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Moult-related reduction of aerobic scope in passerine birds

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Abstract

It is well established that the nutrient and energy requirements of birds increase substantially during moult, but it is not known if these increased demands affect their aerobic capacity. We quantified the absolute aerobic scope of house and Spanish sparrows, *Passer domesticus* and *P. hispaniolensis*, respectively, before and during sequential stages of their moult period. The absolute aerobic scope (AAS) is the difference between maximum metabolic rate (MMR) during peak locomotor activities and minimum resting metabolic rate (RMR min), thus representing the amount of aerobic power above that committed to maintenance needs available for other activities. As expected, RMR min increased over the moult period by up to 40 and 63% in house and Spanish sparrows, respectively. Surprisingly, the maximum metabolic rates also decreased during moult in both species, declining as much as 25 and 38% compared with pre-moult values of house and Spanish sparrows, respectively. The concurrent changes in RMR min and MMR during moult resulted in significant decreases in AAS, being up to 32 and 47% lower than pre-moult levels of house and Spanish sparrows, respectively, during moult stages having substantial feather replacement. We argue that the combination of reduced flight efficiency due to loss of wing feathers and reduced aerobic capacity places moulting birds at greater risk of predation. Such performance constraints likely contribute to most birds temporally separating moult from annual events requiring peak physiological capacity such as breeding and migration.

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Moult-related Reduction of Aerobic Scope in Passerine Birds

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Keywords: avian moult energetics, aerobic scope, resting metabolic rate, maximum metabolic rate, *Passer domesticus*, *Passer hispaniolensis*

Summary: We provide the first evidence that birds experience significant reductions in aerobic capacity while moulting. This outcome exacerbates performance limitations due to known decreased flight efficiency during feather replacement.

Running Title: Aerobic Scope and Avian Moul

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Abstract

It is well established that the nutrient and energy requirements of birds increase substantially during moult, but it is not known if these increased demands affect their aerobic capacity. We quantified the absolute aerobic scope of house and Spanish sparrows, *Passer domesticus* and *P. hispaniolensis*, respectively, before and during sequential stages of their moult period. The absolute aerobic scope (AAS) is the difference between maximum metabolic rate (MMR) during peak locomotor activities and minimum resting metabolic rate (RMR_{min}), thus representing the amount of aerobic power above that committed to maintenance needs available for other activities. As expected, RMR_{min} increased over the moult period by up to 40 and 63% in house and Spanish sparrows, respectively. Surprisingly, the maximum metabolic rates also decreased during moult in both species, declining as much as 25 and 38% compared with pre-moult values of house and Spanish sparrows, respectively. The concurrent changes in RMR_{min} and MMR during moult resulted in significant decreases in AAS, being up to 32 and 47% lower than pre-moult levels of house and Spanish sparrows, respectively, during moult stages having substantial feather replacement. We argue that the combination of reduced flight efficiency due to loss of wing feathers and reduced aerobic capacity places moulting birds at greater risk of predation. Such performance constraints likely contribute to most birds temporally separating moult from annual events requiring peak physiological capacity such as breeding and migration.

INTRODUCTION

Reproduction and feather replacement are two of the most energy- and nutrient-demanding periods in the annual cycle of birds (Murphy 1996) and most seasonally breeding birds have hormonal mechanisms that temporally separate breeding and moult periods (Schleussner et al. 1985; Dawson and Sharp 1998; Dawson 2006). Such segregation better assures provision of critically needed nutrients during each of these life stages, which has important fitness implications. In the case of moult, inadequate energy and nutrient supply or elevated physiological stress during moult could result in compromised quality of feathers (Murphy et al. 1988; DesRochers et al. 2009). This has negative consequences for a diverse range of functions that feathers serve, including aerodynamic, insulation, sexual signalling, and protection from environmental exposure. Because structural flaws in feathers will persist until they are replaced in the next moult period (usually a year later), birds will be functionally penalised for a full year, which reduces their likelihood of surviving until the next breeding opportunity (e.g., Nilsson and Svensson 1996) and, if they do survive, potentially having less success in attracting a mate due to poorer sexual signalling (Fitzpatrick 1998; Bortolotti et al. 2006).

The nutrient requirements of moult are substantial, as feathers contain more than 90% protein, mainly β -keratins (McKittrick et al. 2012), comprise about 7% of a bird's body mass (Turček 1965), and about 25% of its total protein content (Murphy 1996). In addition to the increased need for protein, the process of moult provokes significant elevations in the maintenance energy requirements of birds in inverse relation to their body mass (Hoye and Buttemer 2011). The minimum energy needs of endothermic animals can be evaluated by measuring rates of oxygen consumption when the animal is post-absorptive, asleep during the

rest-phase of its daily cycle, and exposed to thermoneutral temperatures. These rates represent an animal's minimum maintenance costs and are termed basal metabolic rate (BMR) when animals are non-growing and non-reproductive (Hulbert and Else 2004), but are called minimum resting metabolic rate (RMR_{min}) at other life-history stages. Moulting is associated with significant increases in RMR_{min} and differences between these rates and those measured as BMR prior to moulting are typically used to determine the cost of feather synthesis (Dietz et al. 1992; Lindström et al. 1993; Klaassen 1995; Høye and Buttemer 2011).

Another aspect of increase in RMR_{min} that has not been addressed is its potential to affect the absolute aerobic scope (AAS) of moulting birds. Absolute aerobic scope is the difference between maximum metabolic rate (MMR) during peak locomotor activity and resting metabolic rate, thus representing the amount of aerobic power above that committed to maintenance needs available for other activities (Brett 1972). This expression of aerobic scope differs from factorial aerobic scope (FAS), which is a unitless ratio of MMR to RMR_{min} . Although FAS is typically used for comparison of aerobic expansibility between species (e.g., Hinds et al. 1993), it has far less functional relevance for quantifying the aerobic performance consequences of intraspecific variation of RMR_{min} and MMR. For example, a doubling of RMR_{min} with no change in MMR has the same FAS as a halving of MMR with an invariant RMR_{min} . By contrast, the AAS associated with these conditions would differ and reveal the actual amount of aerobic power in excess of resting requirements that is available for activity.

Decreases in AAS will directly result from rises in RMR_{min} unless MMR is proportionately increased. This could result in greater reliance on anaerobic metabolism during activities requiring maximal power and, consequently, a higher likelihood of fatigue and slower rate of recovery (Marras et al. 2010; Killen et al. 2014). Although raising MMR during moulting will

overcome decreases in AAS, there are potential constraints for birds achieving this. Much of the rise in RMR_{min} of birds is attributed to greatly increased rates of protein turnover that facilitate uninterrupted daily feather growth (Murphy 1996). Measurements of protein turnover in white-crowned sparrows (*Zonotrichia leucophrys gambelii*) reveal 72% higher rates of daily protein turnover in moulting versus non-moulting birds, with the majority of the protein sourced from skeletal muscles (Taruscio and Murphy 1995). Because MMR of endotherms is highest during locomotion (Hinds et al. 1993; Bishop 1999), it requires maximal functioning of a suite of biochemical, physiological and morphological processes to meet the peak aerobic demands of locomotor muscles. Thus, significant rises of protein turnover rates during moult that include myofibrillar proteins of locomotor muscles and which might also affect mitochondrial function may limit attainment of MMR at this time. We address questions of how RMR_{min} , MMR, and AAS covary during a moult cycle of two sympatric congeneric species, the house sparrow *Passer domesticus* and the Spanish sparrow *P. hispaniolensis*. In sparrows, primary feathers are moulted and replaced in sequence, starting at primary 1 and finishing with primary 10, with the primary replacement period largely coincident with replacement of all feathers during prebasic moult (Zeidler 1966). The sparrow species in our study have the same moult cycle, exhibit similar morphometry (Alonso 1984), and breed sympatrically in southeastern Europe. They differ substantially, however, in one aspect of their life history: Spanish sparrows breeding in this region undertake obligatory migration after completing moult, whereas house sparrows are sedentary (Hahn et al. 2018). As the physical qualities of feathers of migrating species are superior to those of sedentary birds (Weber et al. 2004; de la Hera et al. 2010), this raises the possibility of different protein requirements for feather replacement and other post-breeding morphological adjustments

between these two species and, thus, potential differences in extent of variation in RMR_{min} and MMR during moult.

MATERIAL and METHODS

Experimental Animals

All house and Spanish sparrows used for this study were captured using mistnets near the Biological Experimental Station Kalimok and the adjacent village of Nova Cherna (44.00°N 26.46°E, lower Danube basin in northern Bulgaria). These local birds were captured between early July and late August in three distinct life-stage periods: Period 1 from 1-18 July representing late-breeding, pre-moult; Period 2 from 25 July-10 August representing early moult; and Period 3 from 17-30 August representing mid- through late-moult stages (Fig. 1). We tried to confine our collections to adult male birds to minimise potential confounding effects due to sex or age (100% males in *P. hispaniolensis*; 72 adults and 5 fully grown first-year birds; 86% males in *P. domesticus*, 42 adults and 6 fully grown first-year birds). We measured wing length and scored body fat and flight muscle using standardised procedures (*sensu* Eck et al 2011), with fat scored on a scale from 0 to 8 and muscle from 0 to 3. Additionally, we determined the stage of primary (flight) feather moult using a simple eleven-level score (primary moult score; MS_p), where zero indicates no primary moult/all feathers are old, and score 10 indicates that the 10th primary is renewed. Following measurements, birds were fitted with uniquely identifiable leg rings and then transferred to an outdoor aviary (2.8 x 2.6 x 1.8m) that had vegetation providing multiple perches and free access to commercial finch seed mix and water. Due to the logistical constraints of running several experiments concurrently, birds differed in the duration of their post-capture

maintenance in the aviary. Of the 124 sparrows used for this experiment, 81 had metabolic measurements made on the day of capture, 21 one day post capture (DPC), nine two DPC, three after three DPC, and nine after four DPC.

Experimental Protocols

Prior to measuring resting metabolic rate (RMR_{min} , see below), birds were transferred to small holding cages permitting free access to water, but without seed. They remained in these cages for 3 hours before they were weighed and placed in respirometers at ca. 21:00 h to ensure they were post-absorptive during the period of RMR_{min} determination. Upon removal from the respirometers at ca. 06:00, birds were reweighed and placed in holding cages and given free access to seed and water for a minimum of 4 h and a maximum of 6 h before evaluating their maximum metabolic rates (MMR; see below). At the end of the MMR measurement, we reweighed the birds, measured their blood haemoglobin content using a HemoCue Hb201 following puncture of a brachial vein with a 26-gauge needle, confirmed their moult status, and then released them about 10-min later.

Metabolic Rate Measurements

We measured RMR_{min} at chamber air temperatures regulated at 30°C, which is known to be thermoneutral for house sparrows (Hudson and Kimzey 1966). Birds were placed in 4-litre respirometer chambers, each fitted with a perch and provided compressed atmospheric air dried through a silica gel column and maintained at 500 ml min⁻¹ by mass flow controllers (Tylan Model FC-280S). All flow controllers were periodically calibrated using a Gilian Gilibrator (Sensidyne, St. Petersburg, Fla., USA). Oxygen consumption rates ($\dot{V}O_2$) were evaluated by comparing oxygen content of inlet and outlet air for each chamber after removal

of water vapour and CO₂ by passing air through Drierite and soda lime, respectively, before being directed to 2 Oxzilla oxygen analysers (Sable Systems) by an electronic stream selector (Sable Systems Respirometer Multiplexer V 2.0). Voltage outputs from the oxygen analysers were recorded at 5-s intervals and each bird was sampled continuously for 27 min h⁻¹. Inlet air was sampled between each 27-min period of bird metabolic measurement to establish a reference baseline and assigned a value of 20.95% oxygen as found in dry CO₂-free air. All reported values of oxygen consumption rates are based on readings taken at least 6 h after feeding and have been calculated using Eqn. 2 of Hill (1972):

$$\dot{V}O_2 = \text{STP flow} * ((\text{FiO}_2 - \text{FeO}_2) / (1 - \text{FeO}_2)), \text{ where STP flow} = 0.9996 \text{ (mass flow values)}$$

to account for 0.04% atmospheric CO₂, FiO₂ = fractional oxygen content of inlet chamber air, and FeO₂ = fractional oxygen content of effluent chamber air. Resting oxygen consumption rate for each bird represents the lowest 5-min average of $\dot{V}O_2$ recorded from sampling intervals displaying stable readings for the majority of the 27-min measurement period.

Maximum metabolic rates (MMR) were determined from $\dot{V}O_2$ during exercise in a hop-flutter wheel (Chappell et al. 1999; McKechnie and Swanson 2010), where birds were encouraged to repeatedly take off. The effective volume of the MMR system was 9.25 l, with compressed atmospheric air dried through a silica gel column supplied at a rate of 5000 ml min⁻¹ using a calibrated mass-flow controller (MKS Instruments). The rotation speed of the wheel was manually adjusted to each bird's behaviour and fully stopped when the bird could not hold its position in the wheel. Oxygen consumption during exercise was continuously recorded with an Oxzilla II differential oxygen analyser or an FC1 oxygen analyzer (both

212 Sable Systems, NV, U.S.A.), using inlet air as a reference at the start and end of each
213 measurement. Sampled air was drawn through a tube containing Drierite and soda lime to
214 remove water vapour and CO₂ respectively, before reaching the oxygen analyser. The
215 maximum $\dot{V}O_2$ was computed from the highest instantaneous oxygen consumption values
216 measured over a 30s interval of exercise, after baseline correction and smoothing the data to
217 remove electrical noise (1s smoothing interval over 3 cycles). All data were processed using
218 LabAnalystX (<http://warthog.ucr.edu>) software to obtain instantaneous oxygen consumption
219 rates. Absolute aerobic scope (AAS) for each bird was computed as the difference between
220 MMR and its RMR_{min}.

221 **Data Selection**

222 Our requirement that RMR_{min} be limited to 5-min averages measured within periods of stable
223 $\dot{V}O_2$ recordings lasting more than half of the 27-min measurement period revealed that 12
224 Spanish sparrows and 3 house sparrows were less restful than their counterparts.
225 Consequently, data from these birds were eliminated from our RMR_{min} and associated AAS
226 determinations. As all other measurements made on these birds were valid, data from them
227 are included in MMR results and in all of the morphological analyses. One Spanish sparrow
228 was unreactive during exercise measurements and was therefore not included in the MMR
229 analyses.

230

231 **Statistical analyses**

232 We ran linear models using the lm package of R (version 3.4.3; 2017) to relate MSp to blood
233 haemoglobin, body mass, fat and muscle scores. The lm procedures evaluate the statistical
234 contribution each predictor covariate makes in the linear model by determining estimates and

standard errors for each covariate and the resultant t values. Because metabolic rate is known to scale as a power function of body mass (Hulbert and Else 2004), RMR_{min} , and MMR and body masses accompanying these measurements were log-transformed. In addition to body mass, linear models examining the relation of MS_p to RMR_{min} , MMR, and AAS included the binary factor DPC to distinguish birds measured on the day of capture from those measured 1 or more days later. Linear models for MMR had an additional covariate to account for differences in body mass gained between the end of RMR_{min} measurements and the start of MMR determinations. Preliminary models found sex did not affect RMR or MMR of house sparrows ($t_{41}=0.513$, $p=0.611$ and $t_{42}=0.609$, $p=0.546$, respectively) and that age had no effect on RMR ($t_{40}=-0.482$, $p=0.633$; $t_{60}=0.085$, $p=0.933$), MMR ($t_{43}=-0.338$, $p=0.700$; $t_{71}=-0.409$, $p=0.684$), or AAS ($t_{40}=-0.188$, $p=0.852$; $t_{59}=0.199$, $p=0.843$) of house or Spanish sparrows, respectively.

RESULTS

The primary moult score (MS_p) varied substantially between periods of bird collection, with both species showing no moult during period one, but substantial moult over the next two periods. Spanish sparrows had higher MS_p than house sparrows during periods 2 and 3, which indicates they had an earlier onset of moult and, thus, were at different phases of their overall moult cycle (Fig. 1A).

Fasted body mass (fM_b) measured after overnight RMR_{min} determinations varied significantly in relation to MS_p in both house sparrows ($t_{46}=2.878$, $p=0.006$) and Spanish sparrows ($t_{75}=3.682$, $p=4.34 \times 10^{-4}$), with fM_b of both species increasing above pre-moult levels at later moult stages (Fig. 2A).

257 Fat scores of house sparrows were invariant throughout moult ($t_{46}=0.947$, $p=0.349$) but
 258 increased significantly for Spanish sparrows in relation to MS_p ($t_{75}=3.912$, $p=2.0 \times 10^{-4}$),
 259 particularly at late-moult stages.

260 Muscle scores did not vary with MS_p for either species ($t_{46}=1.378$, $p=0.175$ and $t_{75}=1.126$,
 261 $p=0.264$ for house and Spanish sparrows, respectively).

262 Blood haemoglobin content was unaffected by moult status of house sparrows ($t_{46}=-0.171$,
 263 $p=0.865$) but showed a significant relation with primary moult score (MS_p) in Spanish
 264 sparrows ($t_{75}=3.15$, $p=0.003$), falling below pre-moult levels after the start of moult, but
 265 rising above pre-moult levels from MS_p 6 onwards (Fig. 2B).

266 A shared pattern of variation in RMR_{min} and MMR in relation to MS_p is clearly evident in
 267 Fig. 3. For both species, RMR_{min} rises moderately after primary 2 is being replaced, and then
 268 is substantially elevated from primary 6 onwards (Fig. 3A). Linear modeling revealed that
 269 RMR_{min} of house sparrows was significantly affected by MS_p ($t_{41}=5.549$, $p=1.89 \times 10^{-6}$) but
 270 not by body mass ($t_{41}=1.835$, $p=0.074$) or number of days post capture (DPC, $t_{41}=-1.341$,
 271 $p=0.187$). For Spanish sparrows, RMR_{min} was significantly affected by MS_p ($t_{61}=6.16$,
 272 $p=6.28 \times 10^{-8}$), body mass ($t_{61}=4.864$, $p=8.46 \times 10^{-6}$), as well as DPC ($t_{61}=-2.028$, $p=0.047$).

273 Comparison of premoult and moult RMR_{min} values of birds measured the day of capture,
 274 house sparrows had a 40% increase at MS_p 6 and Spanish sparrows showed a 63% increase at
 275 MS_p 7.

276 MMR declined from pre-moult levels between replacement of primaries 4 and 7 by house
 277 sparrows and between 4 and 8 in Spanish sparrows (Fig. 3A). MMR of house sparrows was
 278 significantly affected by body mass ($t_{43}=2.298$, $p=0.026$) and DPC ($t_{43}=3.302$, $p=0.002$),
 279 marginally by MS_p ($t_{43}=-2.004$, $p=0.051$), but not by differences in amount of body mass
 280 gained between the end of RMR_{min} and start of MMR measurements ($t_{43}=-1.272$, $p=0.210$).

For Spanish sparrows, MMR was strongly affected by both MS_p ($t_{71}=-3.889$, $p=2.24 \times 10^{-4}$) and body mass ($(t_{71}=3.015$, $p=0.004)$ but not DPC ($(t_{71}=1.888$, $p=0.063$, or body mass changes between RMR_{min} and MMR determinations ($(t_{71}=-0.163$, $p=0.871)$. The coincidence of falling MMR with rises in RMR_{min} during moult progression resulted in AAS being lower than pre-moult values from replacement of primary 4 onwards in both species (Fig. 3B), with MS_p significantly affecting AAS of both house and Spanish sparrows ($t_{41}=-2.311$, $p=0.026$ and $t_{60}=-4.667$, $p=1.76 \times 10^{-5}$), respectively. DPC strongly influenced AAS in house ($(t_{41}=3.429$, $p=0.001)$ and Spanish sparrows ($(t_{60}=2.736$, $p=0.008)$, whereas body mass had a significant effect on AAS of Spanish sparrows ($t_{60}=2.325$, $p=0.023$), but only a marginal effect on house sparrow AAS ($t_{41}=2.011$, $p=0.051$). Both MMR and AAS reached their nadirs during replacement of primary 8 in Spanish sparrows and primaries 7 and 8 in house sparrows (combined due to low sample sizes). Comparison of individual measurements at these late stages of moult with those of their pre-moulting counterparts sharing the same DPC reveal MMR and AAS to respectively average 38 and 47% lower than pre-moult levels in Spanish sparrows, and 25 and 32% lower in house sparrows (Figs. 3A and B).

DISCUSSION

Avian moult has long been associated with significant rises in protein and energy requirements (Murphy 1996), however this is the first study to demonstrate that moulting birds also experience substantially reduced aerobic scope due to coincident increases in RMR_{min} and declines in MMR. Our conclusion rests on the assumption that MMR elicited by our exercise method is representative on an individual's aerobic capacity. Peak levels of

exercise performance can be influenced as much by a subject's motivation as by their physiological capability, which makes identification of underlying causes of performance variation problematic. Our use of freshly captured free-living birds alleviates these uncertainties as most birds were very reactive to our presence. Although the hop-flutter wheel had transparent sides, birds settled quickly when it was covered with a dark cloth. Following this adjustment period, the cover was removed and most birds responded immediately with maximal escape flights interspersed with hopping until becoming fatigued. The MMR we measured for house sparrows is higher than that reported for birds residing in SE Australia (Chappell et al. 1999; Buttemer et al. 2008) but in agreement with measurements of sparrows from South Dakota (Zhang et al. 2015). These MMR differences partly reflect the larger body sizes associated with sparrows from highly seasonal habitats (Murphy 1985). Nevertheless, a question remains regarding the extent to which such MMR measurements (sensu McKechnie and Swanson 2010) reflect the aerobic capacity of birds.

Aerobic scope of endothermic animals is traditionally based on the difference between $\dot{V}O_{2\max}$ and $RMR_{\min}$ (or BMR), where $\dot{V}O_{2\max}$ is the highest rate of oxygen consumption that an animal can achieve during progressively increased locomotory effort (Taylor 1980). These measurements are typically made by varying treadmill speed and inclination for running animals, but measuring upper limits of flight costs are mechanically constrained by difficulties in attaining the laminar-flow wind speeds that are required. Studies successful in provoking $\dot{V}O_{2\max}$ in birds include metabolic rate measurements of ruby-throated hummingbirds (*Archilochus colubris*) while hovering in a hypodense atmosphere (Chai & Dudley 1999) and budgerigars flying at a 5° angle of ascent at 42 km h⁻¹ (Tucker 1968).

327 Coupling their results with BMR measurements for the same species (Lasiewski 1963,
 328 Buttemer et al. 1986) reveals FAS values of 15.2 for the hummingbirds and 19.8 for the
 329 budgerigars. In our study, FAS was 13.5 and 11.6 in pre-moulting house and Spanish
 330 sparrows, respectively. Although the MMR levels reached by birds in the hop-flutter wheel
 331 are likely lower than $\dot{V}O_2$ max values that they can attain under free-living conditions, we are
 332 confident that our MMR measurements are indicative of individual aerobic capability for the
 333 following reasons: firstly, individual MMR measurements have been shown to be highly
 334 repeatable over time, implying that individuals tested with the hop-flutter method reach
 335 consistent aerobic limits (Chappell et al. 2010; Careau et al. 2015); secondly, the MMR of
 336 free-living sparrows transferred to small cages restricting locomotor activities progressively
 337 declined as found for $\dot{V}O_2$ max of humans after reducing aerobic exercise training (Neufer
 338 1989); thirdly, the FAS of sparrows in our study were comparable to the $\dot{V}O_2$ /BMR ratios of
 339 14.4 measured for budgerigars flying level at 42 km h⁻¹ (Tucker 1968; Buttemer et al. 1986)
 340 and 11.4 for starlings (*Sturnus vulgaris*) flying level at 50 km h⁻¹ (Torre-Bueno and
 341 Larochelle 1978; Geluso and Hayes 1999); and lastly, all sparrows tested were physically
 342 exhausted at the conclusion of their exercise bout.
 343 The extent of RMR_{min} increase that we measured in moulting sparrows was fully expected
 344 and is consistent with the 54% increase in RMR_{min} of similarly sized white-crowned sparrows
 345 during peak moult (Schieltz and Murphy 1997). By contrast, no study has explicitly
 346 examined moult effects on avian MMR and AAS and we were surprised by the magnitude of
 347 their decline. Spanish sparrows had greater moult-related declines in MMR and AAS than
 348 house sparrows, but our limited sample size for house sparrows in advanced stages of moult

precludes firm conclusions or assignment of functional significance to the interspecific differences we found. Nevertheless, both species had greatest decreases in AAS and MMR at moult stages associated with substantial feather replacement, which suggests common underlying causes. Identifying the factors functionally responsible for these variations will require additional investigation, but it is worthwhile considering possible mechanisms. The onset of moult in birds is associated with significant increases in thyroxine (T_4) secretion (McNabb 2007) and plasma T_4 levels of up to 54% above pre-moult values have been reported for house sparrows (Smith 1982). Combining evidence that plasma T_4 levels vary with moult intensity in red knots (*Calidris canutus canutus*; Jenni-Eiermann et al. 2002; Vezina et al. 2009) with research finding that T_4 and its deiodinated derivative T_3 directly affect rates of protein synthesis and degradation in avian muscles (Hayashi et al. 1991; Hayashi et al. 2009), locomotor muscle protein turnover rates would be expected to vary directly with moult intensity. Concurrent rises in T_3 would promote increased RMR through its actions on multiple cellular processes that are identified as major components of maintenance energy costs (Rolfe and Brown 1997). Measurements of 3-methylhistidine (3-MH) daily excretion rates, a biomarker of myofibrillar protein breakdown, were 72% and 63% higher during peak moult in white-crowned sparrows and house sparrows, respectively, than in non-moulting birds (Taruscio and Murphy 1995; Buttemer, Addison, and Klasing, unpubl. data). Our study found that declines in MMR were most pronounced between replacement of primary feathers 4 through 8 (Fig. 3), which corresponds with the period of greatest extent of overall feather replacement for both sparrow species (Zeidler 1966; Alonso 1984). While the correspondence between heightened muscle protein turnover rates and decreased MMR remains correlative, it does warrant examination of muscle protein turnover

rates in relation to locomotor muscle composition and mitochondrial characteristics pertinent to aerobic capabilities throughout moult.

Another way by which moult could affect AAS is through associated decreases in activity. Some bird species undergoing extensive moult confine their daily activities to foraging early and late in the day and seek concealment at most other times (e.g., Vega Rivera et al. 1998, Heise and Rimmer 2000). A field study of bullfinches (*Pyrrhula pyrrhula*) found that capture rates were substantially lower for moulting adults with primary moult scores between 4 and 8, signifying a dramatic decrease in daily activity ((Newton 1966). Newton (1966) also noted that captive non-moulting bullfinches were actively flying and singing in aviaries throughout the day, whereas moulting birds were active only while feeding, spending most of the day resting. Although we did not characterise activity patterns of either sparrow species at our study site, aerobic capacity is expected to decrease with reduction in the frequency and extent of locomotor activity. This is evidenced by the aerobic scope of wild-caught house sparrows declining with time being held in small cages, whereas the FAS of sparrows maintained in flight aviaries did not differ from free-living counterparts (Buttemer et al. 2008). Furthermore, the leg and pectoral muscle masses of control sparrows in that experiment were unaffected by cage size, confirming that substantial changes in AAS can occur in the absence of changes in locomotor muscle size. The extent to which changes in activity affect AAS during moult could be examined by fitting free-living or flight-cage housed birds prior to moult with 3-axis accelerometers and comparing the relations between frequency and extent of activity level with RMR_{min} and MMR determinations over the course of moult. Irrespective of the mechanisms responsible for decreased AAS in moulting sparrows, this outcome exacerbates performance limitations associated with reduced flight efficiency during flight feather replacement. During moult, loss of remiges reduces wing surface area, resulting

396 in less effective transfer of metabolic power input to mechanical power output (Chai and
397 Dudley 1999). This imposes greater aerobic demand for the same level of flight performance
398 at a time when the aerobic scope for such activity is reduced, thus placing moulting birds at
399 greater risk of predation. Our findings invite further exploration of moult-related effects on
400 AAS and how these vary in relation to moult rate and body size. Such studies will provide
401 further insight into the functional significance of temporal separation of moult from annual
402 events requiring peak physiological capacity such as breeding and migration (Wingfield
403 1998).
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Author contributions: S.H., S.B. and W.A.B. developed the conceptual framework and analysed the data. S.H., T.E. and W.A.B. made metabolic measurements. S.H., S.B. and W.A.B. wrote the manuscript. D.D., S.P., S.H., T.E. and W.A.B. captured birds and made morphological measurements. All authors discussed and approved the manuscript and have no competing interests.

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Figure Legends

Figure 1. Upper panel: Distribution of primary feather moult scores of *Passer hispaniolensis* and *P. domesticus* in relation to our three periods of sampling. The proportion of birds having a particular moult score is represented by the relative size of each sphere, with the total area of spheres in each period totaling 100% for each species. **Lower panel:** Number of birds sampled for each period in relation to date of sampling.

Figure 2. Upper panel: Body mass of *Passer hispaniolensis* and *P. domesticus* at conclusion of overnight RMR_{min} determinations. **Lower panel:** Blood haemoglobin content (mg/l) in relation to primary feather moult score. Values are depicted as medians with vertical bars representing 25th and 75th percentiles.

Figure 3. Upper panel: Minimum resting metabolic rate (RMR_{min}) and maximum metabolic rate (MMR) of *Passer domesticus* and *Passer hispaniolensis* in relation to primary feather moult score. **Lower panel:** Absolute aerobic scope (ml O₂ min⁻¹) of *Passer domesticus* and *Passer hispaniolensis* in relation to primary feather moult score. Values are depicted as medians with vertical bars representing 25th and 75th percentiles.

Figure 1

Figure 2

Figure 3