SHORT COMMUNICATION

Coping with captivity: takeoff speed and load-lifting capacity are unaffected by substantial changes in body condition for a passerine bird

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ABSTRACT

Captivity presumably challenges the physiological equilibrium of birds and thus influences flight ability. However, the extent to which captive birds exhibit altered features underpinning maximum flight performance remains largely unknown. Here, we studied changes in physiological condition and load-lifting performance in the Eurasian tree sparrow (*Passer montanus*) over 15, 30 and 45 days of captivity. Sparrows showed body mass constancy over time but also an increased hematocrit at 15 days of captivity; both relative pectoralis mass and pectoralis fat content increased at 30 days. However, maximum takeoff speed and maximum lifted load remained largely unchanged until 45 days of captivity. Wingbeat frequency was independent of captivity duration and loading condition, whereas body angle and stroke plane angle varied only with maximum loading and not with duration of captivity. Overall, these results suggest that captive birds can maintain maximum flight performance when experiencing dramatic changes in both internal milieu and external environment.

KEY WORDS: Biomechanics, Captivity stress, Eurasian tree sparrow, Kinematics, Flight performance

INTRODUCTION

Flight performance is relevant to avian survival (Hedenström, 2002), and birds have evolved a suite of morphological, behavioral and physiological traits that enhance aerial ability (Altshuler et al., 2015; Butler, 2016). Maximum flight performance can vary with features of the underlying flight musculature (Veasey et al., 2000; Yap et al., 2018; Whelan et al., 2020), and there may also be multiple trade-offs between physiological condition and flight biomechanics (Chai and Dudley, 1999; Lind, 2001; Lind and Jakobsson, 2001; Altshuler et al., 2015). How birds may adjust features of flight physiology to maintain maximum performance in response to a challenge remains largely unexplored, but can inform us about basic compensatory mechanisms following environmental perturbation over various time scales.

Captivity is a means of studying physiological responses to a controlled environment, and may potentially affect nutritional equilibrium, muscular condition and ultimately the flight ability of birds (Dickens et al., 2009; Mason, 2010; Mason et al., 2013; Dickens and Bentley, 2014; Angelier et al., 2016). Both behavior and physiology of free-living birds can dramatically change in response to acute capture handling (i.e. within several minutes: Romero, 2002), and to short- and long-term captivity ranging in duration from one day to a year (Dickens et al., 2009; Fokidis et al., 2011; Thompson et al., 2015; Li et al., 2019). Spatial restriction may impede free-flight behavior and thus be detrimental to locomotor ability, e.g. captivity reduces takeoff speed of escaping female blue tits (*Cyanistes caeruleus*) (Kullberg et al., 2002). Captivity stress can also result in reduced activity, changes in body condition (e.g. effects on body mass and hematocrit), abdominal fat deposition, metabolic disorders (Sánchez-Guzmán et al., 2004; Dickens et al., 2009; Fokidis et al., 2011) and impaired reproductive output and immune defenses (Morgan and Tromborg, 2007).

When faced with short-term physiological challenges, volant taxa can compensate kinematically to augment flight performance, e.g. through increases in stroke amplitude and wingbeat frequency, and adjustment of body and stroke plane angle, to transiently increase force output (e.g. Altshuler and Dudley, 2003; Hedrick et al., 2004; Yu and Tong, 2005; Sun et al., 2016). Maximum load lifting during vertical ascent represents an extreme aerodynamic and energetic challenge, and has been widely used to study limits to animal flight performance (Altshuler et al., 2004; 2010; Buchwald and Dudley, 2010; Sun et al., 2016; Wang et al., 2020). Load-lifting ability varies considerably with body size, phylogeny, developmental stage, air composition, environmental temperature and behavioral strategy (e.g. Lind, 2001; Lind and Jakobsson, 2001; Altshuler and Dudley, 2003; Kullberg et al., 2002, 2005; Sun et al., 2016; Wang et al., 2020), and thus should clearly demonstrate potential effects of captivity on maximum flight performance, if present. Here, we asked whether captive birds can maintain load-lifting capacity (and if so, by what alterations in flight kinematics) when confronted with chronic captivity and associated changes in physiological condition.

The Eurasian tree sparrow, *Passer montanus* (Linnaeus 1758), is a widely distributed human-commensal bird that is sensitive to captivity (Dixit and Singh, 2013; Li et al., 2019). Previous studies have shown that maximum flight performance in this species can vary with a number of morphological and physiological features (Wang et al., 2019), and also with elevation (Sun et al., 2016); maximum takeoff speed can be similarly variable (Wang et al., 2020). Here, we employed Eurasian tree sparrows to investigate trade-offs between maximum flight performance and physiological condition deriving from multiday captivity. We evaluated dynamic changes in body condition (via measurements of body mass, pectoralis mass and hematocrit), along with lipid deposition in the pectoralis and liver, and correlated these changes with flight

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kinematics and flight performance under both load-free takeoff and maximum load-lifting conditions.

MATERIALS AND METHODS
Animal collection
Eurasian tree sparrows were caught opportunistically with mist nets on the campus of Hebei Normal University (38°0.24′N, 114°31.50′E, elevation: 75 m), Hebei Province, China, in late April and early May of 2016. This period corresponds to the early breeding stage of this species (Li et al., 2012). To exclude effects of age and sex, only first-year female sparrows were studied, i.e. those with small yellow spots on the base of the beak and with a female-specific brood patch. After capture, 40 birds were individually weighed with a portable electronic balance and then transported to the laboratory.

All capture, handling, and experimental protocols were approved by the Ethics and Animal Welfare Committee and the Institutional Animal Care and Use Committee of Hebei Normal University, China. Scientific collecting permits were issued by the Department of Wildlife Conservation (Forestry Bureau) of Hebei Province, China.

Experimental design and captivity stress
Birds were individually housed in cages (38 cm×27 cm×30 cm) provided with abundant food [foxtail millet (*Setaria italica*) mixed with mealworms] and water. Lighting conditions (13.3 h of daylight on average) and temperature (18.5°C on average) in the husbandry room (which was open to outdoor ventilation) were similar to average conditions of the birds' natural environment in springtime. To determine the effects of continuous captivity on physiological indicators of overall condition (i.e. hematocrit, pectoralis and hepatic fat content, and the relative mass of the heart, liver and pectoralis), three groups of birds were kept in cages for 15, 30 or 45 days (i.e. experimental treatments of captivity stress; n=10 per group); a control group of 10 birds was sampled on the day of capture (day 0). Individual birds were randomly assigned to each of these four categories. Individuals from the 45 day group were tested in flight trials at each of the 0, 15, 30 and 45 day captivity periods to determine temporal variation in body mass and flight kinematics parameters (i.e. wing stroke amplitude, wingbeat frequency, body angle, stroke plane angle, relative downstroke duration, maximum takeoff speed and maximum load).

Maximum flight performance and flight kinematics
A rectangular flight chamber (45 cm×45 cm×155 cm) made of transparent Plexiglas was used to determine flight-related parameters during vertical ascent, as used in previous experiments (Sun et al., 2016; Wang et al., 2019, 2020). Birds were first tested for flight performance under the load-free condition (i.e. simulating flight behavior under natural conditions). After a 5 min recovery period, birds were then tested for flight performance under maximum load lifting using a string of plastic beads tied to their left tarsometatarsus. Two synchronized high-speed video cameras (GCP100BAC, JVC Kenwood Corporation, Yokohama, Japan) were used to record all flights. One camera (operated at 50 frames s^{-1}) positioned laterally at a distance of 80 cm to the chamber was used to record features of maximum flight performance (i.e. body position through time and maximum lifted load, along with body and stroke plane angles). The other camera (operated at 250 frames s^{-1}) was placed on the top of the chamber to obtain the stroke amplitude, wingbeat frequency and relative duration of the downstroke (see Sun et al., 2016).

Maximum sustained mass in load lifting was calculated from the total weight of beads and string suspended from the ground when peak ascent height was reached. A mean of 5.3 ascending flights was recorded for each bird, and the maximum weight lifted within each series was assumed to indicate the limit to vertical load lifting. Maximum vertical takeoff speeds were approximated, for comparative purposes, from velocity estimates (i.e. displacement per time) calculated for consecutive transits spanning the full ascent over either six or seven 20 cm segments for the load-free condition, and over three or four 20 cm segments for the load-lifting condition. Because of the low filming speed and the small number of transited segments per ascent, the number of possible values for estimated flight speeds was fairly low, and we accordingly view these values to reflect only comparative and not absolute performance. The highest estimate for these speeds in each individual flight bout was assumed to approximate the maximum takeoff speed.

Stroke amplitude was indicated by extremes of the wing angles between upstroke and downstroke within each bout of the final 0.5 s of either maximum load lifting or unloaded ascent (see Wang et al., 2019). Wingbeat frequency was estimated from the number of frames required to complete four wingbeats during the second half of each ascent. The relative downstroke duration was calculated as the ratio of the downstroke to the wingbeat period. Body angle and stroke plane angle were calculated as the inclinations of the body axis and of the wing stroke plane relative to the horizontal plane, respectively.

Measurement of physiological parameters
Following experiments, the alar vein of each bird was punctured with a 26-gauge needle, and ~80 µl of blood was collected into heparinized microhematocrit capillary tubes. All blood samples were stored on ice for 3–4 h until they could be centrifuged at 855 g for 10 min; hematocrit was measured as the relative volume of red blood cells per total blood volume. Birds were then euthanized via subcutaneous injection of phenobarbitone anesthetic (7.5 µl g^{-1} body mass). The pectoralis (i.e. the major flight muscle), liver and heart (which was blotted to remove blood) were immediately excised and weighed with a precision of 0.1 mg (Ohaus CPJ603 balance, Parsippany, NJ, USA). Dry weights of the flight muscle and liver were obtained following desiccation in an oven at 65°C; lipid content of the flight muscle and liver was determined following extraction by a 2:1 chloroform–methanol mixture (see Folch et al., 1957). The relative pectoralis mass, and also the heart and liver indices, were calculated as the body mass-corrected pectoralis mass, heart and liver mass, respectively.

Data analysis
We examined treatment differences relative to hematocrit, heart index, liver index and pectoralis fat content using one-way analysis of variance (ANOVA). We examined treatment differences relative to hepatic fat content and relative pectoralis mass using a Kruskal–Wallis one-way ANOVA because these variables violated the equality of homogeneity of variance (Levene's test). Effects of treatment duration (i.e. 0, 15, 30 and 45 days of captivity) on body mass and maximum load were assessed using one-way repeated measures ANOVA, with individual identity as a repeated factor. Effects of loading (i.e. either load lifting or the load-free condition), duration of captivity, and the interaction between loading and captivity duration on maximum takeoff speed, wingbeat frequency, stroke amplitude, relative downstroke duration, body angle and stroke plane angle were analyzed with a two-way repeated-measures ANOVA, using individual identity as a repeated factor. Differences between pairs of means were identified by Bonferroni-adjusted tests. *Post hoc* comparisons were made by either Bonferroni-

adjusted tests or Mann–Whitney U tests. Differences in flight kinematic parameters between load-free and load-lifting conditions at the same captivity stage were assessed using paired-sample t-tests. Statistical analysis was performed using SPSS software (version 21.0; IBM, New York, NY, USA), and figures were generated in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). The fiducial level for significant differences was set as $P<0.05$.

RESULTS AND DISCUSSION
Changes in physiological parameters
Body mass and both heart and liver indices were invariant with experimental treatment. However, hematocrit, relative pectoralis mass, pectoralis fat content and hepatic fat content varied significantly with duration of captivity (Table 1). Our results showed that those birds experiencing 15, 30 and 45 day captivity had significantly higher hematocrit relative to the control group (Fig. 1A). Such increased hematocrit values may reflect transition from acute stress to chronic adaptation (Dickens et al., 2009). Higher hematocrit yields enhanced oxygen delivery during either fight or flight responses (Yap et al., 2018), and may indicate both longer-term behavioral changes (decreased daily activity) and adjustments in metabolic substrate (Thompson et al., 2015).

Those birds experiencing 30 and 45 day captivity exhibited a significantly higher relative pectoralis mass compared with the control and the 15 day captivity groups (Fig. 1B), and also had a higher pectoralis fat content relative to controls (Fig. 1C; Table S1).

Table 1. Effects of captivity duration on physiological variables and maximum load, and of captivity duration, load condition and their interaction on flight kinematics in Eurasian tree sparrows

Variable	Factor	d.f.	F-value	P-value
Hematocrit (%)	Captivity	3,36	9.206	**<0.001**
Heart index (%)	Captivity	3,36	0.099	0.960
Liver index (%)	Captivity	3,36	1.378	0.265
Pectoralis fat content (%)	Captivity	3,35	4.777	**0.007**
Hepatic fat content (%)	Captivity	3,36	9.806	**0.020**
Relative pectoralis mass (%)	Captivity	3,36	21.312	**<0.001**
Body mass (g)	Captivity	3,36	2.655	0.069
Maximum load (g)	Captivity	3,36	9.566	**<0.001**
Maximum takeoff speed	Captivity	3,72	2.595	0.062
(m s^{-1})	Load	1,72	68.844	**<0.001**
	Load×Captivity	3,72	3.552	**0.020**
Wingbeat frequency (Hz)	Captivity	3,72	0.659	0.581
	Load	1,72	0.068	0.798
	Load×Captivity	3,72	1.443	0.240
Stroke amplitude (deg)	Captivity	3,72	7.284	**0.002**
	Load	1,72	6.184	**0.023**
	Load×Captivity	3,72	3.425	**0.023**
Relative downstroke	Captivity	3,72	5.664	**0.002**
duration (%)	Load	1,72	3.159	0.092
	Load×Captivity	3,72	4.721	**0.005**
Body angle (deg)	Captivity	3,72	1.136	0.331
	Load	1,72	126.834	**<0.001**
	Load×Captivity	3,72	0.408	0.748
Stroke plane angle (deg)	Captivity	3,72	2.639	0.059
	Load	1,72	118.195	**<0.001**
	Load×Captivity	3,72	0.119	0.948

Effects of captivity duration (0, 15, 30 and 45 days) on physiological variables and maximum load were analyzed using one-way analysis of variance (ANOVA; repeated-measures for maximum load). Effects of captivity duration, load condition (load-free and load-lifting flight) and their interaction on flight kinematics were analyzed using two-way repeated-measures ANOVA. Significant values ($P<0.05$) are shown in bold.

The 15 day captivity group showed an increased hepatic fat content relative to the control and 30 and 45 day captivity groups (Fig. 1D). These dynamic changes in body condition and energy storage suggest important effects of captivity on glucolipid metabolism and overall physiological capacity. The liver, the anatomical crossroads for lipid synthesis and metabolism, is highly susceptible to external stressors up to the point of disrupting lipid homeostasis (Emami et al., 2021). The vast majority of fatty acids in birds are synthesized within the liver, and fat can accumulate rapidly in the pectoralis and subcutaneous tissue via efficient cytosolic transport (Guglielmo, 2018; Emami et al., 2021). Here, our results indicate both tissue- and duration-dependent variation in lipid deposition, presumably as a consequence of restricted locomotion and of enhanced food quality and reliability under captive conditions (Morgan and Tromborg, 2007; Thompson et al., 2015). However, both relative pectoralis mass and pectoralis fat deposition increased only after 30 days of captivity, suggesting a trade-off between pectoralis mass and associated fuel accumulation as a result of mechanical constraints preventing unrestricted fuel accumulation (Bayly et al., 2021). Pathways of protein catabolism and synthesis in flight muscle are regulated according to energetic conditions, and birds typically defend a specific protein-to-fat ratio to maintain flight performance (Lindstrom et al., 2000; Van den Hout et al., 2010). Routine flight is required to upregulate those genes involved in mitochondrial metabolism and fat utilization in the avian pectoralis (DeMoranville et al., 2020), and lack of activity is closely linked to adiposity and visceral fat accumulation (Pedersen, 2009). Unlike mammals, birds do not increase pectoralis mass through strength training (see Lindstrom et al., 2000). Here, reduced locomotor opportunity resulted in long-term lipid deposition in the flight muscle, but yielded no changes in whole-animal flight performance.

Changes in flight performance and kinematics: effects of captivity duration and loading conditions
The maximum lifted load declined significantly with captivity duration (Fig. 2A). Maximum takeoff speed changed significantly with load condition, and with the interaction between lifted load and captivity duration (Table 1). Stroke amplitude varied significantly with load condition and duration of captivity, and with the interaction between load and captivity duration, but wingbeat frequency did not vary significantly. The relative duration of the downstroke changed significantly with the duration of captivity, and with the interaction between load and captivity duration. Body angle and stroke plane angle varied with loading condition, but neither parameter varied either with captivity duration or with the interaction between loading condition and captivity duration. Our results showed that those individuals experiencing a 45 day captivity lifted a significantly lower maximum load than did the control, and 15 and 30 day captivity groups. The maximum takeoff speed decreased substantially in the 45 day captivity group relative to the control and to the 30 day captivity groups under the load-free condition (one-way repeated measures ANOVA: $F_{3,36}=5.490$; $P=0.004$), but remained unchanged under maximum load lifting ($F_{3,36}=0.840$; $P=0.481$; Table S2). The stroke amplitude did not vary with the duration of captivity for the load-free condition (one-way repeated measures ANOVA: $F_{3,36}=1.392$; $P=0.267$), whereas it increased substantially in the 45 day captivity group relative to the control and the 30 day captivity groups ($F_{3,36}=6.212$; $P=0.002$; Table S2). Those birds experiencing the 45 day captivity exhibited a significantly reduced relative downstroke duration in the load-free condition compared with the control and 15 day captivity groups (one-way repeated
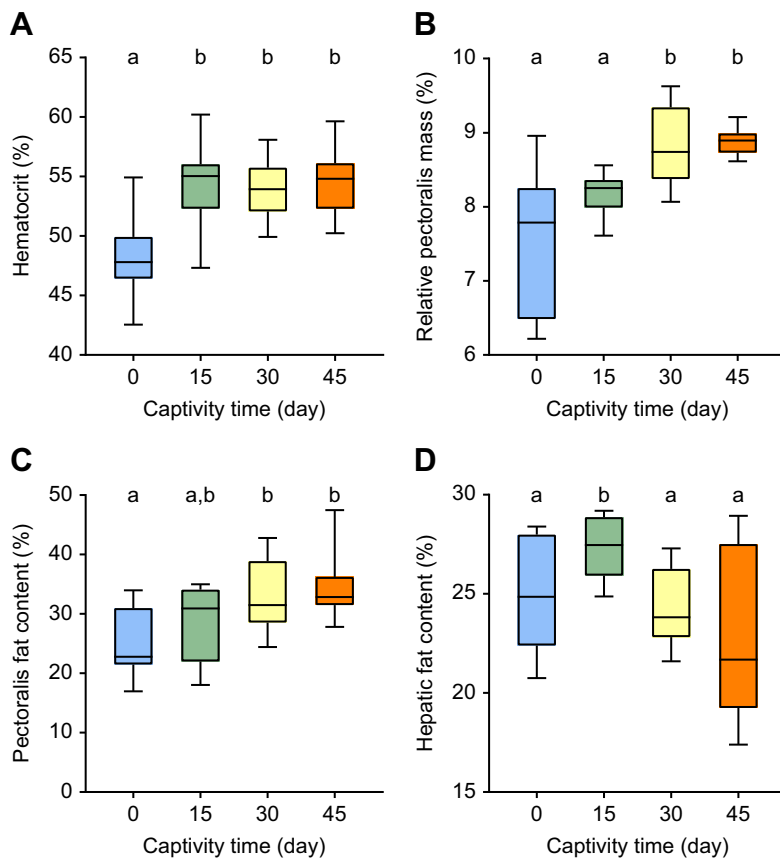
Fig. 1. Comparison of physiological parameters under 0, 15, 30 and 45 day captivity in Eurasian tree sparrows. (A) Hematocrit, (B) relative pectoralis mass, (C) pectoralis fat content and (D) hepatic fat content. Values depicted are the median for each group, with the upper and lower limits of the box representing the 75th and 25th percentiles, respectively. Error bars represent the 95th and the 5th percentiles. Different letters indicated significant differences (*P*<0.05) between groups (see Table S1 for further details).

measures ANOVA: $F_{3,36}=10.873$; $P<0.001$). However, there were no significant differences in relative downstroke duration among all groups when undergoing maximum load lifting ($F_{3,36}=0.536$; $P=0.661$; Table S2).

Although the 30 day captivity led to a suite of physiological changes, maximum lifted load and maximum takeoff speed (in both load-free and load-lifting conditions) were unchanged. Our results differ from those of well-trained human athletes, who exhibit decreased endurance and isokinetic strength within 4 weeks of detraining (Mujika and Padilla, 2000, 2001). Significant deprivation of muscle contractility and locomotor capacity also characterizes other mammalian model taxa following activity restriction (Hortobágyi et al., 2000; Lohuis et al., 2007). To maintain maximum flight performance, the sparrows here kept unchanged various features of flight kinematics (i.e. stroke amplitude, wingbeat frequency and relative downstroke duration), and also of body and wing stroke orientation during load lifting (i.e. body angle and stroke plane angle). More generally, maximum flight capacity of birds at particular life-history stages or in different environments can be impaired by altered body condition, e.g. blue tits (Kullberg et al., 2002) and zebra finches (Kullberg et al., 2005; Criscuolo et al., 2011). Specifically, our result indicates that sparrows maintained maximum loading and takeoff performance in spite of captivity-induced physiological changes.

The unloaded maximum takeoff speed and the maximum lifted mass significantly declined at 45 days relative to controls (and in parallel with physiological changes). Birds often adopt alternative behavioral strategies to compensate for reduced flight ability when encountering abnormal physiological conditions, and in particular during certain life-history stages in extreme weather (e.g. Macleod,

2006; Dietz et al., 2007; Ortega-Jimenez et al., 2016; Walters et al., 2017). Although Eurasian tree sparrows here increased stroke amplitude and decreased downstroke duration at 45 days, they apparently could not offset an overall decline in flight power during vertical ascent. It is important to note that long-term captivity is clearly an unnatural condition, and one that may fall outside the bounds of typical environmental stressors.

Our results showed that maximum takeoff speed decreased significantly under load lifting relative to the load-free condition, independent of duration of captivity (Fig. 2B; Table S3). Stroke amplitude did not change as a function of load in the control, and 15 and 30 day captivity groups, but increased significantly in the 45 day captivity group under load-lifting relative to load-free conditions (Fig. 2C). There were also no significant differences in the relative downstroke duration between load-free and load-lifting conditions in any group except for the 45 day captivity condition, which showed an increase in the relative downstroke duration (Fig. 2D). Sparrows flew with significantly decreased body angle and increased stroke plane angle when load lifting relative to load-free conditions, independent of captivity duration (Fig. 2E,F).

An extra load significantly reduced maximum speed during takeoff, as predicted. Unexpectedly, there were no significant differences in stroke amplitude, wingbeat frequency and relative downstroke duration between load-free and load-lifting conditions until 30 days of captivity. These flight kinematic parameters were thus maintained in spite of dramatic changes in physiological condition, and presumably other (and here unmeasured) features of the wingbeat (such as wing angle of attack), which can be altered to yield increased vertical forces during load lifting. At 45 days, however, Eurasian tree sparrows did increase stroke amplitude and
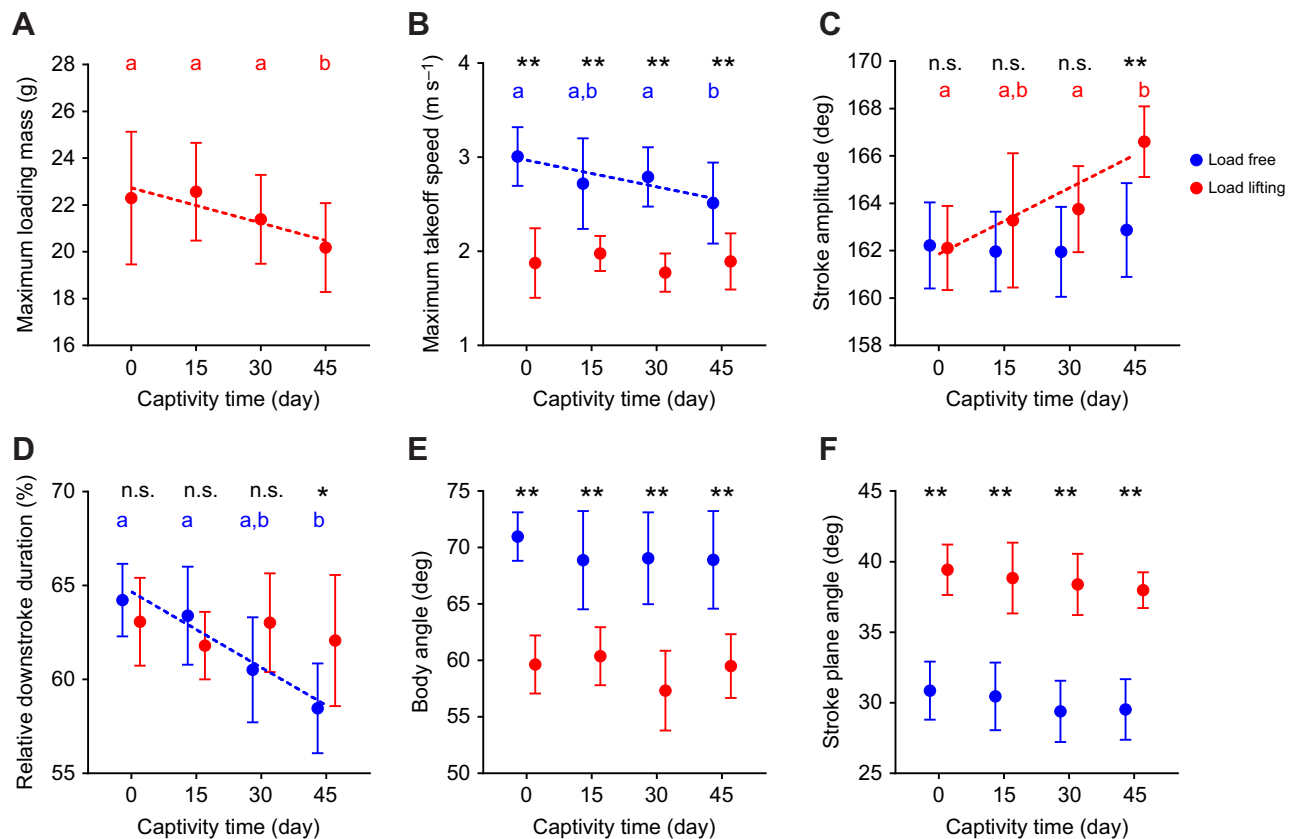
Fig. 2. Comparison of flight kinematics parameters between load-free and load-lifting conditions under 0, 15, 30 and 45 day captivity in Eurasian tree sparrows. (A) Maximum loading mass, (B) maximum takeoff speed, (C) stroke amplitude, (D) relative downstroke duration, (E) body angle and (F) stroke plane angle. A dotted line of the corresponding color represents a significant change in response to captivity in load-free and load-lifting conditions. Different letters represent significant differences between groups with the corresponding color (see Table S2 for further details). Asterisks indicate significant differences between load-free and load-lifting conditions in a particular group (*$P<0.05$, **$P<0.001$; see Table S3 for further details). Error bars represent s.d. of means.

relative downstroke duration in maximum load lifting. An increase in stroke amplitude is broadly characteristic of hummingbirds when challenged aerodynamically (Altshuler and Dudley, 2003; Altshuler et al., 2004, 2005), but birds other than hummingbirds flex their wings during the upstroke and predominantly use the downstroke to generate force, suggesting that relative stroke timing is also important (Berg and Biewener, 2010; Muijres et al., 2012). Variation in downstroke duration can correspondingly influence the costs of muscle activation and of power output (see Bruderer et al., 2001; Usherwood, 2016). Independent of the duration of captivity, Eurasian tree sparrows also increased stroke plane angle and decreased body angle to increase vertical forces via rotation of the net force vector (e.g. Tobalske et al., 2007; Berg and Biewener, 2010; Chin and Lentink, 2019). By contrast, wingbeat frequency did not vary with either duration of captivity or loading condition. Wingbeat frequency in Eurasian tree sparrows tends to be conserved and is not linked with maximum lifting capacity among individuals, either within a population (Wang et al., 2019) or for distinct populations at different altitudes (Sun et al., 2016).

In summary, this study is the first to investigate trade-offs between maximum flight performance and physiological condition for birds exposed to varying durations of captivity stress. Eurasian tree sparrows defended flight capacity over 30 days, as reflected by invariant maximum takeoff speed and maximum lifted mass, but total lifted mass declined after 45 days. The sparrows reduced relative downstroke duration to yield greater vertical forces in the load-free condition, and also increased stroke amplitude to increase

force production when challenged by extra load. Wingbeat frequency was disassociated from the duration of captivity and loading condition, and body angle and stroke plane angle only changed with load condition. Notably, our results indicate that the sparrows can maintain relatively stable maximum locomotor performance when experiencing dramatic changes in both their internal milieu and external environment, although the specific mechanisms of physiological and molecular adjustment responsible for achieving such invariance are unclear. Whether such a capability would enhance survival and ultimately fitness of free-living birds when confronted with unfavorable conditions over varying ecological time scales also remains to be determined.

Author contributions
Conceptualization: D.L.; Methodology: D.L.; Validation: D.L.; Formal analysis: G.K., Y. Wang, R.D.; Data curation: G.K.; Writing - original draft: G.K., Y. Wang, Y. Wu, D.L.; Writing - review & editing: R.D., D.L.; Visualization: G.K.

References

Altshuler, D. L. and Dudley, R. (2003). Kinematics of hovering hummingbird flight along simulated and natural elevational gradients. *J. Exp. Biol.* **206**, 3139-3147. doi:10.1242/jeb.00540

Altshuler, D. L., Dudley, R. and Mcguire, J. A. (2004). Resolution of a paradox: hummingbird flight at high elevation does not come without a cost. *Proc. Natl. Acad. Sci. USA* **101**, 17731-17736. doi:10.1073/pnas.0405260101

Altshuler, D. L., Dickson, W. B., Vance, J. T., Roberts, S. P. and Dickinson, M. H. (2005). Short-amplitude high-frequency wing strokes determine the aerodynamics of honeybee flight. *Proc. Natl. Acad. Sci. USA* **102**, 18213-18218. doi:10.1073/pnas.0506590102

Altshuler, D. L., Dudley, R., Heredia, S. M. and McGuire, J. A. (2010). Allometry of hummingbird lifting performance. *J. Exp. Biol.* **213**, 725-734. doi:10.1242/jeb.037002

Altshuler, D. L., Bahlman, J. W., Dakin, R., Gaede, A. H., Goller, B., Lentink, D., Segre, P. S. and Skandalis, D. A. (2015). The biophysics of bird flight: functional relationships integrate aerodynamics, morphology, kinematics, muscles, and sensors. *Can. J. Zool.* **93**, 961-975. doi:10.1139/cjz-2015-0103

Angelier, F., Parenteau, C., Trouvé, C. and Angelier, N. (2016). Does the stress response predict the ability of wild birds to adjust to short-term captivity? A study of the rock pigeon (*Columbia livia*). *R. Soc. Open Sci.* **3**, 160840. doi:10.1098/rsos.160840

Bayly, N. J., Rosenberg, K. V., Norris, D. R., Taylor, P. D. and Hobson, K. A. (2021). Rapid recovery by fat-and muscle-depleted Blackpoll Warblers following trans-oceanic migration is driven by time-minimization. *Auk* **138**, ukab055. doi:10.1093/ornithology/ukab055

Berg, A. M. and Biewener, A. A. (2010). Wing and body kinematics of take-off and landing flight in the pigeon (*Columba livia*). *J. Exp. Biol.* **213**, 1651-1658. doi:10.1242/jeb.038109

Bruderer, L., Liechti, F. and Bilo, D. (2001). Flexibility in flight behaviour of barn swallows (*Hirundo rustica*) and house martins (*Delichon urbica*) tested in a wind tunnel. *J. Exp. Biol.* **204**, 1473-1484. doi:10.1242/jeb.204.8.1473

Buchwald, R. and Dudley, R. (2010). Limits to vertical force and power production in bumblebees (Hymenoptera: *Bombus impatiens*). *J. Exp. Biol.* **213**, 426-432. doi:10.1242/jeb.033563

Butler, P. J. (2016). The physiological basis of bird flight. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **371**, 20150384. doi:10.1098/rstb.2015.0384

Chai, P. and Dudley, R. (1999). Maximum flight performance of hummingbirds: capacities, constraints, and trade-offs. *Am. Nat.* **153**, 398-411. doi:10.1086/303179

Chin, D. D. and Lentink, D. (2019). Birds repurpose the role of drag and lift to take off and land. *Nat. Commun.* **10**, 5354. doi:10.1038/s41467-019-13347-3

Criscuolo, F., Monaghan, P., Proust, A., Škorpilová, J., Laurie, J. and Metcalfe, N. B. (2011). Costs of compensation: effect of early life conditions and reproduction on flight performance in zebra finches. *Oecologia* **167**, 315-323. doi:10.1007/s00442-011-1986-0

DeMoranville, K. J., Carter, W. A., Pierce, B. J. and McWilliams, S. R. (2020). Flight training in a migratory bird drives metabolic gene expression in the flight muscle but not liver, and dietary fat quality influences select genes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **319**, R637-R652. doi:10.1152/ajpregu.00163.2020

Dickens, M. J. and Bentley, G. E. (2014). Stress, captivity, and reproduction in a wild bird species. *Horm. Behav.* **66**, 685-693. doi:10.1016/j.yhbeh.2014.09.011

Dickens, M. J., Earle, K. A. and Romero, L. M. (2009). Initial transference of wild birds to captivity alters stress physiology. *Gen. Comp. Endocrinol.* **160**, 76-83. doi:10.1016/j.ygcen.2008.10.023

Dietz, M. W., Piersma, T., Hedenström, A. and Brugge, M. (2007). Intraspecific variation in avian pectoral muscle mass: constraints on maintaining maneuverability with increasing body mass. *Funct. Ecol.* **21**, 317-326. doi:10.1111/j.1365-2435.2006.01234.x

Dixit, A. S. and Singh, N. S. (2013). Environmental control of seasonal reproduction in the wild and captive eurasian tree sparrow (*Passer montanus*) with respect to variations in gonadal mass, histology, and sex steroids. *Can. J. Zool.* **91**, 302-312. doi:10.1139/cjz-2012-0190

Emami, N. K., Jung, U., Voy, B. and Dridi, S. (2021). Radical response: effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. *Antioxidants* **10**, 35. doi:10.3390/antiox10010035

Fokidis, H. B., Hurley, L., Rogowski, C., Sweazea, K. and Deviche, P. (2011). Effects of captivity and body condition on plasma corticosterone, locomotor behavior, and plasma metabolites in curve-billed thrashers. *Physiol. Biochem. Zool.* **84**, 595-606. doi:10.1086/662068

Folch, J., Lees, M. and Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497-509. doi:10.1016/S0021-9258(18)64849-5

Guglielmo, C. G. (2018). Obese super athletes: fat-fueled migration in birds and bats. *J. Exp. Biol.* **221**, jeb165753. doi:10.1242/jeb.165753

Hedenström, A. (2002). Aerodynamics, evolution and ecology of avian flight. *Trends Ecol. Evol.* **17**, 415-422. doi:10.1016/S0169-5347(02)02568-5

Hedrick, T. L., Usherwood, J. R. and Biewener, A. A. (2004). Wing inertia and whole-body acceleration: an analysis of instantaneous aerodynamic force production in cockatiels (*Nymphicus hollandicus*) flying across a range of speeds. *J. Exp. Biol.* **207**, 1689-1702. doi:10.1242/jeb.00933

Hortobágyi, T., Dempsey, L., Fraser, D., Zheng, D., Hamilton, G., Lambert, J. and Dohm, L. (2000). Changes in muscle strength, muscle fibre size and myofibrillar gene expression after immobilization and retraining in humans. *J. Physiol.* **524**, 293-304. doi:10.1111/j.1469-7793.2000.00293.x

Kullberg, C., Houston, D. C. and Metcalfe, N. B. (2002). Impaired flight ability—a cost of reproduction in female blue tits. *Behav. Ecol.* **13**, 575-579. doi:10.1093/beheco/13.4.575

Kullberg, C., Jakobsson, S. and Lind, U. K. (2005). Impaired flight ability prior to egg-laying: a cost of being a capital breeder. *Funct. Ecol.* **19**, 98-101. doi:10.1111/j.0269-8463.2005.00932.x

Li, D., Zhang, X., Li, Y., Hao, C., Zhang, J. and Wu, Y. (2012). Stress responses of testosterone and corticosterone-binding globulin in a multi-brooded species, Eurasian Tree Sparrows (*Passer montanus*): does CBG function as a mediator? *Horm. Behav.* **61**, 582-589. doi:10.1016/j.yhbeh.2012.02.007

Li, M., Zhu, W., Wang, Y., Sun, Y., Li, J., Liu, X., Wu, Y., Gao, X. and Li, D. (2019). Effects of capture and captivity on plasma corticosterone and metabolite levels in breeding Eurasian Tree Sparrows. *Avian Res.* **10**, 16. doi:10.1186/s40657-019-0155-8

Lind, J. (2001). Escape flight in moulting tree sparrows (*Passer montanus*). *Funct. Ecol.* **15**, 29-35. doi:10.1046/j.1365-2435.2001.00497.x

Lind, J. and Jakobsson, S. (2001). Body building and concurrent mass loss: flight adaptations in tree sparrows. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **268**, 1915-1919. doi:10.1098/rspb.2001.1740

Lindstrom, A., Kvist, A., Piersma, T., Dekinga, A. and Dietz, M. W. (2000). Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. *J. Exp. Biol.* **203**, 913-919. doi:10.1242/jeb.203.5.913

Lohuis, T. D., Harlow, H. J., Beck, T. D. I. and Iaizzo, P. A. (2007). Hibernating bears conserve muscle strength and maintain fatigue resistance. *Physiol. Biochem. Zool.* **80**, 257-269. doi:10.1086/513190

Macleod, R. (2006). Why does diurnal mass change not appear to affect the flight performance of alarmed birds? *Anim. Behav.* **71**, 523-530. doi:10.1016/j.anbehav.2005.04.020

Mason, G. J. (2010). Species differences in responses to captivity: stress, welfare and the comparative method. *Trends Ecol. Evol.* **25**, 713-721. doi:10.1016/j.tree.2010.08.011

Mason, G., Burn, C. C., Dallaire, J. A., Kroshko, J., Kinkaid, H. M. and Jeschke, J. M. (2013). Plastic animals in cages: behavioural flexibility and responses to captivity. *Anim. Behav.* **85**, 1113-1126. doi:10.1016/j.anbehav.2013.02.002

Morgan, K. N. and Tromborg, C. T. (2007). Sources of stress in captivity. *Appl. Anim. Behav. Sci.* **102**, 262-302. doi:10.1016/j.applanim.2006.05.032

Muijres, F. T., Johansson, L. C. and Hedenström, A. (2012). Leading edge vortex in a slow-flying passerine. *Biol. Lett.* **8**, 554-557. doi:10.1098/rsbl.2012.0130

Mujika, I. and Padilla, S. (2000). Detraining: loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus. *Sports Med.* **30**, 79-87. doi:10.2165/00007256-200030020-00002

Mujika, I. and Padilla, S. (2001). Muscular characteristics of detraining in humans. *Med. Sci. Sports Exerc.* **33**, 1297-1303. doi:10.1097/00005768-200108000-00009

Ortega-Jimenez, V. M., Badger, M., Wang, H. and Dudley, R. (2016). Into rude air: hummingbird flight performance in variable aerial environments. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **371**, 20150387. doi:10.1098/rstb.2015.0387

Pedersen, B. K. (2009). The diseasome of physical inactivity - and the role of myokines in muscle-fat cross talk. *J. Physiol.* **587**, 5559-5568. doi:10.1113/jphysiol.2009.179515

Romero, M. L. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* **128**, 1-24. doi:10.1016/S0016-6480(02)00064-3

Sánchez-Guzmán, J. M., Villegas, A., Corbacho, C., Morán, R., Marzal, A. and Real, R. (2004). Response of the haematocrit to body condition changes in Northern Bald Ibis *Geronticus eremita*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **139**, 41-47. doi:10.1016/j.cbpb.2004.06.018

Sun, Y., Ren, Z., Wu, Y., Lei, F., Dudley, R. and Li, D. (2016). Flying high: limits to flight performance by sparrows on the Qinghai-Tibet plateau. *J. Exp. Biol.* **219**, 3642-3648. doi:10.1242/jeb.142216

Thompson, L. J., Brown, M. and Downs, C. T. (2015). The effects of long-term captivity on the metabolic parameters of a small Afrotropical bird. *J. Comp. Physiol. B* **185**, 343-354. doi:10.1007/s00360-015-0888-6

Tobalske, B. W., Warrick, D. R., Clark, C. J., Powers, D. R., Hedrick, T. L., Hyder, G. A. and Biewener, A. A. (2007). Three-dimensional kinematics of hummingbird flight. *J. Exp. Biol.* **210**, 2368-2382. doi:10.1242/jeb.005686

Usherwood, J. R. (2016). Physiological, aerodynamic and geometric constraints of flapping account for bird gaits, and bounding and flap-gliding flight strategies. *J. Theor. Biol.* **408**, 42-52. doi:10.1016/j.jtbi.2016.07.003

Van den Hout, P. J., Mathot, K. J., Maas, L. R. and Piersma, T. (2010). Predator escape tactics in birds: linking ecology and aerodynamics. *Behav. Ecol.* **21**, 16-25. doi:10.1093/beheco/arp146

Veasey, J. S., Houston, D. C. and Metcalfe, N. B. (2000). Flight muscle atrophy and predation risk in breeding birds. *Funct. Ecol.* **14**, 115-121. doi:10.1046/j.1365-2435.2000.00391.x

Walters, B. T., Cheng, T. N. N., Doyle, J., Guglielmo, C. G., Clinchy, M. and Zanette, L. Y. (2017). Too important to tamper with: predation risk affects body mass and escape behaviour but not escape ability. *Funct. Ecol.* **31**, 1405-1417. doi:10.1111/1365-2435.12851

Wang, Y., Yin, Y., Ge, S., Li, M., Zhang, Q., Li, J., Wu, Y., Li, D. and Dudley, R. (2019). Limits to load-lifting performance in a passerine bird: the effects of intraspecific variation in morphological and kinematic parameters. *PeerJ* **7**, e8048. doi:10.7717/peerj.8048

Wang, Y., Yin, Y., Ren, Z., Jiang, C., Sun, Y., Li, J., Nabi, G., Wu, Y. and Li, D. (2020). A comparison of flight energetics and kinematics of migratory Brambling and residential Eurasian Tree Sparrow. *Avian Res.* **11**, 25. doi:10.1186/s40657-020-00211-y

Whelan, S., Brown, K., Patterson, A., Jimenez, A. G., Hatch, S. A. and Elliott, K. H. (2020). Accelerometry predicts muscle ultrastructure and flight capabilities in a wild bird. *J. Exp. Biol.* **223**, jeb234104. doi:10.1242/jeb.234104

Yap, K. N., Dick, M. F., Guglielmo, C. G. and Williams, T. D. (2018). Effects of experimental manipulation of hematocrit on avian flight performance in high-and low-altitude conditions. *J. Exp. Biol.* **221**, jeb191056. doi:10.1242/jeb.191056

Yu, Y. and Tong, B. (2005). A flow control mechanism in wing flapping with stroke asymmetry during insect forward flight. *Acta Mech. Sin.* **21**, 218-227. doi:10.1007/s10409-005-0032-z