

# Moult-related Reduction of Aerobic Scope in Passerine Birds

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**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 during exercise 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  $\beta$ -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. Moults are associated with significant increases in  $RMR_{min}$  and differences between these rates and those measured as BMR prior to moult 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 moult 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 possibly affect mitochondrial components 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 visually 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 10<sup>th</sup> 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

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

## 170 **Experimental Protocols**

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

## 182 **Metabolic Rate Measurements**

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

of water vapour and CO<sub>2</sub> 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 for 27 min h<sup>-1</sup>. 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 CO<sub>2</sub>-free air. All reported values of oxygen consumption rates are based on readings taken at least 8 h after feeding and have been calculated using Eqn. 2 of Hill (1972):

$$\dot{V}O_2 = \text{STP flow} * ((FiO_2 - FeO_2)/(1 - FeO_2)), \text{ where STP flow} = 0.9996 \text{ (mass flow values)}$$

to account for 0.04% atmospheric CO<sub>2</sub>, FiO<sub>2</sub> = fractional oxygen content of inlet chamber air, and FeO<sub>2</sub> = 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<sup>-1</sup> 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 CO<sub>2</sub> 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<sub>min</sub>.

#### 221 **Data Selection**

222 Our requirement that RMR<sub>min</sub> 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<sub>min</sub> 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 the hop-flutter period and therefore was 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. Because metabolic rate is known to scale as  
234 a power function of body mass (Hulbert and Else 2004), RMR<sub>min</sub>, and MMR and body

masses accompanying these measurements were log-transformed. In addition to body mass, linear models examining the relation of  $R_{\min}$  and AAS to  $MSp$  included covariates to account for time in captivity (days post capture, DPC). 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. The lm procedures evaluate the statistical contribution each predictor covariate makes in the linear model by determining estimates and standard errors for each covariate and the resultant t values.

## RESULTS

The primary moult score ( $MSp$ ) 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  $MSp$  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 with  $MSp$  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).

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

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

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

262 A shared pattern of variation in  $RMR_{min}$  and MMR in relation to  $MS_p$  is clearly evident in  
 263 Fig. 3. For both species,  $RMR_{min}$  rises moderately after primary 2 is being replaced, and then  
 264 is substantially elevated from primary 6 onwards (Fig. 3A). Linear modeling revealed that  
 265  $RMR_{min}$  of house sparrows was significantly affected by  $MS_p$  ( $t_{41}=5.549$ ,  $p=1.89 \times 10^{-6}$ ) but  
 266 not by body mass ( $t_{41}=1.835$ ,  $p=0.073$ ), number of days post capture (DPC,  $t_{41}=-1.341$ ,  
 267  $p=0.187$ ), or sex ( $t_{41}=0.513$ ,  $p=0.611$ ). For Spanish sparrows,  $RMR_{min}$  was significantly  
 268 affected by  $MS_p$  ( $t_{61}=6.16$ ,  $p=6.28 \times 10^{-8}$ ), body mass ( $t_{61}=4.864$ ,  $p=8.46 \times 10^{-6}$ ), as well as DPC  
 269 ( $t_{61}=-2.028$ ,  $p=0.047$ ). Comparison of premoult and moult  $RMR_{min}$  values of birds measured  
 270 the day of capture, house sparrows had a 40% increase at  $MS_p$  6 and Spanish sparrows  
 271 showed a 63% increase at  $MS_p$  7.

272 MMR declined from pre-moult levels between replacement of primaries 4 and 7 by house  
 273 sparrows and between 4 and 8 in Spanish sparrows (Fig. 3A). MMR of house sparrows was  
 274 significantly affected by body mass ( $t_{43}=2.298$ ,  $p=0.026$ ) and DPC ( $t_{43}=3.302$ ,  $p=0.002$ ),  
 275 marginally by  $MS_p$  ( $t_{43}=-2.004$ ,  $p=0.051$ ), but not by differences in amount of body mass  
 276 gained between the end of  $RMR_{min}$  and start of MMR measurements ( $t_{43}=-1.272$ ,  $p=0.210$ ).  
 277 For Spanish sparrows, MMR was strongly affected by both  $MS_p$  ( $t_{71}=-3.889$ ,  $p=2.24 \times 10^{-4}$ )  
 278 and body mass ( $t_{71}=3.015$ ,  $p=0.004$ ) but not DPC ( $t_{71}=1.888$ ,  $p=0.063$ , or body mass  
 279 changes between  $RMR_{min}$  and MMR determinations ( $t_{71}=-0.163$ ,  $p=0.871$ ).

280 The coincidence of falling MMR with rises in  $RMR_{min}$  during moult progression resulted in  
 281 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. Because peak levels of exercise performance can be influenced as much by a subject's motivation as by their physiological capability, attempting to identify underlying causes of performance variation can be 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. Surprisingly, MMR in these birds did not differ from that of birds initiating their period of MMR 30 sec or more after the start of the measurements. Thus our protocol appeared to elicit similar periods of escape effort among birds, but with varying times of onset. 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 attaining the laminar-flow wind speeds required to elicit peak flight costs. 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<sup>-1</sup> (Tucker 1968). Coupling their results with BMR measurements for the same species (Lasiewski 1963, Buttemer et al. 1986) reveals FAS values of 15.2 for the hummingbirds and 19.8 for the

328 budgerigars. In our study, FAS was 13.5 and 11.6 in pre-moulting house and Spanish  
 329 sparrows, respectively. Although the MMR levels reached by birds in the hop-flutter wheel  
 330 are likely lower than  $\dot{V}O_2$ max values that they can attain under free-living conditions, we  
 331 believe our MMR measurements are indicative of individual aerobic capability for the  
 332 following reasons: firstly, individual MMR measurements have been shown to be highly  
 333 repeatable over time, implying that individuals tested with the hop-flutter method reach  
 334 consistent aerobic limits (Chappell et al. 2010; Careau et al. 2015); secondly, the MMR of  
 335 free-living sparrows transferred to small cages restricting locomotor activities progressively  
 336 declined as found for  $\dot{V}O_2$ max of humans after reducing aerobic exercise training (Neufer  
 337 1989); thirdly, the FAS of sparrows in our study were comparable to the  $\dot{V}O_2$ /BMR ratios of  
 338 14.4 measured for budgerigars flying level at 42 km h<sup>-1</sup> (Tucker 1968; Buttemer et al. 1986)  
 339 and 11.4 for starlings (*Sturnus vulgaris*) flying level at 50 km h<sup>-1</sup> (Torre-Bueno and  
 340 Larochelle 1978; Geluso and Hayes 1999); and lastly, all sparrows tested were physically  
 341 exhausted at the conclusion of their exercise bout.

342 The extent of RMR<sub>min</sub> increase that we measured in moulting sparrows was fully expected  
 343 and is consistent with the 54% increase in RMR<sub>min</sub> of similarly sized white-crowned sparrows  
 344 during peak moult (Schieltz and Murphy 1997). By contrast, no study has explicitly  
 345 examined moult effects on avian MMR and AAS and we were surprised by the magnitude of  
 346 their decline. Spanish sparrows had greater moult-related declines in MMR and AAS than  
 347 house sparrows, but our limited sample size for house sparrows in advanced stages of moult  
 348 precludes firm conclusions or assignment of functional significance to the interspecific  
 349 differences we found. Nevertheless, both species had greatest decreases in AAS and MMR at

350 moult stages associated with substantial feather replacement, which suggests common  
351 underlying causes. Identifying the factors functionally responsible for these variations will  
352 require additional investigation, but it is worthwhile considering possible mechanisms.  
353 The onset of moult in birds is associated with significant increases in thyroxine ( $T_4$ ) secretion  
354 (McNabb 2007) and plasma  $T_4$  levels of up to 54% above pre-moult values have been  
355 reported for house sparrows (Smith 1982). Combining evidence that plasma  $T_4$  levels vary  
356 with moult intensity in red knots (*Calidris canutus canutus*; Jenni-Eiermann et al. 2002;  
357 Vezina et al. 2009) with research finding that  $T_4$  and its deiodinated derivative  $T_3$  directly  
358 affect rates of protein synthesis and degradation in avian muscles (Hayashi et al. 1991;  
359 Hayashi et al. 2009), locomotor muscle protein turnover rates would be expected to vary  
360 directly with moult intensity. Concurrent rises in  $T_3$  would promote increased RMR through  
361 its actions on multiple cellular processes that are identified as major components of  
362 maintenance energy costs (Rolfe and Brown 1997). Measurements of 3-methylhistidine (3-  
363 MH) daily excretion rates, a biomarker of myofibrillar protein breakdown, were 72% and  
364 63% higher during peak moult in white-crowned sparrows and house sparrows, respectively,  
365 than in non-moulting birds (Taruscio and Murphy 1995; Buttemer, Addison, and Klasing,  
366 unpubl. data). Our study found that declines in MMR were most pronounced between  
367 replacement of primary feathers 4 through 8 (Fig. 3), which corresponds with the period of  
368 greatest extent of overall feather replacement for both sparrow species (Zeidler 1966; Alonso  
369 1984). While the correspondence between heightened muscle protein turnover rates and  
370 decreased MMR remains correlative, it does warrant examination of muscle protein turnover  
371 rates in relation to locomotor muscle composition and mitochondrial characteristics pertinent  
372 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 in less effective transfer of metabolic power input to mechanical power output (Chai and Dudley 1999). This imposes greater aerobic demand for the same level of flight performance



397 at a time when the aerobic scope for such activity is reduced, thus placing moulting birds at  
398 greater risk of predation. Our findings invite further exploration of moult-related effects on  
399 AAS and how these vary in relation to moult rate and body size. Such studies will provide  
400 further insight into the functional significance of temporal separation of moult from annual  
401 events requiring peak physiological capacity such as breeding and migration (Wingfield  
402 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.

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**Compliance and ethical standards:**

**Conflict of interest:** 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<sub>min</sub> determinations. **Lower panel:** Blood haemoglobin content (mg/l) in relation to primary feather moult score. Values are depicted as medians with vertical bars representing 25<sup>th</sup> and 75<sup>th</sup> percentiles.

**Figure 3. Upper panel:** Minimum resting metabolic rate (RMR<sub>min</sub>) and maximum metabolic rate (MMR) of *Passer domesticus* and *Passer hispaniolensis* in relation to primary feather moult score. **Lower panel:** Absolute aerobic scope (ml O<sub>2</sub> min<sup>-1</sup>) of *Passer domesticus* and *Passer hispaniolensis* in relation to primary feather moult score. Values are depicted as medians with vertical bars representing 25<sup>th</sup> and 75<sup>th</sup> percentiles.



Figure 1

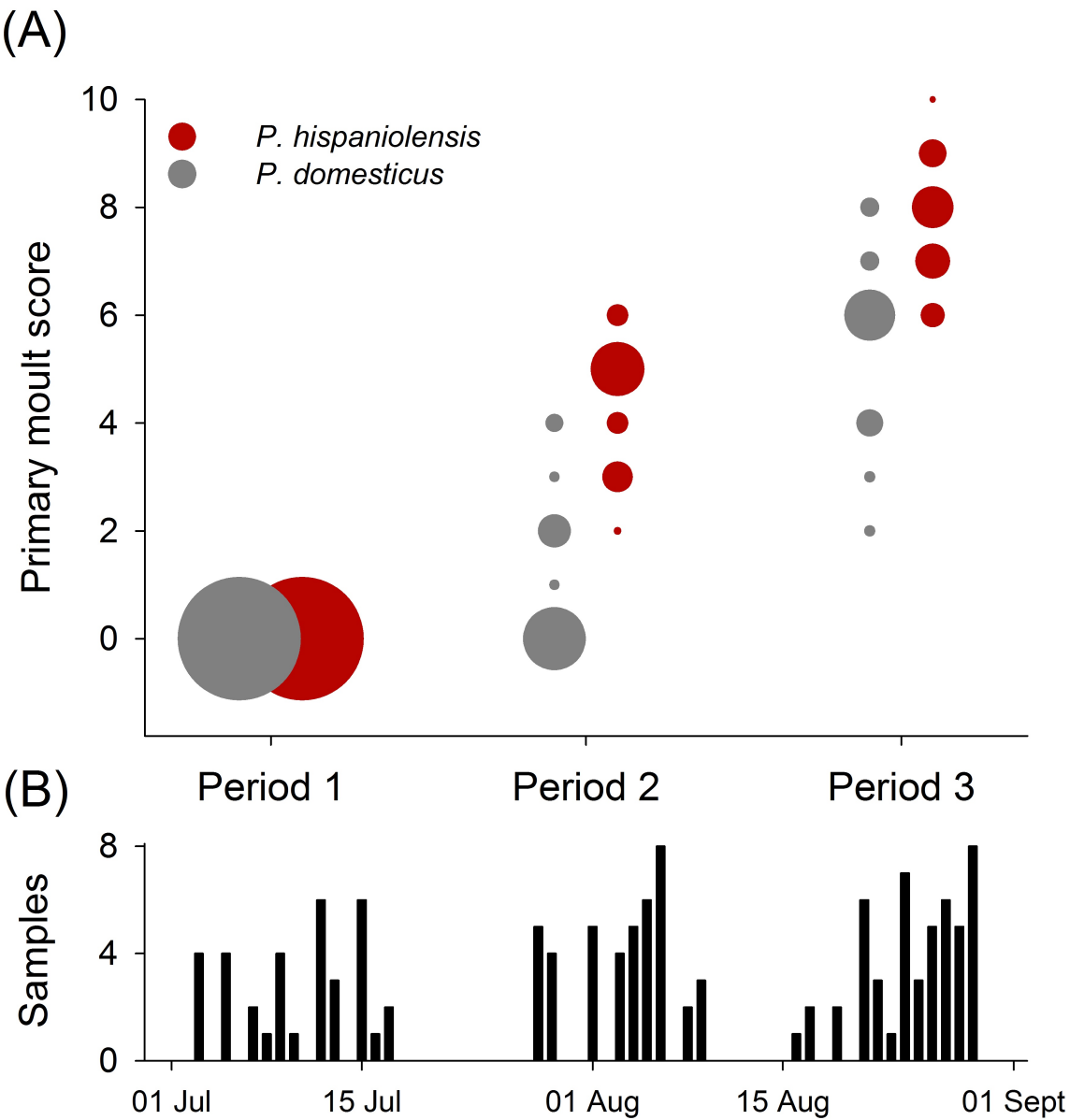


Figure 2

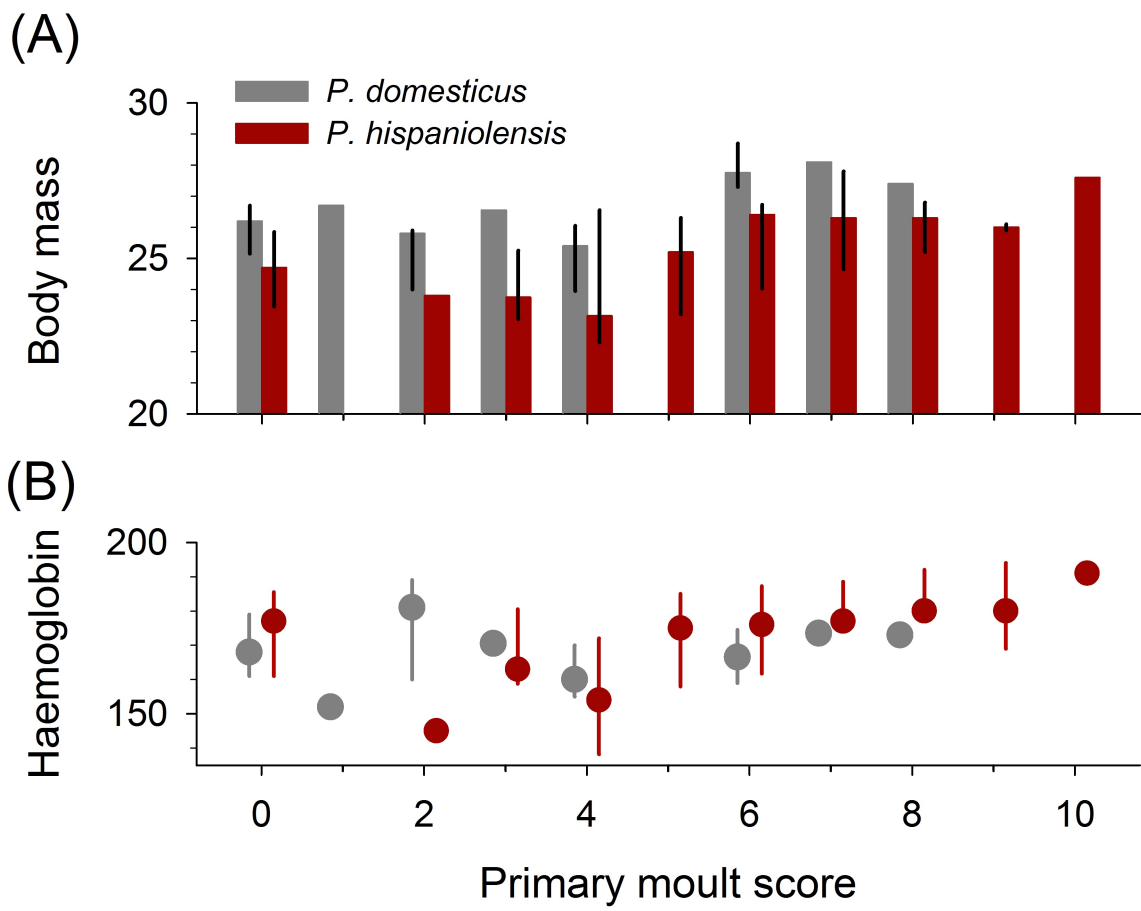


Figure 3

