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1 **Rapid stress-induced inhibition of plasma testosterone in free-**

2 **ranging male rufous-winged sparrows, *Peucaea carpalis*:**

3 **characterization, time course, and recovery**

4

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23 Figures: 5; Tables: 1.

24

25 ABSTRACT

26 Chronic stress generally inhibits the activity of the reproductive system. Acute stress also is

27 often inhibitory, but the mechanism involved and its persistence of action once animals are no

28 longer exposed to the stressor are poorly understood. We investigated the effect of capture and

29 restraint stress on plasma testosterone (T), luteinizing hormone (LH), and corticosterone

30 (CORT) in free-ranging male rufous-winged sparrows, *Peucaea carpalis.* Stress decreased

31 plasma T between 10 and 30 min after capture and restraint but did not influence plasma LH,

32 the main hormone that controls T secretion, suggesting that stress did not decrease plasma T

33 by inhibiting LH secretion. The stress-induced decrease in plasma T was associated with

34 elevated plasma CORT, but there was no evidence that these effects were functionally related.

35 Plasma stress-induced T was positively related to plasma initial T measured within 2 min of

36 capture. This relationship was, however, complex as plasma T decreased proportionally more in

37 response to stress in sparrows with high than low plasma initial T. The relative sensitivity to a

38 same stressor was, therefore, individually variable and this variation was related to initial plasma

39 T. Birds caught and restrained for 30 min, and then released on their breeding territory before

40 recapture up to 6 hours later, maintained depressed plasma T, indicating that the effect of acute

41 stress on this hormone persists after the stressor removal. These studies provide new

42 information on the effects of acute stress on plasma T in free-ranging birds. In particular, they

43 are among the first to characterize the time course and to describe the persistence of these

44 effects. The findings also contribute to identifying factors that are associated with individual

45 differences in plasma hormone levels.

46

47

48 **Keywords**: Androgen - Bird - Corticosterone - Field endocrinology - Luteinizing Hormone –

49   Reproduction.

50

1. Introduction

Vertebrates generally respond to adverse conditions ("stressors") by activation of the hypothalamo-pituitary gland-adrenal (HPA) axis, resulting in elevated secretion and plasma concentrations of glucocorticoids such as cortisol or corticosterone (CORT). Chronic (long-term) and acute (i.e., within minutes to hours) activation of the HPA axis is often associated with complex biochemical, behavioral, and physiological responses including changes in the activity of the reproductive system [60,63]. Chronic stress often suppresses reproductive function [7,53,19]. In males, acute stress likewise suppresses plasma testosterone (T). Examples include fish (brown trout, *Salmo trutta*: [47], amphibians (Ocoee salamander, *Desmognathus ocoee*): [70], and several bird species (*Zonotrichia* spp.: [69,43]; zebra finch, *Taeniopygia guttata*: [44]; house sparrows, *Passer domesticus*, Abert's towhees, *Melozone aberti*, and Cassin's sparrows, *Peucaea cassinii*; Deviche, Gao, and Davies, personal observations). These observations indicate that the phenomenon is widespread across vertebrate taxa. However, other studies on birds found either an increase in plasma T (cockerel: [25]; European starling, *Sturnus vulgaris*: [61]) in response to stress or a decrease or increase depending on plasma T levels before stress (semipalmated sandpiper, *Calidris pusilla*: [21]). The factors (e.g., type of stress: [25]) that account for interspecies differences in the plasma T response to acute stress are largely unknown. Identifying these factors and elucidating their role should benefit from a detailed characterization of the time course, magnitude, and duration of stress effects on plasma T.

Previous studies imply that stress may directly affect testicular function. Acute stress in the white-crowned sparrow, *Zonotrichia leucophrys* decreases plasma T but not luteinizing hormone (LH; [69]). The same was observed in turkeys, *Meleagris gallopavo* [15] and in the male rufous-winged sparrow, *Peucaea carpalis*, in which acute stress also did not affect the plasma T response to a LH injection [13]. These results suggest that acute stress does not decrease plasma T by impairing LH secretion or attenuating the testicular sensitivity to this hormone [13]. Acute stress in birds typically increases plasma CORT in 5-10 min [44,33,69,13] and in the rufous-winged sparrow, plasma T decreases by 30% - 50% after capture and confinement for 15-30 min [13]. Avian testes contain glucocorticoid receptors [32] and in mammals, glucocorticoids influence T production through direct actions on interstitial (Leydig) testicular cells [14,24,27]. Direct actions of CORT on the avian testes may, therefore, also

83   mediate rapid effects of acute stress on plasma T, but this hypothesis has not been

84   investigated. One objective of the present investigation was to address these issues by

85   determining and comparing the time course of changes in plasma CORT, LH, and T in response

86   to acute stress. We hypothesized that plasma T begins to decline after less than 15 min of

87   stress and that the magnitude of the decrease increases as a function of the stress duration.

88   This hypothesis was tested by determining the effects of capture and restraint for 5, 10, or 20

89   min.

90   No study has, to our knowledge, investigated the time course of endocrine recovery from

91   mild acute stress in wild, free-ranging birds. The *second objective* of the present work was to

92   address this question by exposing sparrows to acute stress to decrease their plasma T,

93   releasing them on their breeding territory, and then recapturing them at various times and

94   sampling them again.

95

96

97   **2. Material and methods**

98

99   *2.1. Study species and location*

100

101   The studies used a sedentary, year-round territorial and socially monogamous Sonoran

102   Desert songbird, the adult male rufous-winged sparrow, and were performed in and in the

103   vicinity of the Santa Rita Experimental Range, Pima County, Arizona, USA, where the bird

104   commonly breeds [36]. Sparrows were sampled during 27th – 31st July 2010 (n = 40; Studies 1a

105   and 2a, see below) and 4th – 8th August 2011 (n = 55; Studies 1b and 2b). Seasonal

106   reproduction in Rufous-winged Sparrows is associated with the summer monsoon and birds

107   during the study period were in breeding condition [36,12,57].

108

109   *2.2. Capture and blood sample collection*

110

111   Sparrows were captured in response to simulated territorial intrusion (STI: conspecific

112   song playback), while they were on their breeding territory and using a Japanese mist net. As

113   shown in a previous study on this species, plasma T in males in breeding condition is not

114   influenced by exposure to STI for durations similar to those in the present investigation [12].

4

115 Captures took place between 5:30 AM and 17:15 PM. Within two min of capture, a blood

116 sample was collected from a jugular vein of each bird into a heparinized plastic syringe to

117 determine *plasma initial* (= baseline) hormone concentrations. In other bird species, plasma

118 CORT does not increase markedly until birds are exposed to the stress of capture and restraint

119 for at least 2 min ([50,52], but see [8]). Birds were then confined to individual breathable cloth

120 bags for 5, 10, 20 (Studies 1a,b) or 30 min (Studies 2a,b; details below) after which a second

121 blood sample was obtained (except Study 2a, see below) to determine plasma *stress-induced*

122 hormone concentrations. Capture followed with mild restraint is commonly used in wild birds to

123 acutely and non-invasively stimulate the HPA axis and elevate plasma CORT [5,3,13]. This

124 method does not induce maximum secretory activity of the adrenal glands, as shown by the

125 observation in acutely stressed birds that administration of adrenocorticotropic hormone further

126 elevates plasma CORT ([17] and references therein). Individuals were randomly assigned to

127 experimental groups. At the end of the 30 minute period of confinement, sparrows in Studies 2a

128 and b were released and, if possible, subsequently recaptured (details below), and a blood

129 sample was collected from each bird within two min of recapture. The volume of individual blood

130 samples approximated 120 μl (2 blood samples collected from a bird: Studies 1a,b and 2a) or

131 80 μl (3 blood samples collected from a bird; Study 2b). Following collection, samples were

132 immediately placed on ice until processed later the same day in the laboratory. Plasma was

133 separated by centrifugation, collected, and frozen until assayed (see below).

134 Prior to release at the capture site, each sparrow received a uniquely numbered metal

135 leg band (US Geological Survey) and an intramuscular injection of 0.9 % NaCl in distilled water

136 (volume equal to that of blood taken). Standard measurements (wing chord, $\pm$ 1 mm; weight, $\pm$

137 0.1 g) were taken from each individual and served to calculate individual body condition indices,

138 defined as the residuals of a reduced major axis linear regression of wing chord over body mass

139 [22].

140 All activities were pre-approved by the Arizona State University Institutional Animal Care

141 and Use Committee and conducted under appropriate permits issued by the Bird Banding

142 Laboratory (US Geological Survey), the US Fish and Wildlife Service, the Arizona Game and

143 Fish Department, and the Santa Rita Experimental Range.

144

145 *2.3. Sample sizes*

146   Rufous-winged sparrows weigh on average 15-16 g (Table 1) and the volume of blood

147 collected from one bird in one day could, therefore, not exceed ~250 μl [16], yielding

148 approximately 125 μl of plasma. As a result of the limited volume of plasma available, individual

149 plasma samples could be assayed only for two hormones (either LH and T or T and CORT; see

150 below). The individual sample volume (80 μl, yielding approximately 40 μl of plasma) of blood

151 collected from Study 2b birds (see below) did not make it possible to assay these samples for

152 more than one hormone (i.e., T).

153

154

155 *2.3.1. Studies 1a and 1b*

156   The objective of Studies 1a (2010) and 1b (2011) was to characterize the acute (within

157 20 min of capture) effects of capture and restraint on plasma LH (Study 1a), T (Studies 1a,b),

158 and CORT (Study 1b). Sparrows were caught as described above, bled, placed in a cloth bag

159 for 5 min (2010: $n = 10$; 2011: $n = 11$), 10 min (2010: $n = 10$; 2011: $n = 10$), or 20 min (2010: $n = 8$; 2011: $n = 8$), and then bled again.

160 8; 2011: $n = 8$), and then bled again.

161

162 *2.3.2. Studies 2a and 2b*

163   The main objective of Studies 2a and 2b was to determine the time course of recovery of

164 plasma T following release after birds had been restrained for 30 min as described above. In

165 Study 2a (2010), 12 sparrows were caught, bled, restrained for 30 min, and released on site.

166 Attempts to recapture these birds later the same day resulted in 8 recaptures (= 67% of initial

167 captures) between 1 hr 03 min and 1 hr 41 min (average: 1 hr 19 min) of release. The study was

168 repeated in 2011 (Study 2b) to include a broader range of durations between release and

169 recapture and to include collection of a blood sample after the 30 min period of restraint. In

170 Study 2b, 17 of 26 sparrows (= 65% of initial captures) were recaptured between 0 hr 38 min

171 and 6 hrs 41 min (average: 3 hrs 08 min) of release.

172

173

174 *2.4. Hormone assays*

175

176 *2.4.1. Luteinizing hormone*

177   Plasma LH concentrations were determined using a micromodification of a chicken LH

178   radioimmunoassay described previously [54]. The assay has been previously validated for use

179   in the Rufous-winged Sparrow [11]. Briefly, the reaction volume was 60 μl, comprising 20 μl of

180   plasma sample or standard, 20 μl of primary rabbit LH antibody, and 20 μl of I[125]-labelled LH.

181   The primary antibody was precipitated to separate free and bound I[125] label using 20 μl of

182   donkey anti-rabbit precipitating serum and 20 μl of non-immune rabbit serum. All samples were

183   measured in a single assay. The intra-assay coefficient of variation was 7.3 % and the minimum

184   detectable dose was 0.15 ng/ml.

185

186   *2.4.2. Testosterone and corticosterone*

187   Plasma T and CORT were measured as previously described [12,13], using commercial

188   enzyme-linked immunoassay kits (Enzo Life Sciences, Ann Arbor, MI) according to the

189   manufacturer's recommended procedure. Samples were assayed in duplicate and were

190   randomly assigned to assay plates except that for each hormone samples (2 or 3 depending on

191   the study) from a given sparrow were assayed on the same plate.

192   For the T assay, all samples collected during the same year were assayed together after

193   10x (2010) or 8x (2011) dilution in assay buffer. The primary antibody used in the T assay has

194   less than 5% crossreactivity with 17β-estradiol, dihydrotestosterone, CORT, and progesterone

195   (manufacturer's specifications). The mean interassay and intrassay coefficients of variation

196   were 12.3% (3 samples assayed on each plate) and 2.5% (n = 126 samples), respectively, and

197   the assay sensitivity was 15 pg/ml.

198   For the CORT assay (only samples collected in 2011) samples were assayed together

199   following 15x dilution in assay buffer. The primary antibody used in the CORT assay has less

200   than 2% crossreactivity with progesterone, T, aldosterone, and 17β-estradiol. The mean

201   interassay and intrassay coefficients of variation were 12.1% (3 samples assayed on each

202   plate) and 2.0% (n = 58 samples), respectively, and the assay sensitivity was 142 pg/ml.

203

204   *2.5. Statistical analyses*

205

206   Data were analyzed using Student's t-tests, simple and multiple linear regressions,

207   Spearman rank order correlations, and analyses of variance (ANOVA) followed, when

208   appropriate, with multiple pair-wise comparison tests (Student-Newman-Keuls tests (SNK) or, in

209 the case of one-way repeated factor ANOVAs, Bonferroni t-tests). Data sets to be analyzed by

210 ANOVA and that were not normally distributed were either normalized by transformation to Log

211 X or ranked before analysis [6]. Data that were not transformed or transformed to Log X before

212 analysis are presented as means $\pm$ standard errors (s.e.'s). Data that were ranked before

213 analysis are presented as medians $\pm$ 0.5 interquartile intervals. The statistical significance level

214 of all tests was set at $p = 0.05$. Data were analyzed using SigmaPlot Version 11.0 (Systat

215 Software Inc., San Jose, CA), Statistica Version 10 (StatSoft. Inc., Tulsa, OK), and GraphPad

216 Prism Version 5.04 (GraphPad Software, Inc., La Jolla, CA).

217

218

219 **3. Results**

220

221 *3.1. Endocrine effects of capture and restraint*

222

223 *3.1.1. Plasma corticosterone (Study 1b)*

224 We analyzed plasma CORT data using repeated measure ANOVA with stress (initial *vs.*

225 stress-induced) and restraint duration (5, 10, or 20 min) as independent factors. Plasma initial

226 CORT was similar in the three experimental groups of sparrows (p's > 0.05, SNK) and

227 increased within 5 min of capture (stress effect: $F_{1,57} = 87.243$, $p < 0.001$; Fig. 1). There was no

228 overall restraint duration effect ($p > 0.3$). Even though there was an interaction between this

229 factor and stress ($F_{2,57} = 5.738$, $p = 0.009$), plasma stress-induced CORT in the three groups of

230 birds did not differ (p's > 0.05, SNK).

231

232 *3.1.2. Plasma luteinizing hormone (Study 1a)*

233 We analyzed plasma LH data as described for plasma CORT. Neither stress nor

234 restraint duration influenced plasma LH (p's > 0.25), and there was no interaction between

235 these factors ($p > 0.80$; Fig. 2).

236

237 *3.1.3. Plasma testosterone (Studies 1a, b)*

238 To determine whether capture and restraint influenced plasma T, we combined 2010

239 and 2011 data into a single data set that we analyzed by repeated measure ANOVA with year

240 (2010 *vs.* 2011), stress, and restraint duration as independent factors. Plasma T was, on

241 average, higher in 2010 than 2011 ($F_{1,51}$ = 20.60, p < 0.0001). As samples collected in 2010 and
242 2011 were assayed independently, it is unknown whether this difference reflects genuine year
243 differences in plasma T or interassay differences. There was, however, no year x stress, year x
244 restraint duration, or year x stress x restraint duration interaction (p's > 0.075) and data for the
245 two years were, therefore, combined in further analyses. Plasma T decreased in response to
246 stress ($F_{1,51}$ = 54.62, p < 0001) and was influenced by the restraint duration ($F_{2,51}$ = 4.71, p =
247 0.013), but there was a stress x restraint duration interaction ($F_{2,51}$ = 5.74, p = 0.006; Fig. 3).
248 Plasma initial T was similar in the three experimental groups (SNK: p's > 0.25). Plasma stress-
249 induced T was lower than corresponding plasma initial T after restraint for 10 or 20 min, but not
250 5 min.
251   To further characterize the time course of stress effects on plasma T, we used two-way
252 ANOVA for repeated measures to compare hormone levels in sparrows sampled in 2011 after
253 restraint for 20 min (Study 1b) or 30 min (Study 2b). Plasma stress-induced T was lower than
254 plasma initial T (stress effect: $F_{1,67}$ = 65.391, p < 0.001), but restraint for 30 min did not
255 decrease plasma T more than restraint for 20 min (stress effect x restraint duration interaction: p
256 > 0.100).
257
258 *3.1.4. Further characterization of plasma initial and stress-induced testosterone (Studies 1a,b*
259 *and 2b)*
260   Plasma initial T in 2010 and 2011 was individually variable (2010: n = 40; range: 1.14 –
261 58.03 ng/ml; coefficient of variation (CV): 98 %; 2011: n = 55: range: 1.05 – 25.12 ng/ml; CV: 69
262 %). We used multiple linear regression with four independent factors (year, capture time, body
263 size (as estimated by wing chord), and body condition index) to investigate potential sources of
264 this variation. None of these factors contributed significantly to accounting for the observed
265 individual variation in plasma initial T (p's $\geq$ 0.065).
266   We combined 2010 and corresponding 2011 data to determine the relationship between
267 plasma initial T and plasma T measured after restraint for 10 min (n = 20), 20 min (n = 16), or 30
268 min (n = 26) using linear regressions. Plasma T data in birds that we restrained for 5 min were
269 not analyzed as stress at this time did not affect the circulating concentration of this hormone
270 (Section 3.1.3). Plasma stress-induced T was in all cases positively associated with plasma
271 initial T (10 min: slope = 0.52 $\pm$ 0.06, coefficient of determination, r^2 = 0.81; 20 min: slope = 0.21
272 $\pm$ 0.04, r^2 = 0.64; 30 min: slope = 0.21 $\pm$ 0.04, r^2 = 0.59, p's $\leq$ 0.0002; Fig. 4, left panels).

273　Extending other findings (Section 3.1.3), the decrease in plasma T associated with stress was

274　proportionally larger in sparrows that we restrained for 20 min or 30 min than for 10 min

275　(comparisons of linear regression line slopes: 10 min *vs.* 20 min: $F_{1,32}$ = 18.188, $p < 0.001$; 10

276　min *vs.* 30 min: $F_{1,42}$ = 21.285, $p < 0.0001$), but in this respect birds that we restrained for 20 or

277　30 min did not differ (id., $p > 0.90$).

278　　　Further analyses revealed complex relationships between plasma initial and stress-

279　induced T. The percentage decrease in plasma T relative to plasma initial T in response to

280　capture and restraint was a function of plasma initial T (Fig. 4, right panels). In birds that were

281　restrained for 10 min and especially 20 or 30 min, plasma T decreased proportionally more in

282　response to stress when plasma initial T was high than low. In all cases, experimental data fit

283　the equation of a three parameter exponential decay curve ($y = y_0 + ae^{-bx}$; r^2's > 0.22, p's <

284　0.05).

285

286　*3.1.5. Correlation between plasma testosterone and corticosterone (Study 1b)*

287　　　We used the Spearman rank order correlation test to research associations between

288　plasma T and CORT in sparrows in which both hormones were measured immediately after

289　capture and then after restraint for 10 min or 20 min (Study 1b, $n = 18$). Samples from sparrows

290　that were sampled after restraint for 5 min were not included in these analyses as this

291　manipulation did not influence plasma T (Section 3.1.3). Plasma initial T was not correlated to

292　plasma initial CORT ($r = 0.267$, $p > 0.05$). There also was no correlation between plasma stress-

293　induced T and CORT ($r = -0.201$, $p > 0.05$). These data provide no evidence that the CORT

294　stress response accounted for the stress-induced reduction in plasma T.

295

296　*3.2. Plasma testosterone after on-site release (Study 2b)*

297

298　　　We compared plasma initial, stress-induced, and at recapture T to determine whether

299　the stress-induced decrease in plasma T dissipated after release. Birds at these three times had

300　different plasma T levels (repeated measure ANOVA: $F_{2,50}$ = 14.4, $p < 0.001$; Fig. 5a).

301　Consistent with the results of Studies 1a, b (see above), restraint for 30 min decreased plasma

302　T (Bonferroni t-test: $p < 0.05$). Plasma T at recapture was still lower than at initial capture

303　(Bonferroni t-test: $p < 0.05$) and did not differ from plasma T at the time of release (id., $p > 0.05$).

304　The difference between plasma initial T and plasma T at the time at recapture was not related to

305 how long a bird had been released (Fig. 5b; linear regression: $p > 0.5$). The difference between
306 plasma stress-induced (i.e., at release) T and plasma T at recapture was likewise unrelated to
307 how long a bird had been released (Fig. 5c; linear regression: $p > 0.3$).
308   Collectively, the data offer no evidence that the stress-induced inhibition of plasma T
309 dissipated between release and recapture up to almost 7 hours later.
310
311 *3.3. Comparison of recaptured and not recaptured males (Studies 2a, b)*
312
313   Following initial capture and release, approximately one third of the birds sampled in
314 Studies 2a and 2b either were not re-sighted or were re-sighted in the vicinity of the capture site
315 but could not be recaptured. We analyzed whether recaptured males differed morphologically
316 and/or physiologically from those we did not recapture. For this, we compared data for five
317 parameters (capture time, plasma initial T, wing chord, body mass, and body condition index)
318 between the two male groups using two-way ANOVAs (independent factors: year (2010 *vs.*
319 2011) and recapture *vs.* no recapture). Males studied in 2010 were not bled at the end of the 30
320 min restraint period and before release (see Materials and Methods). Therefore, Student's t-test
321 was used to compare plasma stress-induced T in recaptured *vs.* not recaptured males sampled
322 during that year. In 2011 recaptured males were, on average, initially caught earlier in the day
323 than males that we did not recapture (year x recapture *vs.* no recapture interaction: $F_{1,37} = 6.43$,
324 $p = 0.016$; Table 1). Except for this difference, males that we recaptured did not differ in any
325 respect from males that we did not recapture (p's > 0.1). These results suggest that individuals
326 that we did or not recapture were similar morphologically and physiologically.
327
328
329 **4. Discussion**
330
331   Consistent with previous studies on rufous-winged sparrows and some other species,
332 acute stress resulting from capture and handling decreased plasma T (see Introduction for
333 references). This decrease was observed within 10 min of capture and its magnitude increased
334 as a function of the duration of exposure to the stressor until birds were released 30 min after
335 capture. The stress-induced decrease in plasma T persisted for at least 6 hours after release
336 and was, therefore, relatively long-lasting. The present study is to our knowledge the first to

337   describe this persistence in any free-ranging avian species. A single previous study on free-

338   ranging birds other than rufous-winged sparrows investigated whether acute stress influences

339   plasma LH (white-crowned sparrow: [69]). As found here, the inhibitory effect of stress on

340   plasma T in these sparrows was not associated with a decline in plasma LH. The stress-induced

341   decline in plasma T was not related to the stress-induced increase in plasma CORT, indicating

342   that the decline in plasma T was not simply a function of increased CORT secretion.

343   Furthermore, the plasma T response to stress was relatively larger in males with high than low

344   plasma initial T, revealing a complex, plasma initial T-related T response to stress. These data

345   provide new insights on interactions between acute stress and reproductive hormones in free-

346   ranging birds and on the mechanisms that potentially mediate these interactions, and they

347   contribute to our understanding of the bases of individual differences in circulating androgen

348   levels in intact birds.

349

350   *4.1. Inhibitory effect of acute stress on plasma testosterone: characterization and mechanisms*

351

352   As previously reported for white-crowned [69] and rufous-winged sparrows [13], acute

353   stress in the present study decreased plasma T without affecting plasma LH. In a previous

354   investigation, acute stress also did not attenuate the LH response of male Rufous-winged

355   Sparrows to an injection of the gonadotropin-releasing hormone (GnRH) secretagogue N-

356   methyl-D,L-aspartate (NMA) or of GnRH itself, or the T response to LH administration [13].

357   These results suggest that acute stress in this species does not decrease plasma T by acting on

358   the hypothalamo-pituitary axis. Furthermore, the effect of stress was time-dependent: plasma T

359   decreased as little as 10 min after capture and restraint and then further decreased as the

360   restraint duration increased. The short latency for stress to affect plasma T and the time-

361   dependency of the T response suggest mediation of the response by one or several non-

362   genomic mechanisms. One such mechanism may consist of a rapid direct inhibition of testicular

363   function by glucocorticoids [14,24,27,39]. Alternatively, glucocorticoids may influence plasma T

364   by accelerating its clearance through interactions with plasma corticosterone-binding globulin,

365   which in birds binds CORT and T reversibly, competitively, and with high affinity [9]. To our

366   knowledge, no study has, however, investigated whether CORT influences T clearance in any

367   species.

368   Acute stress in rufous-winged sparrows had overall opposite effects on plasma CORT
369 (increase) and T (decrease; compare Figs. 1 and 3). However, and similar to the situation in the
370 black-legged kittiwake, *Rissa tridactyla* [19], sparrows showed no individual correlation between
371 plasma initial CORT and T. We also found no correlation between the plasma concentrations of
372 these hormones in stressed sparrows. These data do not refute the possibility that CORT
373 influences the plasma T response to stress, but they indicate that if present, this influence is
374 complex and not reflected simply in the plasma concentrations of these hormones.
375   Acute stress may, alternately, decrease plasma T through a glucocorticoid-independent
376 mechanism. One such mechanism may consist of a gonadotropin-inhibitory hormone- (GnIH)
377 mediated impairment of T production [41] and/or a suppression of testicular endocrine function
378 resulting from stress-mediated activation of a sympathetic nervous pathway terminating in the
379 gonads. This pathway and its inhibitory influence on T secretion during stress have been
380 defined in mammals [26,28]. Avian testes receive sympathetic innervation [65] but the function
381 of this innervation in birds has not been investigated.
382
383 *4.2. Persistence of the stress-induced plasma testosterone decrease: mechanism and*
384 *consequences*
385
386   Previous studies found that the adrenocortical CORT response to stress changes during
387 repeated stress exposure. For example, free-ranging female eastern bluebirds, *Sialis sialis*, had
388 similar plasma initial CORT but increased their plasma level of this hormone more when caught
389 and restrained for the first time than a second time weeks later [37]. Similarly, the effect of acute
390 stress on plasma CORT in captivity decreased during repeated exposure to stressors in rats
391 [18] and American kestrels, *Falco sparverius* [35]. These observations indicate that acute stress
392 can alter the HPA sensitivity to subsequent stress exposure, but do not indicate how long the
393 endocrine effects of acute stress persist following a single stressful event and once subjects are
394 no longer exposed to the stressor. To our knowledge the present investigation is the first to
395 address this issue in free-ranging birds. In rufous-winged sparrows that we caught and
396 restrained for 30 min and then released on site, plasma T decreased by approximately 50% and
397 then remained low for at least 6 hours. During this period, plasma T levels did, on average, not
398 differ from levels at the time of release. Thus, the post-release endocrine effect of acute stress
399 persisted for several hours during which birds exhibited no sign of recovery.

400   Sparrows were recaptured using conspecific song playbacks and while still on their
401   breeding territory. Acute stress did, therefore, not lead to territory abandonment or eliminate
402   aggressive responses. However, as we did not study the behavior of the experimental birds, it
403   cannot be excluded that post-release low plasma T was associated with a partial inhibition of
404   spontaneous or STI-induced song rate or aggressivity. This hypothesis would be consistent with
405   the mounting body of evidence demonstrating that T can exert rapid effects (min to hours;
406   review: [42]). For example, in the White-crowned Sparrow T withdrawal after chronic treatment
407   with this hormone reduced the size of and increased the density of neurons in one brain region
408   involved in song production, the HVC, within 12 hours [59]. In the castrated male Japanese
409   quail, T treatment for one day sufficed to increase the size of the preoptic nucleus, which
410   controls reproductive behavior, and the expression of aromatase in this region [4]. In fish, an
411   opportunity to increase social status was followed within 30 min by increased expression of
412   reproductive behavior and plasma androgen levels [40], and T administration stimulated males
413   to approach females within 45 min [34]. In light of these findings, low plasma T for several hours
414   after release, as seen in the present study, may have been associated with subtle behavioral
415   and/or physiological changes.
416   Whether this was the case requires further investigation because it is unknown whether
417   a decrease in plasma T such as found here (average ~50%), and irrespective of the duration of
418   this decrease, is behaviorally or physiologically consequential. Two commonly used
419   experimental approaches to investigate the effects of T consist of (a) castration and (b)
420   hormone replacement to castrates or administration to intact subjects with naturally low plasma
421   T. Castration eliminates the main source of T, resulting in the steroid circulating at negligible or
422   undetectable concentrations. This manipulation in birds negatively influences the brain
423   production of T-sensitive enzymes such as aromatase [64], the size and neuronal
424   characteristics of androgen-sensitive brain regions [58], and the expression of androgen-
425   dependent behaviors [1,23,48]. In the second approach, T is often administered chronically to
426   result in circulating levels of the hormone that are similar to naturally maximum levels [2,49].
427   However, we know little about the shape of the relationship between plasma T concentrations
428   varying within the physiological range and the expression of androgen-dependent behavioral,
429   morphological, and physiological traits. As is commonly the case in other endocrine systems
430   [45], this relationship appears to be non-linear [30,31] and this non-linearity may contribute to

431   the frequently reported absence of correlation in intact birds between plasma T and the

432   expression of T-dependent behavior (e.g., [56]).

433

434   *4.3. Plasma testosterone: individual variability and relationship between plasma initial and*

435   *stress-related hormone levels*

436

437   Plasma T in most high and middle latitude breeders undergoes large seasonal changes

438   and is generally highest during the breeding season [46,62,55]. This pattern can be modified by

439   social factors, which in some circumstances rapidly elevate plasma T above seasonal levels

440   [66,68,51]. As a result, plasma T at any given time reflects the combined influence of seasonal

441   and facultative regulatory factors [20]. The multiplicity and complexity of mechanisms that

442   regulate plasma T usually result in large inter-individual variability, as noted in previous studies

443   [29] as well as in the present investigation. Individual variation in plasma initial T in rufous-

444   winged sparrows was not accounted for by the capture time, the body size, the body condition

445   index, or exposure to STI [12]. We found plasma initial and stress-related T concentrations to be

446   positively related (Fig. 4, left panels). However, close examination of the data revealed this

447   relationship to be complex: when restrained for 10, 20, or 30 min, sparrows with initially low

448   plasma T decreased their hormone level proportionally less than birds with initially high plasma

449   T (Fig. 4, right panels). For example, after restraint for 20 or 30 min plasma T had, on average,

450   decreased by approximately 20% in birds with initially low ($\leq$ 5 ng/ml) plasma T, but by

451   approximately 70% in birds with initially high ($\geq$ 10 ng/ml) plasma T. Thus, individuals with low

452   plasma initial T were relatively more resistant to the effects of acute stress than those with high

453   plasma initial T. A somewhat similar situation was observed in the semipalmated sandpiper [21].

454   In this species, plasma T in response to capture and restraint stress increased and decreased in

455   birds with initially low and high plasma T, respectively. What is the potential significance of

456   these observations?

457   Seasonally (i.e., in many bird species, photoperiodically) regulated plasma T levels are

458   thought to be necessary and sufficient to maintain androgen-dependent physiological and

459   behavioral functions such as reproductive behavior, spermatogenesis, and secondary sexual

460   characters [10,20]. In previous avian and non-avian studies showing that acute stress inhibits

461   plasma T [43,47,69,70], this inhibition was only partial, resulting in stress-induced plasma T

462   remaining within 25% - 75% of plasma initial T. We found here that when exposed to a same

463   stressor, birds with high plasma initial T decreased their plasma T proportionally more than birds

464   with low plasma initial T. Furthermore, restraint for 10 min decreased plasma T less than

465   restraint for longer durations, but the effect of restraint for 20 min or 30 min on plasma T were

466   similar. These findings, along with data indicating that social interactions can in some situations

467   increase T secretion within minutes, confirm that plasma T levels are labile and prone to rapid

468   changes. The available results are consistent with the hypothesis that when faced with acute

469   stress, organisms decrease their plasma T, but not below the seasonally appropriate level

470   necessary to maintain essential androgen-dependent functions. Further research is warranted

471   to identify the putative mechanism that controls the balance between inhibition and maintenance

472   of plasma T above physiologically and behaviorally necessary levels.

473

474

475   **Acknowledgments**

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480 **References**
481

1. Arnold, A. P. The effects of castration and androgen replacement on song, courtship, and aggression in zebra finches (*Poephila guttata*). Journal of Experimental Zoology (1975) 309-326.

2. Ashley, N. T., Hays, Q. R., Bentley, G. E., Wingfield, J. C. Testosterone treatment diminishes sickness behavior in male songbirds. Hormones and Behavior (2009) 169-176.

3. Brewer, J. H., O'Reilly, K. M., Dean, K. S., Loren, B. C. Interannual variation in the adrenal responsiveness of black-legged kittiwake chicks (*Rissa tridactyla*). General and Comparative Endocrinology (2008) 361-368.

4. Charlier, T. D., Ball, G. F., Balthazart, J. Rapid action on neuroplasticity precedes behavioral activation by testosterone. Hormones and Behavior (2008) 488-495.

5. Cockrem, J. F., Silverin, B. Variation within and between birds in corticosterone responses of great tits (*Parus major*). General and Comparative Endocrinology (2002) 197-206.

6. Conover, W. J., Iman, R. L. Rank-transformations as a bridge between parametric and nonparametric statistics. American Statistician (1981) 124-129.

7. Cyr, N. E., Romero, L. M. Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. General and Comparative Endocrinology (2007) 82-89.

8. Dawson A., Howe, P. D. Plasma corticosterone in wild starlings (*Sturnus vulgaris*) immediately following capture and in relation to body weight during the annual cycle. General and Comparative Endocrinology (1983) 303-308.

9. Deviche, P., Breuner, C., Orchinik, M. Testosterone, corticosterone, and photoperiod interact to regulate plasma levels of binding globulin and free steroid hormone in dark-eyed juncos, *Junco hyemalis*. General and Comparative Endocrinology (2001) 67-77.

10. Deviche, P., Hurley, L. L., Fokidis, B. H. Avian testicular structure, function, and regulation. In "Hormones and Reproduction of Vertebrates", Vol. 4 (D.O. Norris and K.H. Lopez, Eds), pp. 27-69. Academic Press, San Diego, CA (2011).

511   11. Deviche, P., Sabo, J., Sharp, P. J. Glutamatergic stimulation of luteinising hormone
512   secretion in relatively refractory male songbirds. Journal of Neuroendocrinology
513   (2008) 1191-1202.

514   12. Deviche, P., Small, T., Sharp, P., Tsutsui, K. Control of luteinizing hormone and
515   testosterone secretion in a flexibly breeding male passerine, the rufous-winged
516   sparrow, *Aimophila carpalis*. General and Comparative Endocrinology (2006)
517   226-235.

518   13. Deviche, P. J., Hurley, L. L., Fokidis, H. B., Lerbour, B., Silverin, B., Silverin, B., Sabo,
519   J., Sharp, P. J. Acute stress rapidly decreases plasma testosterone in a free-
520   ranging male songbird: potential site of action and mechanism. General and
521   Comparative Endocrinology (2010) 82-90.

522   14. Dong, Q., Salva, A., Sottas, C. M., Niu, E., Holmes, M., Hardy, M. P. Rapid
523   glucocorticoid mediation of suppressed testosterone biosynthesis in male mice
524   subjected to immobilization stress. Journal of Andrology (2004) 973-981.

525   15. El Halawani, M. E., Silsby, J. L., Fehrer, S. C., Behnke, E. J. The influence of acute or
526   repeated immobilization on plasma prolactin levels in the turkey (*Meleagris*
527   *gallopavo*). General and Comparative Endocrinology (1985) 410-415.

528   16.   Fair, J. M., Paul, E., Jones, J., Clark, A. B., Davie, C., Kaiser, G. Guidelines to the use
529   of wild birds in research. Ornithological Council, Washington, D.C. (2010), 209
530   pp.

531   17. Fokidis, H. B., Deviche, P. Plasma corticosterone of city and desert curve-billed
532   thrashers, *Toxostoma curvirostre*, in response to stress-related peptide
533   administration. Comparative Biochemistry and Physiology A: Molecular and
534   Integrative Physiology (2011) 32-38.

535   18. Gadek-Michalska, A., Bugajski, J. Repeated handling, restraint, or chronic crowding
536   impair the hypothalamic-pituitary-adrenocortical response to acute restraint
537   stress. Journal of Physiology and Pharmacology (2003) 449-459.

538   19. Goutte, A., Angelier, F., Chastel, C. C., Trouve, C., Moe, B., Bech, C., Gabrielsen, G.
539   W., Chastel, O. Stress and the timing of breeding: glucocorticoid-luteinizing
540   hormones relationships in an arctic seabird. General and Comparative
541   Endocrinology (2010) 108-116.

542   20. Goymann, W. Social modulation of androgens in male birds. General and Comparative
543   Endocrinology (2009) 149-157.

544   21. Gratto-Trevor, C. L., Oring, L. W., Fivizzani, A. J. Effects of blood sampling stress on
545   hormone levels in the semipalmated sandpiper. Journal of Field Ornithology
546   (1991) 19-27.

547   22. Green, A. J. Mass/length residuals: measures of body condition or generators of
548   spurious results? Ecology (2001) 1473-1483.

549   23. Hagelin, J. C. Castration in Gambel's and scaled quail: Ornate plumage and dominance
550   persist, but courtship and threat behaviors do not. Hormones and Behavior
551   (2001) 1-10.

552   24. Hardy, M. P., Gao, H. B., Dong, Q., Ge, R., Wang, Q., Chai, W. R., Feng, X., Sottas, C.
553   Stress hormone and male reproductive function. Cell and Tissue Research
554   (2005) 147-153.

555   25. Heiblum, R., Arnon, E., Gvaryahu, G., Robinzon, B., Snapir, N. Short-term stress
556   increases testosterone secretion from testes in male domestic fowl. General and
557   Comparative Endocrinology (2000) 55-66.

558   26. Herman, M., Rivier, C. Activation of a neural brain-testicular pathway rapidly lowers
559   Leydig cell levels of the steroidogenic acute regulatory protein and the
560   peripheral-type benzodiazepine receptor while increasing levels of neuronal nitric
561   oxide synthase. Endocrinology (2006) 624-633.

562   27. Hu, G. X., Lian, Q. Q., Lin, H., Latif, S. A., Morris, D. J., Hardy, M. P., Ge, R. S. Rapid
563   mechanisms of glucocorticoid signaling in the Leydig cell. Steroids (2008) 1018-
564   1024.

565   28. James, P., Rivier, C., Lee, S. Presence of corticotrophin-releasing factor and/or tyrosine
566   hydroxylase in cells of a neural brain-testicular pathway that are labelled by a
567   transganglionic tracer. Journal of Neuroendocrinology (2008) 173-181.

568   29. Kempenaers, B., Peters, A., Foerster, K. Sources of individual variation in plasma
569   testosterone levels. Philosophical Transactions of the Royal Society B: Biological
570   Sciences (2008) 1711-1723.

571   30. Klint, T., Johnson, A. L., Cheng, M. F. Factors in the dose-response effect of
572   testosterone in the male ring dove. Physiology and Behavior (1984) 1037-1040.

573   31. Klukowski, L. A., Cawthorn, J. M., Ketterson, E. D., Nolan, V., Jr. Effects of
574   experimentally elevated testosterone on plasma corticosterone and
575   corticosteroid-binding globulin in dark-eyed juncos (*Junco hyemalis*). General
576   and Comparative Endocrinology (1997) 141-151.

577   32. Kwok, A. H., Wang, Y., Wang, C. Y., Leung, F. C. Cloning of chicken glucocorticoid
578   receptor (GR) and characterization of its expression in pituitary and extrapituitary
579   tissues. Poultry Science (2007) 423-430.

580   33. Lendvai, A. Z., Giraudeau, M., Chastel, O. Reproduction and modulation of the stress
581   response: an experimental test in the house sparrow. Proceedings of the Royal
582   Society B: Biological Sciences (2007) 391-397.

583   34. Lord, L. D., Bond, J., Thompson, R. R. Rapid steroid influences on visually guided
584   sexual behavior in male goldfish. Hormones and Behavior (2009) 519-526.

585   35. Love, O. P., Shutt, L. J., Silfies, J. S., Bird, D. M. Repeated restraint and sampling
586   results in reduced corticosterone levels in developing and adult captive American
587   kestrels (*Falco sparverius*). Physiological and Biochemical Zoology (2003) 753-
588   761.

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

592   37. Lynn, S. E., Prince, L. E., Phillips, M. M. A single exposure to an acute stressor has
593   lasting consequences for the hypothalamo-pituitary-adrenal response to stress in
594   free-living birds. General and Comparative Endocrinology (2010) 337-344.

595   38. Lynn, S. E., Stamplis, T. B., Barrington, W. T., Weida, N., Hudak, C. A. Food, stress, and
596   reproduction: short-term fasting alters endocrine physiology and reproductive
597   behavior in the zebra finch. Hormones and Behavior (2010) 214-222.

598   39. Martin, L. J., Tremblay, J. J. Glucocorticoids antagonize cAMP-induced Star transcription
599   in Leydig cells through the orphan nuclear receptor NR4A1. Journal of Molecular
600   Endocrinology (2008) 165-175.

601   40. Maruska, K. P., Fernald, R. D. Behavioral and physiological plasticity: rapid changes
602   during social ascent in an African cichlid fish. Hormones and Behavior (2010)
603   230-240.

604   41.   McGuire, N. L., Bentley, G. E. A functional neuropeptide system in vertebrate gonads:
605   Gonadotropin-inhibitory hormone and its receptor in testes of field-caught house
606   sparrow (*Passer domesticus*). General and Comparative Endocrinology (2010)
607   565-572.

608   42.   Michels, G., Hoppe, U. C. Rapid actions of androgens. Frontiers in Neuroendocrinology
609   (2008) 182-198.

610   43.   Moore, I. T., Perfito, N., Wada, H., Sperry, T. S., Wingfield, J. C. Latitudinal variation in
611   plasma testosterone levels in birds of the genus *Zonotrichia*. General and
612   Comparative Endocrinology (2002) 13-19.

613   44.   Newman, A. E., Pradhan, D. S., Soma, K. K. Dehydroepiandrosterone and
614   corticosterone are regulated by season and acute stress in a wild songbird:
615   jugular versus brachial plasma. Endocrinology (2008) 2537-2545.

616   45.   Norris, D. O. Vertebrate Endocrinology. Academic Press, Amsterdam, 550 pp. (2007).

617   46.   Penfold, L. M., Wildt, D. E., Herzog, T. L., Lynch, W., Ware, L., Derrickson, S. E.,
618   Monfort, S. L. Seasonal patterns of LH, testosterone and semen quality in the
619   northern pintail duck (*Anas acuta*). Reproduction, Fertility and Development
620   (2000) 229-235.

621   47.   Pickering, A. D., Pottinger, T. G., Carragher, J., Sumpter, J. P. The effects of acute and
622   chronic stress on the levels of reproductive hormones in the plasma of mature
623   male brown trout, *Salmo trutta* L. General and Comparative Endocrinology
624   (1987) 249-259.

625   48.   Pinxten, R., De, R. E., Balthazart, J., Eens, M. Context-dependent effects of castration
626   and testosterone treatment on song in male European starlings. Hormones and
627   Behavior (2002) 307-318.

628   49.   Plumari, L., Plateroti, S., Deviche, P., Panzica, G. C. Region-specific testosterone
629   modulation of the vasotocin-immunoreactive system in male dark-eyed junco,
630   *Junco hyemalis*. Brain Research (2004) 1-8.

631   50.   Romero, L. M., Reed, J. M. Collecting baseline corticosterone samples in the field: is
632   under 3 min good enough? Comparative Biochemistry and Physiology A:
633   Molecular and Integrative Physiology (2005) 73-79.

634   51. Ros, A. F. H., Dieleman, S. J., Groothuis, T. G. G. Social stimuli, testosterone, and
635   aggression in gull chicks: Support for the challenge hypothesis. Hormones and
636   Behavior (2002) 334-342.

637   52. Schoech, S. J., Ketterson, E. D., Nolan, V., Jr. Exogenous testosterone and the
638   adrenocortical response in dark-eyed juncos. The Auk (1999) 64-72.

639   53. Schoech, S. J., Rensel, M. A., Bridge, E. S., Boughton, R. K., Wilcoxen, T. E.
640   Environment, glucocorticoids, and the timing of reproduction. General and
641   Comparative Endocrinology (2009) 201-207.

642   54. Sharp, P. J., Dunn, I. C., Talbot, R. T. Sex differences in the LH responses to chicken
643   LHRH-I and -II in the domestic fowl. Journal of Endocrinology (1987) 323-331.

644   55. Shi, Z. D., Huang, Y. M., Liu, Z., Liu, Y., Li, X. W., Proudman, J. A., Yu, R. C. Seasonal
645   and photoperiodic regulation of secretion of hormones associated with
646   reproduction in Magang goose ganders. Domestic Animal Endocrinology (2007)
647   190-200.

648   56. Silverin, B., Baillien, M., Balthazart, J. Territorial aggression, circulating levels of
649   testosterone, and brain aromatase activity in free-living pied flycatchers.
650   Hormones and Behavior (2004) 225-234.

651   57. Small, T. W., Sharp, P. J., Deviche, P. Environmental regulation of the reproductive
652   system in a flexibly breeding Sonoran Desert bird, the rufous-winged sparrow,
653   *Aimophila carpalis*. Hormones and Behavior (2007) 483-495.

654   58. Strand, C. R., Deviche, P. Hormonal and environmental control of song control region
655   growth and new neuron addition in adult male house finches, *Carpodacus*
656   *mexicanus*. Developmental Neurobiology (2007) 827-837.

657   59. Thompson, C. K., Bentley, G. E., Brenowitz, E. A. Rapid seasonal-like regression of the
658   adult avian song control system. Proceeding of the National Academy of
659   Sciences USA (2007) 15520-15525.

660   60. Tilbrook, A. J., Turner, A. I., Clarke, I. J. Effects of stress on reproduction in non-rodent
661   mammals: the role of glucocorticoids and sex differences. Reviews in
662   Reproduction (2000) 105-113.

663   61. Van Hout, A. J., Eens, M., Darras, V. M., Pinxten, R. Acute stress induces a rapid
664   increase of testosterone in a songbird: implications for plasma testosterone
665   sampling. General and Comparative Endocrinology (2010) 505-510.

666   62. Van, D. E., Pinxten, R., Eens, M. Seasonal fluctuations in plasma testosterone levels
667   and diurnal song activity in free-living male great tits. General and Comparative
668   Endocrinology (2003) 1-9.

669   63. Viau, V. Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal
670   axes. Journal of Neuroendocrinology (2002) 506-513.

671   64. Vockel, A., Prove, E., Balthazart, J. Effects of castration and testosterone treatment on
672   the activity of testosterone-metabolizing enzymes in the brain of male and female
673   zebra finches. Journal of Neurobiology (1990) 808-825.

674   65. Wakita, T., Ohmori, Y., Watanabe, T., Fukuta, K. Location of sympathetic postganglionic
675   and sensory neurons innervating the testis in the male chicken. Anatomy
676   Histology and Embryology (1999) 299-302.

677   66. Wingfield, J. C. Environmental and endocrine control of reproduction in the song
678   sparrow, Melospiza melodia. I. Temporal organization of the breeding cycle.
679   General and Comparative Endocrinology (1984) 406-416.

680   67. Wingfield, J. C. Influence of weather on reproduction. Journal of Experimental Zoology
681   (1984) 589-594.

682   68. Wingfield, J. C., Hahn, T. P. Testosterone and territorial behaviour in sedentary and
683   migratory sparrows. Animal Behaviour (1994) 77-89.

684   69. Wingfield, J. C., Smith, M. J., Farner D.S. Endocrine responses of white-crowned
685   sparrows to environmental stress. The Condor (1982) 399-409.

686   70. Woodley, S. K., Lacy, E. L. An acute stressor alters steroid hormone levels and activity
687   but not sexual behavior in male and female Ocoee salamanders (*Desmognathus*
688   *ocoee*). Hormones and Behavior (2010) 427-432.

689

Figure legends

Fig. 1. Plasma corticosterone (CORT; medians + 0.5 interquartile intervals) of adult male

rufous-winged sparrows, *Peucaea carpalis*, within 2 min of capture (Initial) and after 5, 10, or 20

min of restraint (Stress-induced). Sample sizes are indicated within columns. An asterisk

denotes a statistically significant increase relative to plasma initial T ($p \leq 0.05$; Student-

Newman-Keuls test).

Fig. 2. Plasma luteinizing hormone (LH; means + s.e.'s) of adult male rufous-winged sparrows,

Peucaea carpalis measured within 2 min of capture (= Initial) and after restraint for 5, 10, or 20

min (= Stress-induced). Sample sizes are indicated within columns.

Fig. 3. Plasma testosterone (T; means + s.e.'s) of adult male rufous-winged sparrows, *Peucaea*

carpalis measured within 2 min of capture (= Initial) and after restraint for 5, 10, or 20 min (=

Stress-induced). An asterisk denotes a statistically significant decrease relative to plasma initial

T ($p < 0.05$; Student-Newman-Keuls test); n.s. = $p > 0.05$. Sample sizes are indicated within

columns.

Fig. 4. Relationships in adult male rufous-winged sparrows, *Peucaea carpalis*, between plasma

initial testosterone (T) and plasma T after capture and restraint for 10, 20, or 30 min (left

panels); and plasma initial T and the percentage decrease in plasma T associated with capture

and restraint for 10, 20, or 30 min (right panels). On each panel, each point represents one

different individual.

Fig. 5. (a) Plasma testosterone (means $\pm$ s.e.'s; n = 17) of adult male rufous-winged sparrows,

Peucaea carpalis, at capture (Initial), after 30 min of restraint (30 min stress), and at recapture

0.5 – 7 hrs after release (Recapture). Means with the same letter do not differ significantly ($P >$

0.05; Bonferroni t-test). (b) Difference between plasma T at recapture and plasma initial T of the

same males as in panel (a). (c) Difference between plasma T at recapture and at release of the

same males as in panel (a). Each point on panels b and c represents one individual.

Figure 1.

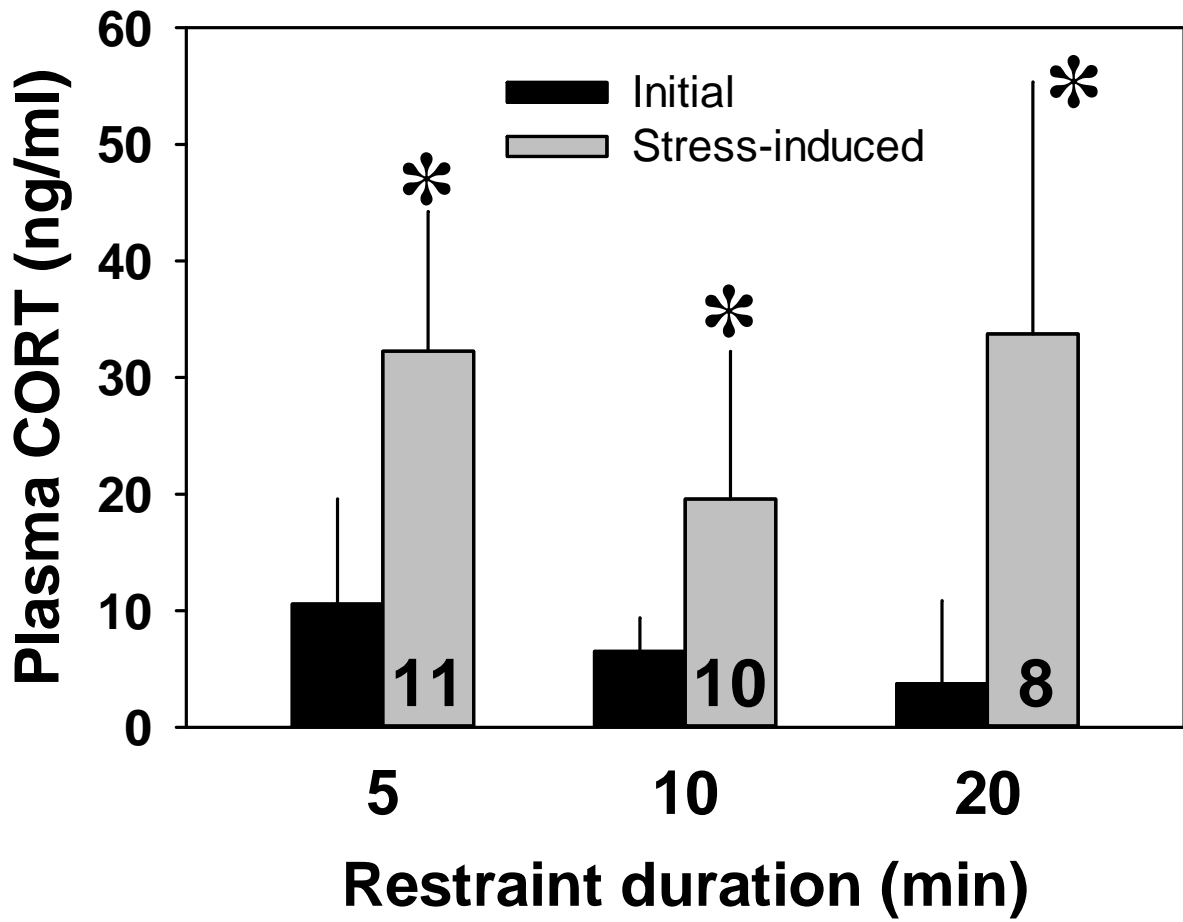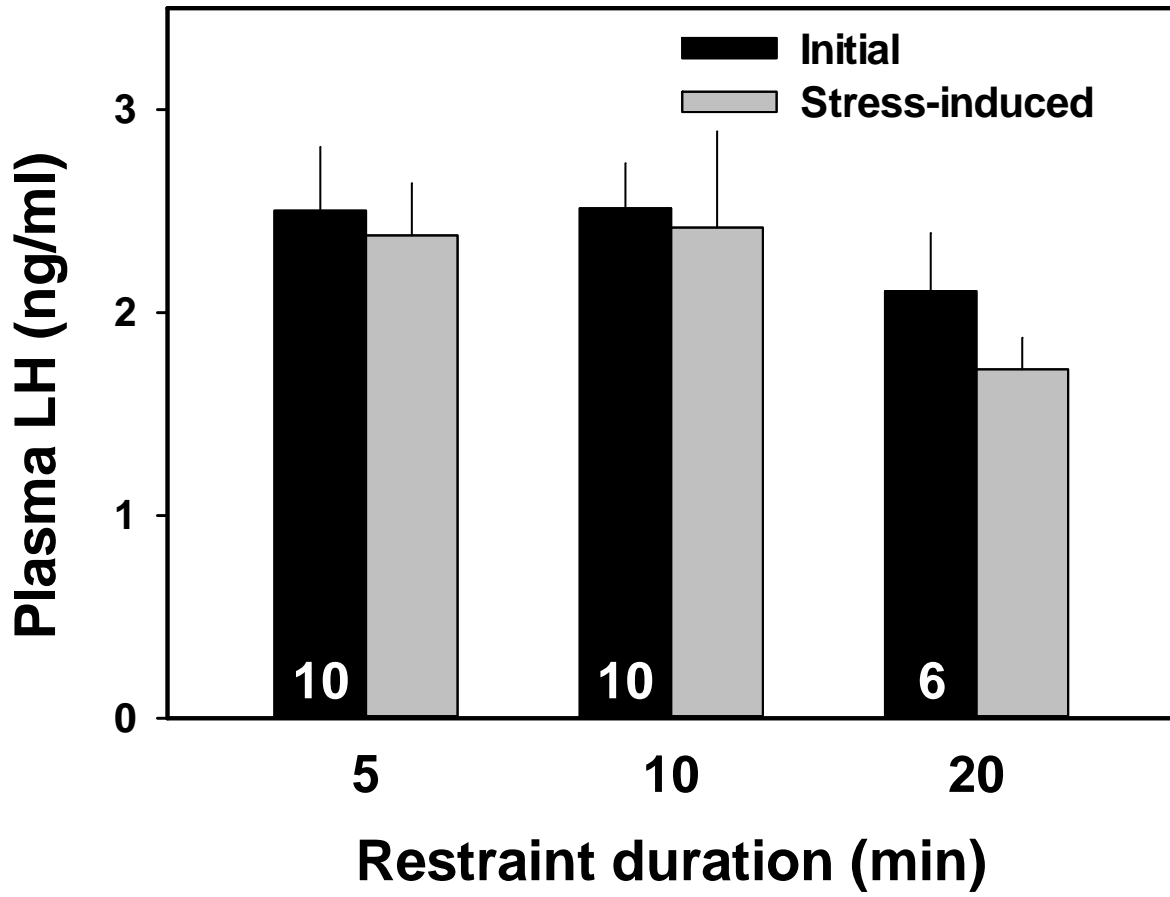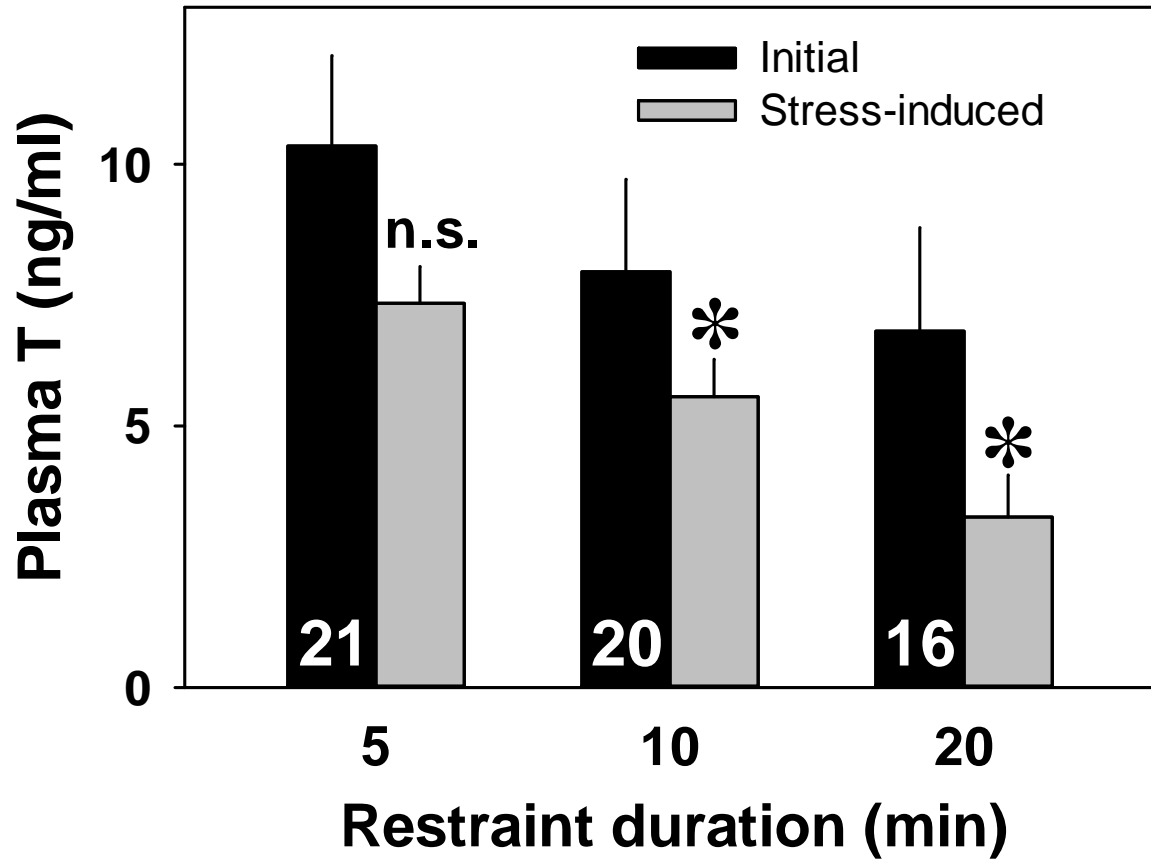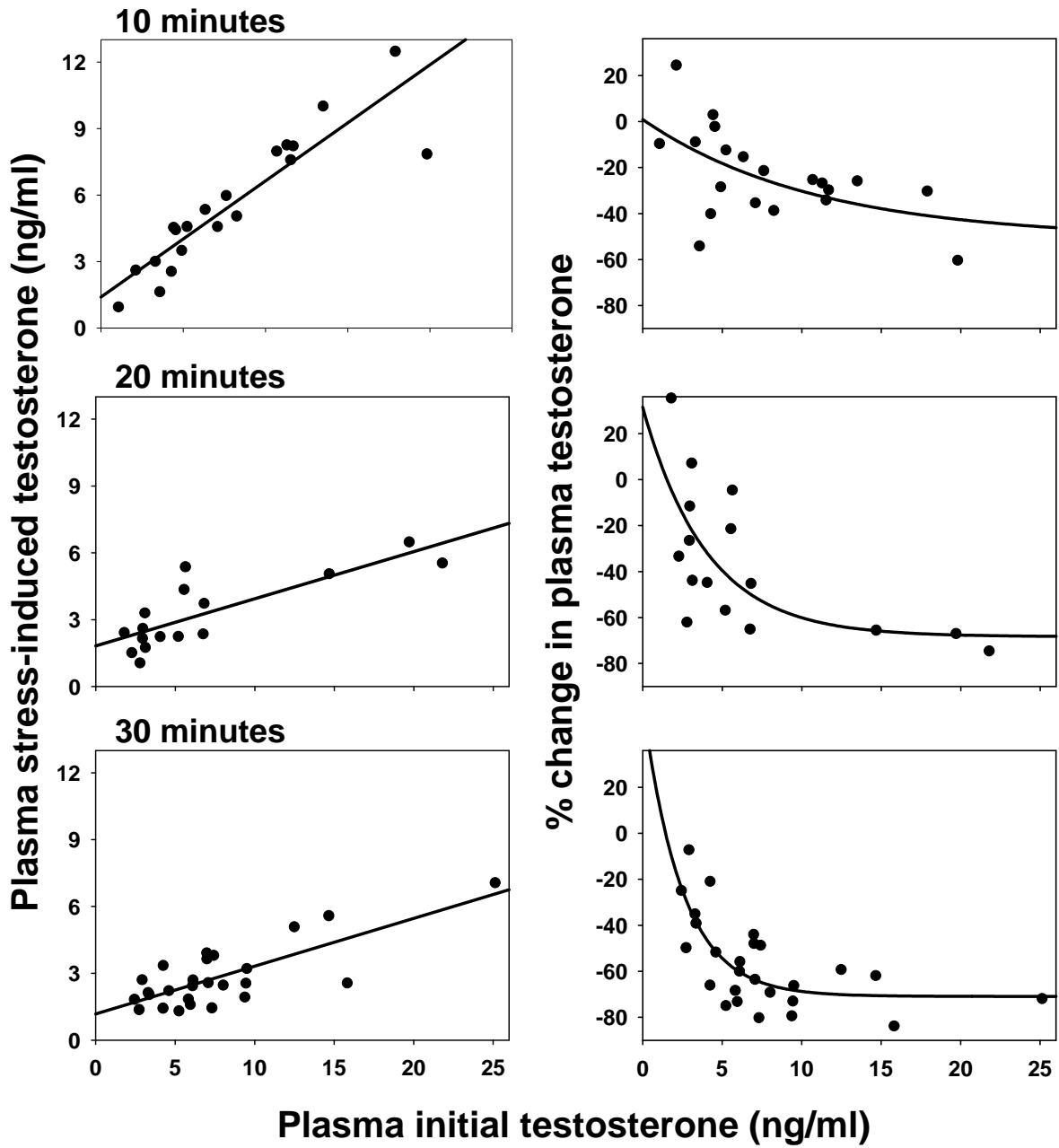Figure 2.

Figure 3.

Figure 4.

10 minutes

20 minutes

30 minutes

Plasma stress-induced testosterone (ng/ml)

% change in plasma testosterone

Plasma initial testosterone (ng/ml)
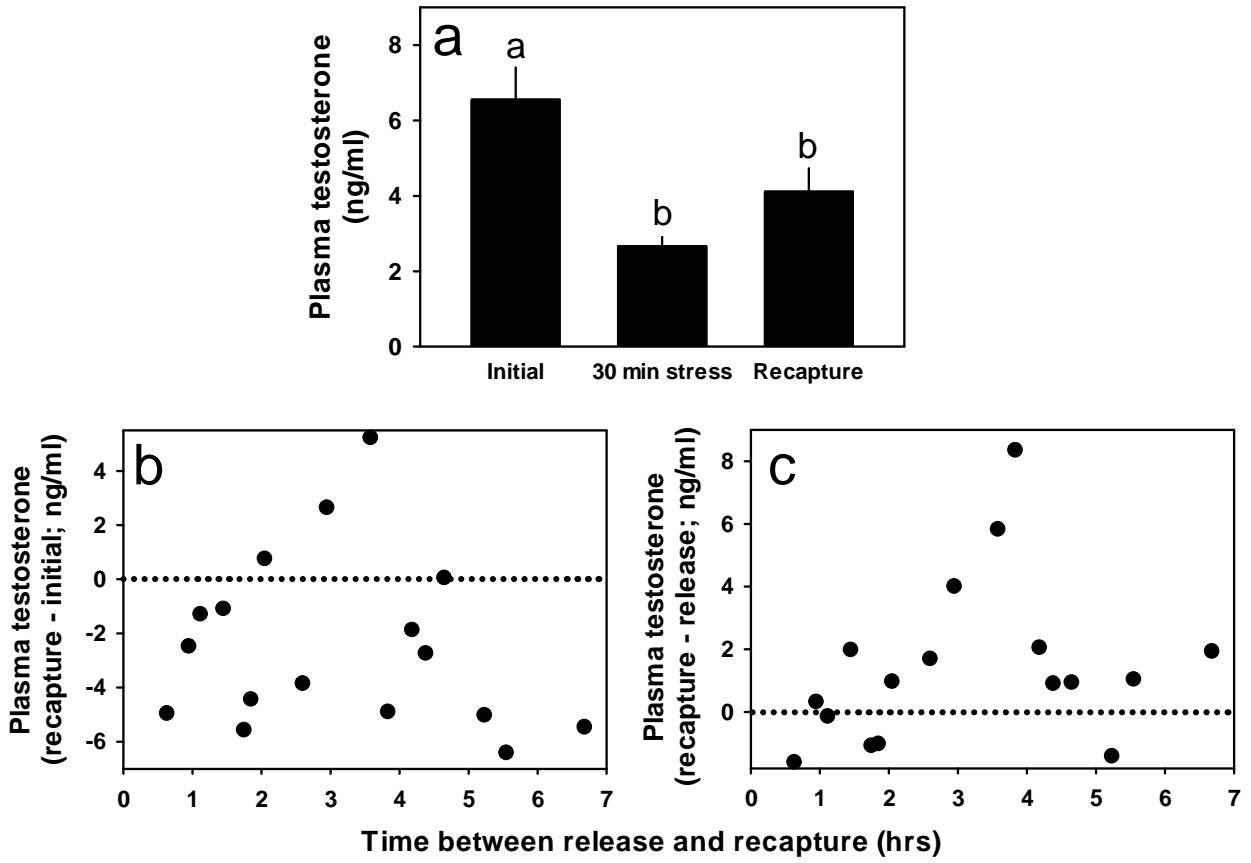
Figure 5.

Table 1: Comparison (means ± s.e.'s unless otherwise indicated) of various parameters between adult male rufous-winged sparrows, *Peucaea carpalis*, which were or not recaptured within hours of an initial capture in 2010 and 2011.

Year	Recapture?	*n*	Initial capture time (hrs, decimal) [a]	Plasma initial T (ng/ml) *	30 min stress plasma T (ng/ml) *	Wing chord (mm) [a]	Body mass (g)	Body condition index**
2010	Yes	8	9.9 ± 3.3	4.61 ± 1.22	2.80 ± 0.55	62 ± 1	15.7 ± 0.1	-0.12 ± 0.14
	No	4	7.8 ± 2.2	6.09 ± 1.53	N/A	61 ± 0	15.2 ± 0.3	-0.49 ± 0.31
2011	Yes	17	6.8 ± 0.9	6.55 ± 0.86	2.66 ± 0.25	62 ± 1	15.9 ± 0.2	-0.06 ± 0.22
	No	9	11.7 ± 2.6	9.53 ± 2.25	3.06 ± 0.65	62 ± 1	16.3 ± 0.2	0.44 ± 0.21
Recaptured *vs.* not recaptured males			Recap. < Not Recap. (2011 only)	n.s.	n.s.	n.s.	n.s.	n.s.

*T = testosterone;

** body condition index expressed as the residual of a reduced major axis linear regression of wing chord over body mass;

[a]: medians + 0.5 interquartile intervals;

n.s. = $p > 0.05$ (analysis of variance).