



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Effects of short-term fasting on stress physiology, body condition, and locomotor activity in wintering white-crowned sparrows

Citation for published version:

Krause, JS, Pérez, JH, Meddle, S & Wingfield, JC 2017, 'Effects of short-term fasting on stress physiology, body condition, and locomotor activity in wintering white-crowned sparrows' *Physiology & Behavior*, vol. 177, pp. 282-290. DOI: 10.1016/j.physbeh.2017.04.026

Digital Object Identifier (DOI):

[10.1016/j.physbeh.2017.04.026](https://doi.org/10.1016/j.physbeh.2017.04.026)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Physiology & Behavior

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.





Effects of short-term fasting on stress physiology, body condition, and locomotor activity in wintering male white-crowned sparrows



Jesse S. Krause^{a,*}, Jonathan H. Pérez^{a,1}, Simone L. Meddle^b, John C. Wingfield^a

^a Department of Neurobiology, Physiology and Behavior, University of California Davis, One Shields Avenue, Davis, CA 95616, USA

^b The Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Midlothian EH25 9RG, Scotland, UK

ARTICLE INFO

Keywords:

Corticosterone
Hypothalamic-pituitary-adrenal (HPA) axis
Emergency life history stage
Food removal
Allostasis

ABSTRACT

For wild free-living animals the availability of food resources can be greatly affected by environmental perturbations such as weather events. In response to environmental perturbations, animals activate the hypothalamic-pituitary-adrenal (HPA) axis to adjust physiology and behavior. The literature asserts that during weather events food intake declines leading to changes in HPA axis activity, as measured by both baseline and stress-induced glucocorticoid concentrations. Here we investigated how body condition, locomotor activity, and stress physiology were affected by varying lengths of a fast (1, 2, 6, and 24 h; similar to that experienced by free-living birds) compared to when food was provided *ad libitum* in captive wintering male white-crowned sparrows, *Zonotrichia leucophrys gambelii*, exposed to a short day photoperiod. Baseline corticosterone concentrations were increased for all fasting durations but were highest in 6 and 24 h fasted birds. Stress-induced corticosterone was elevated in 1 h fasted birds with a trend for the 2 h of fast; no other differences were found. Baseline corticosterone concentrations were negatively related to both total fat scores and body mass. All birds lost body mass regardless of fast length but birds fasted for 24 h lost the most. Fat scores declined in the 6 and 24 h groups, and no measureable changes were detected in pectoralis muscle profile. Locomotor activity was increased over the entire period in which food was removed regardless of fasting duration. Together this suggests that reduced food availability is responsible, at least in part, for the rapid elevation both baseline corticosterone under any duration of fast and stress-induced concentrations during short-term fasts.

1. Introduction

In an ever changing world, animals are becoming exposed to frequent perturbations to their environment [1,2]. One of the most common stressors endured by free-living animals are reductions in food availability, which can occur due to inclement weather, trophic mismatches, and changes in predator prey dynamics [3–5]. A reduction in food supply, promotes the mobilization and utilization of energy reserves which results in the overall decline in body condition [6]. During these stressors, vertebrates activate the hypothalamic-pituitary-adrenal (HPA) axis resulting in elevation in the stress hormone corticosterone, often above basal concentrations, which then modifies physiology and behavior [7,8]. Baseline concentrations of corticosterone are thought to control metabolic processes (i.e., protein and lipid catabolism) while stress-induced concentrations, in response to environmental perturbations, are thought to mediate life history trade-offs [9,10]. Corticosterone would generally be considered to be at a baseline state until environmental perturbations elevate stress-induced concen-

trations [8]. Baseline corticosterone concentrations often fully saturate high affinity mineralocorticoid receptors (MR) while low affinity glucocorticoid receptors (GR) are occupied when concentrations rise [11]. The presence of both high and low affinity receptors allows the system to operate in a two-tiered fashion enabling differential regulation of physiology and behavior under unstressed and stressed conditions [11,12].

The specific environmental stimuli that control rapid changes in HPA activity in response to severe perturbations remain largely unknown but have been attributed to declines in food availability and increased severity of the environment. In birds, corticosterone can increase in response to increasing snow [5,13] and rain fall [14–17], fasting in incubating birds [18–20], natural declines in prey availability [21], or increased predator pressure [3] and is most often attributed to a decline in caloric intake. Captive studies corroborate findings in the field that restriction or removal of food results in elevated baseline concentrations of corticosterone and this is directly related to body condition [21–26]. To date, studies investigating the relationship

* Corresponding author.

E-mail address: jskrause@ucdavis.edu (J.S. Krause).

¹ Co-first authors.

between food intake and corticosterone concentrations have found mixed results for stress-induced concentrations of corticosterone [21,24,27].

The allostasis model suggests that the degree of HPA axis activity both at baseline and stress-induced concentrations is intricately tied to the energetic state of the individual, which in turn mediates the effects of environmental conditions and resource availability [28]. Activation of the emergency life history stage is thought to occur when animals enter into allostatic overload; the point at which both internal energetic stores and available food in the environment can no longer meet the demands of metabolism. Individuals in poor condition are thus most likely to rapidly increase HPA axis activity, triggering the emergency life history stage and devoting resources towards self-maintenance [29,30]. However this threshold has remained a nebulous and arbitrary level in the literature as so many unmeasurable factors affect an animal's overall energetic balance. During periods of stress and possible allostatic overload, corticosterone concentrations have been linked with protein catabolism [10,31,32], lipid mobilization [33,34], escape behavior [35], hyperactivity in cages [22,24,36,37], suppression of reproduction [38,39], increased feeding activity [32,37], and enhanced immune function [40] all of which are thought to promote survival. However, corticosterone does not appear to be directly linked to glucose metabolism in birds [24,41]. In addition, it is not entirely understood how changes in corticosterone during these stressors leads to changes in feeding rates following the disturbance which help to restore body condition after metabolic stores have been drawn upon.

The aim of this study was to investigate the relationships between food intake, body condition, daily activity, and HPA axis activity in a long-distance migratory songbird, the white-crowned sparrow, *Zonotrichia leucophrys gambelii*, which breed in Alaska and Canada and winters in the American southwest [42]. Using the same captive experimental paradigm described by [22] we investigated the effects of varying durations of fasting on the relationships between activity, body condition, and corticosterone concentrations. This paradigm is ecologically relevant to disturbances experienced by birds in the wild. As the length of the fast increased, we predicted that the degree of HPA activation would increase, measured both at baseline and stress-induced concentrations, and body condition would continue to decline. We predicted a negative relationship between corticosterone concentrations with both fat scores and body mass. In addition, we predicted that food removal would promote increased perch hopping activity and this would be positively correlated with corticosterone concentrations. Finally, we hypothesized that the change in body condition and food intake either on the day of the fast or recovery day would be related to circulating corticosterone.

2. Methods

2.1. Birds

A total of 36 male white-crowned sparrows were caught from late October through early November 2015 in the vicinity of Davis,

California. During this time of year, birds are highly social and form flocks ranging in size from a few to several hundred individuals [36]. Free-living birds exhibit the highest feeding intensity in the early morning and late afternoon, while in captivity foraging intensity is more evenly distributed; although both feed throughout the day [36].

Birds were housed in individual cages (35 cm (w) × 40 cm (l) × 45 cm (h)) in three separate indoor aviaries held at 20 ± 2 °C and exposed to a constant photoperiod of 11.5 L and 12.5 D to mimic daylength at the time of capture (lights on 6:30 AM and off at 6:00 PM). A diet of 60% Mazuri maintenance chow and 40% wild bird seed and water was provided *ad libitum*. Cages were equipped with an infrared photodetection system to monitor activity *via* beam breaks as previously described [43,44]. All experiments were approved by University of California, Davis Institutional Animal Care and Use Committee under protocol 16283.

2.2. Experimentation

Data were collected for four major periods which include when birds were caught (field), the days leading up to the experiment (pre-fasted), the day of the experiment (fasted), and the day following the experiment (recovery). Birds were acclimated to captivity for a period of two and a half weeks prior to collection of pre-fasted corticosterone and body condition data when food and water was provided *ad libitum*. Collection of pre-fasted data allowed birds to serve as their own control. The birds were given a one week recovery period prior to the onset of experimentation. Birds were split into four different experimental groups of 9 birds each in which food was removed from their cages for 1, 2, 6 and 24 h. On the day of experimentation, two investigators entered the three rooms at 8:30 AM, replaced the cage floor liners (ensured removal of any residual food in the cage), and removed the food for the 1, 2 and 24 h groups. The next day, the same process was repeated for the 6 h group which was housed in the same room as the 24 h group. Food was returned immediately after the birds were bled and returned to their cages. This experimental design has been previously demonstrated to induce changes in baseline corticosterone and behavior in white-crowned sparrows ([22], see Fig.1).

2.3. Morphometrics

Body mass was measured to the nearest 0.01 g using an Ohaus digital scale. Pectoralis muscle profile was assessed visually on a scale of 0 to 3 [45], while subcutaneous fat deposits were visually scored from 0 (absent) to 8 (bulging) in both furcular and abdominal regions [46]. All muscle and fat scores were performed by the same observer throughout the experiment to minimize observer effects and treatment was unknown to the observer.

2.4. Food intake

Daily food consumption over a 24 h period was measured at 8:30 AM, 2 h after lights on, using an Ohaus digital scale

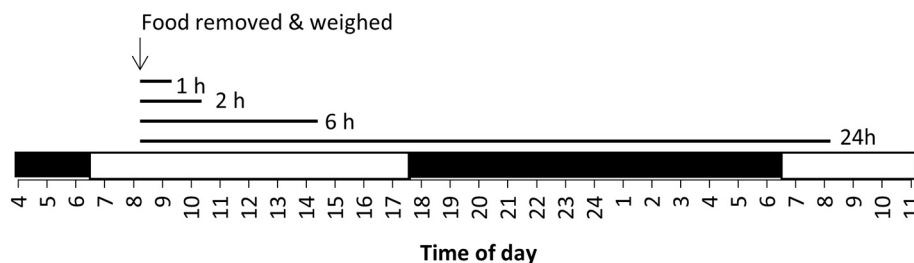


Fig. 1. Study design for captive male white-crowned sparrows (n = 36) held on short days (L:D 11.5:12.5). Time of day is indicated by the numbers with lights on period indicated by the open bar (day) and the lights off by the solid bar (night). The duration of the fast varied by experimental group (n = 9) and is indicated by the black solid lines. At the onset of the food manipulation period, researchers entered the room to remove food and grit cups and remove the pan liner to ensure no food was left.

(± 0.01 g). Food intake was measured for 3 days immediately following transfer to captivity, 3 days prior to, the day of, and 1 day post food removal. This allowed us to assess changes in food consumption over the entirety of the experiment.

2.5. Physiological and behavioral sampling

At the end of each food restriction period, the investigators entered the room to collect a baseline corticosterone sample with a mean ± SD time to sample of 117 ± 39 s of disturbance. The mean time to sample under *ad libitum* conditions was 121 ± 42 s. Previous studies on wintering white-crowned sparrows have shown that sample collection within 3 min of capture represented baseline or near baseline concentrations of corticosterone [47]. A standardized handling restraint protocol was used to assess HPA axis activity by placing a bird into an opaque cloth bag until the second sampling point 30 min post removal from the cage [48]. Approximately 80 µL of whole blood was collected into a heparinized capillary tube at each time point and placed into a cooler until later processing. Blood was centrifuged at 10,000 RPM for a period of 5 min and hematocrit was measured using the baseline sample only, as previously described [49]. Plasma was aspirated using a Hamilton syringe, placed into a microcentrifuge tube and then stored at -20 °C until the assay date.

A total of 34 activity monitoring systems were used to register individual bird activity levels. All birds in each group were recorded except for the 1 hour group in which only 4 individuals were monitored because not enough activity monitors were available for every individual. Activity was quantified by the number of infrared beam breaks that occurred as the bird hopped about the perch over a 15 minute interval. Data were recorded using a mini-mitter activity system and VitaView software. All entry into and out of the experimental room was noted to control for investigator induced disturbances and were excluded from the analyses. The room was entered once a day for animal husbandry. On the day of experimentation the room was entered to remove the food and then to capture the bird for a blood sample.

2.6. Corticosterone assay

Corticosterone previously described by Wingfield et al. [48]. In brief, 10–30 µL of plasma was measured and then combined with ~2000 CPM of radioactive corticosterone for later determination of recovery efficiency. Steroids were extracted from the aqueous phase for a period of 3 h using dichloromethane (redistilled within 24 h of use). Extracts were dried in a 35 °C water bath under a stream of N₂. Steroids were reconstituted using 550 µL phosphate-buffered saline with gelatine (PBSG). Next, 200 µL aliquots were placed into test tubes in duplicate and combined with 100 µL of 10⁴ CPM of tritiated corticosterone (Perkin Elmer NET399250UC) and 100 µL of antiserum (MP Biomedical antibodies: 07–120,016, Lot 3R3-PB-20E). A 100 µL aliquot was placed into individual scintillation vial to determine percent recoveries. Each scintillation vial received 3.5 mL of scintillation fluid (Perkin Elmer; Ultima gold 6,013,329) and then counted for 5 m using a Beckman 6500 liquid scintillation counter. Unbound steroid was stripped from solution by adding 500 µL of dextran coated charcoal followed by centrifugation at 2800 RPM for 10 min. Final hormone values were corrected using the individual recovery for each sample. The mean detection limits of the assay were 8.60 pg/tube and the mean recoveries were 85.62 ± 4.12. The intra- and inter-assay variation was 5.05 and 6.14%, respectively. The bound to free ratio was 0.31. The antibody was validated by checking for parallelism between diluted plasma pool that was spiked with a known amount of corticosterone and standard curve (Supp. Fig. 1).

2.7. Statistical analyses

Statistical analyses were performed using JMP 11 Pro (SAS Institute

Inc., Cary, NC, 1989–2007). Hormonal data were analyzed using two separate approaches. First we used a paired *t*-test to examine the effects of food removal at both baseline and stress-induced time points to understand how concentrations of corticosterone fluctuated at the individual level. We used a linear mixed effects model, with individual included as a random effect, to investigate the effects of acute restraint (stress), food removal duration (group), and their interaction on circulating corticosterone. The residuals were saved from the linear mixed effects model and were found to be normally distributed for both control (W = 0.98, P = 0.35) and experimental (W = 0.96, P = 0.07) groups. The relationship between baseline corticosterone and fat scores were investigated using nonparametric ANOVA – Kruskal-Wallis test.

Changes in corticosterone, fat, and body mass were determined by subtracting values measured under *ad libitum* conditions from fasted conditions. Changes in the various parameters were compared using a Pearson's correlation analyses for both baseline and stress-induced concentrations of corticosterone in relation to changes in body measurement from *ad libitum* to fasted conditions or from fasted to recovery conditions.

Body mass was analyzed using a linear mixed effects model with individual included as a random factor and the main effects of food removal duration (group) and day of the study. Body condition index was calculated by using a principal components analysis using skull and tarsus lengths and the PC1 scores were saved. PC1 scores were then regressed against body mass and the residuals were saved yielding body condition index score. We calculated the change in body mass during the day of the fast and on the day of recovery. A repeated measures analysis of variance (ANOVA) was used to investigate the main effects of food removal duration and day of study along with their interaction. *Post hoc* analyses for repeated measures ANOVA and linear mixed models were conducted using Tukey's Honestly Significance Difference Test (HSD).

Since fat scores and pectoralis muscle profiles are ordinal data they were analyzed with an ordinal logistic model. Hematocrit levels were analyzed using a Paired *t*-test to compare pre-fasted to fasted levels within each fasted group. A one-way ANOVA was used to investigate differences in hematocrit levels across groups at both pre-fasted and fasted levels. To determine if fasting had an effect on activity during the fasting period total day time activity data was analyzed using one-way ANOVA.

3. Results

3.1. Corticosterone concentrations

Prior to the food removal experiment, there were no differences in baseline (F_{3,34} = 1.77, P = 0.17) or stress-induced (F_{3,34} = 0.66, P = 0.58; Table 1 & Fig. 2.) concentrations of corticosterone between the groups that were later food restricted. Fasting caused measureable increases in baseline concentrations of corticosterone after 1 (t = 2.81, P = 0.02), 2 (t = 8.31, P < 0.001), 6 (t = 2.84, P = 0.03), and 24 (t = 2.94, P = 0.03; Fig. 2) hours of fasting compared to the same

Table 1
Results summary table for each measured parameter.

Variable	Fasting duration			
	1 h	2 h	6 h	24 h
Baseline CORT	↑	↑	↑	↑
Peak CORT	↔	↔	↔	↔
Total fat	↔	↔	↓	↓
Muscle profile	↔	↔	↔	↓
Locomotor	↑	↑	↑	↑
Body mass	↓	↓	↓	↓
Hematocrit	↔	↔	↑	↓
Food intake	↓	↓	↓	↓

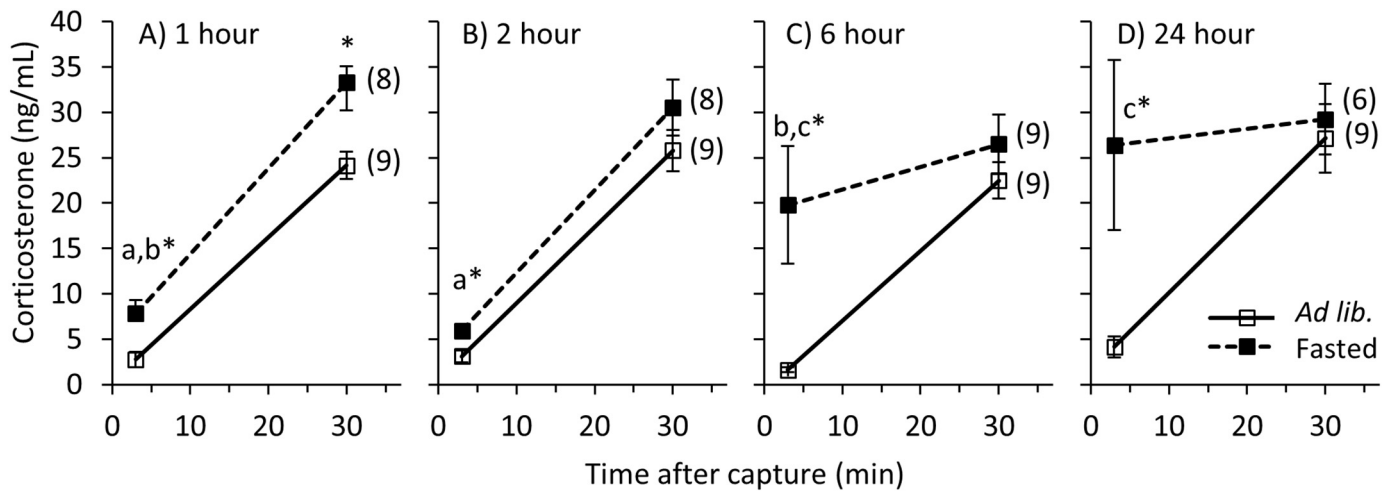


Fig. 2. Plasma corticosterone at both baseline and stress-induced concentrations in white-crowned sparrows after having fasted (filled symbols) for (A) 1, (B) 2, (C) 6 and (D) 24 h compared to the same individuals when food provided *ad libitum* (open symbols). Asterisks (*) indicated significant differences between *ad libitum* and fasted individuals. Letters indicate significant differences in baseline concentrations of corticosterone across groups varying in the duration of fasting. Stress-induced concentrations of corticosterone were not different across any group. Values are presented as means \pm SEM. Significance was determined at $P < 0.05$.

individuals provided food *ad libitum* collected one week earlier. A comparison of baseline corticosterone concentrations across groups showed that birds fasted for 24 h had higher baseline corticosterone concentrations than 1 ($t = 2.13, P = 0.03$) and 2 ($t = 1.94, P = 0.05$) hour groups while the 6 h group had higher baseline concentrations than the 2 ($t = 2.64, P = 0.008$) hour group.

Fasted birds exposed to acute stress increased corticosterone concentrations ($F_{1,56} = 55.82, P < 0.001$) and concentrations differed between *ad libitum* and fasted groups by treatment ($F_{3,56} = 5.18, P = 0.003$; Fig. 1). Stress-induced concentrations were significantly higher in fasted compared to *ad libitum* birds for the 1 h group ($t = 7.01, P = 0.003$) but not 2 ($t = 2.18, P = 0.06$), 6 ($t = 1.53, P = 0.16$), or 24 ($t = 1.59, P = 0.17$) hour groups.

3.2. Corticosterone, condition, and activity relationships

Baseline corticosterone was affected by total fat scores during fasting ($\chi^2 = 16.77, P = 0.01$) but not when food was provided *ad libitum* ($\chi^2 = 2.92, P = 0.71$; Fig. 3). Baseline corticosterone concentrations were significantly higher in birds with a fat score of 0 compared to 3–8 although not different from a score of 1 or 2. Baseline concentrations of corticosterone, when food was provided *ad libitum*, was not significantly related to body mass using a linear regression

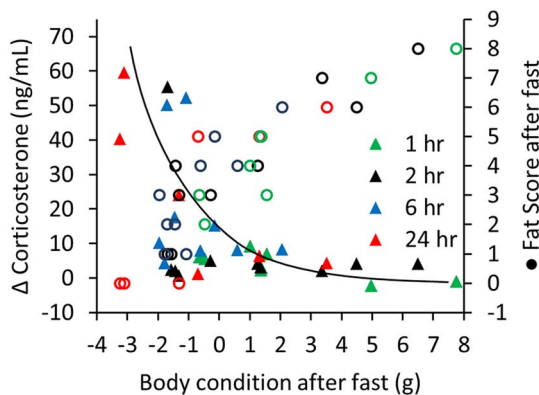


Fig. 3. The relationship between body conditions and total fat scores (open circles) and baseline corticosterone concentrations (filled triangles) in white-crowned sparrows fasted for 1, 2, 6, and 24 h. There was an insufficient sample size was available to analyze each fasting duration separately. The relationship between body condition and corticosterone was shown using an exponential decay curve for fasted birds.

($R^2 = 0.002, P = 0.99$) while during fasting an exponential decay curve proved to be the best fit ($R^2 = 0.33, P = 0.006$; Fig. 3). There was no relationship between activity data and baseline concentrations of corticosterone during fasting ($R^2 = 0.004, P = 0.91$) or in the presence of food ($R^2 = 0.008, P = 0.64$).

The amount of food consumed on the recovery day was positively correlated with the change in baseline corticosterone ($R^2 = 0.64, P = 0.0006$; Fig. 4a). Change in fat from *ad libitum* to fasted conditions was negatively correlated with the change in baseline corticosterone concentrations ($R^2 = 0.33, P = 0.046$, Fig. 4b). Change in body mass from *ad libitum* to fasting conditions was positively correlated with the change in stress-induced corticosterone concentrations ($R^2 = 0.39, P = 0.02$, Fig. 4c). No other relationships were found for either baseline or stress-induced corticosterone concentrations when compared to changes in fat, mass, food intake, or activity from *ad libitum* to fasted conditions or fasted to recovery conditions.

3.3. Body mass

Following transfer from the field to captivity, body mass increased in the 1 ($t = 3.28, P = 0.003$), 2 h groups ($t = 4.19, P < 0.001$), remained unchanged for the 6 h group ($t = 0.81, P = 0.42$) and declined in 24 h group ($t = 3.07, P = 0.004$; Table 1 & Fig. 5a). There was a significant main effect of the duration of the fast ($F_{3,124} = 6.65, P < 0.001$), day of the study ($F_{2,124} = 11.87, P < 0.001$) but the interaction was not significant ($F_{6,124} = 2.73, P = 0.06$). Body mass was lower in all fasted individuals, regardless of treatment group, compared to when food was provided *ad libitum* ($t = 4.83, P < 0.001$). Mass loss during the fast was greatest in the 24 h group and least in the 1 h group although not significantly different from the 2 h group (Fig. 5b). Mass gain on the day of recovery was not sufficient to return mass to pre-fasted levels for the 1 ($t = 7.28, P < 0.001$), 2 ($t = 4.33, P < 0.001$), 6 ($t = 13.42, P < 0.001$) and 24 ($t = 10.14, P < 0.001$) hour groups, respectively. The greatest mass gain occurred in the 24 h food restricted birds while 6 h group had the lowest mass gain on the recovery day ($F_{3,28} = 27.53, P < 0.001$; Tukey's HSD $P < 0.05$; Fig. 3b).

3.4. Total fat scores

Fat scores significantly increased in all groups following initial capture ($\chi^2 = 63.5, DF = 7, P < 0.001$; Fig. 5c). Fat scores were lowest in the 24 h group both at pre-fasted ($\chi^2 = 36.83, P = 0.04$) and

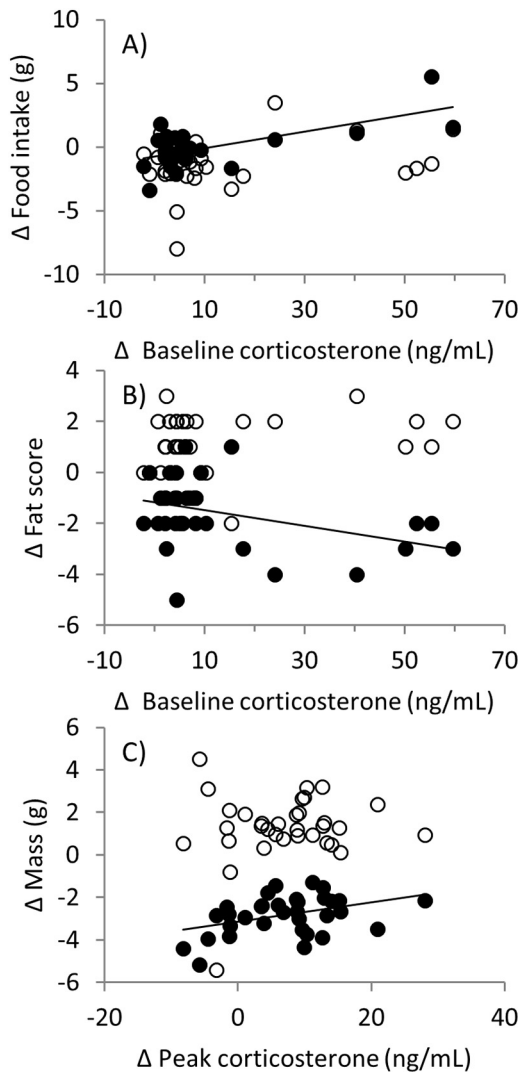


Fig. 4. Relationships between changes in either baseline or stress-induced corticosterone concentrations in relationship to changes in food intake, fat, and mass for *ad libitum* to fasted conditions and fasted to recovery conditions. Filled circles indicated changes from *ad libitum* to fasted conditions and open circles indicate fasted to recovery.

fasted ($\chi^2 = 45.5$, $P = 0.005$) time points. Fasting induced measurable declines in fat at 6 ($\chi^2 = 16.36$, $DF = 6$, $P = 0.01$) and 24 h ($\chi^2 = 16.13$, $DF = 4$, $P = 0.02$) but not for 1 ($\chi^2 = 10.80$, $DF = 7$, $P = 0.14$) or 2 ($\chi^2 = 4.49$, $P = 0.72$; Table 1 & Fig. 5c) hours. Comparison across fasted groups showed that greater fat loss occurred in the 24 h group compared to 1 ($\chi^2 = 14.90$, $DF = 6$, $P = 0.01$) and 2 ($\chi^2 = 9.40$, $DF = 6$, $P = 0.05$) while not different from 6 h group ($\chi^2 = 5.58$, $DF = 6$, $P = 0.13$; Fig. 5d). Fat gain on the recovery day was not different across the groups ($\chi^2 = 21.57$, $DF = 6$, $P = 0.11$; Fig. 5d). However, total fat scores on recovery day, post food removal, were significantly lower in the group fasted for 6 h ($\chi^2 = 11.37$, $DF = 7$, $P = 0.04$) compared to when food was provided *ad libitum*, while the 1 ($\chi^2 = 8.31$, $DF = 7$, $P = 0.21$), 2 ($\chi^2 = 4.49$, $DF = 7$, $P = 0.72$), 24 ($\chi^2 = 8.89$, $DF = 4$, $P = 0.11$) hour groups were indistinguishable (Fig. 5c).

3.5. Muscle profile

Upon transfer to captivity muscle profile significantly declined in the 24 ($\chi^2 = 9.34$, $P = 0.009$) hour group while no differences were detected for the 1 ($\chi^2 = 1.74$, $P = 0.41$), 2 ($\chi^2 = 0.40$, $P = 0.52$), or 6 ($\chi^2 = 0.41$, $P = 0.81$; Fig. 5e) hour groups. While birds were provided

food *ad libitum* muscle size was not different across the groups used in the food removal study ($\chi^2 = 4.19$, $DF = 3$, $P = 0.65$). Muscle profiles were unaffected by fasting ($\chi^2 = 4.21$, $DF = 3$, $P = 0.12$; Table 1 & Fig. 5e). If the 24 h group is run in a paired *t*-test then it is significant ($t = 3.16$, $P = 0.01$) hours. The change in muscle scores were not different across groups either on the day of the fast ($\chi^2 = 10.63$, $P = 0.10$) or on the recovery day ($\chi^2 = 7.54$, $DF = 3$, $P = 0.27$; Fig. 3f). Muscle profiles were the same between recovery day and *ad libitum* except for the 24 h group in which muscle profiles were larger ($\chi^2 = 6.08$, $P = 0.05$). Muscle profiles did not differ across groups on the recovery day ($\chi^2 = 6.79$, $DF = 3$, $P = 0.07$).

3.6. Food intake

Food intake did not differ between the groups when food was provided *ad libitum* ($F_{3,32} = 0.30$, $P = 0.82$; Fig. 5g). Food intake during the 24 h sampling period was significantly reduced following food removal in the 1 ($t = 3.07$, $P = 0.01$), 2 ($t = 4.46$, $P = 0.002$), and 6 ($t = 2.96$, $P = 0.01$) and 24 h group ($t = 5.02$, $P = 0.001$; Table 1 & Fig. 5g). A comparisons across groups showed that food intake was lowest in the 24 ($F_{3,29} = 13.57$, $P = 0.01$; Fig. 5h). During the recovery day food intake was not different compared to pre-fasted levels in the 1 ($t = 1.97$, $P = 0.08$), 2 ($t = 0.25$, $P = 0.80$) but was higher in the 24 h group ($t = 2.69$, $P = 0.04$) and the level was greater than all other groups.

3.7. Hematocrit levels

Hematocrit levels were not different across groups during the pre-fasted *ad libitum* blood sampling period ($F_{3,35} = 1.77$, $P = 0.17$). Hematocrit levels significantly increased following fasting in the 6 h group ($t = 2.46$, $P = 0.03$) but did not change in the 1 ($t = 0.58$, $P = 0.57$), 2 ($t = 0.61$, $P = 0.56$) or the 24 ($t = 0.61$, $P = 0.56$) hour groups. Total hematocrit levels were different across groups following fasting ($F_{3,32} = 2.91$, $P = 0.05$). Hematocrit levels were significantly lower in the 24 compared to the 1 ($t = 3.15$, $P = 0.01$) and 6 h group ($t = 5.16$, $P = 0.009$).

3.8. Daily activity

During the food removal period, activity was increased in the 1 ($F_{2,69} = 3.2$, $P = 0.04$), 2 ($F_{2,242} = 41.35$, $P < 0.001$), 6 ($F_{2,706} = 176$, $P < 0.001$), and 24 ($F_{2,1860} = 75.19$, $P < 0.001$) hour fasted groups compared to control and recovery days (Table 1 & Fig. 6). The 24 h fasted group had lower total day time activity on the day of recovery compared to both control and experimental days while no other differences were detected for the other groups.

4. Discussion

4.1. Effects of fasting on baseline corticosterone concentrations

Baseline corticosterone concentrations increased in response fasting, regardless of the duration, but the variance was greatest in the 6 and 24 h groups. High corticosterone was best explained by an exponential decay curve in which overall body condition that was lower than the population average and coupled to fat scores that were lower than two. Birds with very high corticosterone (> 20 ng/mL) concentrations were found in 2, 6 and 24 h groups which suggests that high concentrations are not solely dependent upon the duration of the fast. The relationships between corticosterone, fat scores, and body condition provides evidence in support of the allostasis model in that corticosterone concentrations are not elevated until energetic stores are nearly exhausted and energy cannot be gained in sufficient quantity from the environment to support daily metabolic needs [2]. Similar nonlinear relationships between corticosterone and body condition

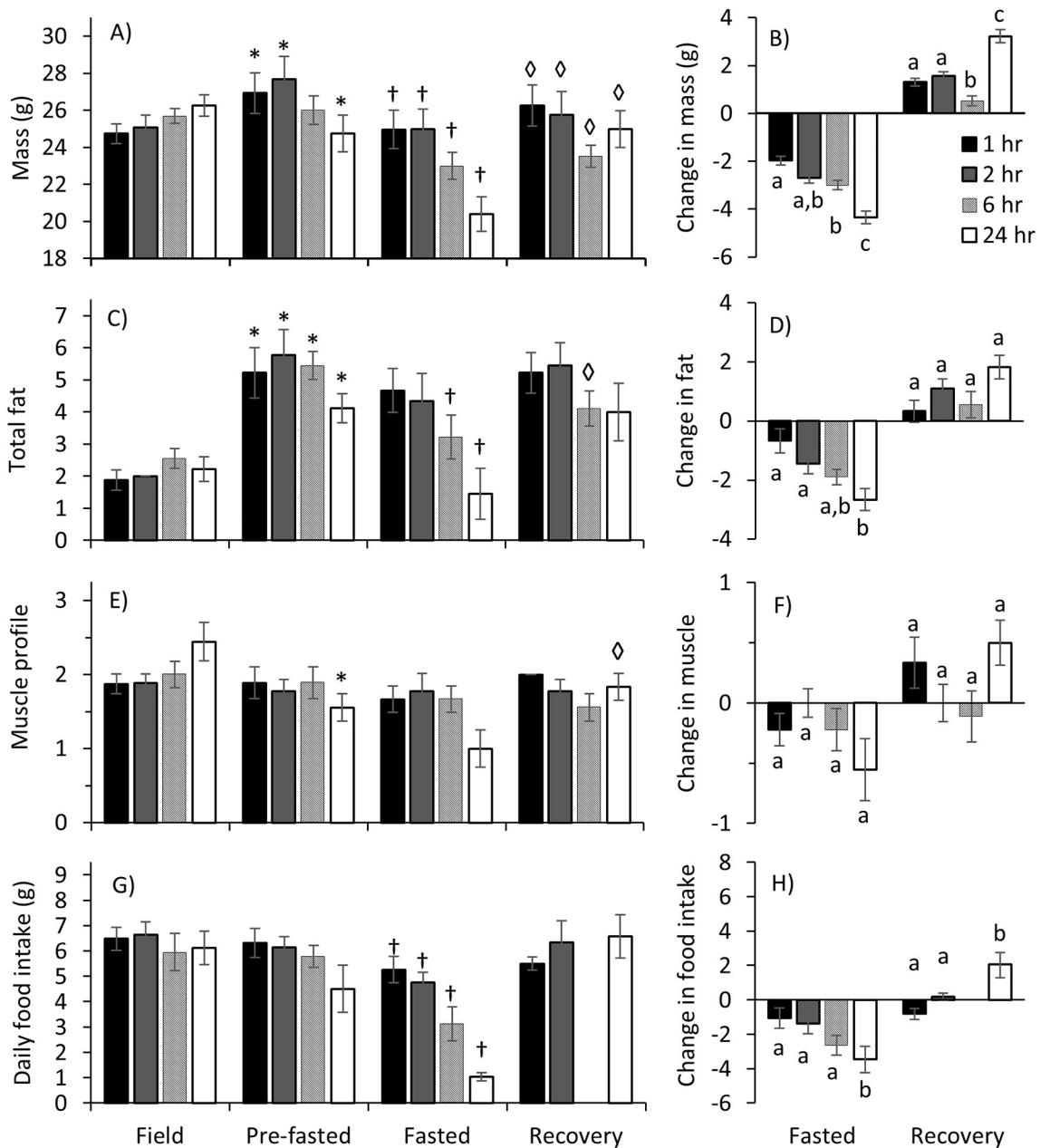


Fig. 5. The effects of fasting duration on the raw measurements and the relative change in (A, B) body mass, (C, D) total fat, (E, F) muscle profile, and (G, H) food intake. Significant differences between the days of the experiment are indicated by asterisks (*) for differences between field caught and pre-fasted (*ad libitum*); daggers (†) for pre-fasted (*ad libitum*) and fasted individuals; and diamonds (◇) for pre-fasted and recovery. Food intake data for 6 h group on the day of recovery was not collected due logistical problems. Letters that are different indicate significant differences across groups varying in the duration of fasting.

have been reported in previous studies [15,24,25,50].

The time course for the elevation in baseline corticosterone concentrations over the rather short period of 1–2 h is of note as gastric emptying rates for small songbirds has been estimated at 2–3 h [36,51] and birds had access to food before the experiment started. The rapid elevation in baseline corticosterone suggests a psychological, as opposed to a metabolic, stressor that is responsible in birds fasted over short durations. While high fasted baseline corticosterone may identify birds that were in stage 3 fasting (the point at which fat stores are nearing exhaustion and protein catabolization is reinitiated which is known to elicit increases in corticosterone concentrations across taxa). The degree to which baseline corticosterone can change in the wild can be quite variable during poor weather conditions with concentrations often being elevated [22,23,25–27,32,37] but can also remain low near baseline concentrations compared to good weather [52], which may

suggest that if ample forage is available and body condition can be maintained that baseline corticosterone concentrations may not significantly rise. Similar in this study no relationships between body condition and corticosterone could be identified when food was provided *ad libitum*. Ecological perturbations that have a negative impact on an animal's ability to forage often result in increased corticosterone concentrations which is likely to activate the emergency life history stage [29].

The hormone data corroborate the findings Lynn et al. [22] for the groups fasted for 1, 2, and 6 h but 24 h whose baseline concentrations were the same as pre-fasted conditions. Our study along with several other studies use a similar design in which measurements are taken prior to food removal and then on the day of removal for all individuals and cannot fully remove the confound of duration of time in captivity. Despite this shortcoming, consistent patterns of elevated corticosterone

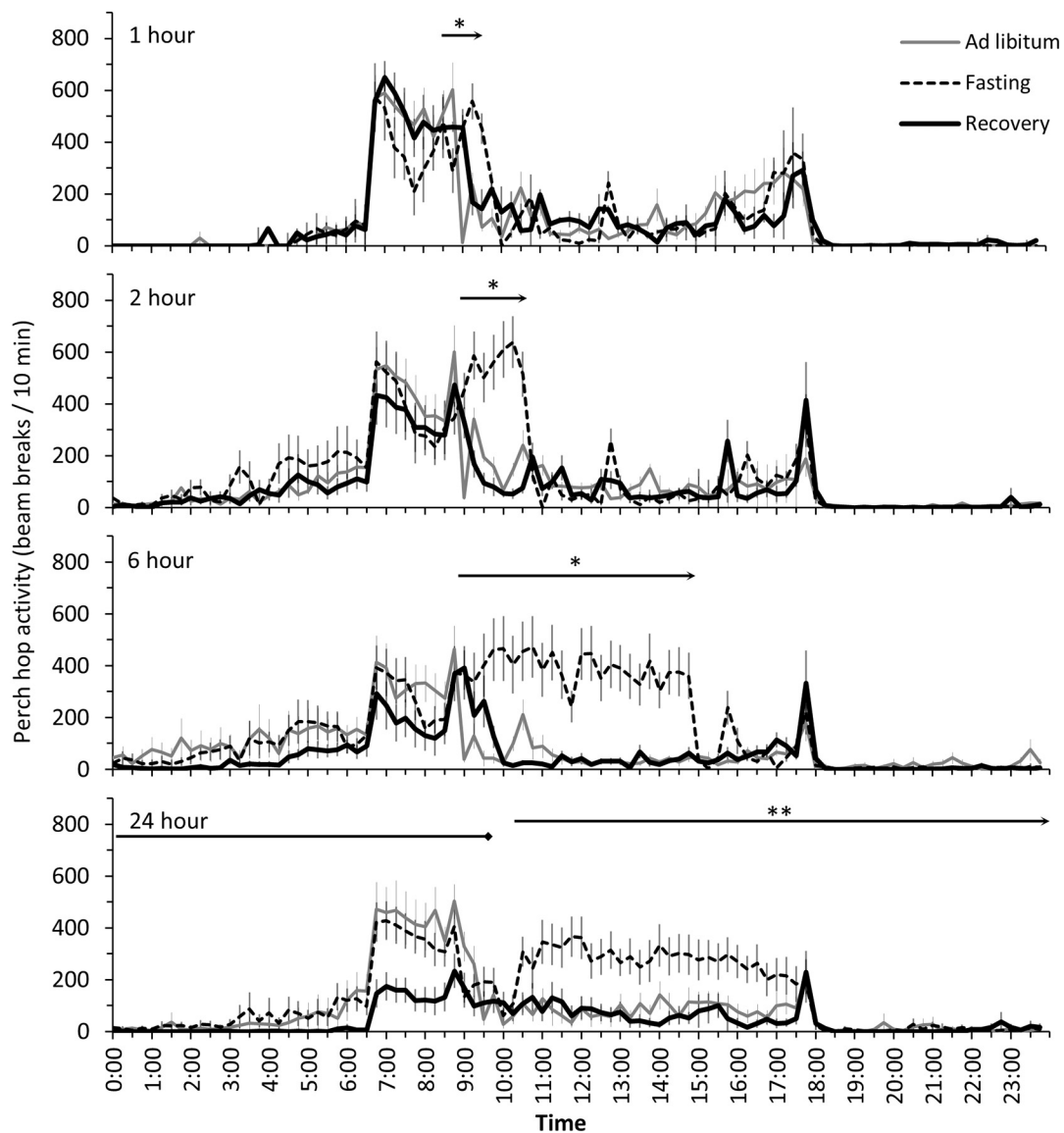


Fig. 6. Effects of fasting for 1, 2, 6, and 24 h on locomotor activity on days prior to the fast, during the fast, and on the recovery day. Asterisks (*) indicate a significant difference between fasting and both pre-fasted and recovery periods. Total activity was significantly lower on the recovery day compared to pre-fasted in the 24 h group, only.

in response to food removal would suggest that it is not solely an artifact of the study design [22,24,26,32,53]. The data suggest that if good overall body condition is maintained during the fast then baseline corticosterone concentrations will remain relatively low. It is difficult to make direct comparisons because body condition measures were not reported and the experiment was performed on long day birds as compared to our short day birds which may have influenced circadian patterns of corticosterone secretion.

4.2. Effects of fasting on stress-induced corticosterone concentrations

The current study identified differential modulation of stress-induced corticosterone concentrations over a short time frame following food removal in which the system becomes hyper-sensitized to acute restraint stress for the 1 h group and a trend for the 2 h group. This might be explained by an initial strong psychological stress when the food is first removed which later subsides after six hours. The regulation of three stages of fasting and *ad libitum* conditions may likely be linked to unique stress profiles that differ in how rapidly corticosterone concentrations are elevated. The HPA axis has the greatest capacity for activation, as measured by stress-induced corticosterone,

during breeding [54] and this has been hypothesized to promote a more rapid response to a perturbation [2]. How the rapid changes in HPA axis activation are achieved remain enigmatic despite several studies. Adrenal activation in white-crowned sparrows appears to be maximal during the winter months as injections of adrenocorticotrophic hormone (ACTH), corticotropin releasing hormone (CRH), or arginine vasotocin (AVT) failed to elevate corticosterone concentrations above restraint handling alone [55]. Other possible mechanism by which HPA axis modulation can occur may be related to control of negative feedback through receptor expression [56,57], sympathetic input to the adrenal gland [58,59], expression of 11-β hydroxysteroid dehydrogenase [60], local negative feedback mechanisms [61] and the endocannabinoid system [62] can all regulate corticosterone secretion.

4.3. Locomotor activity

Locomotor activity was increased following food removal and remained elevated until food was returned. Hyperactivity in response to food removal has previously been documented in birds and was described as a food seeking behavior [22,24,36,37]. We predicted that the degree of hyperactivity would be related to the corticosterone

concentrations but we failed to find this relationship. Exogenous administration of corticosterone has identified relationships with locomotion in the field [35] and in the laboratory during *ad libitum* [63] and fasted conditions [37]. Following the fast, locomotor activity was reduced on the recovery day in the 24 h food removal group only.

4.4. Body condition

Body mass and fat scores were reduced by fasting, although dependent upon the duration of the fast, while pectoralis muscle profiles and hematocrit were not negatively affected. Birds with the highest baseline concentrations of corticosterone during the fast also had the greatest decline in total fat scores. Exposure to exogenous corticosterone under *ad libitum* conditions can reduce body mass, fat scores, and muscle scores in white-crowned sparrows suggesting that corticosterone, under fasting conditions, is likely adding in the production metabolites to fuel metabolism [31,64]. In addition, blocking the glucocorticoid receptor decreases circulating free fatty acids in the blood of white-crowned sparrows [34]. Taken together this may suggest that elevated concentrations of corticosterone are driving fat metabolism in white-crowned sparrows.

A somewhat paradoxical result was the positive relationship between stress-induced corticosterone concentrations and body condition although this has been previously reported in birds [24]. It is possible that there is a two tiered system for regulating changes in body condition during the food stressor in which the second tier is selected if conditions become even worse as mimicked by the restraint stress. Following the fast, body mass did not fully recover while total fat scores and pectoralis muscle profiles were returned to normal except for lower fat in the 6 h group and greater muscle profile in the 24 h group. Overall, the 24 h group endured the greatest change in overall body condition and even increased pectoralis scores on the recovery day beyond estimates on the pre-fasted day. The return of food following the fast and the rapid deposition of fat is not surprising given the rate at which fat is often lost and gained over the course of the 24 h period in small passerines [65]. The failure to regain total body mass is likely attributable to muscle loss. In the wild, some individuals are able to increase body condition during harsh conditions [66] while others able to maintain body condition despite harsh conditions [5].

4.5. Food intake

Fasting and acute restraint stress caused measurable declines in food intake in all groups. The reduction in food intake is rather surprising for the 1–2 h group given the minimal relative disturbance within the lights on period and ample time to become hyperphagic for the remaining part of the day. Morton [41] in 1967, showed the white-crowned sparrows tend to eat at a consistent rate throughout the day and the degree of decline in food intake may be a linear function of time that food was not present. The 24 h group increased food intake following the fast and had the greatest mass gain during the recovery period. The change in baseline corticosterone concentrations from *ad libitum* to fasted conditions were positively correlated with the change in food intake on the day of recovery. Contrary to our study, a significant negative relationship was found between baseline corticosterone levels and food intake on the recovery days in Northern wheatears [67]. In wild birds corticosterone might prime feeding circuits in the brain on the recovery day following the disturbance to reestablish body condition but this may context dependent. Whether or not corticosterone is the direct signal for hyperphagia following fasting is still under debate as opioids and cholecystokinin have also been implicated in regulating feeding in white-crowned sparrows [37,68,69].

5. Conclusion

Wild free-living animals are often exposed to severe environmental

conditions that limit access to food. This current study suggests that the lack of food is responsible, at least in part, for the rapid elevation both baseline corticosterone under any duration of fast and stress-induced concentrations during short-term fasts. Whether or not these rapid changes in HPA axis activation are the result of metabolic or psychological stressors still needs to be fully explored. Corticosterone signaling is likely regulating metabolic processes, along with other hormones, to facilitate the mobilization of fuel stores as reflected through reductions mass and fat scores. The degree to which the HPA axis was activated was linked to body condition as lighter and leaner individuals displayed the most robust response to food removal. The rapid changes in body mass can occur in as little as one hour while longer duration fasts are required to reduce fat scores. The reduction in body condition is likely exacerbated by the hyperactivity associated with food seeking behavior. Overall, this study provides new insights in the rapid changes in physiology, body condition, and activity in response to ecologically relevant challenges such as food deprivation occurring over various durations.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.physbeh.2017.04.026>.

Acknowledgements

This work was supported by the National Science Foundation Office of Polar Programs ARC 0909133 and Integrative Organismal Systems IOS1558049 to John C. Wingfield. Jonathan H. Pérez was supported by National Science Foundation Graduate Research Fellowships (NSF GFRP 1148897). Simone L. Meddle acknowledges Roslin Institute strategic grant funding from the Biotechnology and Biological Sciences Research Council (BB/J004316/1 and BB/J004332/1). We would like to thank everyone the helped with blood sampling: Thomas Hahn, Gabby Names, Emilie Graves, Caroline Newell, Karen Word, Helen Chmura, Rebecca Calisi, Jeffrey Cheah, Ariela Reyna, Marilyn Ramenofsky, and Guilherme Marinho.

References

- [1] M.D. Smith, An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research, *J. Ecol.* 99 (2011) 656–663.
- [2] J.C. Wingfield, J.S. Krause, J.H. Perez, H.E. Chmura, Z. Németh, K.R. Word, R.M. Calisi, S.L. Meddle, A mechanistic approach to understanding range shifts in a changing world: what makes a pioneer? *Gen. Comp. Endocrinol.* 222 (2015) 44–53.
- [3] M.J. Sheriff, C.J. Krebs, R. Boonstra, The ghosts of predators past: population cycles and the role of maternal programming under fluctuating predation risk, *Ecology* 91 (2010) 2983–2994.
- [4] C. Both, R.G. Bijlsma, M.E. Visser, Climatic effects on timing of spring migration and breeding in a long-distance migrant, the pied flycatcher *Ficedula hypoleuca*, *J. Avian Biol.* 36 (2005) 368–373.
- [5] J.S. Krause, J.H. Pérez, H.E. Chmura, S.L. Meddle, K.E. Hunt, L. Gough, N. Boelman, J.C. Wingfield, The stress response is attenuated during inclement weather in parental, but not in pre-parental, Lapland longspurs (*Calcarius lapponicus*) breeding in the Low Arctic, *Horm. Behav.* 83 (2016) 68–74.
- [6] J.C. Wingfield, M. Ramenofsky, Hormone-behavior interrelationships of birds in response to weather, *Adv. Study Behav.* 43 (2011) 93.
- [7] S.J. Schoech, M.A. Rensel, E.S. Bridge, R.K. Boughton, T.E. Wilcoxon, Environment, glucocorticoids, and the timing of reproduction, *Gen. Comp. Endocrinol.* 163 (2009) 201–207.
- [8] M.J. Sheriff, B. Dantzer, B. Delehanty, R. Palme, R. Boonstra, Measuring stress in wildlife: techniques for quantifying glucocorticoids, *Oecologia* 166 (2011) 869–887.
- [9] J.C. Wingfield, R.M. Sapolsky, Reproduction and resistance to stress: when and how, *J. Neuroendocrinol.* 15 (2003) 711–724.
- [10] M.M. Landys, M. Ramenofsky, J.C. Wingfield, Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes, *Gen. Comp. Endocrinol.* 148 (2006) 132–149.
- [11] E.R. de Kloet, From receptor balance to rational glucocorticoid therapy, *Endocrinology* 155 (2014) 2754–2769.
- [12] M. Joëls, H. Karst, R. DeRijk, E.R. de Kloet, The coming out of the brain mineralocorticoid receptor, *Trends Neurosci.* 31 (2008) 1–7.
- [13] L.B. Astheimer, W.A. Buttemer, J.C. Wingfield, Seasonal and acute changes in adrenocortical responsiveness in an arctic-breeding bird, *Horm. Behav.* 29 (1995) 442–457.
- [14] S.A. Raouf, L.C. Smith, M.B. Brown, J.C. Wingfield, C.R. Brown, Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows, *Anim.*

- Behav. 71 (2006) 39–48.
- [15] S. Jenni-Eiermann, E. Glaus, M. Grüebler, H. Schwab, L. Jenni, Glucocorticoid response to food availability in breeding barn swallows (*Hirundo rustica*), Gen. Comp. Endocrinol. 155 (2008) 558–565.
- [16] G.T. Smith, J.C. Wingfield, R.R. Veit, Adrenocortical response to stress in the common diving petrel, *Pelecanoides urinatrix*, Physiol. Zool. 67 (1994) 526–537.
- [17] J. Ouyang, Á. Lendvai, R. Dakin, A. Domalik, V. Fasanello, B. Vassallo, M. Haussmann, I. Moore, F. Bonier, Weathering the storm: parental effort and experimental manipulation of stress hormones predict brood survival, BMC Evol. Biol. 15 (2015) 1–8.
- [18] F. Angelier, J.C. Wingfield, C. Parenteau, M. Pellé, O. Chastel, Does short-term fasting lead to stressed-out parents? A study of incubation commitment and the hormonal stress responses and recoveries in snow petrels, Horm. Behav. 67 (2015) 28–37.
- [19] M. Spée, M. Beaulieu, A. Dervaux, O. Chastel, Y. Le Maho, T. Raclot, Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adélie penguin, Horm. Behav. 58 (2010) 762–768.
- [20] A.-M. Thierry, S. Masmélin, Y. Handrich, T. Raclot, Elevated corticosterone levels and severe weather conditions decrease parental investment of incubating Adélie penguins, Horm. Behav. 63 (2013) 475–483.
- [21] A.S. Kitaysky, J.F. Piatt, J.C. Wingfield, M. Romano, The adrenocortical stress-response of black-legged kittiwake chicks in relation to dietary restrictions, J. Comp. Physiol. B. 169 (1999) 303–310.
- [22] S.E. Lynn, C.W. Breuner, J.C. Wingfield, Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird, Horm. Behav. 43 (2003) 150–157.
- [23] S.E. Lynn, T.B. Stamps, W.T. Barrington, N. Weida, C.A. Hudak, Food, stress, and reproduction: short-term fasting alters endocrine physiology and reproductive behavior in the zebra finch, Horm. Behav. 58 (2010) 214–222.
- [24] H.B. Fokidis, L. Hurley, C. Rogowski, K. Sweazea, P. Deviche, Effects of captivity and body condition on plasma corticosterone, locomotor behavior, and plasma metabolites in curve-billed thrashers, Physiol. Biochem. Zool. Ecol. Evol. Approaches 84 (2011) 595–606.
- [25] Á.Z. Lendvai, J.Q. Ouyang, L.A. Schoenle, V. Fasanello, M.F. Haussmann, F. Bonier, I.T. Moore, Experimental food restriction reveals individual differences in corticosterone reaction norms with no oxidative costs, PLoS One 9 (2014) e110564.
- [26] J.M. Cornelius, C.W. Breuner, T.P. Hahn, Under a neighbour's influence: public information affects stress hormones and behaviour of a songbird, Proc. Biol. Sci. 277 (2010) 2399–2404.
- [27] A.S. Kitaysky, E.V. Kitaiskaia, J.C. Wingfield, J.F. Piatt, Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks, J. Comp. Physiol. B. 171 (2001) 701–709.
- [28] B.S. McEwen, J.C. Wingfield, The concept of allostasis in biology and biomedicine, Horm. Behav. 43 (2003) 2–15.
- [29] J.C. Wingfield, D.L. Maney, C.W. Breuner, J.D. Jacobs, S. Lynn, M. Ramenofsky, R.D. Richardson, Ecological bases of hormone—behavior interactions: the “Emergency Life History Stage”, Am. Zool. 38 (1998) 191–206.
- [30] J.C. Wingfield, A.S. Kitaysky, Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr. Comp. Biol. 42 (2002) 600–609.
- [31] L.B. Astheimer, W.A. Buttemer, J.C. Wingfield, Corticosterone treatment has no effect on reproductive hormones or aggressive behavior in free-living male tree sparrows, *Spizella arborea*, Horm. Behav. 37 (2000) 31–39.
- [32] H.B. Fokidis, M.B. des Roziers, R. Sparr, C. Rogowski, K. Sweazea, P. Deviche, Unpredictable food availability induces metabolic and hormonal changes independent of food intake in a sedentary songbird, J. Exp. Biol. 215 (2012) 2920–2930.
- [33] M.M. Landys, T. Piersma, M. Ramenofsky, J.C. Wingfield, Role of the low-affinity glucocorticoid receptor in the regulation of behavior and energy metabolism in the migratory red knot *Calidris canutus islandica*, Physiol. Biochem. Zool. 77 (2004) 658–668.
- [34] M.M. Landys, M. Ramenofsky, C.G. Guglielmo, J.C. Wingfield, The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*, J. Exp. Biol. 207 (2004) 143–154.
- [35] C.W. Breuner, T.P. Hahn, Integrating stress physiology, environmental change, and behavior in free-living sparrows, Horm. Behav. 43 (2003) 115–123.
- [36] M.L. Morton, Diurnal feeding patterns in white-crowned sparrows, *Zonotrichia leucophrys gambelii*, Condor 69 (1967) 491–512.
- [37] L.B. Astheimer, W.A. Buttemer, J.C. Wingfield, Interactions of corticosterone with feeding, activity and metabolism in passerine birds, Ornis Scand. 23 (1992) 355–365.
- [38] R.M. Calisi, N.O. Rizzo, G.E. Bentley, Seasonal differences in hypothalamic EGR-1 and GnIH expression following capture-handling stress in house sparrows (*Passer domesticus*), Gen. Comp. Endocrinol. 157 (2008) 283–287.
- [39] P.J. Deviche, L.L. Hurley, H.B. Fokidis, B. Lebour, B. Silverin, J. Sabo, P.J. Sharp, Acute stress rapidly decreases plasma testosterone in a free-ranging male songbird: potential site of action and mechanism, Gen. Comp. Endocrinol. 169 (2010) 82–90.
- [40] R.M. Sapolsky, L.M. Romero, A.U. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, Endocr. Rev. 21 (2000) 55–89.
- [41] L. Remage-Healey, L.M. Romero, Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird, Am. J. Physiol. Regul. Integr. Comp. Phys. 281 (2001) R994–R1003.
- [42] G. Chilton, M. Baker, C. Barrentine, M. Cunningham, White-crowned sparrow no. 183, The Birds of North America. The American Ornithologist's Union, Academy of Natural Sciences of Philadelphia, 1995.
- [43] J.H. Pérez, J.D. Furlow, J.C. Wingfield, M. Ramenofsky, Regulation of vernal migration in Gambel's white-crowned sparrows: role of thyroxine and triiodothyronine, Horm. Behav. 84 (2016) 50–56.
- [44] M. Ramenofsky, Z. Németh, Regulatory mechanisms for the development of the migratory phenotype: roles for photoperiod and the gonad, Horm. Behav. 66 (2014) 148–158.
- [45] F. Bairlein, D. Simons, Nutritional adaptations in migrating birds, Israel J. Zool. 41 (1995) 357–367.
- [46] A. Kaiser, A new multi-category classification of subcutaneous fat deposits of songbirds (Una Nueva Clasificación, con multi-categorías, para los Depósitos de Grasa en Aves Canoras), J. Field Ornithol. (1993) 246–255.
- [47] L.M. Romero, J.M. Reed, Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 140 (2005) 73–79.
- [48] J.C. Wingfield, C.M. Vleck, M.C. Moore, Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert, J. Exp. Zool. 264 (1992) 419–428.
- [49] J.S. Krause, Z. Németh, J.H. Pérez, H.E. Chmura, M. Ramenofsky, J.C. Wingfield, Annual hematocrit profiles in two subspecies of white-crowned sparrow: a migrant and a resident comparison, Physiol. Biochem. Zool. 89 (2016) 51–60.
- [50] L.M. Romero, M. Wikelski, Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Niño events, Proc. Natl. Acad. Sci. 98 (2001) 7366–7370.
- [51] J. Stevenson, Experiments on the digestion of food by birds, Wilson Bull. 45 (1933) 155–167.
- [52] J.S. Krause, J.H. Pérez, H.E. Chmura, S.K. Sweet, S.L. Meddle, K.E. Hunt, L. Gough, N. Boelman, J.C. Wingfield, The effect of extreme spring weather on body condition and stress physiology in Lapland longspurs and white-crowned sparrows breeding in the Arctic, Gen. Comp. Endocrinol. 237 (2016) 10–18.
- [53] A. Kitaysky, J. Wingfield, J. Piatt, Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes, Funct. Ecol. 13 (1999) 577–584.
- [54] M.L. Romero, Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates, Gen. Comp. Endocrinol. 128 (2002) 1–24.
- [55] L.M. Romero, J.C. Wingfield, Alterations in hypothalamic-pituitary-adrenal function associated with captivity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 122 (1999) 13–20.
- [56] J.S. Krause, M.A. McGuigan, V.R. Bishop, J.C. Wingfield, S.L. Meddle, Decreases in mineralocorticoid but not glucocorticoid receptor mRNA expression during the short Arctic breeding season in free-living Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*), J. Neuroendocrinol. 27 (2015) 66–75.
- [57] Z.G. Hodgson, S.L. Meddle, M.L. Roberts, K.L. Buchanan, M.R. Evans, R. Metzendorf, M. Gahr, S.D. Healy, Spatial ability is impaired and hippocampal mineralocorticoid receptor mRNA expression reduced in zebra finches (*Taeniopygia guttata*) selected for acute high corticosterone response to stress, Proc. R. Soc. B Biol. Sci. 274 (2007) 239–245.
- [58] Y.M. Ulrich-Lai, W.C. Engeland, Adrenal splanchnic innervation modulates adrenal cortical responses to dehydration stress in rats, Neuroendocrinology 76 (2002) 79–92.
- [59] Y.M. Ulrich-Lai, M.M. Arnhold, W.C. Engeland, Adrenal splanchnic innervation contributes to the diurnal rhythm of plasma corticosterone in rats by modulating adrenal sensitivity to ACTH, Am J Physiol Regul Integr Comp Physiol. 290 (2006) 1128–1135.
- [60] H.J. Harris, Y. Kotelevtsev, J.J. Mullins, J.R. Seckl, M.C. Holmes, Intracellular regeneration of glucocorticoids by 11 β -hydroxysteroid dehydrogenase (11 β -HSD)-1 plays a key role in regulation of the hypothalamic-pituitary-adrenal axis: analysis of 11 β -HSD-1-deficient mice, Endocrinology 142 (2001) 114–120.
- [61] J.J. Walker, F. Spiga, R. Gupta, Z. Zhao, S.L. Lightman, J.R. Terry, Rapid intra-adrenal feedback regulation of glucocorticoid synthesis, J. R. Soc. Interface 12 (2015).
- [62] N.K. Evanson, J.G. Tasker, M.N. Hill, C.J. Hillard, J.P. Herman, Fast feedback inhibition of the hpa axis by glucocorticoids is mediated by endocannabinoid signaling, Endocrinology 151 (2010) 4811–4819.
- [63] C.W. Breuner, J.C. Wingfield, Rapid behavioral response to corticosterone varies with photoperiod and dose, Horm. Behav. 37 (2000) 23–30.
- [64] D.S. Busch, T.S. Sperry, E. Peterson, C.-T. Do, J.C. Wingfield, E.H. Boyd, Impacts of frequent, acute pulses of corticosterone on condition and behavior of Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*), Gen. Comp. Endocrinol. 158 (2008) 224–233.
- [65] E. Lehikoinen, Seasonality of the daily weight cycle in wintering passerines and its consequences, Ornis Scand. (Scand. J. Ornithol.) 18 (1987) 216–226.
- [66] I. Krams, D. Cirule, V. Suraka, T. Krama, M.J. Rantala, G. Ramey, Fattening strategies of wintering great tits support the optimal body mass hypothesis under conditions of extremely low ambient temperature, Funct. Ecol. 24 (2010) 172–177.
- [67] C. Eikenaar, F. Bairlein, M. Stöwe, S. Jenni-Eiermann, Corticosterone, food intake and refueling in a long-distance migrant, Horm. Behav. 65 (2014) 480–487.
- [68] D.L. Maney, J.C. Wingfield, Central opioid control of feeding behavior in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*, Horm. Behav. 33 (1998) 16–22.
- [69] R.D. Richardson, T. Boswell, S.C. Weatherford, J.C. Wingfield, S.C. Woods, Cholecystokinin octapeptide decreases food intake in white-crowned sparrows, Am. J. Physiol. Regul. Integr. Comp. Phys. 264 (1993) R852–R856.