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Effects of capture and captivity on plasma corticosterone and metabolite levels in breeding Eurasian Tree Sparrows

Mo Li[1], Weiwei Zhu[1], Yang Wang[1], Yanfeng Sun[2], Juyong Li[1], Xuelu Liu[1], Yuefeng Wu[1], Xuebin Gao[3*] and Dongming Li[1*]

Abstract

Background: Bringing free-living animals into captivity subjects them to the stress of both capture and captivity, leading to the alteration of normal physiological processes and behaviors through activation of the hypothalamic–pituitary–adrenal axis. In free-living birds, although elevated plasma corticosterone (CORT) is an important adaptation regulating physiological and behavioral responses during the process of capture and captivity stress, little information is currently available on the effects of such stress on plasma metabolite levels.

Methods: We examined the effects of immediate capture and 24-h captivity on body mass, body condition, plasma CORT, and metabolite levels including glucose (Glu), triglyceride (TG), total cholesterol (TC), uric acid (UA), in breeding Eurasian Tree Sparrows (*Passer montanus*).

Results: CORT and Glu levels were increased significantly by the stress of capture, whereas TC and UA levels decreased. Body mass, body condition declined notably after 24 h in captivity, but CORT, Glu, and UA levels increased. Furthermore, male sparrows had lower TG levels after both capture and captivity than those of females. The relationships between plasma CORT and metabolite levels varied between sexes.

Conclusions: Our results revealed that the metabolic status of Eurasian Tree Sparrows could be dramatically altered by capture and captivity. Monitoring the dynamic effects of both capture and captivity on plasma CORT, metabolite levels in a free-living bird contributes to a better understanding of the stress-induced pathways involved in sex-dependent energy mobilization.

Keywords: Capture stress, Captivity stress, Corticosterone, Plasma metabolites, Free-living birds

Background

Bringing free-living animals into captivity is frequently required for conservation, and for research in animal ecology, environmental physiology, and conservation biology (Dickens et al. 2010; Mason 2010; Mason et al. 2013; Dickens and Bentley 2014). This process subjects animals to both the acute stress of immediate capture and the chronic stress of captivity, resulting in the alteration of normal physiology and behavior (Dickens et al. 2010; Angelier et al. 2016). In vertebrates, capture stress may acutely activate the hypothalamic–pituitary–adrenal (HPA) axis to increase glucocorticoid (GC) levels that can enhance survival by regulating behavioral and physiological responses (Romero and Wingfield 1999; Sapolsky et al. 2000; Wingfield and Kitaysky 2002). However, captivity stress may chronically increase GC levels, which may reduce fitness through a suite of deleterious effects such as suppressing parental behavior, compromising reproductive success and promoting metabolic disorders (Dallman 1993; Wingfield et al. 1995; Vegiopoulos and Herzig 2007). Some previous studies have demonstrated the effects of capture and captivity stress on the HPA axis

*Correspondence: gaoxb63@163.com; lidngmng@gmail.com
[1] Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, China[3] Shaanxi Institute of Zoology, Xi'an 710032, China
Full list of author information is available at the end of the article

and behavioral responses of free-living animals (Rich and Romero 2005; Cyr et al. 2007, 2009; Dickens and Romero 2009; Dickens et al. 2009; Lattin et al. 2012; Fischer et al. 2018). To date, however, there has been little research on the dynamic changes of both plasma GCs and metabolites during the first hours, or days, free-living animals have spent in captivity (Angelier et al. 2016).

In mammals, stress-induced GCs promote hydrolysis of triglycerides (TG) stored in adipocytes except inducing an increase in circulating glucose (Glu) levels through hepatic gluconeogenesis and maintain hyperglycemia by suppressing the uptake of Glu into peripheral tissues (Grégoire et al. 1991; Yamada et al. 1993). Although increasing GC levels is a general adaptive characteristic used to mobilize energy stores in a variety of metabolic pathways (i.e., increased energy expenditure), the regulatory mechanisms and physiological consequences of capture stress and captivity stress differ (Sapolsky et al. 2000). Compared to mammals, birds can maintain higher, better-controlled, plasma Glu levels (Braun and Sweazea 2008; Li 2017). Although an increase in corticosterone (CORT, the main GC in birds) in response to acute stress is ubiquitous in free-living birds, alteration of plasma Glu levels is not universal. For example, plasma Glu was found to increase in response to capture stress in Abert's Towhees (*Melozone aberti*) and King Penguin (*Aptenodytes patagonicus*) chicks (Corbel et al. 2010; Davies et al. 2013), but not in Curve-billed Thrashers (*Toxostoma curvirostre*) (Fokidis et al. 2011). Moreover, plasma Glu response to capture stress varies with life-history stages in Rufous-winged Sparrows (*Peucaea carpalis*), i.e. not change in breeding stage, and decreased in molting and non-breeding stages (Deviche et al. 2016a). The reason for this variability in plasma Glu regulation in free-living birds remains largely unknown. In birds, plasma TG has been widely used as an indicator of body mass or condition (Guglielmo et al. 2005). A rapid decrease in the TG of free-living birds can be detected as soon as 20 min after capture (Guglielmo et al. 2002). The confinement, unfamiliar and unnatural conditions experienced by free-living animals in captivity can lead to the secretion of plasma GC and metabolites (e.g. Glu and TG), resulting in metabolic disorders, energetic stress and reduced fitness (Morgan and Tromborg 2007; Dickens et al. 2009, 2010; Mason 2010; Fokidis et al. 2011).

Uric acid (UA) is a product of protein degradation (Costantini 2008) and is also a potent antioxidant (Stinefelt et al. 2005; Cohen et al. 2007; Braun and Sweazea 2008). Capture stress can lead to decreased plasma UA in some bird species, which is believed to function as an antioxidant defense against increased free radicals during oxidative stress (Cohen et al. 2007; Davies et al. 2013). However, chronic stress of captivity may generally raise UA levels, which is thought to promote gluconeogenesis by increasing the use of amino acids (Klasing 1998). Cholesterol is an essential constituent of cell membranes modulating their fluidity and is also a precursor of steroid hormones. Hypocholesterolemia may be associated with low serum antioxidant reserves and may increase susceptibility to oxidative stress (Muldoon et al. 1996). It has, therefore, been hypothesized that cholesterol may act as an antioxidant (Schroepfer 2000). Even though oxidative stress typically triggers the stress response process (Costantini 2008), few studies have linked it to the modulation of stress response mediated by GC release.

Male and female birds exhibit sex-specific physiology associated with their differentiated reproductive functions, e.g., development of the testis and spermatogenesis in males and development of the ovary and oogenesis in females. In free-living birds, the effects of both capture and captivity stress on sex-specific CORT responses have been well documented (Dickens et al. 2010; Breuner 2011). However, the influences of capture and captivity stress on sex-specific metabolites have received little attention.

To determine the dynamic effects of both capture stress and subsequent captivity stress on plasma CORT and metabolites, we studied changes in plasma CORT, metabolite levels, including Glu, TG, total cholesterol (TC), and UA, of breeding Eurasian Tree Sparrows (*Passer montanus*) that were subject to both the stress of capture in the field and a subsequent 24-h period in captivity. The Eurasian Tree Sparrow is a typical human commensal species that distributes throughout the Eurasian continent (Summers-Smith 2014). In recent years, the reproductive biology and endocrinology of the Eurasian Tree Sparrow has been relatively well studied, e.g. their adrenocortical responses to capture stress during the breeding season in relation to body condition, testosterone (T), corticosteroid-binding globulin (CBG; Li et al. 2008, 2011, 2012, 2016; Zhao et al. 2017a, b). We hypothesized that (1) Glu would increase, and TG decrease in breeding Eurasian Tree Sparrows, concomitantly with increased CORT levels in response to both capture and captivity stress; (2) plasma UA would decrease and UA increase after capture and 24-h captivity stress; (3) male and female sparrows would differ in both baseline and stress-induced (capture and captivity) metabolite levels.

Methods

Animals and study sites

A total of 22 Eurasian Tree Sparrows (12 males and 10 females) were captured opportunistically with mist nets between 06:00 and 10:00 at the Shijiazhuang Academy of Agriculture and Forestry Sciences (38°06.71′N, 114°31.49′E, elevation: 73 m), and at the old (38°01.83′N,

114°31.50′E, elevation: 75 m), and new (37°59.88′N, 114°31.18′E, elevation: 72 m) campuses of Hebei Normal University, Hebei Province, China. All capture sites were situated in similar habitat near farmlands and buildings. All sparrows were captured during the breeding season: May 15–June 9, 2012–2013.

Experiment design of capture and captivity stress

All wild-caught sparrows were subject to a standardized capture-handling-restraint stress protocol (Wingfield et al. 1992; Li et al. 2008, 2011) within 1 h after capture in the field, followed by 24 h of captivity stress in cages. After capture and sampling in the field, each bird was individually housed in cages (40 cm × 30 cm × 30 cm) and provided with foxtail millet (*Setaria italica*) mixed with mealworms and water ad libitum, and transferred to a laboratory at Hebei Normal University. To reduce confounding variables the temperature and photoperiod in the laboratory were kept as close as possible to those in the birds' natural environment (temperature: 17–29 °C; photoperiod: 14.2–14.9 h).

Blood sampling and morphological measurements

In the field, baseline levels of plasma CORT and metabolites were sampled within 3 min of capture, and capture stress-induced levels were sampled 30 and 60 min post-capture. Briefly, a wing vein was punctured with a 26 gauge needle, and approximately 80 μL of blood was collected into heparinized microhematocrit capillary tubes. Birds were then placed in an opaque cloth bag, and additional blood samples were collected from them after another 30 and 60 min post-capture. In the laboratory, captivity stress-induced levels of plasma CORT and metabolites were sampled at the end of the 24 h period of captivity within 3 min of extracting birds from their cages. All blood samples were stored on ice for 3–4 h until they could be centrifuged in the laboratory at 855 × *g* for 10 min. Plasma samples were stored at − 20 °C until assayed.

After blood sampling was completed, each bird was weighed to ± 0.1 g, and its wing length, tarsus length, and the width and height of the cloacal protuberance, measured to ± 1 mm. Following Zhao et al. (2017b), we used the ratio of an individual bird's body mass (g) to wing length $(mm)^3$ as an index of its body condition. Birds were sexed by presence or absence of a brood patch (only females have a brood patch), and by cloacal protuberance size and wing length (males have significantly larger cloacal protuberances and longer wings than females).

Assays of plasma CORT and metabolites

Plasma CORT levels were measured using enzyme immunoassay kits according to the manufacturer's instructions (Cat No. ADI-901-097, Enzo Life Sciences) with minor modifications (Li et al. 2011). Briefly, 7 μL of plasma was diluted with 42 μL of 2% steroid displacement buffer. After 15 min, 230 μL of assay buffer was added to each sample, vortexed and a 100 μL aliquot of this mixture placed in an individual well. Standard curves with six dilutions ranging from 12,500 to 4 pg of CORT were obtained from each sample. An extra standard of 100 pg of CORT was run in each plate to assess inter-assay variation. All samples were run in duplicate. Intra- and inter-assay variation were 4.5% and 7.9%, respectively. Assay sensitivity was 0.9 ng/mL.

Plasma metabolites (Glu, TG, TC, UA) were measured in 25 μL plasma samples diluted with dH_2O (1:39) using an automatic biochemical analyzer (Mindray BS-180) with commercially available kits (Mindray Corp., Shenzhen, China). All samples were run in duplicate. Intra- and inter-assay variation were 3.1% and 8.2% (Glu), 9.6% and 10.1% (TG), 7.9% and 9.2% (TC), 8.4% and 11.6% (UA), respectively. Assay sensitivity was 0.3 mmol/L (Glu), 0.03 mmol/L (TG), 0.04 mmol/L (TC), 14.2 μmol/L (UA).

Statistical analysis

To identify the factors that affected body mass, plasma CORT, metabolites, and enzyme activity, a generalized linear mixed model (GLMM) was used to assess the significance of the fixed effects of repeated-measures sampling (time), sex, and the interaction between time and sex, and the random effects of sampling year and site. We used SPSS 24.0 software to fit GLMMs and estimate *F* statistics, degrees of freedom and *p*-values. The statistical significance of differences between pairs of means was assessed using Bonferroni-adjusted post hoc tests based on the means estimated by the GLMMs or using independent *t*-tests. Spearman correlations were used to investigate relationships between baseline (within 3 min of capture) or stress-induced CORT levels (30 and 60 min post-capture and after 24 h in captivity) and metabolites. p values < 0.05 were considered significant.

Results

Effects of capture and 24-h captivity stress on body mass and plasma CORT

Both body mass and CORT levels varied markedly with sampling time, independent of sex, and the interaction between time and sex (Table 1). Body condition index varied with sample time and sex, independently of the interaction between time and sex (Table 1). Post hoc results showed that both body mass and body condition decreased significantly during the 24-h period birds were held in captivity (Fig. 1a, b), and that female sparrows were in better condition than males (Fig. 1b). Both the stress of capture and captivity caused CORT levels to

Table 1 Results of a generalized linear mixed model (GLMM) of the effects of sampling time, sex, and interaction between time and sex (fixed factors), and sampling year, site, and individual (random factors) on the body mass (Mass), body condition index (BCI), plasma corticosterone (CORT), glucose (Glu), triglyceride (TG), total cholesterol (TC), uric acid (UA), levels of Eurasian Tree Sparrows (*Passer montanus*)

Variable	Factor	*F*	*df*	*p*	Variable	Factor	*F*	*df*	*p*	Variable	Factor	*F*	*df*	*p*
Mass	Intercept	2.834	3,32	0.054	BCI	Intercept	4.162	3,32	0.013	CORT	Intercept	2.817	7,81	0.011
	Time	6.688	1,32	*0.014*		Time	4.163	1,32	*0.050*		Time	6.052	3,81	*0.001*
	Sex	0.071	1,32	0.792		Sex	5.645	1,32	*0.024*		Sex	0.52	1,81	0.473
	Time × sex	0.132	1,32	0.719		Time × sex	0.019	1,32	0. 893		Time × sex	0.5	3,81	0.683
UA	Intercept	6.808	7,72	< 0.001	Glu	Intercept	5.552	7,72	< 0.001	TG	Intercept	2.855	7,72	0.011
	Time	15.172	3,72	*< 0.001*		Time	11.274	3,72	*< 0.001*		Time	1.852	3,72	0.146
	Sex	0.632	1,72	0.429		Sex	0.935	1,72	0.337		Sex	13.735	1,72	*< 0.001*
	Time × sex	0.09	3,72	0.965		Time × sex	0.941	3,72	0.426		Time × sex	0.138	3,72	0.937
TC	Intercept	4.600	7,71	< 0.001										
	Time	7.506	3,71	*< 0.001*										
	Sex	3.142	1,71	0.081										
	Time × sex	1.105	3721	0.353										

Body mass was measured 3 min after capture and after 24 h in captivity and other physiological variables were measured 3, 30 and 60 min after capture and after 24 h in captivity. Significant factors ($p < 0.05$) are shown in italics

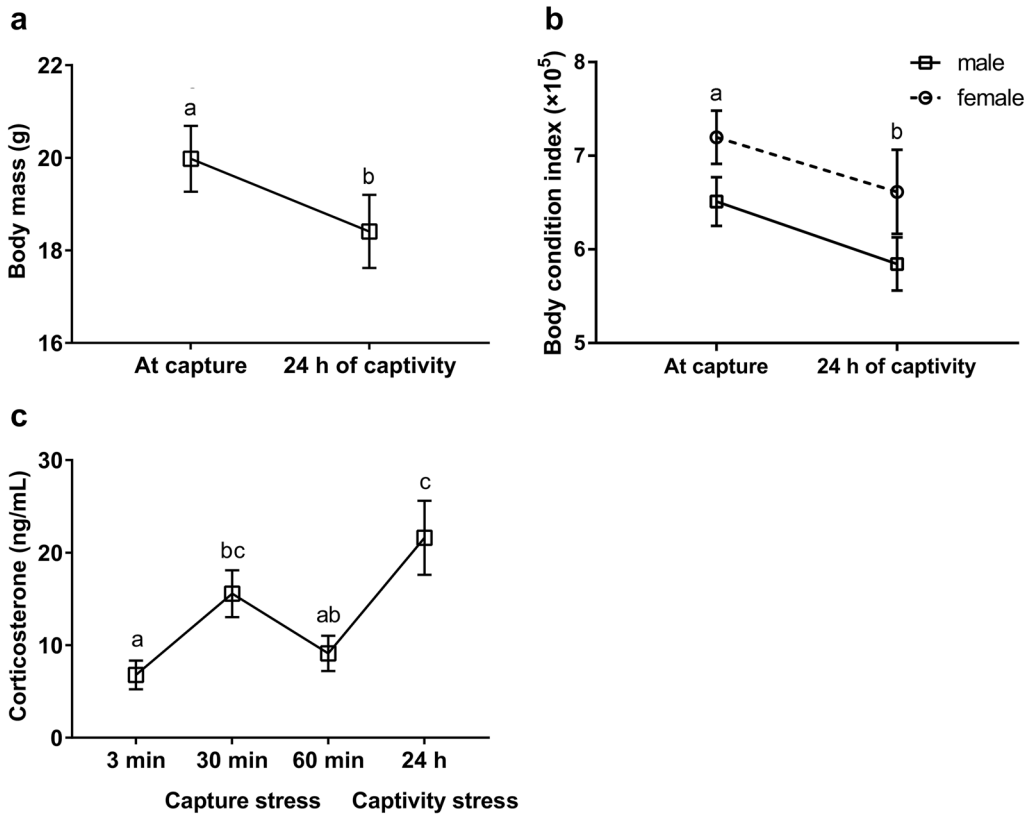

Fig. 1 Change in body mass (mean ± SEM; **a** and body condition (mean ± SEM; **b**; male: $n = 12$; female: $n = 10$) index in free-living Eurasian Tree Sparrows (*Passer montanus*) after 24 h in captivity and plasma corticosterone levels (CORT, mean ± SEM; **c** measured within 3, 30 and 60 min of capture and after 24 h in captivity ($n = 22$). Different letters indicate statistically significant differences between groups ($p < 0.05$)

increase significantly relative to baseline levels (Fig. 1c; Additional file 1: Table S1). There was no significant difference between CORT levels measured 30 min after capture and those measured after 24 h in captivity (Fig. 1c; Additional file 1: Table S1).

Effects of capture and 24-h captivity on plasma Glu, TC, TG, and UA

Plasma Glu, TC, and UA levels varied significantly with sampling time, independent of sex, and the interaction between time and sex (Table 1). Post hoc test results revealed that Glu levels measured 30 and 60 min after capture were significantly higher than baseline levels (Fig. 2a; Additional file 1: Table S1). TC levels decreased notably at 60 min after capture relative to baseline levels (Fig. 2c; Additional file 1: Table S1). UA levels were significantly lower at 30 min and 60 min post-capture than baseline levels (Fig. 2b; Additional file 1: Table S1). Glu levels measured after 24 h in captivity were significantly higher than both baseline levels and those measured 60 min post-capture, and UA levels were also markedly higher after 24 h in captivity than both the baseline level and those measured 30 min and 60 min post-capture (Fig. 2a, b; Additional file 1: Table S1). However, TC levels after 24 h in captivity did not differ from those of baseline and stress-induced levels after capture.

Plasma TG levels did not vary with sampling time but varied significantly with sex, independent of the interaction between time and sex (Table 1). Male sparrows had significantly lower TG levels than females (Fig. 2d).

Correlations between CORT and metabolites

Baseline plasma CORT levels did not correlate with baseline levels of Glu, TG, TC, and UA in both male and female sparrows (Table 2). Stress-induced CORT levels at 30 min and 60 min after capture were not correlated with any variables except for a positive relationship between stress-induced CORT and TG levels 30 min post-capture in females (Table 2; Fig. 3a). Captivity stress-induced CORT levels in females were negatively correlated Glu, TG, and positively correlated with UA levels (Table 2; Fig. 3b, c, d). Furthermore, although baseline TG levels were neither correlated with body mass (male: $r=-0.291$, $p=0.385$; female: $r=-0.587$, $p=0.126$) nor

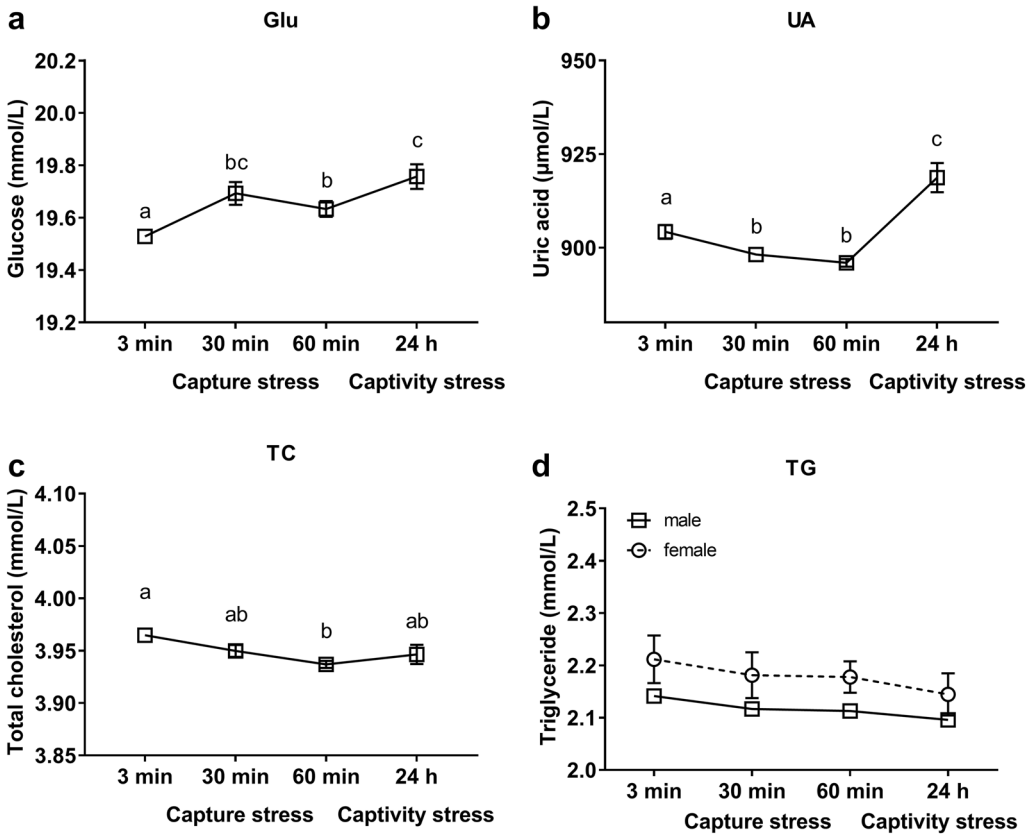

Fig. 2 Changes in plasma glucose (Glu), mean ± SEM; **a** uric acid (UA), mean ± SEM; **b** total cholesterol (TC), mean ± SEM; **c** and triglyceride (TG), mean ± SEM; **d** levels in male ($n=12$) and female ($n=10$) Eurasian Tree Sparrows (*Passer montanus*) measured within 3, 30 and 60 min of capture, and after 24 h in captivity. Groups with different letters represent statistically significant differences between groups ($p<0.05$)

Table 2 Results of Spearman correlation between plasma corticosterone (CORT) and, glucose (Glu), triglyceride (TG), total cholesterol (TC), uric acid (UA), levels in male and female Eurasian Tree Sparrows (*Passer montanus*)

Variable	Male				Female			
	Glu	TG	TC	UA	Glu	TG	TC	UA
Baseline CORT								
r	0.173	0.236	0.509	0.318	0.630	0.371	0.037	0.037
p	0.612	0.484	0.110	0.340	0.129	0.413	0.937	0.937
n	11	11	11	11	7	7	7	7
CORT at 30 min after capture								
r	−0.079	0.273	0.006	−0.067	0.714	*0.786*	0.321	0.750
p	0.829	0.446	0.987	0.855	0.071	*0.036*	0.482	0.052
n	10	10	10	10	7	*7*	7	7
CORT at 60 min after capture								
r	0.006	−0.127	−0.624	0.042	0.464	0.214	0.071	0.179
p	0.987	0.726	0.054	0.907	0.294	0.645	0.879	0.702
n	10	10	10	10	7	7	7	7
CORT after 24-h of captivity								
r	−0.417	0.100	−0.233	0.567	*−0.714*	*−0.762*	−0.524	*0.738*
p	0.265	0.798	0.546	0.112	*0.047*	*0.028*	0.183	*0.037*
n	9	9	9	9	*8*	*8*	8	*8*

All variables were measured at 3 (baseline levels), 30 and 60 min after capture and after 24 h in captivity. *r* represents Spearman correlation coefficient and significant factors ($p < 0.05$) are shown in italics

body condition (male: $r = -0.064$, $p = 0.853$; female: $r = -0.214$, $p = 0.610$), baseline TC levels were positively correlated with UA levels in both sexes (male: $r = 0.682$, $p = 0.012$; female: $r = 0.905$, $p = 0.039$).

Discussion

In Eurasian Tree Sparrows, although both the stress of capture and 24 h in captivity elevated plasma CORT and Glu levels, they had different effects on TC and UA levels. Furthermore, TG levels of males and females differed. Free-living Eurasian tree sparrows were caught and sampled on the day of capture and re-sampled the day after bringing them to the laboratory. Therefore, the observed changes in plasma CORT and metabolites may have been due to the combined effects of initial capture stress followed by 24 h in captivity.

Effects of capture stress

Capture stress protocol has been widely used in evaluating acute stress on the physiological changes in free-living animals, which includes not only an unpredictable capture-handling stimulus but also a stimulus of short-term fasting (Wingfield et al. 1992). In the present study, free-living Eurasian tree sparrows showed an increase in plasma CORT levels 30 min after capture. Such acute responses are generally thought to allow animals to cope physiologically and behaviorally with perturbations in the environment (Romero 2002). Meanwhile, plasma Glu levels followed a similar pattern to CORT. This result is consistent with the increase in Glu observed in king penguin chicks and Abert's towhees (Corbel et al. 2010; Davies et al. 2013), and pre-breeding rufous-winged sparrows (Deviche et al. 2016a). During capture stress, mobilizing carbohydrate stores as the principal energy source has been considered the most efficient way to transition to the emergency life history stage (Wingfield et al. 1998; Landys et al. 2006). Increased stress-induced CORT is believed to be essential to maintain hyperglycemia by suppressing the Glu uptake of peripheral tissues (Landys et al. 2004) and promoting hepatic gluconeogenesis (Jenni-Eiermann et al. 2002). However, recent avian studies demonstrated that increased plasma CORT is not necessarily associated with hyperglycemia (Fokidis et al. 2011; Deviche et al. 2014, 2016a, b). Therefore, the underlying mechanism of plasma CORT in regulating Glu levels in free-living animals may differ from previous findings.

TG levels in Eurasian tree sparrows did not change in response to capture stress and were not correlated with body mass or body condition. Consistent with our results, capture stress did not reduce the TG plasma levels of urban curve-billed thrashers (Fokidis et al. 2011). However, capture stress has been found to decrease TG levels in both free-living desert curve-billed thrashers and western sandpipers (*Calidris mauri*), which suggests TG catabolism and inhibition of lipogenesis (Guglielmo

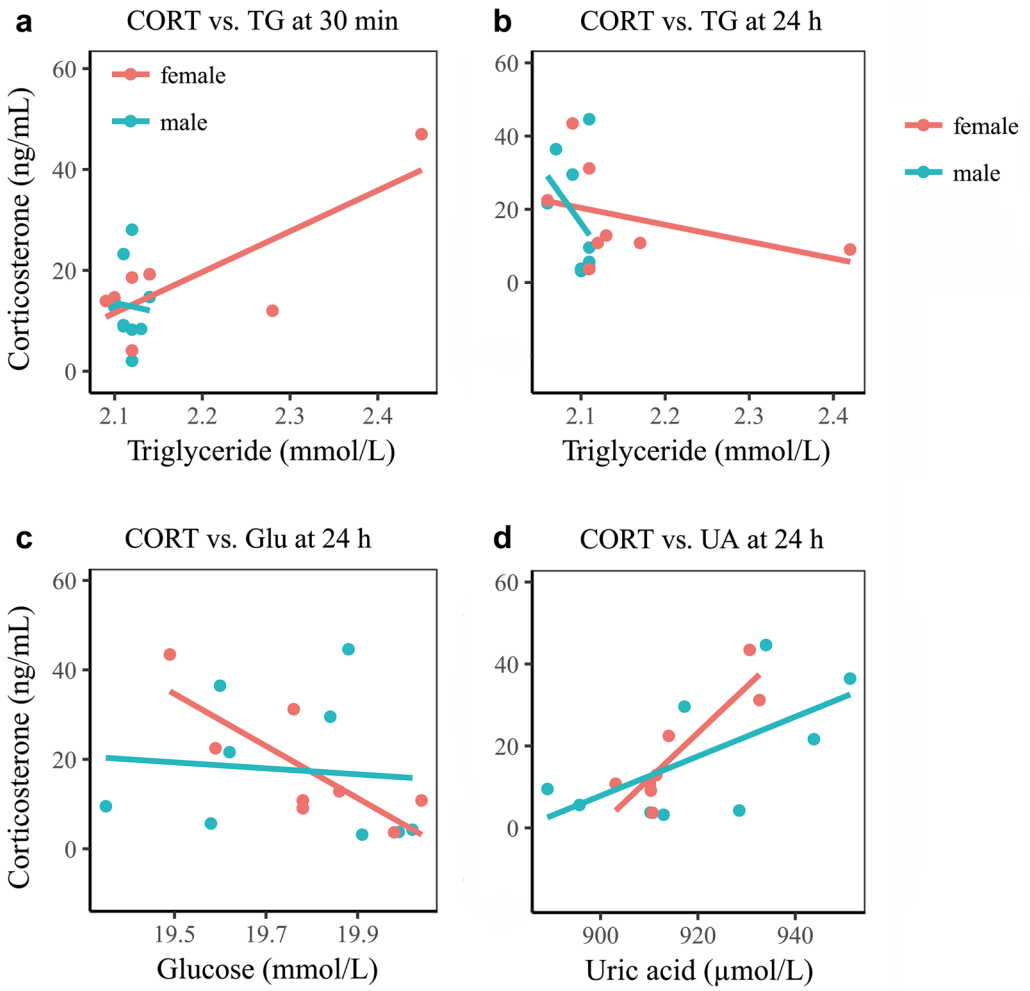

Fig. 3 Correlations between plasma corticosterone (CORT) and triglyceride (TG) measured 30 min of capture (**a**), and CORT and TG (**b**), glucose (Glu, **c**), and uric acid (UA, **d**) after 24 h in captivity in male and female Eurasian Tree Sparrows (*Passer montanus*), respectively

et al. 2002; Fokidis et al. 2011). It was demonstrated that the changes of TG levels during capture stress might be dependent on body condition, i.e., those individuals with better body condition do not necessarily decrease their plasma TG levels (Fokidis et al. 2011). Therefore, the effect of capture stress on TG levels may depend on an individuals' energy status. Further research is required to determine the correlation between plasma TG and energy status in free-living birds, and the modulation of lipid metabolism in response to capture stress.

Consistent with the results of studies on some other species (Cohen et al. 2007; Davies et al. 2013; Deviche et al. 2014, 2016a), the UA levels of Eurasian tree sparrows decreased 30 min and 60 min after capture (Fig. 2b) suggesting an inhibitory effect of acute stress on plasma UA. UA, a non-enzymatic antioxidant, may act as the first line of defense against the proliferation of free radicals (Cohen et al. 2007). Increasing tissue uptake of UA during capture stress may, therefore, be especially beneficial with regard to improving antioxidant capacity (Cohen et al. 2007; Strazzullo and Puig 2007; Sautin and Johnson 2008; Deviche et al. 2014).

In birds, plasma cholesterol is obtained from the diet or hepatic production (Hazelwood 1972), and may preferentially be directed toward steroidogenesis (e.g., CORT, T, and estradiol) and spermatogenesis (Orgebin-Crist and Tichenor 1973; Tift et al. 2011). To the best of our knowledge, our results provide the first evidence of reduced TC levels in response to capture stress, and of a positive correlation between baseline TC levels and UA levels in a free-living animal. These findings indicate that the regulation of cholesterol metabolism may be associated with enhanced catabolism and oxidative stress response induced by the stress of capture. Although

this observation is consistent with reduced TC levels in humans suffering from depression (Partonen et al. 1999), the acute stress of immobilization did not affect the TC levels of experimental rats (Hershock and Vogel 1989). Why the TC levels of Eurasian tree sparrows decreased in conjunction with reduced UA levels and how this is physiologically related to the oxidative stress response remains unclear. Further research is therefore required to investigate the change in TC levels in response to capture stress in free-living animals.

Effects of 24-h captivity stress

Eurasian Tree Sparrows experienced prolonged effects of the initial capture and restraint stress of captivity and underwent significant weight loss in captivity despite water and food were provided ad libitum. The underlying causes of weight loss may directly result from 24 h of captivity stress that not only subjected birds to the stress of confinement but also deprived them of their natural diet, which may have caused them to fast or reduce their food intake. Although we did not measure food consumption or energy expenditure, we found that plasma CORT levels increased after 24 h in captivity. This result is consistent with the reduced weight and elevated baseline CORT observed in captive, wild-caught Chukar (*Alectoris chukar*) (Dickens et al. 2009), increased CORT levels and reduced body weight after 4–10 h of fasting in the Zebra Finch (*Taeniopygia guttata*) (Lynn et al. 2010), and in Rock Pigeons (*Columbia livia*) after 24 h in captivity (Angelier et al. 2016). Reduced body weight is a hallmark of the elevated baseline CORT (Sapolsky et al. 2000) secreted in response to the chronic stress associated with captivity, e.g., confinement, altered light conditions and diet (Morgan and Tromborg 2007).

GCs are known to inhibit TG synthesis and promote the availability of lipid energy from adipose tissue stores (Dallman 1993; Bentley 1998; Landys et al. 2006), thus providing substrates for continued gluconeogenesis (Grégoire et al. 1991; Landys et al. 2004). Previous studies have shown increased plasma UA as a result of protein degradation after flight or exercise in some avian species (Shmueli et al. 2000; Jenni-Eiermann et al. 2002; Tsahar et al. 2006). In addition, exogenous GCs have also been found to induce proteolysis and muscle atrophy in several species (Landys et al. 2006), which is critical for replenishing amino acid substrates for gluconeogenesis. Along with elevated CORT, increased Glu and UA levels in conjunction with reduced body weight, leads us to speculate that Eurasian Tree Sparrows relied heavily on gluconeogenesis and protein catabolism, during the 24 h they were in captivity. Given that 24 h of captivity did not induce the changes of TC and TG levels compared with their baseline levels in the field, whether the lipids catabolism was enhanced in response to captivity stress remains unclear.

Sexual differences in stress responses

There were no significant differences between the sexes with respect to changes in body mass, plasma CORT, Glu, TC, and UA levels following both capture and captivity. Furthermore, plasma CORT was not correlated with Glu and UA levels following capture stress. These results are consistent with those of previous studies that also found no sexual differences in acute CORT response to capture stress in this species during the breeding season (Li et al. 2008, 2011, 2016). This may reflect the fact that both male and female Eurasian tree sparrows incubate eggs and feed nestlings (Summers-Smith 2014). However, sex-specific relationships between plasma CORT and metabolites after capture and 24 h in captivity (Table 2; Fig. 3) suggest condition-dependent variations between sexes, e.g., insufficient glucose, and thus enhanced protein degradation in the females, but not in male sparrows. The underlying cause of this sex-specific correlation between stress-induced CORT and, Glu, and UA levels remains to be further determined.

Our results show that female sparrows were not only in better condition but also had higher plasma TG levels than males. Consistent with our results, male Canada Geese (*Branta canadensis*) during the spring post-migratory phase also had significantly lower serum TG than females (Mori and George 1978). We further found that stress-induced (30 min post-capture) CORT levels in female sparrows were positively correlated with TG levels whereas this correlation became negative after 24 h in captivity. This trend was not observed in male sparrows. Male sparrows have significantly higher baseline testosterone (T), which is thought to be a mediator of catabolism, than females during breeding (Li et al. 2012). It remains unclear if the higher TG levels of females can be explained by their better body condition when captured, and if such sex-specific differences are due to an interaction between energy metabolism and reproductive physiology.

Limitations

It should be pointed out that our experimental design does not permit us to determine whether or to what extent the initial capture stress contributed to the endocrine, and metabolic differences observed in the 24-h captivity. It is worthy of mentioning that, initial capture stress-induced elevated plasma Glu levels in Rufous-winged Sparrows, which persisted until the following day when they were re-capture, but plasma CORT and UA levels returned to initial levels (Deviche et al. 2016b). As we mentioned above, the elevated Glu in Eurasian Tree

Sparrows observed here may have attributed to the combined effects of initial capture stress followed by 24 h in captivity. How do initial capture stress influence on the plasma Glu and other metabolites on the following day in free-living Eurasian Tree Sparrows, remains to be further investigated.

Conclusions

In the present study, we identified that while the plasma CORT and Glu levels of free-living Eurasian Tree Sparrows increased significantly in response to capture stress, UA and TC levels decreased markedly. These results suggest that wild animals can rapidly regulate their plasma CORT and metabolite levels when subject to acute stress. Furthermore, sparrows subject to 24-h captivity had decreased body mass but increased plasma CORT, Glu, and UA levels. Male sparrows had lower TG after capture and captivity than females, which indicates that there are sex-specific differences in the physiology of this species. Overall, our results show that the stress of capture and captivity can have different effects on plasma CORT, metabolite levels in a passerine bird, which contributes to better understanding of the stress-induced pathways involved in sex-dependent energy mobilization. Our findings can also be applicable to illustrate the physiological alterations of free-living birds, especially endangered specie when bringing them into captivity during the process of ex situ conservation.

Additional file

Additional file 1: Table S1. Multiple post hoc comparisons of differences in mean plasma corticosterone (CORT), glucose (Glu), triglyceride (TG), total cholesterol (TC), uric acid (UA), levels of Eurasian Tree Sparrows (*Passer montanus*) measured 3, 30 and 60 min after capture, and after 24 h in captivity.

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Authors' contributions
DL and XG conceived the ideas and designed the study; ML, WZ, YS, JL, and XL conducted the experiment and collected the data; YW carried out the statistical analyses with helps of DL and YW; DL wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All procedures were approved by the Institutional Animal Care and Use Committee of Hebei Normal University and carried out under the auspices of scientific collecting permits issued by the Hebei Provincial Forestry Bureau's Department of Wildlife Conservation.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, China. [2] Ocean College of Hebei Agricultural University, Qinhuangdao 066003, China. [3] Shaanxi Institute of Zoology, Xi'an 710032, China.

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