**ENDOCRINE, METABOLIC, AND BEHAVIORAL EFFECTS OF AND RECOVERY FROM ACUTE
STRESS IN A FREE-RANGING BIRD**

Pierre Deviche¹, Stephanie Bittner¹, Scott Davies², Shelley Valle¹, Sisi Gao¹, and Elodie Carpentier³

¹ School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

² Present address: Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA

³ Universite de Poitiers, Faculte des Sciences Fondamentales et Appliquees, Poitiers, F-86022,
France

* Corresponding author's email: deviche@asu.edu

Abstract

Acute stress in vertebrates generally stimulates the hypothalamo-pituitary-adrenal axis and is often associated with multiple metabolic changes, such as increased gluconeogenesis, and with behavioral alterations. Little information is available, especially in free-ranging organisms, on the duration of these reversible effects once animals are no longer exposed to the stressor. To investigate this question, we exposed free-ranging adult male Rufous-winged Sparrows, *Peucaea carpalis*, in breeding condition to a standard protocol consisting of a social challenge (conspecific song playback) followed with capture and restraint for 30 min, after which birds were released on site. Capture and restraint increased plasma corticosterone (CORT) and decreased plasma testosterone (T), glucose (GLU), and uric acid (UA). In birds that we recaptured the next day after exposure to conspecific song playback, plasma CORT and UA levels no longer differed from levels immediately after capture the preceding day. However, plasma T was similar to that measured after stress exposure the preceding day, and plasma GLU was markedly elevated. Thus, exposure to social challenge and acute stress resulted in persistent (≥ 24 hrs) parameter-specific effects. In recaptured sparrows, the territorial aggressive response to conspecific song playback, as measured by song rate and the number of flights over the song-broadcasting speakers, did, however, not differ between the first capture and the recapture, suggesting no proximate functional association between plasma T and conspecific territorial aggression. The study is the first in free-ranging birds to report the endocrine, metabolic, and behavioral recovery from the effects of combined social challenge and acute stress.

Keywords

Antioxidant; acute stress; corticosterone; glucose; glycemia; oxidative stress; passerine; song playback; sparrow; testosterone; uric acid.

1. Introduction

In vertebrates, acute stressors activate the hypothalamo-pituitary-adrenal (HPA) axis, resulting in elevated plasma glucocorticoids (GCs; primarily corticosterone [CORT] in birds; Astheimer et al., 1995; Cockrem et al., 2016; Deviche et al., 2014; O'Reilly and Wingfield 2001). Stressors can also induce metabolic changes including an increase in glycemia (cat, *Felis catus*: Rand et al., 2002; Japanese Quail, *Coturnix coturnix japonica*: Gildersleeve et al., 1985; turkey, *Meleagris gallopavo*: Donaldson et al., 1991; chicken, *Gallus gallus*: Debut et al., 2005) and so enhance the amount of glucose (GLU) available to metabolically active tissues. Stimulatory effects of stress on glycemia are, however, not observed universally. In particular, several avian studies found that plasma GLU did not increase (Curve-billed Thrasher, *Toxostoma curvirostre*: Fokidis et al., 2011; European Starling, *Sturnus vulgaris*: Ramage-Healey and Romero, 2002; Abert's Towhee, *Melospiza aberti*: Davies et al., 2013) or even decreased (Rufous-winged Sparrow, *Peucaea carpalis* [RWSP]: Deviche et al., 2014, 2016; see below) during acute stress. Furthermore, GCs are generally considered to play an important role in mediating the stimulatory effects of stress on glycemia, but the generality of this conclusion in birds is not established. Indeed, the increase in plasma CORT that is observed during stress was associated with elevated plasma GLU in some cases (Debut et al., 2005; Herring Gull, *Larus argentatus*: Jeffrey et al., 1985) but not others (pigeon, *Columba livia*: Viswanathan et al., 1987; chicken: Xie et al., 2015). These differences may stem from CORT stimulating glycemia in some species and not others. Alternately, methodological differences between studies may account for the observed variation in relationship between plasma CORT and GLU. For example, in the European Starling, acute stress elevated plasma CORT, but plasma GLU increased during stress at night and not during daytime (Ramage-Healey and Romero, 2001). In the same study CORT administration at supraphysiological dose did by itself not influence plasma GLU, but this treatment hastened recovery from the hypoglycemic effect of concurrent insulin injection.

Acute stress can also promote oxidative damage (Lin et al., 2006; Huang et al., 2015) and in some avian species, it decreases the plasma level of the main circulating antioxidant, uric acid (UA; Cohen et al., 2007). At the individual level, the decrease in plasma UA during acute stress correlates with the corresponding increase in plasma CORT (Deviche et al., 2016). This correlation suggests a causal relationship whereby elevated plasma CORT lowers plasma UA, but whether this is the case remains unclear. Indeed, UA is a main product of protein degradation and in the chicken, CORT treatment during growth decreased muscle mass and increased UA excretion, suggesting increased UA production (Siegel and Van Kampen, 1984). Furthermore, CORT treatment to chickens increased plasma UA within hours (Simon 1984). Acute stress also often reduces the plasma levels of reproductive hormones such as testosterone (T; Lynn et al., 2010; Deviche et al., 2014; Davies et al., 2016). In mammals, GCs are thought to contribute to this reduction by acting on gonads (Orr and

Mann, 1992; Hardy et al., 2005; Dong et al., 2004; Monder et al., 1994), but few studies have researched this subject in non-mammalian vertebrates.

Most of the avian studies described above investigated captive subjects or domesticated species, and so we know little about relationships between plasma CORT, T, UA, and GLU in free-ranging birds. To address this question, our recent work used free-ranging adult male RWSP, the same species as used in the present work. Exposing RWSP to acute stress (capture and restraint for 30 min) consistently increased plasma CORT and decreased plasma UA (Deviche et al., 2012, 2014, 2016). However, acute stress influenced plasma T and GLU in a life history stage-dependent manner. During stress plasma T decreased in pre-breeding and breeding condition males, when plasma T was initially (i.e., before experimental stress) high, but not in molting and non-breeding males, when levels of this hormone were lower (Deviche et al., 2016). Furthermore, in the same study GLU increased during acute stress in pre-breeding birds, did not change in late-breeding birds, and decreased in birds sampled during post-breeding molt and in winter. We also found that blood hormone and metabolites were selectively influenced by social factors: a social challenge consisting in conspecific song playback exposure for 30 min increased plasma CORT and decreased plasma UA (Deviche et al., 2014) as effectively as restraint for the same duration, but did not affect plasma T or GLU. The *first objective* of the present work was to confirm and extend these findings. For this, we exposed males to song playback, caught them and took a blood sample, restrained them for 30 min, and took a second blood sample. If song playback exposure increases plasma CORT and decreases plasma UA maximally, we predicted that subsequent acute stress exposure would have no additional effect on these parameters. Alternatively, if song playback exposure does not maximally stimulate and depress plasma CORT and UA, respectively, we predicted acute stress to further elevate plasma CORT and decrease plasma UA. The present investigation was conducted using sparrows in breeding condition and so based on the above-described studies, which also used birds in breeding condition, we predicted acute stress to decrease plasma T and either to decrease or to not influence plasma GLU.

Once individuals are no longer exposed to an acute stressor, endocrine effects of stress generally subside. The latency to restore homeostasis has been investigated in humans (Annerstedt et al., 2013) and domesticated animals (Ericsson et al., 2014). For example, in the chicken, plasma CORT increased within 10 min of restraint and had returned to pre-restraint level 50 min after birds were released into their cages (Ericsson et al., 2014). In captive European Starlings, plasma CORT returned to levels approximating initial (pre-stress) levels three hours after stress exposure (Remage-Healey and Romero, 2001). It should be pointed out that these studies provide no information about the time course of recovery from a singular stressful event in free-ranging vertebrates and only one study has, to our knowledge, investigated this question in birds. In this

study on male RWSP, plasma T decreased within 10 min of capture and restraint, and had not recovered after birds were released and recaptured, on average, three hours later (Deviche et al., 2012). The *second objective* of the present work was to further characterize how birds hormonally and metabolically recover from acute stress. To this aim, we measured plasma CORT, T, GLU, and UA in free-ranging adult male RWSP one day after birds were socially challenged, caught, exposed to acute stress as described above, and released on site. Based on the above studies on chickens and captive European Starlings, we predicted plasma CORT levels to be similar at recapture to those measured at the time of first capture the preceding day. If so and if plasma CORT regulates plasma T, GLU, and UA on a short-term basis, we predicted the values of these parameters at the time of recapture to also be similar to those at the time of initial capture. Alternatively, if CORT, but not T, GLU, and/or UA, had returned to initial levels at the time of recapture, this would suggest that changes in plasma T, GLU, and/or UA are proximately controlled by factors other than or in addition to plasma CORT.

Across vertebrates, testosterone (T) generally increases the expression of aggressive behavior (Wingfield et al. 2006). An association between T and the expression of aggressive behavior is supported by the increase in aggression coincident with elevated T during transition to the breeding period, but the correlation between plasma T and within- as well as between-individual variation in aggression is less clear (Wingfield et al., 1987). The *third objective* of the present investigation was to address this question. To this aim we determined the relationships between plasma T and territorial aggression toward conspecific song playback before initial capture and again immediately before recapture the next day. If, at the time of recapture, plasma T was still low compared to initial levels the previous day, and if plasma T proximately regulates territorial aggression, we predicted that birds would be less responsive to song playback one day after the initial capture than on the previous day. To our knowledge, this is the first study to examine the time course of endocrine and metabolic recovery from acute stress in free-ranging birds, as well as the territorial aggressive behavioral correlates of this recovery.

2. Materials and Methods

2.1. Species and Study Site

We sampled 17 experimentally naïve adult male RWSP (17th – 20th July 2014; 6 am – 3 pm MST) during the breeding period of the species at the Santa Rita Experimental Range (Pima Co., AZ, USA; altitude: 979 m.a.s.l.; latitude: 31° 49' N; longitude: 110° 55' W). Breeding in this species closely follows the onset of the summer monsoon which, in the region where the study was

conducted, usually begins in early July (Lowther et al., 1999). Rufous-winged Sparrows are sedentary, males are territorial year-round, and they have been investigated extensively (Lowther et al., 1999; Deviche et al., 2006, 2014, 2016).

2.2. Behavioral Quantification, Capture, and Blood Sampling

On Sample Day 1, we located males based on spontaneous singing or brief (≤ 2 min) response to conspecific song playback and exposed them to a standard protocol consisting of song playback followed with capture and restraint. For this, a mist net was erected but not opened in the vicinity of where the bird was found and the song playback system was placed below the net. The song played for 5 min to give the target bird time to locate the source of the song. Immediately thereafter, we played the song for another 15 min during which we recorded the behavior (the number of flights over the net and the number of songs; Deviche et al., 2014) of the target bird. Behavior data were collected by three observers working in pairs (SD paired either with SB [$n = 8$ birds] or with SV [$n = 9$ birds]). Data collected by pairs of observers were in all cases correlated (Pearson product moment correlations: number of flights: correlation coefficients > 0.99 , $P_s < 0.0001$; number of songs: correlation coefficients > 0.83 , $P_s \leq 0.009$) and they did not differ (Wilcoxon signed ranks tests: $P_s > 0.100$) except that one observer (SD) counted more songs (median ± 0.5 interquartile interval: 70 ± 4) than one of the other two observers (SV; 64 ± 14 ; $P = 0.008$). Given the overall consistency of data collected by pairs of observers, average values of corresponding scores were used for statistical analyses.

At the end of the 15 min behavior observation period, the net was unfurled and left open until the male was caught (~ 5 min). Caught birds had, therefore, been exposed to song playback continuously for approximately 25 min. Within 2 min of capture, we collected a blood sample ($70 \mu\text{l}$) from the jugular vein of each bird into a heparinized syringe as described by Deviche et al. (2012). In a previous study on RWSP belonging to the same population as the present birds and also studied during their breeding period, exposure to song playback for 30 min increased plasma CORT and decreased plasma UA, but did not influence plasma T or GLU (Deviche et al., 2014). Assuming that the same occurred in the present study, we refer to hormone and metabolite concentrations in the first blood sample collected from each bird as initial rather than baseline (i.e., implying prior to song playback exposure and capture) concentrations. After initial blood collection, we stimulated a physiological stress response using a common protocol (Astheimer et al., 1995; Romero and Wingfield, 1998; Rensel and Schoech, 2011) consisting of placing the bird into a cloth bag for 30 min and then collecting a second blood sample ($70 \mu\text{l}$; 30 min stress). We then measured the body mass (± 0.1 g), wing chord (± 1 mm), and cloacal protuberance (CP; ± 0.1 mm) width of each bird (Deviche et al., 2014) and marked it with a US Geological Survey uniquely numbered tarsal

aluminum band plus two colored plastic tarsal bands before release on site. Wing chords and body masses were used to calculate body condition using the scale mass index method (Peig and Green, 2009). Blood samples were immediately placed on ice until centrifuged the same day. Plasma was harvested and stored at -80°C until assayed (see below).

Assuming that approximately 70% of the body mass consists of water (i.e., 10.5 ml water for a 15 g RWSP; Table 1), the total volume of blood taken on Sample Day 1 (140 μl , i.e., approximately 70 μl plasma) represented a relatively negligible (0.7%) loss of body water. Furthermore, the decrease in blood volume (13% of initial volume assuming blood volume equal to 7% of the body mass; McGuill and Rowan, 1989) associated with blood removal was presumably rapidly compensated for by movement of water from other tissues into the blood to restore blood volemia. Still, as RWSP inhabit hot and arid environments where access to drinking water is limited and frequently impossible, we sought to minimize changes in body water volemia and sodium balance resulting from blood sampling by giving each bird an intramuscular injection of saline solution (200 μl ; 0.9 % NaCl in water) before release. A second objective of this treatment was to attenuate potentially stressful effects of blood volume depletion. In the female rat, blood collection decreases plasma prolactin but no such decrease occurs after saline solution therapy immediately following blood removal (Lawson and Gala, 1974). Saline solution administration to sparrows did not compensate for the loss of blood cells resulting from blood removal, which can have numerous biological effects (McGuill and Rowan 1989). However, it is worth noting in mice that repeated blood sampling (up to 15% of the estimated total blood volume weekly for 6 weeks) induced no apparent change in body mass or behavior, and neither did it result in clinically significant anemia (Raabe et al., 2011), suggesting that this manipulation stimulates effective compensatory physiological mechanisms.

The next day (Sample Day 2), we re-visited all of the sites where birds were caught on Sample Day 1 and repeated the above-described behavioral and blood sampling methods. Due to the limited maximum volume of blood that can be collected from a given bird, a single blood sample was collected from each male on Sample Day 2 within 2 min of recapture. Each recaptured sparrow was weighed as above before release. We re-sighted 12 of the 17 sparrows sampled on Sample Day 1 and succeeded in re-capturing seven of these males (6 am - 1 pm; MST), but could not recapture the other five males. Based on these results, we divided the experimental birds into three groups: males that we recaptured ($n = 7$), males that we re-sighted but did not recapture ($n = 5$), and males we did not re-sight ($n = 5$).

All procedures were preapproved by the Arizona State University Institutional Animal Care and Use Committee, and were conducted under current scientific collecting permits (Arizona Game

and Fish Department permit SP673434, US Fish and Wildlife Service permit MB019542-0, and Bird Banding Laboratory permit 22640).

2.3. *Plasma assays*

We assayed plasma samples for CORT (enzyme-linked immunoassay [ELISA]: Enzo Life Sciences, Farmingdale, NY), T (ELISA: Enzo Life Sciences, Farmingdale, NY), GLU (colorimetric assay: Cayman Chemical Co., Ann Arbor, MI), and UA (colorimetric assay: BioAssay Systems, Hayward, CA) concentrations using previously validated methods (Deviche et al., 2006, 2010, 2014). For each blood parameter, all samples were assayed in duplicate, using 96 well plastic plates, and in a single assay. Samples were assigned to plates randomly, but all (max. 3) samples collected from a given male were assayed on the same plate. The assay sensitivities and intra-assay coefficients of variation were as follows: CORT: 0.16 - 0.36 pg/ml (depending on the plate) and 8.04%; T: 0.95 - 1.31 pg/ml and 6.66%; GLU: 6 µg/dL and 1.46%; UA: 70 µg/dL and 3.76%.

2.4. *Statistical analyses*

We compared data collected on Sample Day 1 for birds that were either recaptured on Sample Day 2, re-sighted but not recaptured on Sample Day 2, or not re-sighted on Sample Day 2 using Kruskal-Wallis analysis of variance (ANOVA) on ranks (behavior; fat scores), one-way ANOVA (wing chord; body mass; scaled mass index; CP width), or two way repeated measure RM-ANOVA (initial vs. stress-associated plasma CORT, T, GLU, and UA). We used Student-Newman-Keuls (SNK) tests for pair-wise comparisons following statistically significant ($P \leq 0.05$) ANOVA main effects.

We used one-way RM-ANOVA followed with SNK tests to compare changes over time (initial [Sample Day 1], 30 min stress [Sample Day 1], and Sample Day 2) in blood parameters in sparrows that we recaptured. We used Wilcoxon sign rank tests to compare the behavioral responses of sparrows on Sample Days 1 and 2, and the Mann-Whitney U-test to compare the behavior of sparrows that we re-sighted and then recaptured with that of the birds that we re-sighted but did not recapture on Sample Day 2. The body masses of males that we recaptured on Sample Day 2 were compared to masses on Sample Day 1 using Student's t-test for paired samples.

Associations between plasma hormone and metabolite concentrations were analyzed using Spearman rank correlation coefficients. We also used these coefficients to research associations between capture times on Sample Day 1 and these concentrations.

All data sets used for parametric tests were homoscedastic and did not depart from normal distribution. Morphological, endocrine, and metabolic data are expressed as means $\pm$ standard errors, and behavioral data are expressed as medians $\pm$ 0.5 interquartile intervals. Data were

analyzed using SigmaPlot version 13.0 (Systat Software, Inc., San Jose, CA) and the statistical significance level of all tests was set at $\alpha = 0.05$.

3. Results

3.1. *Endocrine and metabolic effects of acute stress (Sample Day 1)*

There was no relationship on Sample Day 1 between the time of day that birds were caught and any blood parameter concentration (Spearman rank order correlations: $n = 17$, P 's > 0.091).

On Sample Day 1 plasma hormones and metabolites were influenced by the stress of capture and restraint, which increased plasma CORT by 313% and decreased plasma T by 51%, UA by 37%, and GLU by 7% (Table 1 and Fig. 1). There was no indication that birds belonging to the three experimental groups (i.e., recaptured, re-sighted but not recaptured, and not re-sighted on Sample Day 2) differed with respect to plasma hormone or metabolite levels, as shown by non-significant group effects or group x time interactions (RM-ANOVA: Table 1).

We also found no evidence on Sample Day 1 that males belonging to the three groups differed morphologically (wing chord, body mass, body condition, CP width, and fat score) or behaviorally (number of songs and number of flights over net; Table 2).

There was no correlation between initial plasma CORT and initial plasma T, UA, or GLU, between 30 min stress plasma CORT and 30 min stress plasma T, UA, or GLU, or between the change during stress (30 min stress minus initial) in plasma CORT and in plasma T, UA, or GLU (Spearman rank order correlations, all P s > 0.05). There also was no correlation between initial plasma T and initial plasma UA or GLU, 30 min stress plasma T and 30 min stress plasma UA or GLU, or the change during stress in plasma T and in plasma UA or GLU (Spearman rank order correlations, P s all > 0.05). These results provide no support to the hypothesis that there were functional relationships between these parameters either at capture or in response to restraint-related stress. No association was observed on Sample Day 1 between initial plasma T and the number of songs or flights over the net (Pearson product moment correlation: P s > 0.2).

3.2. *Comparison of First Capture (Sample Day 1) vs. Recapture (Sample Day 2) data*

Considering the sparrows that we recaptured, plasma CORT increased in response to capture and restraint on Sample Day 1, but by Sample Day 2 plasma CORT had returned to the initial level measured on Sample Day 1 ($F_{2,12} = 16.05$, $P < 0.001$; Fig. 2). Plasma T decreased in response to capture and restraint, but, contrary to plasma CORT, was still depressed at acute stress level on Sample Day 2 ($F_{2,12} = 9.11$, $P = 0.004$; Fig. 2). Plasma UA followed a different temporal

pattern ($F_{2,12} = 6.83$, $P = 0.01$), decreasing during acute stress and returning to the initial level by Sample Day 2. Plasma GLU also changed over time ($F_{2,12} = 23.05$, $P < 0.001$): in recaptured males, it did not change significantly during acute stress on Sample Day 1 but was markedly elevated on Sample Day 2 relative to Sample Day 1.

The duration of exposure to song playback before capture did not differ between Sample Day 1 (median $\pm$ 0.5 interquartile interval: 25 ± 6 min) and Sample Day 2 (37 ± 22 min; Wilcoxon signed rank test: $P = 0.078$), and birds that we recaptured on Sample Day 2 showed no behavioral difference in response to song playback that day compared to Sample Day 1 (number of songs: Sample Day 1: 62 ± 6 ; Sample Day 2: 84 ± 17 ; number of flights: Sample Day 1: 15 ± 9 ; Sample Day 2: 12 ± 6 ; Wilcoxon sign rank tests: $P_s > 0.29$). As was the case on Sample Day 1, there was no association on Sample Day 2 between plasma T and the number of songs or flights over the net (Pearson product moment correlation: number of songs: $r = 0.09$, $P = 0.846$; number of flights: $r = -0.589$, $P = 0.164$).

Males that we sampled on Sample Day 1 and re-sighted but did not recapture on Sample Day 2 tended to be less active on Sample Day 2 than on Sample Day 1 (number of songs: Sample Day 1: 87 ± 31 ; Sample Day 2: 50 ± 17 ; number of flights over mist net: Sample Day 1: 11 ± 9 ; Sample Day 2: 0 ± 2 ; Wilcoxon sign rank tests: P_s both = 0.063). As a result, on Sample Day 2 these males differed behaviorally from those we recaptured (Mann-Whitney U test: number of songs: $P = 0.030$; number of flights: $P = 0.005$).

The average body masses of birds captured on Sample Days 1 and 2 were identical (15.1 ± 0.3 g; $P > 0.5$).

4. Discussion

To our knowledge, this study is the first in free-ranging birds to concurrently determine behavioral, endocrine, and metabolic correlates of recovery from a brief behavioral challenge followed with acute stress exposure. We found that acute stress increased plasma CORT and decreased plasma T, UA, and GLU. Furthermore, some of these effects were relatively persistent: in birds that we re-sampled one day after on-site release, plasma T was still low and at the level measured during acute stress the previous day. These birds, therefore, exhibited no sign of recovery from exposure to song playback and stress exposure. By contrast, at recapture, plasma CORT and UA had returned to the levels measured at the time of initial capture, and glycemia was markedly elevated. Our observation that plasma CORT did not differ from initial plasma CORT at recapture is consistent with results in captive European Starlings (Remage-Healey and Romero 2001). In

starlings, as in the RWSP, handling and restraint elevates plasma CORT, but this effect dissipates within a few hours even when birds are subjected to repeated handling and restraint.

4.1. *Endocrine and metabolic effects of social challenge and acute stress*

Exposing RWSP in breeding condition to song playback for 30 min increases their plasma CORT and decreases their plasma UA (Deviche et al., 2014). This observation may account for the fact that in the present study initial plasma CORT was apparently higher (36.2 ± 5.0 ng/ml) and plasma UA lower (26.0 ± 2.0 mg/dl) than in birds sampled at the same time of the year, but after exposure to song playback for less than 4 min (Deviche et al., 2014; CORT: 12.8 ± 2.5 ng/ml; UA: 35.9 ± 3.8 mg/dl). Song playback exposure does, however, not stimulate CORT secretion or depress plasma UA maximally because here we found that restraint after song playback exposure further increased plasma CORT and decreased plasma UA. In contrast to plasma CORT and UA, in RWSP plasma T and GLU are not detectably influenced by song playback exposure (Deviche et al., 2014). Consistent with this conclusion, initial plasma GLU in the present study (378 ± 9 mg/dL) appears to be similar to that in sparrows sampled after song playback exposure for less than 4 min (Deviche et al., 2014: 390 ± 7 mg/dL) and the same is true for plasma T (6.80 ± 0.92 ng/ml vs. 5.29 ± 1.69 ng/ml). Therefore, the decrease in plasma T and GLU that was associated with restraint for 30 min in the present study presumably reflects the influence of handling *per se* rather than a cumulative effect of song playback exposure plus capture and handling.

Consistent with our previous work (Deviche et al., 2014, 2016), sparrows in the present study experienced a marked decrease in plasma UA during acute stress. Uric acid is a potent and the most abundant antioxidant in avian blood (Cohen et al., 2007), and stress can induce oxidative damage (Costantini et al., 2008; Chen et al., 2014; Stier et al., 2009; Zeng et al., 2014). During stress, a decrease in plasma UA as observed here may reflect increased tissue uptake and potentially utilization of the metabolite, thereby mitigating stress-related oxidative damage. Further research is, however, necessary to assess the validity of this hypothesis because of evidence indicating UA's role as an antioxidant in plasma, but as a pro-oxidant once within cells (Sautin and Johnson 2008).

Previous avian studies reported a negative association between plasma CORT and UA in some species (Cohen et al., 2007; Davies et al., 2013; Deviche et al., 2014). For example, male RWSP whose plasma CORT increased more during stress also experienced a larger decrease in plasma UA (Deviche et al., 2016). No such relationship at the individual level was found here. This difference with previous studies may relate to the fact that RWSP used by Deviche et al. (2014) were sampled after a brief (on average, less than 4 min) period of exposure to song playback whereas birds in the present study were bled following exposure to two factors (song playback and

then capture and restraint) that presumably both affected their plasma CORT and UA. Nevertheless, it should be pointed out that on Sample Day 1 plasma CORT and UA increased and decreased, respectively, during restraint stress, and on Sample Day 2, both plasma CORT and UA had returned to initial levels. Overall, changes in plasma CORT (increase during stress and decrease after release) were, therefore, associated with changes in opposite direction (decrease during stress and increase after release) in plasma UA. This negative trend may result from CORT lowering plasma UA, but whether this is the case requires further scrutiny. Indeed, in the King Penguin (*Aptenodytes patagonica*) chick, fasting decreases plasma CORT as well as UA, the latter presumably reflecting decreased protein utilization (Le Ninan et al., 1988). In addition, CORT administration to chickens rapidly (within a few hours) elevates plasma UA (Simon, 1984), as does chronic (nine days) CORT treatment (Davison et al., 1983).

In a previous study on RWSP males sampled during their breeding season (July), as was the case in the present study, we also found a modest decrease in plasma GLU during acute stress (Deviche et al., 2014). More recently, however, the effect of acute stress on plasma GLU was found to be life history stage-specific (Deviche et al., 2016): during stress plasma GLU increased in pre-breeding birds (June), did not change in late breeding birds (August), and decreased in males sampled after breeding (September) or that were in non-breeding condition (December; Deviche et al., 2016). In these birds, life history stage-related changes in the plasma GLU response to acute stress were not consistently associated with changes in plasma CORT, which increased during stress at all stages. These results provided little support for the hypothesis that an increase in plasma CORT, as experienced during acute stress, causes hyperglycemia. Importantly, on Sample Day 2 plasma CORT of recaptured RWSP had returned to initial level but these birds had markedly elevated glycemia relative to Sample Day 1. In the European Starling, administration of insulin induced hypoglycemia and concurrent treatment with CORT alleviated this effect (Ramage-Healey and Romero, 2001). However, CORT treatment-associated recovery from the hypoglycemic action of insulin was not noted until 150 min after this treatment, when plasma CORT was approaching basal levels. Similarly, the increase in plasma GLU observed on Sample Day 2 in RWSP in the present study may reflect delayed stimulation by stress-induced elevated plasma CORT. Further research investigating the dynamics of changes in plasma CORT and GLU during stress, and incorporating the potential contribution of other hormones, in particular insulin, are needed to clarify the mechanism leading to delayed hyperglycemia,

In some avian species, CORT may inhibit testicular function, including T synthesis, by acting on gonadally produced gonadotropin-inhibitory hormone (GnIH; Ernst et al., 2015). Here we found no association on Sample Day 1 between plasma CORT and T measured either immediately after capture or after 30 min of restraint. In addition, on Sample Day 2, plasma CORT had returned to

initial level whereas plasma T was still depressed relative to this level. These observations do not support the hypothesis that changes in plasma CORT and T during acute stress are functionally related. Previous work on male RWSP (Deviche et al., 2014) and the Abert's Towhee (Davies et al., 2016) likewise failed to identify consistent associations between changes in plasma CORT and T. Extending this conclusion, the plasma CORT response of sparrows to capture and restraint was similar across life history stages, but depending on the life history stage, plasma T decreased or did not change during stress (Deviche et al., 2016). Furthermore, in that study and similar to the present study, at the individual level there was no correlation between plasma CORT and T either at capture or during acute stress. Previous research on RWSP found that plasma T rapidly (within 10 min: Deviche et al., 2012) decreased during stress. This decrease was not associated with inhibition of plasma luteinizing hormone (Deviche et al., 2012) and the mechanism that mediates it is not identified, but the results available to date provide no evidence that it results from a direct inhibitory action of CORT on T synthesis.

4.2. *Relationships between plasma testosterone and territorial behavior*

The present investigation casts new light on relationships between short-term changes in plasma hormones and the expression of territorial behavior. Previous study on RWSP found no influence of the duration of song playback exposure on plasma T (Deviche et al., 2006), and exposure either to song playback alone (as was the case in the present study) or to song playback plus a live conspecific male provoked a strong aggressive response without altering plasma T (Deviche et al., 2014). Consistent with observations in other songbirds (Goymann, 2009), these results suggest that territorial aggression does not have short-term effects on T secretion in male RWSP, and that plasma T in these birds does not correlate with the expression of territorial behavior. Here we report that despite low (approximately 50% of initial level) plasma T for at least one day, sparrows behaviorally responded to song playback similarly on Sample Days 1 and 2. Furthermore, we found no association between plasma T and territorial behavior during either day. These observations extend the above conclusion. It should, however, be noted that some mammalian studies involving T administration found a positive linear relationship between the dose of administered hormone and its physiological effects (Bhasin et al., 2001), but this relationship was not always present in behavioral studies (Gray et al., 2005; Wood, 2002; Goymann et al., 2015). We cannot exclude in RWSP that plasma T controls territorial behavior expression, but levels that are markedly lower than initial levels and similar to those in experimentally stressed birds on Samples Days 1 and 2, suffice to maintain full behavior expression.

It should finally be pointed out in the present study that on Sample Day 1, all birds were exposed to two stimuli (social challenge followed with restraint stress) that both had the potential to

influence territorial behavior as well as endocrine and metabolic parameters on Sample Day 2. The experimental design does not permit us to determine whether and the extent to which each stimulus contributed to the behavioral, endocrine, and metabolic differences observed between Sample Days 1 and 2. We also note that the analyses of Sample Day 1 data revealed no statistically significant morphological, physiological (plasma hormone and metabolites), or behavioral differences between birds that we recaptured, re-sighted and did not recapture, or did not re-sight on Sample Day 2 (Tables 1 and 2). These results suggest that on Sample Day 1 the three experimental groups were homogenous with regard to the parameters that we measured. Nevertheless, it needs to be recognized that on Sample Day 1 groups may have differed in subtle ways that eluded detection due to relatively modest sample sizes and, consequently, limited statistical power to detect group differences, and that these differences in turn caused the behavioral differences (e.g., between birds that we recaptured and those that we re-sighted but did not recapture) measured on Sample Day 2.

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6. References

- Annerstedt, M., Jonsson, P., Wallergard, M., Johansson, G., Karlson, B., Grahn, P., Hansen, A.M., Wahrborg, P., 2013. Inducing physiological stress recovery with sounds of nature in a virtual reality forest--results from a pilot study. *Physiol. Behav.* 118, 240-250.
- Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1995. Seasonal and acute changes in adrenocortical responsiveness in an arctic-breeding bird. *Horm. Behav.* 29, 442-457.
- Bhasin, S., Woodhouse, L., Casaburi, R., Singh, A.B., Bhasin, D., Berman, N., Chen, X., Yarasheski, K.E., Magliano, L., Dzekov, C., Dzekov, J., Bross, R., Phillips, J., Sinha-Hikim, I., Shen, R., Storer, T.W., 2001. Testosterone dose-response relationships in healthy young men. *Am. J. Physiol. Endocrinol. Metab.* 281, E1172-E1181.
- Chen, H.J., Spiers, J.G., Sernia, C., Anderson, S.T., Lavidis, N.A. 2014. Reactive nitrogen species contribute to rapid onset of redox changes induced by acute immobilization stress in rats. *Stress* 1796, 520-527.
- Cockrem, J.F., Candy, E.J., Barrett, D.P., Agnew, P., Potter, M.A., 2016. Individual variation and repeatability of corticosterone responses of little penguins (*Eudyptula minor*) sampled in two successive years at Oamaru, New Zealand. In press, *Gen. Comp Endocrinol.*

- Cohen, A., Klasing, K., Ricklefs, R., 2007. Measuring circulating antioxidants in wild birds. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 147, 110-121.
- Costantini, D., Fanfani, A., Dell'omo, G., 2008. Effects of corticosteroids on oxidative damage and circulating carotenoids in captive adult kestrels (*Falco tinnunculus*). *J. Comp. Physiol. B.* 178, 829-835.
- Davies, S., Rodriguez, N.S., Sweazea, K.L., Deviche, P., 2013. The effect of acute stress and long-term corticosteroid administration on plasma metabolites in an urban and desert songbird. *Physiol. Biochem. Zool.* 86, 47-60.
- Davies S., Noor, S., Carpentier E., Deviche P., 2016. Innate immunity and testosterone rapidly respond to acute stress, but is corticosterone at the helm. *J. Comp. Physiol. B* DOI 10.1007/s00360-016-0996-y.
- Davison, T.F., Rea, J., Rowell, J.G., 1983. Effects of dietary corticosterone on the growth and metabolism of immature *Gallus domesticus*. *Gen. Comp. Endocrinol.* 50, 463-468.
- Debut, M., Berri, C., Arnould, C., Guemene, D., Sante-Lhoutellier, V., Sellier, N., Baeza, E., Jehl, N., Jegou, Y., Beaumont, C., Le Bihan-Duval, E., 2005. Behavioural and physiological responses of three chicken breeds to pre-slaughter shackling and acute heat stress. *Br. Poult. Sci.* 46, 527-535.
- Deviche, P., Small, T., Sharp, P., Tsutsui, K., 2006. Control of luteinizing hormone and testosterone secretion in a flexibly breeding male passerine, the Rufous-winged Sparrow. *Gen. Comp. Endocrinol.* 149, 226-235.
- Deviche, P., Gao, S., Davies, S., Sharp, P.J., Dawson, A., 2012. Rapid stress-induced inhibition of plasma testosterone in free-ranging male rufous-winged sparrows, *Peucaea carpalis*: characterization, time course, and recovery. *Gen. Comp. Endocrinol.* 177, 1-8.
- Deviche, P., Beouche-Helias, B., Davies, S., Gao, S., Lane, S., Valle, S., 2014. Regulation of plasma testosterone, corticosterone, and metabolites in response to stress, reproductive stage, and social challenges in a desert male songbird. *Gen. Comp. Endocrinol.* 203, 120-131.
- Deviche, P., Valle, S., Gao, S., Davies, S., Bittner, S., Carpentier, E., 2016. The seasonal glucocorticoid response of male Rufous-winged Sparrows to acute stress correlates with changes in plasma uric acid, but neither glucose nor testosterone. In press, *General and Comparative Endocrinology*.
- Donaldson, W.E., Christensen, V.L., Krueger, K.K., 1991. Effects of stressors on blood glucose and hepatic glycogen concentrations in turkey poults. *Comp. Biochem. Physiol. A. Comp. Physiol.* 100, 945-947.

491 Dong, Q., Salva, A., Sottas, C.M., Niu, E., Holmes, M., Hardy, M.P., 2004. Rapid glucocorticoid
 492 mediation of suppressed testosterone biosynthesis in male mice subjected to immobilization
 493 stress. *J. Androl.* 25, 973-981.

494 Ericsson, M., Fallahsharoudi, A., Bergquist, J., Kushnir, M.M., Jensen, P., 2014. Domestication
 495 effects on behavioural and hormonal responses to acute stress in chickens. *Physiol. Behav.*
 496 133, 161-169.

497 Ernst, D.K., Lynn, S.E., Bentley, G.E., 2015. Differential response of GnIH in the brain and gonads
 498 following acute stress in a songbird. In press, *Gen. Comp. Endocrinol.*

499 Fokidis, H.B., Hurley, L., Rogowski, C., Sweazea, K., Deviche, P., 2011. Effects of captivity and
 500 body condition on plasma corticosterone, locomotor behavior, and plasma metabolites in
 501 curve-billed thrashers. *Physiol. Biochem. Zool.* 84, 595-606.

502 Gildersleeve, R.P., Galvin, M.J., Thaxton, J.P., McRee, D.I., 1985. Hematological response of
 503 Japanese quail to acute hemorrhagic stress. *Comp. Biochem. Physiol. A. Comp Physiol.* 81,
 504 403-409.

505 Goymann, W., 2009. Social modulation of androgens in male birds. *Gen. Comp. Endocrinol.* 163,
 506 149-157.

507 Goymann, W., Villavicencio, C.P., Apfelbeck, B., 2015. Does a short-term increase in testosterone
 508 affect the intensity or persistence of territorial aggression? - An approach using an individual's
 509 hormonal reactive scope to study hormonal effects on behavior. *Physiol. Behav.* 149, 310-316.

510 Gray, P.B., Singh, A.B., Woodhouse, L.J., Storer, T.W., Casaburi, R., Dzekov, J., Dzekov, C., Sinha-
 511 Hikim, I., Bhasin, S., 2005. Dose-dependent effects of testosterone on sexual function, mood,
 512 and visuospatial cognition in older men. *J. Clin. Endocrinol. Metab.* 90, 3838-3846.

513 Hardy, M.P., Gao, H.B., Dong, Q., Ge, R., Wang, Q., Chai, W.R., Feng, X., Sottas, C., 2005. Stress
 514 hormone and male reproductive function. *Cell Tissue Res.* 322, 147-153.

515 Huang, C., Jiao, H., Song, Z., Zhao, J., Wang, X., Lin, H., 2015. Heat stress impairs mitochondria
 516 functions and induces oxidative injury in broiler chickens. *J. Anim. Sci.* 93, 2144-2153.

517 Jeffrey, D.A., Peakall, D.B., Miller, D.S., Herzberg, G.R., 1985. Blood chemistry changes in food-
 518 deprived herring gulls. *Comp. Biochem. Physiol. A. Comp. Physiol.* 81, 911-913.

519 Lawson, D.M., Gala, R.R., 1974. The influence of surgery, time of day, blood volume reduction and
 520 anaesthetics on plasma prolactin in ovariectomized rats. *J. Endocrinol.* 62, 75-83.

521 Le Ninan, F., Cherel, Y., Robin, J.P., Leloup, J., Le Maho Y., 1988. Early changes in plasma
 522 hormones and metabolites during fasting in king penguin chicks. *J. Comp. Physiol. B* 158, 395-
 523 401.

524 Lin, H., Decuyper, E., Buyse, J., 2004. Oxidative stress induced by corticosterone administration in
 525 broiler chickens (*Gallus gallus domesticus*) 2. Short-term effect. Comp. Biochem. Physiol. B
 526 Biochem. Mol. Biol. 139, 745-751.

527 Lowther, P.E., Groschupf, K.D., Russell, S.M. Rufous-winged Sparrow (*Aimophila carpalis*). In The
 528 Birds of North America, No. 422 (A. Poole and F. Gill, eds.). The Birds of North America, Inc.,
 529 Philadelphia, PA. 1999.

530 Lynn, S.E., Stamps, T.B., Barrington, W.T., Weida, N., Hudak, C.A., 2010. Food, stress, and
 531 reproduction: short-term fasting alters endocrine physiology and reproductive behavior in the
 532 zebra finch. Horm. Behav. 58, 214-222.

533 McGill, M.W., Rowan, A.N. 1989. Biological effects of blood loss: implications for sampling volumes
 534 and techniques. ILAR News 4, 5-20.

535 Monder, C., Miroff, Y., Marandici, A., Hardy, M.P., 1994. 11 beta-hydroxysteroid dehydrogenase
 536 alleviates glucocorticoid-mediated inhibition of steroidogenesis in rat Leydig cells.
 537 Endocrinology 134, 1199-1204.

538 O'Reilly, K.M., Wingfield, J.C., 2001. Ecological factors underlying the adrenocortical response to
 539 capture stress in arctic-breeding shorebirds. Gen. Comp. Endocrinol. 124, 1-11.

540 Orr, T.E., Mann, D.R., 1992. Role of glucocorticoids in the stress-induced suppression of testicular
 541 steroidogenesis in adult male rats. Horm. Behav. 26, 350-363.

542 Peig, J., Green, A.J. New perspectives for estimating body condition from mass/length data: the
 543 scaled mass index as an alternative method. Oikos 118, 1883-1891. 2009.

544 Raabe, B.M., Artwohl, J.E., Purcell, J.E., Lovaglio, J., Fortman, J.D., 2011. Effects of weekly blood
 545 collection in C57BL/6 mice. J. Am. Assoc. Lab. Anim. Sci. 50, 680-685.

546 Rand, J.S., Kinnaird, E., Baglioni, A., Blackshaw, J., Priest, J., 2002. Acute stress hyperglycemia in
 547 cats is associated with struggling and increased concentrations of lactate and norepinephrine.
 548 J. Vet. Intern. Med. 16, 123-132.

549 Remage-Healey, L., Romero, L.M., 2001. Corticosterone and insulin interact to regulate glucose and
 550 triglyceride levels during stress in a bird. Am. J. Physiol. Regul. Integr. Comp. Physiol 281,
 551 R994-1003.

552 Remage-Healey, L., Michael, R.L., 2002. Corticosterone and insulin interact to regulate plasma
 553 glucose but not lipid concentrations in molting starlings. Gen. Comp. Endocrinol. 129, 88-94.

554 Rensel, M.A., Schoech, S.J., 2011. Repeatability of baseline and stress-induced corticosterone
 555 levels across early life stages in the Florida scrub-jay (*Aphelocoma coerulescens*). Horm.
 556 Behav. 59, 497-502.

557 Romero, L.M., Wingfield, J.C., 1998. Seasonal changes in adrenal sensitivity alter corticosterone
 558 levels in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Comp.
 559 Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 119, 31-36.
 560 Sautin, Y.Y., Johnson, R.J., 2008. Uric acid: the oxidant-antioxidant paradox. Nucleosides.
 561 Nucleotides. Nucleic Acids 27, 608-619.
 562 Siegel, H.S., Van Kampen, M., 1984. Energy relationships in growing chickens given daily injections
 563 of corticosterone. Br. Poult. Sci. 25, 477-485.
 564 Simon, J., 1984. Effects of daily corticosterone injections upon plasma glucose, insulin, uric acid and
 565 electrolytes and food intake pattern in the chicken. Diabetes Metab. 10, 211-217.
 566 Stier, K.S., Almasi, B., Gasparini, J., Piauult, R., Roulin, A., Jenni, L., 2009. Effects of corticosterone
 567 on innate and humoral immune functions and oxidative stress in barn owl nestlings. J. Exp.
 568 Biol. 212, 2085-2091.
 569 Viswanathan, M., John, T.M., George, J.C., Etches, R.J., 1987. Flight effects on plasma glucose,
 570 lactate, catecholamines and corticosterone in homing pigeons. Horm. Metab. Res. 19, 400-
 571 402.
 572 Wingfield, J., Ball, G.F., Dufty, A.M., Hegner, R.E., Ramenofsky, M. 1987. Testosterone and
 573 aggression in birds. Amer. Scientist 75, 602-608.
 574 Wingfield, J.C., Moore, I.T., Goymann, W., Wacker, D., Sperry, T., 2006. Contexts and ethology of
 575 vertebrate aggression: implications for the evolution of hormone-behavior interactions. In:
 576 Biology of aggression (Nelson R.J., editor). New York, NY. Oxford Univ. Press. 179-210.
 577 Wood, R.I., 2002. Oral testosterone self-administration in male hamsters: dose-response, voluntary
 578 exercise, and individual differences. Horm. Behav. 41, 247-258.
 579 Xie, J., Tang, L., Lu, L., Zhang, L., Lin, X., Liu, H.C., Odle, J., Luo, X., 2015. Effects of acute and
 580 chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed
 581 broiler breeders. Poult. Sci. 94, 1635-1644.
 582 Zeng, T., Li, J.J., Wang, D.Q., Li, G.Q., Wang, G.L., Lu, L.Z., 2014. Effects of heat stress on
 583 antioxidant defense system, inflammatory injury, and heat shock proteins of Muscovy and
 584 Pekin ducks: evidence for differential thermal sensitivities. Cell Stress Chaperones. 19, 895-
 585 901.

Table 1. Plasma hormones (testosterone; corticosterone) and metabolites (uric acid, glucose; means $\pm$ s.e.m.'s) of free-ranging adult male Rufous-winged Sparrows, *Peucaea carpalis*, measured on Sampling Day 1 immediately after capture (Initial) and after 30 min of restraint (30 min). On Sample Day 2 birds were either recaptured, re-sighted but not recaptured, or not re-sighted. The table includes results of repeated measure analyses of variance (RM-ANOVA) showing, for each parameter, differences between groups, the effect of time (initial vs. 30 min) and the interaction between these factors. Statistically significant effects ($P < 0.05$) are indicated in bold.

Experimental group	Testosterone (ng/ml)		Uric acid (mg/dl)		Glucose (mg/dl)		Corticosterone (ng/ml)	
	Initial	30 min	Initial	30 min	Initial	30 min	Initial	30 min
Recaptured (n = 7)	6.20 $\pm$ 1.12	3.24 $\pm$ 0.51	28.28 $\pm$ 2.64	17.00 $\pm$ 1.41	352 $\pm$ 12	345 $\pm$ 18	34.95 $\pm$ 7.97	113.98 $\pm$ 15.58
Re-sighted, not recaptured (n = 5)	9.87 $\pm$ 1.75	4.09 $\pm$ 0.49	22.29 $\pm$ 2.96	13.67 $\pm$ 1.15	381 $\pm$ 10	349 $\pm$ 24	33.87 $\pm$ 9.03	87.68 $\pm$ 7.21
Not re-sighted (n = 5)	4.55 $\pm$ 1.44	2.67 $\pm$ 0.56	26.36 $\pm$ 5.08	17.71 $\pm$ 2.50	411 $\pm$ 10	357 $\pm$ 33	40.16 $\pm$ 11.02	138.07 $\pm$ 40.35
RM-ANOVA								
Group	$F_{2,14} = 3.07$ $P = 0.079$		$F_{2,14} = 1.18$ $P = 0.34$		$F_{2,14} = 1.42$ $P = 0.27$		$F_{2,14} = 0.89$ $P = 0.43$	
Time	$F_{1,14} = 35.50$ $P < 0.001$		$F_{1,14} = 26.73$ $P < 0.001$		$F_{1,14} = 5.40$ $P = 0.04$		$F_{1,14} = 36.27$ $P < 0.001$	
Group x Time interaction	$F_{2,14} = 3.50$ $P = 0.059$		$F_{2,14} = 0.26$ $P = 0.78$		$F_{2,14} = 1.11$ $P = 0.36$		$F_{2,14} = 0.91$ $P = 0.43$	

Table 2. Morphological and behavioral characteristics of free-ranging adult male Rufous-winged Sparrows, *Peucaea carpalis*, that were sampled on Sample Day 1 and on the following day (Sample Day 2) recaptured, re-sighted but not recaptured, or not re-sighted.

Experimental group	Wing chord (mm) ¹	Body mass (g) ¹	Body condition (g) ¹	Cloacal protuberance width (mm) ¹	Fat score ²	Number of songs ²	Number of flights ²
Recaptured (n = 7)	62 ± 0	15.1 ± 0.3	15.0 ± 0.3	5.2 ± 0.2	0.5 ± 0.3	62 ± 6	15 ± 9
Re-sighted, not recaptured (n = 5)	62 ± 1	15.7 ± 0.4	15.3 ± 0.3	5.0 ± 0.2	1.5 ± 0.4	87 ± 31	11 ± 9
Not re-sighted (n = 5)	61 ± 1	14.7 ± 0.2	15.2 ± 0.4	4.9 ± 0.3	0.5 ± 0.7	43 ± 17	10 ± 17
Probabilities	0.164 ³	0.066 ³	0.681 ³	0.637 ³	0.135 ⁴	0.058 ⁴	0.687 ⁴

¹: mean ± standard error; ²: median ± 0.5 interquartile interval.

³: one-way analysis of variance; ⁴: Kruskal-Wallis analysis of variance on ranks.

Figure legends

Fig. 1. Capture and restraint of free-ranging adult male Rufous-winged Sparrows, *Peucaea carpalis*, (n = 17) increases plasma corticosterone and decreases plasma testosterone, uric acid, and glucose. The figure shows plasma hormone and metabolite concentrations (means $\pm$ s.e.'s) measured within 2 min of capture (initial) and after 30 min of restraint (30 min stress). A * denotes $P < 0.05$ in comparison with corresponding initial values (Student's t test).

Fig. 2. Capture and restraint of free-ranging adult male Rufous-winged Sparrows, *Peucaea carpalis* (n = 7), results in prolonged suppression of plasma testosterone and hyperglycemia. The figure shows plasma hormone and metabolite concentrations (means $\pm$ s.e.'s) within 2 min of capture (initial), after 30 min of restraint (30 min stress), and the day (Day 2) after birds had been released on their breeding territory. For each parameter, means with different superscript letters differ significantly ($P \leq 0.05$; Student-Newman-Keuls test).

Figure 1. Deviche et al.

Figure 2. Deviche et al.