. 2b) and were in poorer condition, compared to females that were trapped later in the trapping effort ($P = 0.04$ Wald chi-square = 4.33, d.f. = 1, respectively; Fig. 2c. Baseline CORT did not affect the time for females to enter the trap ($P > 0.81$, Wald-Chi square = 0.06, d.f. = 1; Fig. 2a).

3.2. Body condition, HPA-axis activity, and feeding behavior

Females in poor body condition fed at feeders with fewer companions ($P = 0.005$, $t = 2.98$, d.f. = 40; Fig. 3). There were no relationships between an individual's baseline ($P = 0.63$, $t = -0.48$, d.f. = 40) or stress-induced CORT ($P = 0.092$, $t = 0.10$, d.f. = 40) and their number of companions at feeders. There was a non-significant trend for birds in poor condition to prefer to feed at feeders that on average had a lower number of birds visiting them ($P = 0.07$, $t = 1.84$, 1.73, d.f. = 40), but no relationship between either baseline or stress-induced CORT and the proportion of time birds spent feeding at busy feeders ($P = 0.62$, 0.32, $t = 0.51$, 0.32, d.f. = 40, 40). There was a non-significant trend for birds in poor condition to make higher numbers of feeding trips, compared to birds in good condition ($P = 0.09$, $t = -1.73$, d.f. = 40), but no relationship between number of feeding trips and either baseline or stress-induced CORT ($P = 0.26$, 0.49, $t = -1.15$, -0.69, d.f. = 40, 40). There were no relationships between condition or baseline CORT and the time it took birds to first feed in the morning ($P = 0.61$, 0.35, $t = 0.51$, -0.94, d.f. = 40, 40). However, birds with high levels of stress-induced CORT fed later in the morning than birds with low levels of stress-induced CORT ($P = 0.008$, $t = 2.81$, d.f. = 40; Fig. 4).

Table 1
A comparison at two time points of mass, condition, total feeds, and the time females first entered a feeder in the morning (breakfast time). Correlation coefficients and P values are given for sets of variables. Bold values indicate a significance of $P \leq 0.01$.

Variable	Average	St. Dev.	Corr. Coefficient	Significance
Initial mass (g; first)	14.57	1.28		
Mass trapping (g; second)	11.89	1.22	**0.67**	**>0.001**
Initial condition (g; first)	14.57	1.24		
Condition at trapping (g; second)	11.95	1.18	**0.56**	**>0.001**
Total feeds (first)	20.00	5.08		
Total feeds (second)	23.59	6.16	**0.56**	**>0.001**
Breakfast time (first)	750.27	553.41		
Breakfast time (second)	887.31	922.62	**0.36**	**0.01**
Visits to busy feeder (first)	0.58	0.33		
Visits to busy feeder (second)	0.55	0.30	0.05	0.71
Birds accompany (first)	6.48	1.48		
Birds accompany (second)	4.64	0.85	0.08	0.58

3.3. Exploratory behavior, body condition, and HPA-axis activity

After initial release into the aviary, birds in better body condition prior to release into the aviaries took longer to enter the first and second feeders, compared to birds in poor body condition ($P = 0.001$, 0.03; Wald Chi-square = 10.58, 5.045.23, 13.67, d.f. = 1, 1 respectively; Fig. 5). There were no relationships between baseline or stressed induced CORT and the time to enter the first or second feeders ($P > 0.18$ for all).

4. Discussion

Individual foraging strategies represent the behavioral result of trade-offs between physiological control mechanisms that regulate not only behaviors like fearfulness, but also physiological parameters like condition and stress reactivity. Our data clearly show that individuals vary in their tendency to use risky food resources and that this variation is related to their requirement for food and HPA-axis activity. Our results provide further support for the idea that elements of foraging, social, and exploratory behavior vary consistently across the individuals of a population and may be considered to reflect individual 'personality' (Dall et al., 2004). However, we have also shown that these behaviors covary with individual differences in body condition and HPA-axis activity, and our findings suggest that a significant driver of the measured behavioral variation, in this, and earlier studies, may be the motivation to feed. We also demonstrated a significant relationship between HPA-axis activity and the trappability of individuals in a controlled and finite population. We trapped the majority of females in this study within the first two days of trapping. Had we only caught and assayed the birds that were easiest to capture, we would have obtained a strong bias in our measures of HPA-axis activity, because the last trapped birds had significantly lower levels of stress-induced CORT than the birds trapped first.

4.1. HPA-axis activity, condition, and foraging behavior

GCs act as physiological signals to animals to modify their behavior and metabolism to deal with potentially adverse environmental conditions (reviewed in Sapolsky et al., 2000). In birds, elevated CORT is associated with increased foraging and food intake (Astheimer et al., 1992; Crossin et al., 2012), increased locomotor activity (Breuner et al., 1998), increased physiological processes that mobilize stored energy such as gluconeogenesis (Remage-Healey and Romero, 2001) and lipogenesis (Gray et al., 1990), and decreased condition (Breuner and Hahn, 2003). In our experiment, we found that females in poor condition prior to the start of the experiment remained in poor condition throughout the experiment, indicating that condition is a stable trait across environmental and social contexts in this experiment (Table 1). Females in poor condition were the first to enter the feeders after release into the aviaries (Fig. 5), suggesting that the greater exploratory tendencies of these birds were driven by greater motivation to feed resulting from low energy reserves.

4.2. HPA-axis activity condition and sociality

Social interactions can both influence and be affected by hormone systems such as the HPA-axis as well as condition. In non-cooperative breeders, social conflict can increase HPA-axis activity (Carere et al., 2003) and subordinates tend to have higher HPA-axis activity compared to dominant individuals (Creel, 2001). In our

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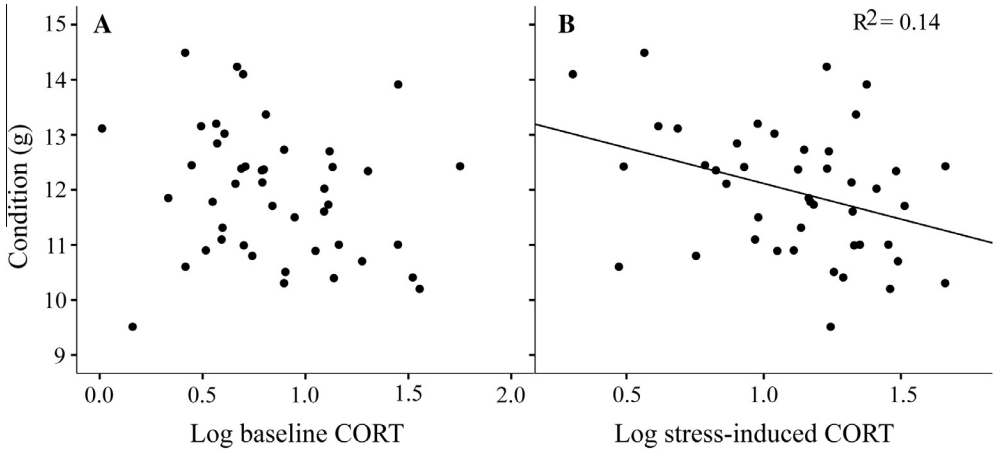

Fig. 1. The relationship between CORT levels (baseline and stress-induced) and condition in female zebra finches.

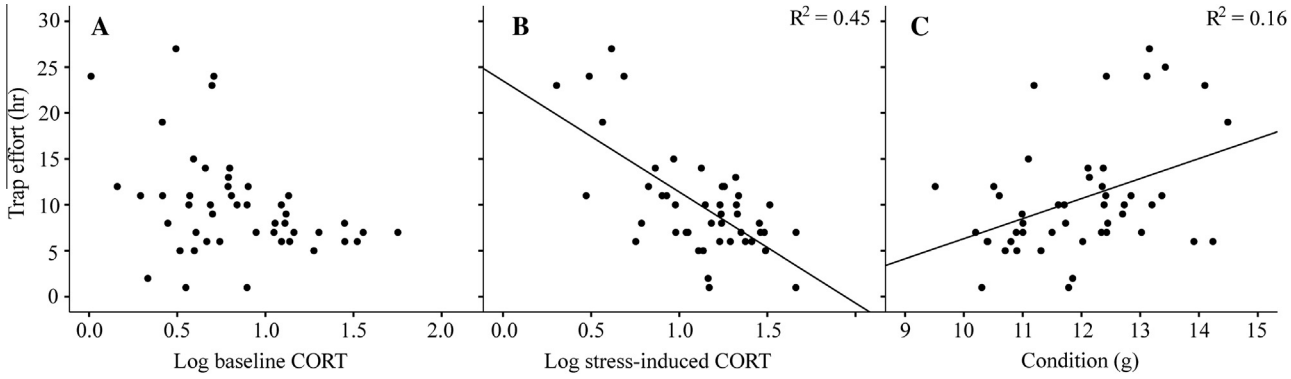

Fig. 2. The relationship between CORT levels (baseline and stress-induced) and condition and trapping effort (h).

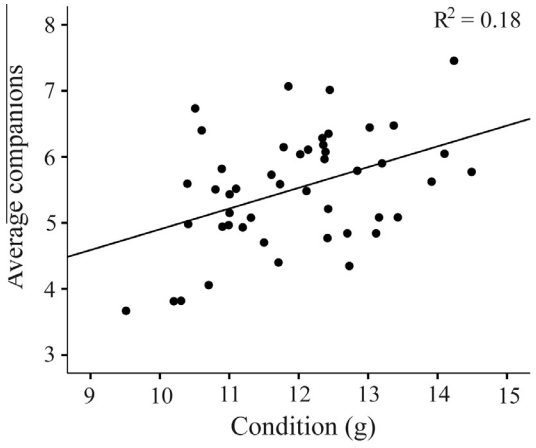

Fig. 3. The relationship between a female's condition and the mean number of companions she fed with at feeders.

study, females with the lowest levels of stress-induced CORT were first to feed in the mornings (Fig. 4). Although we did not directly measure social dominance, it is possible that females with the lowest levels of CORT were most dominant and, thus, were able to monopolize feeders early in the mornings when passerines have the lowest body mass (Metcalfe and Ure, 1995) and, therefore, greatest motivation to feed due to the loss of critical energy reserve overnight. Similar to what has been described in other species, we found a negative correlation between condition and stress-induced CORT, such that females in the worst condition released the highest levels of CORT in response to restraint stress (Breuner and Hahn, 2003; Fig. 1). We also found that the feeding behavior of birds in the aviary was influenced by their condition (measured at the beginning and end of the trial). Individuals in poor condition tended to feed asocially at feeders with fewer other birds (Fig. 3) and make more visits to the feeders throughout the day (although the latter relationship only trended to significance). The causal nature of these relationships is unclear; individuals in poor condition could be restricted from accessing food resources by more dominant individuals, or vice versa, or indeed underlying disease or pathogen effects may have reduced body condition sufficiently to affect foraging strategies and social networks.

4.3. HPA-axis activity and trapping bias

In our study, in common with so many studies of wild animals, we used food to capture individuals, and our results demonstrated that such an approach is likely to produce biases due to incomplete and non-random sampling of a population. Although there have been a number of studies that have previously highlighted the biases that may result in relation to behavioral differences amongst individuals (Biro, 2013; Biro and Dingemanse, 2008; Carter et al., 2012), our study is significant in directly examining trappability in relation to HPA-axis activity and condition. The first females to enter the feeding trap, released the highest levels of CORT in response to restraint stress and were in the poorest condition. In birds, temporary fasting can increase CORT responses and result in greater activity (Lynn et al., 2003, 2015). On trapping days,

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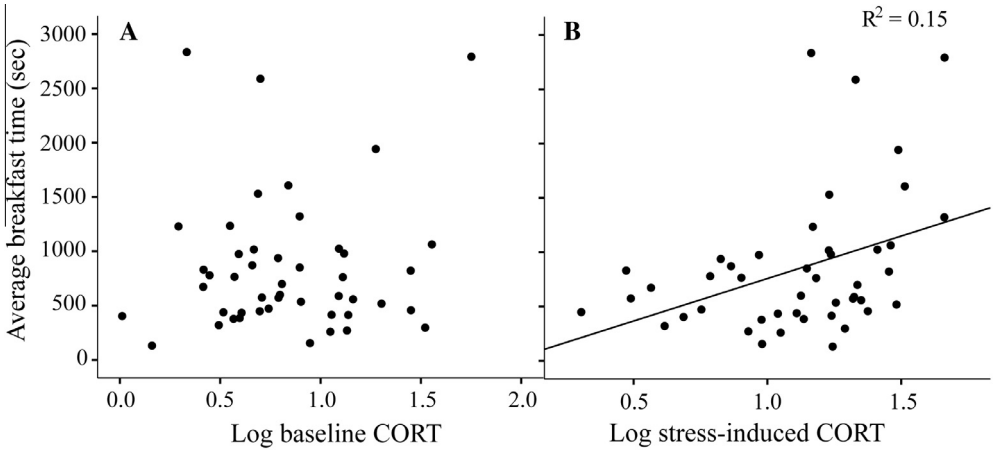

Fig. 4. Relationship between CORT levels (baseline and stress-induced and the average time (s) it took females to feed for the first time in the morning.

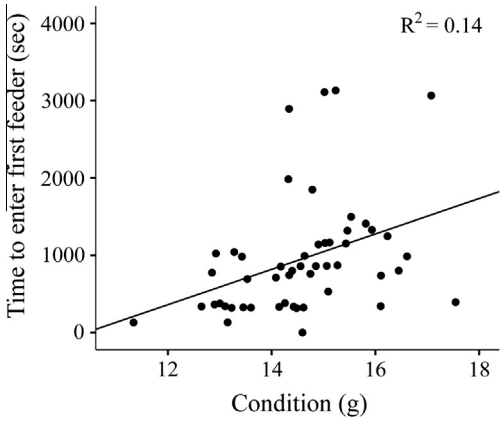

Fig. 5. The relationship between condition and the amount of time (s) it took females to enter the first feeder upon initial release into the aviary.

only one feeder was open to the birds, limiting food availability. Birds in the poorest condition, with the least energy reserves, were potentially more affected by restricted nutrition on trapping days and, thus, responded to restraint stress with higher levels of CORT output compared to birds that were trapped later in the trapping effort that were in better condition.

Although it is not possible to disentangle the causative versus correlative effects in our study, our data nonetheless suggest that trapping efforts could bias physiological data if some individuals are more or less prone to approach traps based on their HPA-axis activity and condition. This may be particularly relevant in field studies with free-living birds where researchers use traps that could be perceived as novel objects, food to bait traps, or when trapping effort is restricted to a short period of time. In a controlled aviary setting, with a known amount of individuals we were eventually able to trap ≈92% of the population to evaluate HPA-axis activity. Trapping success was highest on the second day of trapping (by which time we had trapped almost half of our sample) and decreased throughout the trapping effort. Had we terminated trapping effort after trapping half of the birds on the second day, we would have inadvertently biased our results by trapping individuals in the aviary with the highest HPA-axis activity. This trapping bias may be particularly important to consider in field studies that seek to evaluate temporal changes in CORT across seasons. If individuals with high HPA-axis activity are more likely to be trapped during early trapping effort at the beginning of a season, researchers may reach erroneous conclusions about temporal variation in hormone secretion based on trapping bias. Although this potential bias resulting from non-random sampling has not been investigated, studies have shown that HPA-axis activity can vary in relation to trapping method (passive vs target netting; Angelier et al., 2010) and that some species may be more susceptible to stress caused by trapping than others (Lynn and Porter, 2008) suggesting that researchers should carefully consider how their trapping methodology could inadvertently impact their findings. Future studies could tease apart the interactions between condition, HPA-axis activity, and trappability using implants to manipulate endogenous CORT levels.

4.4. Conclusions

The study of inter-individual behavioral variation in animals has become the focus of a major research drive in the past decade. Many of the studies are focused on single aspects of behavior such as exploratory behavior, or boldness. Although many studies have investigated the 'behavioral syndromes' that link together different elements of behavior, we would get a more comprehensive understanding by integrating these studies of behavior with work on the underlying physiology and condition of individuals. Our work suggests that most of the behavioral variation in traits such as exploratory behavior is driven by an individual's relationship with food, and their ability to access food in a reliable way. The social context in which we have conducted our work contributes to the individual variation in condition and stress. In turn this affects the patterns with which individuals access food across the day, and the ease with which we were able to capture them. We recommend that future work on elements of personality is fully integrated with the study of individual variation in HPA-axis activity and physiological condition.

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