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Experimentally reducing corticosterone mitigates rapid captivity effects on behavior, but not body composition, in a wild bird

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ABSTRACT

Wild animals and captives display physiological and behavioral differences, and it has been hypothesized, but rarely tested, that these differences are caused by sustained elevation of the hormone corticosterone. We used repeated computed tomography (CT) imaging to examine body composition changes in breeding male and female wild house sparrows (*Passer domesticus*; $n = 20$) in response to two weeks of captivity, and assessed behavioral changes using video recordings. Half of the birds received the drug mitotane, which significantly decreased stress-induced corticosterone titers compared to controls. Based on the CT images, fat volumes increased, and pectoralis muscle density and heart and testes volumes decreased, over the two weeks of captivity in both groups of birds. However, beak-wiping, a behavior that can indicate anxiety and aggression, showed increased occurrence in controls compared to mitotane-treated birds. While our results do not support the hypothesis that these body composition changes were primarily driven by stress-induced corticosterone, our data suggest that experimentally reducing stress-induced corticosterone may mitigate some captivity-induced behavioral changes. Broadly, our results emphasize that researchers should take behavioral and physiological differences between free-living animals and captives into consideration when designing studies and interpreting results. Further, time in captivity should be minimized when birds will be reintroduced back to the wild.

1. Introduction

Wild animals are brought into captivity for a variety of conservation purposes, including captive breeding, translocation, and rehabilitation of injuries. Laboratory-housed wild animals are also used in many studies of vertebrate physiology and behavior, which allows researchers to control potentially confounding variables and pinpoint the effects of different experimental manipulations. However, a growing body of research indicates that wild animals undergo profound physiological and behavioral changes in response to captivity (Calisi and Bentley, 2009; Dickens et al., 2009; Mason, 2010). This includes changes in endocrine systems, reproductive behaviors, immune function, brain morphology, and circadian rhythms.

It has been suggested that many captivity-induced changes may be due to chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis, which secretes corticosteroid hormones (cortisol and/or corticosterone, depending on the species; hereafter CORT) in response to stressors (Morgan and Tromborg, 2007). Many features of the captive environment can cause HPA activation, including forced proximity to humans, a lack of environmental enrichment, and exposure to artificial lighting (Buijs et al., 2011; Evans et al., 2012; Nephew et al., 2003). Although short-term HPA activation is important for surviving stressors and re-establishing homeostasis, chronic HPA activation is associated with a number of negative physiological and behavioral effects, including reproductive suppression, immunosuppression and metabolic dysregulation (Dallman et al., 2003; Spencer et al., 2001; Wingfield and Sapolsky, 2003). Although several studies have examined correlations between CORT titers and various behavioral and physiological measures in captive wild animals (Adams et al., 2011; Martin et al., 2011; Moore et al., 1991), researchers rarely experimentally manipulate CORT titers so as to clearly demonstrate a causal role for CORT in captivity-induced physiological or behavioral changes. Birds in particular are capable of rapid tissue remodeling in response to ecological conditions or physiological state; for example, organ size has been shown to fluctuate rapidly in response to changes in diet, breeding stage, ambient temperature and migratory readiness (Dykstra and Karasov, 1992; McWilliams and Karasov, 2001; Piersma et al., 1996; Piersma et al., 1993; Vézina et al., 2006; Vézina and Williams, 2003; Williams, 2004). However, it is currently unknown which captivity-induced changes in body composition may be caused by changing environmental conditions (e.g., ad libitum food) and which are due to HPA activation. To know how to mitigate the effects of captivity on wild animals, it is important to untangle

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which effects are specifically due to prolonged high CORT titers and which effects may be caused by alterations in other physiological and behavioral systems.

In this study, we used computed tomography (CT) imaging to assess body composition of wild house sparrows (*Passer domesticus*, $n = 20$) at capture and after two weeks in a laboratory setting. Imaging studies allow the quantification of tissue volumes in a non-destructive fashion, permitting multiple measurements in the same animal to control for individual variation and study the effects of different experimental manipulations. We also examined plasma corticosterone, fructosamine, body mass and fat scores. Fructosamines are stable ketoamines formed in the blood by glycation reactions between sugars and primary amines in proteins such as albumin, and reflect longer-term changes in blood glucose on the scale of ~2 weeks (Armbruster, 1987). Changes in plasma fructosamine would be helpful in interpreting body composition changes, which could be caused by increased or decreased gluconeogenesis (Dallman et al., 2003). Finally, we also used video recordings to examine the frequency of five different behaviors, including two behaviors (feather ruffling and beak wiping) that can reflect anxiety and aggression in wild birds (Bauer et al., 2011; Dabelsteen, 1984; de Bruijn and Romero, 2011; Evans, 1984), as well as overall activity, feeding and preening behavior.

We used mitotane, a drug that reduces plasma CORT titers in house sparrows (Breuner et al., 2000), to test the hypothesis that captivity-induced behavioral and physiological changes are caused by sustained activation of the HPA axis. Mitotane is metabolically activated by an adrenal-specific cytochrome P450 (Jonsson et al., 1994); in this active form, it blocks cyctochrome P450-mediated reactions, causing selective necrosis of adrenocortical tissue and reducing glucocorticoid production (Maher et al., 1992). Half of the birds ($n = 10$) received injections of mitotane, and the other half ($n = 10$) a vehicle control. We carried out this study during the breeding season, a time when many wild species, including the house sparrow, show an annual peak in baseline and stress-induced plasma CORT concentrations (Lattin et al., 2012). The reactive scope model of stress physiology predicts that these naturally-high CORT titers make it more likely that additional elevations of CORT - such as would be encountered from stressors related to captivity - will push animals beyond their adaptive range into the realm of pathology (Romero et al., 2009). Based on previous studies of the effects of CORT on metabolic, immune and reproductive systems and behavior, we hypothesized that captivity would induce a number of specific changes in wild house sparrows if elevated CORT was directly responsible (Table 1); we also predicted that these changes would be reduced or absent in mitotane-treated animals.

Table 1
Many features of the captive environment cause elevated corticosteroids in wild animals. Based on previous work demonstrating the effects of high corticosteroids on different aspects of physiology and behavior, we had several hypotheses about the effects of 2 weeks of captivity stress on wild house sparrows (all predicted increases/decreases are relative to capture values).

Measure	Predictions (measure affected)	Sources
Metabolic	Increased gluconeogenesis (higher plasma fructosamine, larger liver), increased proteolysis (decreased pectoralis thickness and density, smaller heart), increased lipogenesis (increased fat score, increased fat tissue)	(Dallman et al., 2003; Rebuffé-Scrive et al., 1992; Rogers et al., 1993)
Immune	Immunosuppression (smaller spleen)	(Hull et al., 2007)
Reproductive	Reproductive suppression (smaller testes)	(Hull et al., 2007; Wingfield and Sapolsky, 2003)
Behavior	Increased anxiety-related behaviors (feather ruffling, beak wiping), increased feeding behavior, increased overall activity	(Bauer et al., 2011; Dallman et al., 2003; de Bruijn and Romero, 2011; Rogers et al., 1993)

2. Materials and methods

2.1. Experimental subjects

Animals were collected under Connecticut state permit 1,417,011, and all procedures approved by the Yale University Animal Care and Use Committee under permit 2014-11,648. We used appropriate anesthetics and analgesics and approved methods of euthanasia as specified in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Wild house sparrows were caught in New Haven, West Haven, Branford, and Hamden CT, USA using mist nets near bird feeders. Every day for five consecutive days (21–25 June 2015), starting ~2.5 h after sunrise, we captured 4 birds. We attempted to catch equal numbers of males and females for this study, but slightly more females were caught ($n = 6$ females, $n = 4$ males in each group). All birds used for this study were identified as adults using plumage features (Pyle, 1997). At capture, blood samples were collected from the alar vein using heparinized capillary tubes. Blood samples collected <3 min after birds reached the mist net were considered representative of baseline CORT (Romero and Reed, 2005). Because we were unable to collect plasma samples from 4 birds in <3 min, $n = 16$ at capture. Birds were then put into breathable cloth bags for 30 min, after which blood samples were taken for determination of stress-induced CORT and fructosamine (120 μl total blood drawn). We weighed birds using a spring scale (Pesola AG, Baar, Switzerland) and recorded fat classes according to Kaiser (1993). Blood samples were kept on ice until centrifugation up to 5 h later. Plasma was removed and stored at −80 °C until assay.

In the laboratory, sparrows were housed 2/cage (1 male and 1 female, or 2 females) on natural photoperiod (16L:8D). Access to mixed seeds, grit, water, and a dish of sand for sandbathing was provided ad libitum. Day length in the lab corresponded to natural day length. Immediately after initial CT imaging of birds (see below), we randomly assigned two of the birds caught each day to receive mitotane (ortho, para-DDD; 180 mg/kg body weight; Sigma Aldrich, St. Louis, MO, USA) and the other two to receive a vehicle control (peanut oil). Previous work has demonstrated that twice-daily intramuscular injections of mitotane can reduce circulating CORT to undetectable levels in house sparrows in just 36 h (Breuner et al., 2000), but in this study, we wished to reduce CORT to within the physiological range, because long-term adrenal insufficiency can make animals vulnerable to adrenal crisis and death (Hague et al., 1989). We also wished to avoid muscle damage that can be caused by intramuscular administration. Therefore, we injected mitotane subcutaneously over the breast muscle every other day, which can reduce circulating CORT in house sparrows to the low physiological range (C.M. Bauer, personal communication).

Birds were re-weighed after each week in captivity, and fat classes re-assessed at 2 weeks post-captivity. The person doing fat class assignment (CRL) was blinded to treatment. After 1 and 2 weeks, additional blood samples were collected for baseline and stress-induced CORT, and for fructosamine after 2 weeks (blood taken at 1 week: 75 μl; blood taken at 2 weeks: 120 μl). Total blood drawn was below the ~1% of body weight/2 weeks guideline for house sparrows (which typically weigh 26–29 g). Baseline CORT samples were collected <3 min after entering the bird room, and stress-induced CORT and fructosamine samples collected after 30 min in a cloth bag. No one entered the bird room for at least 1 h prior to blood sampling. One animal from each group died during the second week of captivity of unknown causes, so sample sizes at 2 weeks were $n = 9$ for each group.

2.2. CT imaging

On the day of capture and two weeks later, birds underwent CT imaging (Siemens Inveon micro PET-CT scanner, Siemens Medical Solutions USA, Inc., Malvern, PA, USA). Birds were anesthetized using inhaled isoflurane at a concentration of 2.5–5% and maintained at

39.5 °C with a heating pad (m2 m Imaging, Cleveland, OH, USA). Once birds were deeply anesthetized (did not respond to toe pinch), a sterile intraosseous catheter with a 27 gauge butterfly needle was inserted into the birds' tibiotarsus bone to administer the gadolinium-based contrast agent ExiTron Nano 12,000 (Miltenyi Biotec Inc., Auburn CA, USA). Contrast was diluted 1:1 in sterile saline (50 μL contrast per animal, equivalent to ~640 mg iodine/kg body weight). Because this agent is taken up by cells of the reticuloendothelial system, it provides excellent contrast in liver and spleen lasting several weeks after injection (Supplemental Fig. 1), and it has demonstrated low toxicity in small animal imaging (Boll et al., 2013; Boll et al., 2011). CT scans were performed at three different time points: pre-contrast, 2 h after contrast injection, and 2 weeks after capture. CT acquisitions were performed in a step-and-shoot mode with two bed positions, and used 80 kV, 500 μA, 240 ms exposure time, and 360 projections at low magnification. Each scan lasted ~10 min. Reconstructed pixel size was 0.11 cm × 0.11 cm × 0.11 cm. We chose CT parameters to minimize radiation doses to animals while enhancing soft tissue contrast. The estimated absorbed dose received by animals (~30 mGy/scan) was well below thresholds where negative physiological effects are expected to occur, which are typically first seen at doses of ~400–600 mGy (Boone et al., 2004; Kersemans et al., 2011; Ritman, 2004).

2.3. Hormone and fructosamine assays

Radioimmunoassay (RIA) was used to determine CORT concentrations following the methods of Wingfield et al. (1992). Briefly, samples were allowed to equilibrate overnight with 20 μL of radiolabeled CORT to determine individual recoveries. Each sample was extracted with redistilled dichloromethane, dried under nitrogen gas and resuspended in phosphate-buffered saline with 1% gelatin. Samples were assayed in duplicate using antibody B3–163 (Esoterix, Calabasas Hills, CA) and assay values corrected for individual recoveries following extraction. Average recovery was 78%; detectability was 1 ng CORT/ml plasma. Intra- and inter-assay coefficients of variation were 5% and 23%, respectively. This amount of inter-assay variability is somewhat high for an RIA, but because experimental groups, baseline and stress-induced CORT samples and different time points were completely mixed and randomly distributed across the two runs of the RIA, we do not think this significantly impacted our results.

Fructosamine values were determined using the MaxDiscovery Fructosamine Enzymatic Assay Kit (#5615-01, Bioo Scientific Corporation, Austin, TX, USA). Pooled sparrow plasma diluted with phosphate-buffered saline demonstrated parallelism with fructosamine standards included with the kit (Supplemental Fig. 2). Based on this validation work, we diluted plasma 1:1 in phosphate-buffered saline to ensure fructosamine concentrations of sparrow samples would fall near the middle of the standard curve. Plasma samples were run in duplicate on a Benchmark Plus plate reader (Bio-Rad, Hercules, CA, USA); all samples were run in the same assay, eliminating inter-assay variation. The intra-assay coefficient of variation was 18%.

2.4. Behavioral observations

Each day after CT scanning (i.e., the day after capture, and two weeks later), video was recorded from 0700 to 0930 EST (starting 2 h after lights on) and from 1900 to 2030 EST (starting 2 h before lights off). In the case of female-female pairs, individuals were distinguished by marking one with green felt pen on the breast. Four birds (2 cage pairs) were recorded at a time. The camera was turned on and off remotely so as not to disturb the birds. No humans entered the room for at least 30 min preceding the start of recording.

The first 60 min of each video was analyzed for five different behaviors: number of hops/flights, beak wipes, feather ruffling, preens and time spent feeding. We were also interested in assessing allopreening, copulation behavior and aggressive interactions (pecks of cagemate), but these behaviors were very rarely observed so were not analyzed. All behaviors were measured as single instances (tallied) using a hand tally counter, except for time spent feeding, which was measured using a stop watch. Hops and flights were defined to include hops in place, turns, or hops on the cage floor, as well as any short flights within the cage; more broadly, this measure was counted each time the birds' feet left the cage floor or the perch. Time spent feeding was defined as the amount of time a bird spent on the edge of the food dish or the cage floor pecking for food. Beak wipes were defined as a bird repeatedly rubbing its beak back and forth on a surface, and a single event was counted when separated from the previous incident by ≥2 s. Preening was defined by a bird grooming itself with its beak or scratching itself with its leg; a single event was counted when separated from the previous incident by ≥1 s. Feather ruffling was defined as a bird shaking and fluffing all its feathers. All behaviors were scored by the same person (AVP), who was blinded to the treatment.

2.5. CT data analysis

Images were analyzed using Siemens Inveon Acquisition Workplace software, Version 2.0 (Siemens Healthcare GmbH, Erlangen, Germany). Three-dimensional regions of interest (ROI) of heart, spleen, liver, testes (in males), omental fat (all fat within the abdominal cavity) and subcutaneous fat of the furcula region were manually drawn on CT images using anatomical landmarks. It was not possible to distinguish ovary from surrounding soft tissue, so this tissue was not analyzed. Because pectoralis was difficult to distinguish from surrounding muscles such as the supracoracoideus, we instead used two other measures of pectoralis muscle size: 1) average pectoralis thickness adjacent to the sternum in 5 consecutive frames in the axial view, 2) average attenuation in Hounsfield Units (HU) of left and right pectoralis muscle adjacent to the sternum in the axial view, in areas delineated by a 20 mm^3 sphere. HU are used to quantify CT tissue attenuation based on a linear scale using water as the reference (0 HU); less dense tissues, such as fat, have negative attenuation values whereas denser tissues, such as muscle, have positive attenuation values. Attenuation measures are commonly used in CT muscle studies, where decreases in attenuation can indicate the invasion of muscles by lower-density fat (Goodpaster et al., 2000). For delineation of subcutaneous and omental fat ROIs, image display was set to the range of −300 to −50 HU for easier identification. Total ROI volumes for each tissue, or thickness and CT attenuation in the case of pectoralis muscle, were then used as measures of organ size. In a randomly-chosen subset of bird carcasses that were fully dissected ($n = 5$ male, 9 female, euthanized 2 d after their last CT scan), image-derived measures from 2 week post-captivity CT scans were significantly positively correlated with tissue masses (Supplemental Fig. 3), demonstrating that these measures are good indicators of overall tissue size. Fat scores assessed externally at 2 weeks post-captivity were not significantly correlated with the mass of furcular fat ($r^2 = 0.08$, $p = 0.33$) or omental fat ($r^2 = 0.08$, $p = 0.45$). For each tissue, all measurements were made by the same person (CRL or AVP), who was blinded to the treatment.

2.6. Statistical analysis

Values are presented as means ± SEM. All analyses were run in JMP Pro 11.2.1 (SAS Institute Inc., Cary, NC, USA), except for behavioral analyses, which were run using Stata 14 (StataCorp LP, College Station, TX, USA) and R (R Core Team, 2016). During CT image analysis, two female birds from the mitotane group and one female from the control group were found to have large masses of reproductive tissues and eggs in their body cavities on capture (Supplemental Fig. 4a). One of these females laid an egg in the lab; the other two must have resorbed eggs. Reproductive tissues in these females were completely regressed after two weeks in captivity (Supplemental Fig. 4b). Hypothesizing that the volume of other soft tissues in the body cavity may have been affected by

these large masses of reproductive tissue, we performed heart, liver and spleen analyses with these three females excluded. Female breeding stage (laying vs chick-rearing) has been shown to affect the size of other organs in European starling (*Sturnus vulgaris*), although not always in a consistent manner across different years (Vézina and Williams, 2003).

We evaluated combining behavioral measures using principal components analysis, but these five behaviors were not strongly correlated (Supplemental Table 1), so we analyzed them separately. Because hops/flights, preens, beak wipes and feather ruffling consisted of count data, we analyzed these behaviors using negative binomial linear mixed models in Stata (overdispersion prevented use of the Poisson distribution) (Bolker et al., 2009). Feeding data consisted of values between 0 and 1 (the fraction of total time birds spent feeding), and were analyzed with gamma linear mixed models using the lme4 package in R (Bates et al., 2015; Breslow and Clayton, 1993). There were no zero values for any behavior at any time point, so we did not have to contend with zero-inflated data. For baseline and stress-induced corticosterone, plasma fructosamine, body mass, and tissue volumes, we used Gaussian linear mixed models. All mixed models were based on a restricted maximum likelihood approach, with time, treatment (mitotane vs. control), sex, and time × treatment interactions as fixed effects and individual bird as a random effect to account for the repeated-measures nature of the data (Cudeck, 1996). We checked for homoskedasticity by inspecting plots of studentized residuals against predicted values of dependent variables. Because of the ordinal nature of fat score data, we used ordinal logistic models to analyze these data, with the same model effects. We used Tukey's HSD as a post-hoc test where appropriate, and calculated effect sizes for mixed models as R^2_{GLMM} as described by Nakagawa et al. (2013), using marginal (R^2_m) and conditional (R^2_c) values from the MuMIn package in R (Barton, 2016).

3. Results

3.1. Plasma measures

Baseline CORT varied by time period (Fig. 1a; $F_{2,34} = 3.44$, $p = 0.044$), although there were no significant effects of mitotane treatment or sex on this measure (treatment: $F_{1,47} = 0.10$, $p = 0.75$; time * treatment: $F_{2,34} = 0.62$, $p = 0.55$; sex: $F_{1,16} = 0.32$, $p = 0.58$; $R^2_m = 0.15$, $R^2_c = 0.15$). Tukey's HSD post-hoc tests revealed that baseline CORT at capture was significantly lower than it was after 1 week of captivity ($p = 0.038$). For stress-induced CORT, there were no significant overall effects of time (Fig. 1b; $F_{2,34} = 2.87$, $p = 0.071$), treatment ($F_{1,46} = 0.61$, $p = 0.44$), or sex ($F_{1,16} = 0.065$, $p = 0.80$), but there was a significant time * treatment interaction ($F_{2,34} = 3.88$, $p = 0.031$; $R^2_m = 0.19$, $R^2_c = 0.36$). Post-hoc tests indicated that stress-induced CORT of mitotane birds at 2 weeks post-captivity was significantly lower than their own initial stress-induced CORT values ($p = 0.030$), and from control birds at 1 week post-captivity ($p = 0.025$). Plasma fructosamine concentrations were not affected by captivity (Table 2; $F_{1,20} = 0.21$, $p = 0.65$), mitotane treatment ($F_{1,20} = 0.011$, $p = 0.92$), or sex ($F_{1,20} = 0.28$, $p = 0.60$), and there was no captivity * mitotane interaction ($F_{1,20} = 2.38$, $p = 0.14$; $R^2_m = 0.06$, $R^2_c = 0.18$).

3.2. Body mass and fat score

Birds lost ~8% body mass in captivity (Fig. 2; $F_{2,51} = 13.57$, $p < 0.0001$), and male sparrows were significantly heavier than females (males: 29.2 ± 0.42 g, females: 28.2 ± 0.38 g; $F_{1,51} = 5.51$, $p = 0.022$) although there was no effect of mitotane treatment on body mass ($F_{1,51} = 1.97$, $p = 0.17$) or interaction between captivity and mitotane treatment ($F_{2,51} = 0.31$, $p = 0.74$; $R^2_m = 0.28$, $R^2_c = 0.56$). This effect of captivity on body mass remained even if the three egg-laying females, who had high initial body mass due to unlaid eggs, were excluded from analysis (time: $F_{2,42} = 10.41$, $p = 0.0002$, treatment: $F_{1,42} = 1.75$, $p = 0.19$; sex: $F_{1,42} = 7.23$, $p = 0.010$; time * treatment: $F_{2,42} = 0.15$, $p = 0.86$; $R^2_m = 0.29$, $R^2_c = 0.59$). Tukey's HSD post-hoc tests revealed that body mass was significantly lower after 1 and 2 weeks of captivity compared to mass at capture ($p < 0.001$ and $p = 0.0002$, respectively). Despite a decrease in overall body mass, fat scores increased for birds with time in captivity (Table 2; time: likelihood ratio (LR) $\chi^2 = 14.05$, $p = 0.0002$), and were significantly greater in females compared to males (males: 1.7 ± 0.2, females: 2.2 ± 0.2; LR$\chi^2 = 6.42$, $p = 0.011$), but were not affected by mitotane treatment (treatment: LR$\chi^2 = 0.13$, $p = 2.26$; time * treatment: LR$\chi^2 = 2.77$, $p = 0.10$; $R^2_m = 0.38$, $R^2_c = 0.38$).

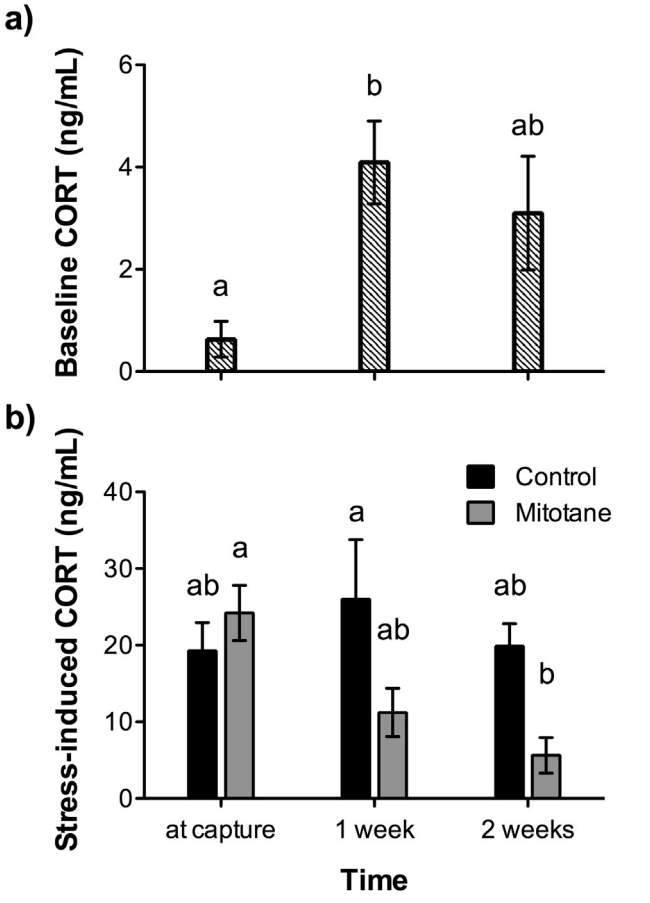

Fig. 1. Plasma corticosterone of wild house sparrows (*Passer domesticus*) at capture and after 1 and 2 weeks in a laboratory setting: a) at baseline (<3 min from time of capture or from entering the bird room), and b) after 30 min of restraint stress in a cloth bag. Half of the birds ($n = 10$) received injections of the drug mitotane, and the other half ($n = 10$) received a vehicle control. One animal from the control group and one animal from the mitotane group died during the second week of captivity, so $n = 9$ for each group at 2 weeks post-captivity. At capture, we were unable to collect baseline samples from several birds in <3 min, so baseline sample size at this time point is slightly lower ($n = 16$). Different letters represent statistical differences among groups as indicated by Tukey's HSD post-hoc tests. Values are presented as mean ± SEM.

3.3. Tissue size

With time in captivity, house sparrows increased volume of subcutaneous fat in the furcula (Fig. 3a; $F_{1,17} = 18.47$, $p = 0.0005$) and omental fat (3b; $F_{1,17} = 5.027$, $p = 0.039$), and decreased volumes of heart (Fig. 3c; $F_{1,14} = 5.57$, $p = 0.033$) and testes (Fig. 3d; $F_{1,5} = 45.81$, $p = 0.0011$), but there was no effect of sex or mitotane treatment on subcutaneous furcular fat (treatment: $F_{1,32} = 0.61$, $p = 0.44$; sex: $F_{1,16} = 3.57$, $p = 0.077$; time * treatment interaction: $F_{1,17} = 0.33$, $p = 0.57$; $R^2_m = 0.41$, $R^2_c = 0.41$), omental fat (treatment: $F_{1,27} = 0.62$, $p = 0.44$; sex: $F_{1,16} = 0.044$, $p = 0.84$; time * treatment

Table 2
House sparrow (*Passer domesticus*) plasma fructosamine and fat scores at capture and after two weeks in a laboratory setting. One animal from the control group and one animal from the mitotane group died during the second week of captivity. Values are presented as mean ± SEM (sample size). See text for more details on statistical analyses.

	Control animals		Mitotane animals	
Parameter (units)	At capture	Week 2	At capture	Week 2
Fructosamine (mmol/l)	2.2 ± 0.3 (10)	2.0 ± 0.2 (9)	1.8 ± 0.1 (10)	2.2 ± 0.2 (9)
Fat score	2.2 ± 0.3 (10)	2.8 ± 0.09 (9)*	1.9 ± 0.3 (10)	2.9 ± 0.07 (9)*

* Significantly different from capture values at $p < 0.05$.

interaction: $F_{1,17} = 0.37$, $p = 0.55$; $R^2_m = 0.09$, $R^2_c = 0.48$), heart (treatment: $F_{1,15} = 0.11$, $p = 0.74$; sex: $F_{1,13} = 0.70$, $p = 0.42$; time * treatment: $F_{1,14} = 0.28$, $p = 0.60$; $R^2_m = 0.08$, $R^2_c = 0.85$) or testes (treatment: $F_{1,5} = 3.09$, $p = 0.14$; time * treatment: $F_{1,5} = 0.17$, $p = 0.70$; $R^2_m = 0.61$, $R^2_c = 0.87$). There were no effects of captivity, sex or mitotane treatment on pectoralis muscle thickness (Fig. 3e; time: $F_{1,17} = 2.73$, $p = 0.12$; treatment: $F_{1,21} = 0.029$, $p = 0.87$; sex: $F_{1,16} = 1.69$, $p = 0.21$; time * treatment interaction: $F_{1,17} = 1.17$, $p = 0.30$; $R^2_m = 0.12$, $R^2_c = 0.77$), but pectoralis muscle density significantly decreased over time (Fig. 3f; time: $F_{1,17} = 42.33$, $p < 0.0001$; treatment: $F_{1,29} = 0.12$, $p = 0.73$; sex: $F_{1,16} = 0.0018$, $p = 0.97$; time * treatment: $F_{1,17} = 2.21$, $p = 0.16$; $R^2_m = 0.44$, $R^2_c = 0.64$). Although there were no main effects of time ($F_{1,14} = 1.66$, $p = 0.22$), sex ($F_{1,13} = 3.01$, $p = 0.11$) or treatment ($F_{1,24} = 2.70$, $p = 0.11$) on liver volume, there was a significant interaction between time and mitotane treatment (Fig. 3g; $F_{1,14} = 6.81$, $p = 0.021$; $R^2_m = 0.24$, $R^2_c = 0.49$), where mitotane birds increased liver volume with time in captivity, while there was no change in controls. There were no effects of captivity, sex or mitotane treatment on spleen volume (Fig. 3h; time: $F_{1,14} = 0.65$, $p = 0.43$; sex: $F_{1,13} = 1.25$, $p = 0.28$; treatment: $F_{1,20} = 2.44$, $p = 0.13$; time * treatment interaction: $F_{1,14} = 0.41$, $p = 0.53$; $R^2_m = 0.17$, $R^2_c = 0.62$).

3.4. Behavioral measures

For number of hops/flights and preening behavior, there were no changes in frequency with time in captivity, or effects of sex or mitotane on these behaviors (Supplemental Fig. 5; hops and flights: time: $t = -0.0048$, $p = 0.99$; treatment: $t = 0.88$, $p = 0.38$; sex: $t = -1.18$, $p = 0.24$; time * treatment interaction: $t = -0.41$, $p = 0.68$; $R^2_m = 0.09$, $R^2_c = 0.09$; preening time: $t = 1.65$, $p = 0.10$; treatment: $t = 0.54$, $p = 0.59$; sex: $t = 0.11$, $p = 0.91$; time * treatment interaction: $t = -1.17$, $p = 0.24$; $R^2_m = 0.06$, $R^2_c = 0.21$). Feeding and feather ruffling behavior both increased with time in captivity (Fig. 4; feeding: time: $t = -2.36$, $p = 0.018$; feather ruffling: time: $t = 2.71$, $p = 0.007$), but there were no effects of mitotane or sex on these behaviors (time feeding: treatment: $t = 0.21$, $p = 0.83$; sex: $t = -0.14$, $p = 0.89$; time * treatment interaction: $t = 0.35$, $p = 0.73$; $R^2_m = 0.19$, $R^2_c = 0.19$; feather ruffling: treatment: $t = -0.44$, $p = 0.66$; sex: $t = -0.18$, $p = 0.86$; time * treatment interaction: $t = -1.45$, $p = 0.15$; $R^2_m = 0.25$, $R^2_c = 0.25$). Beak wiping increased with time in captivity (Fig. 4; $t = 4.90$, $p < 0.001$), and there was a significant interaction with mitotane treatment, where mitotane birds showed smaller increases in this behavior compared to controls (time * treatment interaction: $t = -2.43$, $p = 0.015$; treatment: $t = 0.73$, $p = 0.47$). Beak wiping was not affected by sex ($t = -0.0086$, $p = 0.99$; $R^2_m = 0.30$, $R^2_c = 0.66$).

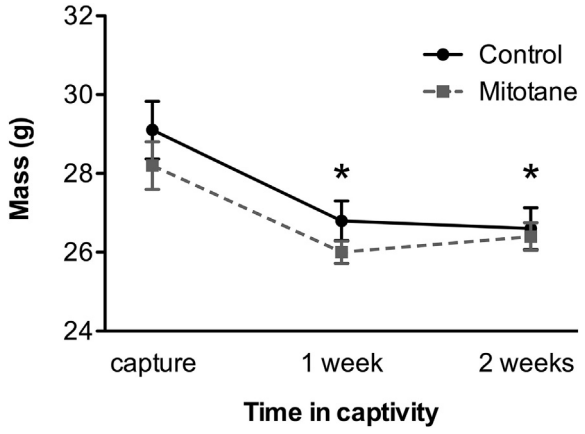

Fig. 2. Wild house sparrows (*Passer domesticus*) lost significant body mass after 1 week in a laboratory setting, and there was no further change in mass by week 2 (* = different from capture mass at $p < 0.05$). Half of the birds ($n = 9$) received injections of the drug mitotane, which reduced stress-induced corticosterone, whereas the other half ($n = 9$) received a vehicle control. Mitotane treatment did not affect body mass. See text for more details on statistical analyses.

4. Discussion

We used a novel method, CT imaging with a gadolinium contrast agent, to demonstrate that rapid and widespread changes in body composition occur in wild birds upon transfer to a laboratory setting. Previous studies have mostly used either external measures of body composition (i.e., fat scores) or invasive methods (i.e., laparotomy, muscle biopsy) to assess the same measures in living animals. Thus, CT imaging opens up new research opportunities by allowing researchers to conduct longitudinal studies of body composition in wild animals (Piersma and Lindström, 1997), similar to other in vivo imaging modalities, including ultrasound and magnetic resonance imaging (Starck et al., 2001; Tinsley et al., 2004).

Birds lost significant body mass (~8%) during their 2 weeks in captivity, and heart and testes also decreased in volume, suggesting that some of the mass loss could be from regression of these tissues. Interestingly, although the thickness of the pectoralis muscle did not change over time, pectoralis muscle density significantly decreased, an effect consistent with replacement of denser muscle tissue with less-dense fat (Goodpaster et al., 2000), which could also be responsible for some of the mass loss. Fat scores and both subcutaneous and omental fat volumes increased with time in captivity. Plasma fructosamine, an integrated (~2 week) measure of glucose metabolism, was not affected by captivity, which was contrary to our predictions (Table 1) but consistent with other studies showing no changes in plasma glucose in wild birds in response to captivity (Fokidis et al., 2011). This suggests that the changes in body composition were either caused by changes in the availability of other energy substrates (e.g., triglycerides - and it should be noted that birds were given ad libitum access to fatty seeds), or by changes in glucose use and storage in the body.

Mitotane reduced stress-induced CORT titers by >50% relative to controls (Fig. 1b), although baseline CORT did not differ between mitotane and control animals. In both groups, baseline CORT titers increased significantly after 1 week of captivity. An increase in circulating baseline CORT has been seen in a wide variety of species in response to captivity, including house sparrows (Dickens and Romero, 2013; Fischer and Romero, 2016). In control birds, stress-induced CORT titers did not significantly change over the two weeks in the lab, similar to what was seen in a previous study examining plasma CORT concentrations in breeding house sparrows in response to captivity (Lattin et al., 2012). Because the CORT response to stressors was lower in mitotane-treated animals, and there are many stressors associated with life in the captive

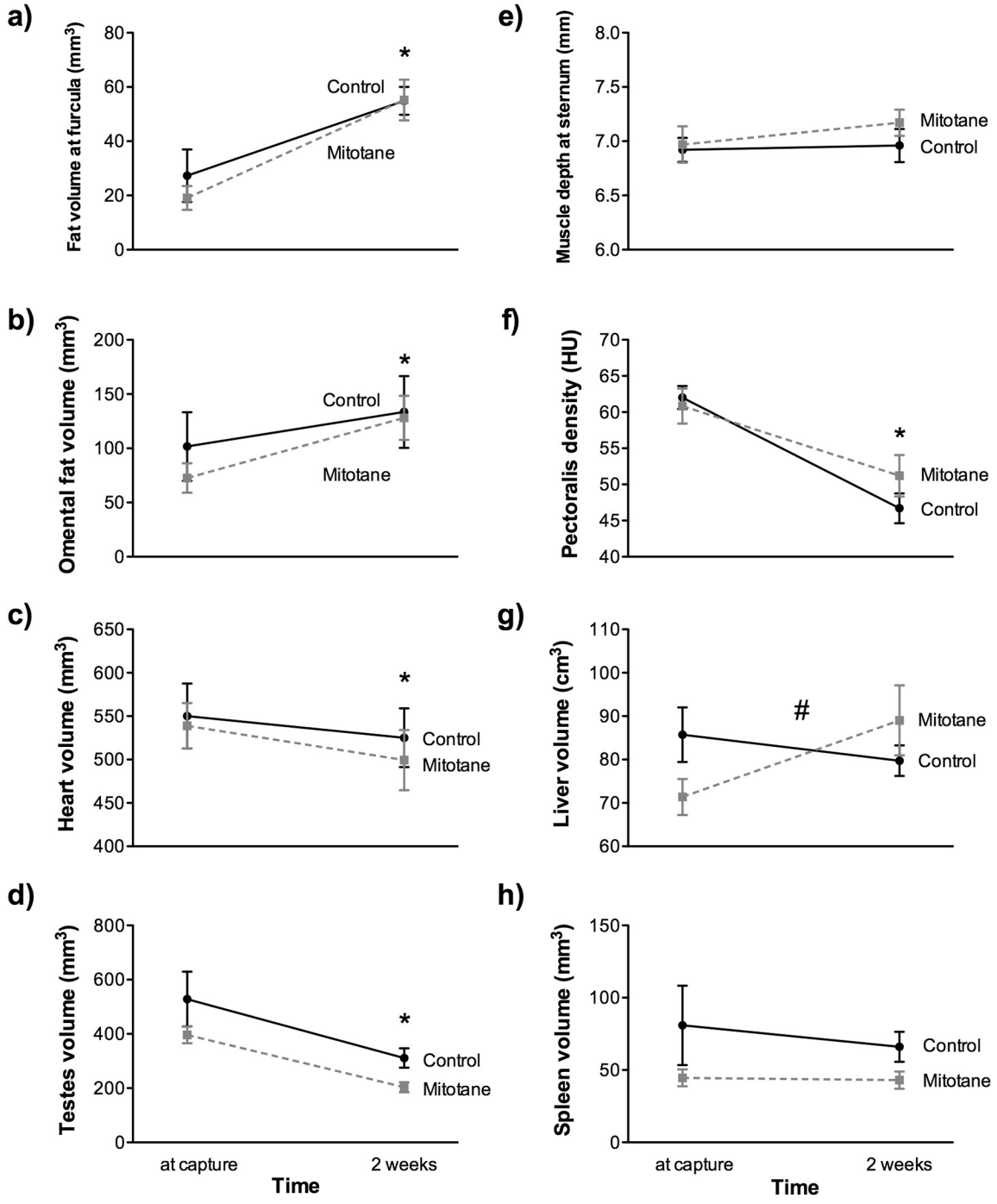

Fig. 3. Body composition of wild-caught house sparrows (*Passer domesticus*) changed after two weeks in captivity, for the most part regardless of whether birds received injections of the corticosterone-blocking drug mitotane ($n = 9$) or a vehicle control ($n = 9$). Size of: a) subcutaneous fat volume, b) omental fat volume, c) heart volume, d) testes volume, e) pectoralis muscle depth at sternum, f) pectoralis muscle density at sternum measured using tissue attenuation in Hounsfield units (HU), g) liver volume, and h) spleen volume measured using computed tomography (CT) images. * = significantly different from capture, # = significant interaction between time and mitotane treatment. See text for more details on sample sizes, image analysis and statistical analyses.

environment (Buijs et al., 2011; Evans et al., 2012; Nephew et al., 2003), this suggests that mitotane-treated animals were exposed to less CORT overall compared to controls. At the very least, animals were captured and handled every other day for mitotane or control injection administration, and animal caretakers entered the bird room daily to replace food and water and clean cages. Both of these stimuli have been shown to significantly increase plasma corticosterone titers in birds (Harvey et al., 1980; Nephew et al., 2003).

It has been hypothesized that many of the physiological changes occurring in wild animals upon transfer to captivity are caused by chronic elevation of CORT in response to stressors of the captive environment (Morgan and Tromborg, 2007). Indeed, some of the changes in body composition we measured were consistent with documented effects of sustained high CORT, such as increased fat depots (Rebuffé-Scrive et al., 1992) and decreased testes size (Hull et al., 2007) (see also Table 1). However, very few of these body composition changes differed between mitotane-treated and control animals, suggesting that stress-induced plasma CORT was not responsible, or, alternatively, that even lowered titers of stress-induced CORT were enough to induce these changes. Also, because

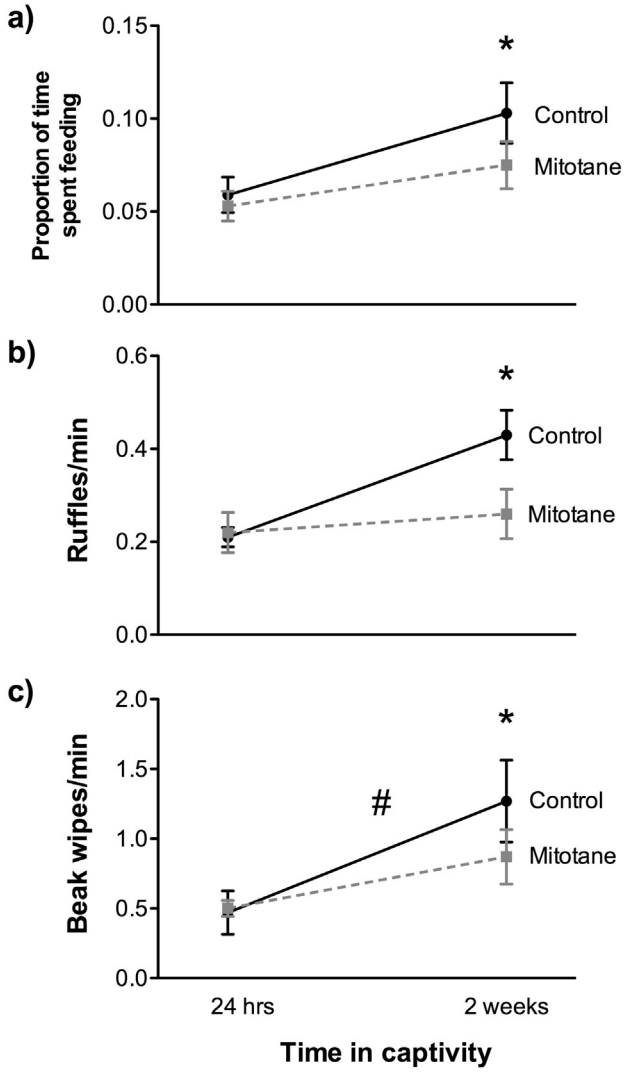

Fig. 4. Wild-caught house sparrows (*Passer domesticus*) significantly increased frequency of three behaviors with time spent in captivity: a) time spent feeding, b) feather ruffling, and c) beak wiping. Birds that received injections of the corticosterone-blocking drug mitotane ($n = 9$) did not increase incidence of beak wiping as much as birds that received a vehicle control ($n = 9$). * = significantly different from capture, # = significant interaction between time and mitotane treatment. See text for more details on statistical analyses.

baseline CORT increased significantly in both groups of animals after 1 week of captivity, it is possible these changes in body composition were driven by increased baseline hormone titers.

The one anatomical difference between mitotane-treated and control animals we observed was in liver volume, where mitotane-treated animals showed an increase in liver size with time in captivity, compared to no change in controls. Mitotane inhibits CORT production by suppressing mitochondrial steroid 11β-hydroxylase and cholesterol side-chain cleavage activity in the zona fasciculata of the adrenals (Sanderson, 2006). Because it is metabolically activated by an adrenal-specific cytochrome P450 (Jonsson et al., 1994), mitotane has been found to be quite selective in its effects - for example, an earlier study found that mitotane had no effects on testis size or testosterone titers in male house sparrows (Breuner et al., 2000). However, there are documented instances of mitotane treatment causing liver damage (Nagai et al., 1999), which can lead to hepatocyte proliferation (Sell, 2003). Therefore, this change in liver size is potentially a side effect of mitotane treatment rather than a meaningful difference related to decreased stress-induced CORT titers in these animals.

Our data suggest that physiological mechanisms other than heightened HPA activity may be responsible for captivity-induced changes in body composition in wild house sparrows. In fact, although CORT is often invoked as the primary cause of stress-related effects, several other physiological mediators have been shown to be involved in creating the phenotype associated with chronic stress, including catecholamines (Selvage et al., 2004), neuropeptide Y (Kuo et al., 2008) and the gut hormone ghrelin (Patterson et al., 2013). Some of the physiological changes caused by captivity may also be unrelated to stress, and instead be caused by features of the captive environment that differ greatly from the wild, such as ad libitum access to high-fat food and a sedentary lifestyle (Dykstra and Karasov, 1992; Piersma et al., 1993; Price et al., 2011; Vézina et al., 2006).

We also found that captivity altered the frequency of two behaviors related to anxiety and aggression (feather ruffling and beak wiping), as well as the amount of time animals spent feeding. In contrast to our physiological results, one of these behavioral changes (beak wiping) was affected by mitotane administration, where control birds increased this behavior more than mitotane birds. Several rodent studies have also found associations between CORT and behaviors related to anxiety and aggression (Mikics et al., 2004; Vallée et al., 1997; Wood et al., 2003), which may be at least partly mediated by CORT-induced increases in corticotropin-releasing factor expression in the amygdala (Shepard et al., 2000). Mitotane treatment did not have any impact on time spent feeding, feather ruffling, hops and flights, or preening behavior, suggesting that the effect of long-term elevated CORT on sparrow behavior was relatively specific. Therefore, our data suggest that experimentally reducing stress-induced CORT titers may mitigate some captivity-induced behavioral changes.

5. Conclusion

The mitotane treatment used in this study was meant to reduce CORT to the lower end of a normal physiological range, rather than complete elimination, which might have made animals vulnerable to adrenal crisis and death (Hague et al., 1989). However, it is important to remember that baseline CORT is generally thought to interact more with the higher-affinity mineralocorticoid receptor, whereas many of the effects of stress-induced CORT are thought to occur via activation of the lower-affinity glucocorticoid receptor (Landys et al., 2006). Thus, a study using specific antagonists for glucocorticoid and mineralocorticoid receptors could reveal more about the role of baseline and stress-induced CORT in driving changes in body composition and behavior in captive birds. Overall, our results demonstrate that even a short period of captivity can have significant effects on body composition and behavior of wild birds, and emphasize that researchers should take behavioral and physiological differences between free-living animals and captives into consideration when designing studies and interpreting results.

From a conservation perspective, this study also suggests that time in captivity should be minimized when birds will be reintroduced back to the wild. For example, because heart mass is strongly associated with aerobic performance in house sparrows (Chappell et al., 1999), sparrows in our study may have had decreased aerobic capacity after 2 weeks in the lab, which could potentially affect their ability to survive if released. This may be one reason that translocation attempts of wild birds have historically had such low incidence of success (Griffith et al., 1989).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2016.12.016.

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