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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

# Corticosterone secretion patterns prior to spring and autumn migration differ in free-living barn swallows (*Hirundo rustica* L.)

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**Abstract** Recent studies of long-distance migratory birds show that behavioural and physiological changes associated with predictable or unpredictable challenges during the annual cycle are distinctively regulated by hormones. Corticosterone is the primary energy regulating hormone in birds. Corticosterone levels are elevated during stresses but they are also modulated seasonally according to environmental conditions and life-history demands. We measured the baseline and stress-induced levels of corticosterone in the barn swallow (*Hirundo rustica* L.) just before spring and autumn migrations in South Africa and Finland, respectively. Barn swallows completing their pre-breeding moult had low body condition (residual body mass) and high baseline corticosterone levels in the wintering grounds. In contrast, baseline corticosterone levels in Finland were low and not related to residual mass. These data contradict the first prediction of the migration modulation hypothesis (MMH) by showing no association with baseline corticosterone levels and pre-migratory fuelling. Yet, the adrenocortical response to the capture and handling stress was notably blunted in South Africa compared to a strong response in Finland. Further, individuals that had

started fuelling in Finland showed a reduced response to the handling stress. Taken together, elevated baseline corticosterone levels and high residual mass may blunt the adrenocortical response in long-distance migrants and aerial feeders such as the barn swallow. This observation lends support to the second prediction of the MMH.

**Keywords** Baseline corticosterone · Adrenocortical response · Migration · Body condition · Molt

## Introduction

Long-distance migratory birds exhibit various behavioural and physiological changes during migratory processes. These hormone-driven changes may help birds to cope with the demands of increased activity during migration. Corticosterone is one of the main stress hormones but also the primary energy-regulating hormone in birds (Holmes and Phillips 1976; Harvey et al. 1984; Norris 2007). Birds are thought to modulate the baseline levels of circulating corticosterone in order to respond to the predictable changes during the annual cycle. When confronting unpredictable, life-threatening situations, birds rapidly increase the levels of circulating corticosterone in a process called the adrenocortical stress response (Wingfield et al. 1997, 1998; Holberton 1999; Landys et al. 2006). These short-term periods of increased corticosterone levels may save an individual's life, whereas chronically elevated corticosterone concentrations may reduce fitness by damaging the immune system, degrading skeletal muscles, and causing retardation of growth and development (Wingfield 1994; Holberton 1999; Sapolsky et al. 2000).

Many studies have shown moderately elevated baseline levels of corticosterone in different bird species during the

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migratory period (Holberton et al. 1996, 2007, 2008; Romero et al. 1997; Piersma and Ramenofsky 1998; Holberton 1999; Piersma et al. 2000; Landys et al. 2004a, b; Long and Holberton 2004). According to the migration modulation hypothesis (MMH), this is related to the development and/or maintenance of migratory condition (Holberton et al. 1996; Holberton 1999; Long and Holberton 2004), since moderately elevated baseline corticosterone levels increase searching behaviour, food intake rate (hyperphagia) and fat reserves (Astheimer et al. 1992; Breuner et al. 1998; Wingfield et al. 1998; Löhms et al. 2003, 2006; Dallman et al. 2004; Landys et al. 2004a, b; Long and Holberton 2004; Holberton et al. 2008). According to the MMH, the acute adrenocortical stress response may also be reduced in order to protect skeletal muscle proteins from corticosterone-induced degradation during up-regulated baseline corticosterone levels (Holberton et al. 1996; Holberton 1999; Long and Holberton 2004). Recent studies have shown that baseline levels of corticosterone do not remain elevated throughout the whole migratory period. Baseline levels can vary notably depending on the stage of the migration (i.e. take-off, flight, landing, stop-over) and/or the energetic condition of the bird (Holberton 1999; Piersma et al. 2000; Landys-Ciannelli et al. 2002; Long and Holberton 2004; Falsone et al. 2009; Raja-aho et al. 2010). Elevated baseline levels of corticosterone may also be associated with the expression of migratory activity and orientation due to high energetic need and unpredictable conditions ahead (Piersma et al. 2000; Landys-Ciannelli et al. 2002; Löhms et al. 2003).

Some studies have indicated that baseline levels of corticosterone before autumn migration were not as high as before spring migration (Romero et al. 1997; Piersma and Ramenofsky 1998). This may be because in spring birds tend to fly faster and longer distances without stopping over (Yohannes et al. 2009b; Tøttrup et al. 2012), and they may therefore need to acquire and mobilise larger energy reserves (Tøttrup et al. 2012). In addition, energetic condition of birds is related to corticosterone secretion. During the migratory fuelling, lean birds with depleted fat reserves, and a great need to increase energy reserves for migratory flight, may have higher baseline corticosterone levels than birds in good body condition, and/or the adrenocortical response might be reduced due to already elevated baseline corticosterone levels (Long and Holberton 2004).

Many studies have shown that the magnitude of the corticosterone peak induced by capture and handling may decrease with increasing energy reserves (Smith et al. 1994; Wingfield et al. 1994a, b; Breuner and Hahn 2003; Landys et al. 2006; Raja-aho et al. 2010). Yet, there are other studies showing no relationship between body

condition and the magnitude of the stress response (Romero et al. 1997; Landys-Ciannelli et al. 2002). Conflicting results from the relationship between body condition and corticosterone secretion may partly be due to different energy/metabolic demands of various life-history stages of birds (Romero 2002). Migratory passerines are known to adopt several different strategies in energy accumulation (Schaub and Jenni 2000; Yohannes et al. 2009a). Therefore, different migration strategies of birds may also cause variation in corticosterone secretion and, thus, in energy management (Romero et al. 1997).

In order to shed light on whether passerine birds exhibit differences in corticosterone secretion, and thus in energy accumulation in the times preceding spring and autumn migrations, we studied the variation in the baseline and stress-induced levels of corticosterone of a long-distance migratory bird, the barn swallow (*Hirundo rustica* L.). Since barn swallows moult in their wintering quarters prior to their spring migration, we also wanted to test whether the phase of moult is associated with corticosterone secretion and body condition (residual mass). Down-regulation of both baseline corticosterone level and adrenocortical response during moult may be essential in preventing the adverse effects on protein deposition while growing new feathers (Romero et al. 1998). Furthermore, we tested how the energetic condition as indicated by the residual mass is associated with the corticosterone secretion.

## Materials and methods

### Study areas and sampling of blood

Barn swallows wintering in eastern part of South Africa (Johannesburg area) most likely belong to the breeding populations of Sweden, Finland, the Baltic countries and further east (Szép et al. 2006, 2007; Ambrosini et al. 2009). This is deduced from the ring recoveries of the Finnish barn swallows where 38 out of 78 (49 %) African recoveries are from eastern South Africa, and 11 of them are close to the Johannesburg area (data from the Finnish Ringing Centre). Barn swallows before spring migration were captured in 2007 (21 February–3 March;  $n = 25$ ) in Potchefstroom (approximately 100 km SW from Johannesburg) South Africa (26°42'S, 27°06'E), whereas barn swallows before autumn migration in 2011 (1 August–10 September;  $n = 25$ ) were captured in Petteby, SW Finland (60°17'N, 22°11'E). Barn swallows were tape-lured (in Finland) and mist-netted (Ecotone) from the night roosts, 0.5–3 h after sunrise (South Africa at 6:30–8:00 am., Finland at 4:30–8:30 am.). According to the Southern African Bird Atlas Project 2, the spring migration in Gauteng

(Johannesburg area) starts around 12 March, and the midpoint of departure is 3 April (Altwegg et al. 2012). In Finland, the midpoint of departure in years 2000–2004 was in the beginning of September (P. Suorsa, unpublished data). Thus, first samplings of blood were made 5–6 weeks before the median date of migration in both South Africa and Finland.

Weather data, including the mean temperature and rainfall of the sampling day and the day prior to sampling, were based on measurements of the Finnish Meteorological Institute in Turku (60°45'N, 22°18'E) and the Weather Station in Potchefstroom North-West Province, South Africa (26°73'S, 27°06'E). Daily temperatures during the sampling day and the preceding day in Finland were  $16.4 \pm 1.88$  (mean  $\pm$  SD) and  $15.9 \pm 1.99$  °C, and in South Africa  $24.1 \pm 1.10$  and  $24.6 \pm 0.88$  °C, respectively. The amount of rainfall during the sampling days and the preceding days were 55 and 5.2 mm in Finland, and 0 mm in South Africa. All in all, the weather conditions were very favourable both in spring and in autumn.

We collected the first blood samples within 2–3 min after capture ( $n = 50$ ). These samples are known to reflect the baseline corticosterone concentrations (Wingfield et al. 1982; Sapolsky et al. 2000; Romero and Reed 2005). We took the second blood sample 30 min after capture ( $n = 50$ ) and the third one 60 min after capture ( $n = 39$ ) to assess the hormonal response to capture and handling stress. In between the blood samplings, we held the individuals in a cotton cloth bag (South Africa), and in a plywood board box (Finland) containing a set of 20 individual cells (10  $\times$  10  $\times$  22 cm). Blood samples were taken in heparinised microhaematocrit capillary tubes after puncture of a brachial vein. Within 4–10 min after the puncture, the tubes were centrifuged at 4,000g for 5 min and the extracted plasma was immediately stored in liquid nitrogen in the field. In the laboratory, all the samples were stored at a controlled temperature below  $-80$  °C in a freezer. South African samples were stored in carbon dioxide ice when transported to Finland.

#### Age, sex and body condition

Ageing is almost impossible on plumage characteristics in the barn swallow after the moult of tail feathers in Africa. The age and sex (only for South African birds) were determined according to Jenni and Winkler (1994) and Svensson (1992). Following Cramp (1988) and BWPi2.0 (2003–2006), we were able to determine the age of the South African barn swallows (adults = 13, juveniles = 12) using the length of their wings and tails, and the amount of wear and colour of non-moulted tail and wing feathers. As part of another study (Raja-aho et al. 2012), the sex of barn swallows was determined from the

gonads (males  $n = 15$ , females  $n = 10$ ). Corticosterone levels of barn swallows in South Africa did not differ between age groups or sexes (generalised linear models;  $n = 25$ ,  $P > 0.05$  in both analyses). In addition, corticosterone levels of Finnish barn swallows captured in August ( $n = 19$ ) did not differ from those captured in September (generalised linear models;  $n = 6$ ,  $P > 0.05$ ).

Before the second (30 min) sampling, we measured the maximum wing length (Svensson 1992), the length of outermost tail feathers (South African birds only), and body mass to the nearest 0.1 g with a spring balance. We used residual mass (deviation from linear regression of body mass on wing length) as a measure of energetic condition in our analyses. The residual mass also takes into account non-lipid energy sources, such as muscles, and is therefore a more general measure of condition than the mere fat score (Long and Holberton 2004).

Barn swallows moult completely in the winter quarters. Their moult is slow, duration 121–185 days, compared to most small passerines, and they may set off to the spring migration while still moulting (Jenni and Winkler 1994). We therefore also checked the moult of wing (primaries, secondaries) and tail feathers of South African barn swallows ( $n = 25$ ). We used a primary score to describe the progress of moult from 0 = old feather to 5 = new feather, summed over nine developed primaries, thus varying from 0 to 45 (Ginn and Melville 1983). With the help of the primary score and the known minimum duration of the moult (121 days), we calculated the minimum number of days each individual still needed to complete its moult:  $(45 - \text{primary score}) / (45 / 121)$ . Primary moult usually extends the whole moult period and can therefore be used as a proxy for the total moult duration (Jenni and Winkler 1994).

Due to ongoing moult of primaries at the tip of the wing, we had to estimate the completed wing length of the South African barn swallows in spring on the basis of fully grown wing feathers of barn swallows captured in May in Finland ( $n = 24$ ; Raja-aho et al. 2012). Complete wing length was needed for the calculation of size-independent residual mass of all barn swallows in the following analyses. We first measured the wing length up to the newly moulted, already complete primaries (5th–8th). We estimated the missing length of moulting wing with help of mean values of length differences between the 5th and 9th, 6th and 9th, etc. primaries of barn swallows in May. We then added the estimate to the measured length in order to get the complete wing length.

#### Corticosterone assay

The corticosterone assay used in this study (in 2007 and 2011) has been validated in earlier avian studies (Love

et al. 2005; Tachibana et al. 2007), and was utilized in our earlier study (Raja-aho et al. 2010). We determined plasma corticosterone concentrations using Correlate-EIA<sup>TM</sup> corticosterone enzyme immunoassay kits (Assay Designs, Ann Arbor, MI, USA) with some modifications due to the small sample quantity (Raja-aho et al. 2010). In brief, 5- $\mu$ l plasma samples were treated with an equal amount of 1 % steroid displacement reagent before the final dilution of the samples. Due to the small plasma volume, the samples were diluted more (1:50) than in the manufacturer's instructions (1:40). The samples were analysed in batches as duplicates. As a result, the calculated sensitivity of the assay was 34 pg ml<sup>-1</sup> corresponding to 1.7 ng ml<sup>-1</sup> plasma corticosterone concentration; 8 out of 49 samples (baseline), 0 out of 50 samples (30 min), and 4 out of 39 samples (60 min) were below the detection limit (DL). We gave the value 1 ng ml<sup>-1</sup> to corticosterone concentrations below DL. This does neither affect the analysis nor the biological conclusions drawn, because the variation in values below DL is very small compared to the total variation of corticosterone concentrations.

In order to account for variation between the batches (plates), we used a plasma sample from one turkey (when analysing the South African samples from 2007) and one barn swallow (when analysing the Finnish samples from 2011) as an internal control (either in duplicate or triplicate). The manufacturer's values for intra-assay coefficient of variation were 6.6–8.0 %. In our study, the inter-assay precision of plates with turkey as a control was 11.1 % (manufacturer's values 7.8–13.1 %). The plates with barn swallow sample as a control did not differ. We calculated ratios between the turkey sample values measured with different plates ( $n = 3$ ), and a mean of these values in order to adjust corticosterone concentrations to compensate for differences between plates. The corticosterone concentrations obtained for each barn swallow were then divided by the ratio specific to the particular plate (range 0.87–1.08).

#### Statistical analyses

Because the distributions of corticosterone concentrations were skewed, we analysed all data by using generalised linear models (GLM) with the GENMOD-procedure of SAS (v.9.2; SAS 2008). We used a negative binomial distribution with log link function for non-normally distributed data, referred to below as *dist negbin*, and normal distribution (*dist normal*) for normally distributed data. We also evaluated the model fit with values of scaled deviance (SCD), which is the residual deviance for the model (the sum of individual deviance contributions). The optimal value for the goodness of fit is one (SAS 2008). In order to establish ranges for the true effect, we calculated 95 % confidence intervals (Steidl and Thomas 2001).

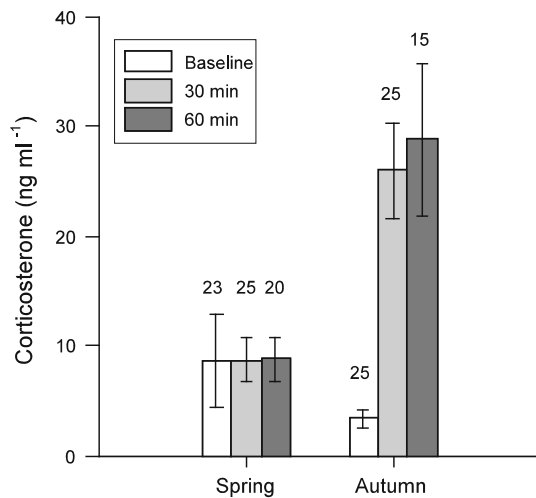
Firstly, we analysed the difference in corticosterone levels (a response variable) between spring and autumn barn swallows across sampling times (baseline, 30 min, 60 min) with a repeated-measures GLM using an individual as the repeated-measures component (birds with  $\geq 2$  samples,  $n = 50$  individuals, 133 blood samples). Season, sampling time and the interaction between season and sampling time were used as explanatory variables in this analysis. We also tested the significance of differences within sampling times of both seasons by comparing the estimated marginal means (EMMs).

Secondly, with GLM, we compared the relationship between body condition (residual body mass), moult completion (South African birds only) and baseline levels of corticosterone for both seasons in a separate model. Residual body mass, moult completion and the interaction between residual mass and the completion of moult were used as explanatory variables in this model. Thirdly, we tested the association between body condition, moult completion (South African birds) and the adrenocortical response (difference between the highest detected corticosterone value and the baseline value) separately for both seasons. Since the maximal level of the corticosterone secretion may have occurred at any time between sampling times, and therefore could be missed, we used the highest detected value in the analysis (Raja-aho et al. 2010). We used residual body mass, baseline corticosterone and the interaction between residual mass and baseline corticosterone as explanatory variables. We also tested whether the moult completion of barn swallows in spring was associated with the adrenocortical response.

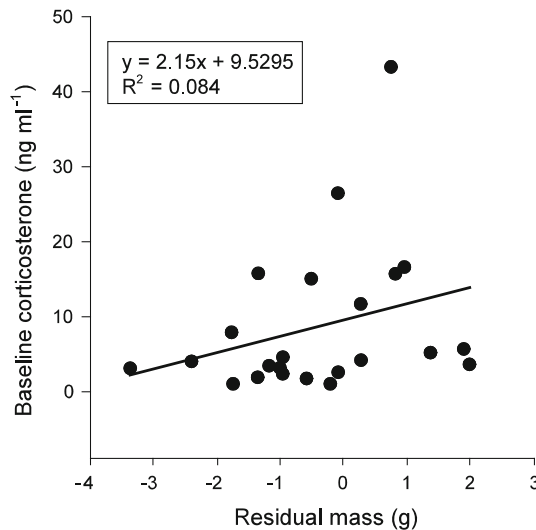
Finally, we tested the differences in residual mass between spring and autumn barn swallows. In addition, we tested if body condition was associated with date (accumulation of mass) in either group or with moult completion in spring birds. In all analyses, we obtained the minimal adequate models by step-down removal of non-significant predictors starting with the interactions (Crawley 1993). Instead of the model estimates, the original data, in which interactions have not been taken into account, are shown in Figs. 2 and 3 (below).

#### Results

The mean levels of corticosterone at different sampling times (baseline, 30 and 60 min) varied between spring and autumn barn swallows (GLM, repeated-measure; interaction season  $\times$  sampling time;  $\chi^2 = 13.14$ ,  $P = 0.0014$ ; Fig. 1). The baseline corticosterone levels of barn swallows in spring (EMM, 95 % CI; 8.7 ng ml<sup>-1</sup>, 5.5–13.8) were higher than those in autumn (3.4 ng ml<sup>-1</sup>, 2.7–4.3,  $\chi^2 = 12.42$ ,  $P = 0.0004$ ). The 30 min corticosterone levels were lower



**Fig. 1** Variation in the plasma corticosterone levels (estimated marginal means and  $\pm 95\%$  CI) between sampling times in free-living barn swallows (*Hirundo rustica* L.) before spring (year 2007) and autumn migrations (year 2011) (individuals with at least two samples)



**Fig. 2** Relationship between the baseline corticosterone level ( $\text{ng ml}^{-1}$ ) and the residual mass (g) in free-living barn swallows before spring migration ( $n = 23$ )

in spring barn swallows ( $8.8 \text{ ng ml}^{-1}$ ,  $7.1\text{--}10.9$ ) than in autumn barn swallows ( $26.0 \text{ ng ml}^{-1}$ ,  $22.0\text{--}30.7$ ,  $\chi^2 = 60.42$ ,  $P < 0.0001$ ). This also held true for the 60 min values, where the corticosterone levels were lower in spring ( $8.8 \text{ ng ml}^{-1}$ ,  $7.1\text{--}11.0$ ) than in autumn ( $28.8 \text{ ng ml}^{-1}$ ,  $22.7\text{--}36.6$ ,  $\chi^2 = 51.07$ ,  $P < 0.0001$ ; Fig. 1).

The baseline corticosterone levels of barn swallows in spring were positively related to residual mass (Fig. 2; Table 1), whereas such a relationship was not found in autumn. The moult completion was not associated either with baseline levels of corticosterone or adrenocortical response. The adrenocortical response (highest level minus baseline level) was higher in autumn than in spring. The

adrenocortical response was negatively associated with the residual mass of barn swallows in autumn but no correlation was found in spring (Fig. 3a; Table 1). In spring, the adrenocortical stress response was negatively associated with baseline level of corticosterone; barn swallows with high levels of baseline corticosterone had lower adrenocortical stress response (Fig. 3b; Table 1).

The residual mass of barn swallows before spring migration in South Africa (absolute mass  $\pm$  SE:  $17.2 \text{ g} \pm 0.29$ ) was lower than before autumn migration in Finland ( $18.0 \text{ g} \pm 0.26$ ; GLM, *dist normal*: SCD = 1.04,  $\chi^2 = 4.72$ ,  $df = 1$ ,  $P = 0.03$ ). The residual mass of barn swallows in spring correlated negatively with capturing date (GLM, *dist normal*: slope  $-0.2 \pm 0.05$ , SCD = 1.09;  $\chi^2 = 7.57$ ,  $df = 1$ ,  $P = 0.006$ ). Body condition was not associated with moult completion (*dist norm*: SCD = 1.09,  $\chi^2 = 3.10$ ,  $df = 1$ ,  $P = 0.0783$ ). We found no association with the corticosterone secretion and weather data (GLM,  $n = 50$ ,  $P > 0.05$  in all analyses).

### Discussion

Corticosterone secretion patterns of both baseline and adrenocortical responses in the barn swallow were clearly different from 5 to 6 weeks before the midpoint of spring (Altwegg et al. 2012) and autumn migration (P. Suorsa, unpublished data). On average, baseline levels of corticosterone were 2.6-fold higher in South Africa than in Finland. This is consistent with earlier studies with red knots (*Calidris canutus*), American redstarts (*Setophaga ruticilla*) and white-crowned sparrows (*Zonotrichia leucophrys*), which showed elevated baseline corticosterone values in spring compared to those in autumn (Romero et al. 1997; Marra and Holberton 1998; Piersma and Ramenofsky 1998). Barn swallows in South Africa were captured having almost finished their primary moult, during which the baseline corticosterone levels could be expected to be low (reviewed in Romero 2002). However, we found no association between the stage of moult and baseline corticosterone level. At the time of sampling in South Africa (21 February–3 March), barn swallows were about to set off to the north within a few weeks: spring migration in Gauteng starts around 12 March (Altwegg et al. 2012). According to Holberton et al. (1996) and Long and Holberton (2004), elevated levels of baseline corticosterone may be related to the development or maintenance of migratory condition. However, residual body masses in South Africa were low in general and in fact had a declining trend during our catching period. In addition, low baseline levels of corticosterone together with higher residual masses in barn swallows in Finland suggest that corticosterone may not have a role in triggering fuelling before autumn migration

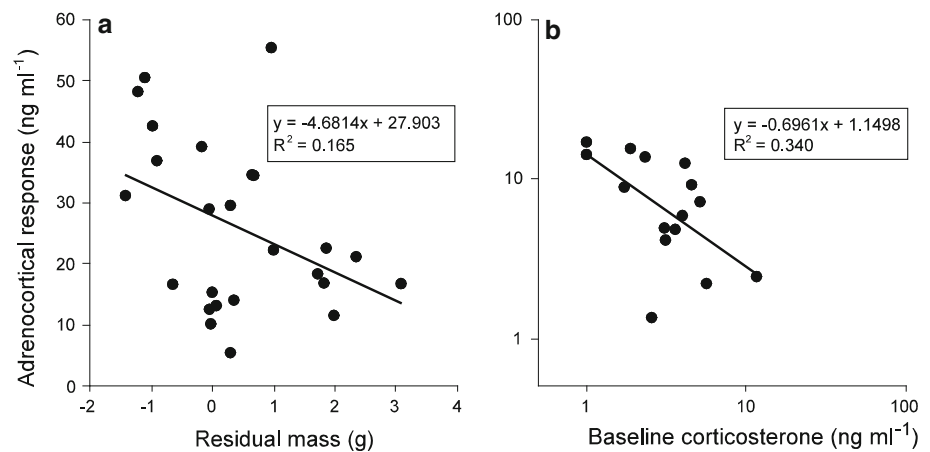
**Table 1** Generalised linear models for the relationship between blood corticosterone levels ( $\text{ng ml}^{-1}$ ) and body condition in the barn swallow (*Hirundo rustica* L.) before spring and autumn migrations

Dependent variable	Independent variable	Slope (95 % CI)	$\chi^2_{df}$	P
Spring				
Baseline cort ( $n = 23$ )				
SCD = 1.12 ( <i>dist negbin</i> )	Residual mass	0.3 (0.01–0.63)	3.77 <sub>1, 21</sub>	0.0523
Magnitude of adrenocortical response ( $n = 15$ )				
SCD = 1.23 ( <i>dist negbin</i> )	Baseline cort	-0.2 (-0.32 to -0.06)	7.05 <sub>1, 13</sub>	0.0079
Autumn				
Magnitude of adrenocortical response ( $n = 25$ )				
SCD = 1.12 ( <i>dist negbin</i> )	Residual mass	-0.2 (-0.34 to -0.02)	4.36 <sub>1, 23</sub>	0.0369

Variables excluded from the model by step-down removal of non-significant predictors: baseline: residual mass  $\times$  completion of moult, completion of moult; response (spring): baseline cort  $\times$  residual mass, residual mass; response (autumn): baseline cort  $\times$  residual mass, baseline corticosterone

SCD Scaled deviances are shown to indicate the fit of the models

**Fig. 3** Relationship between the adrenocortical stress response (highest level–baseline level,  $\text{ng ml}^{-1}$ ) and **a** the residual mass (g) in free-living barn swallows before autumn migration ( $n = 25$ ) and **b** baseline corticosterone ( $\text{ng ml}^{-1}$ , logarithmic X- and Y-axis) in free-living barn swallows before spring migration ( $n = 23$ )



either. Hence, our results do not support the first prediction of the MMH.

One likely explanation for elevated baseline corticosterone levels of South African barn swallows may be the weather conditions. For an aerial feeder rain and temperature are the most important characteristics of weather. In rainy weather barn swallows cannot capture flying insects efficiently, which is reflected in decreasing body mass (Ormerod 1989; Pilaastro and Magnani 1997; Raja-aho 2005; Jenni-Eiermann et al. 2008). On the other hand, a long dry spell may decrease the abundance of insects in general (uMoya-NILU 2007), thus impairing foraging effort despite of the optimal flying conditions. During the sampling period the weather in South Africa was warm with slightly lower than average rainfall of 51.8 mm in February and 30 mm in March. Average rainfall in 1998–2007 in February was 60.5 mm and in March 68.7 mm (Weather Station Potchefstroom North-West Province, South Africa). If barn swallows were suffering from hunger, one would expect the lightest birds having the highest levels of baseline corticosterone. In contrast, our

data showed a positive correlation between baseline levels of corticosterone and residual body mass. However, wetlands such as our sampling site, comprising a river, may contain large amounts of insects even during dry spells. In addition, barn swallows subjected to a chronic lack of food would have suspended their moult. In our data, the ones moulting also had growing new feathers. Therefore, we consider that the lack of food is not the likely reason explaining our general finding of elevated baseline levels prior to spring migration.

More likely, a reason for elevated baseline levels of corticosterone in spring may be the increased activity of biotransformation enzymes and high oxidative stress detected in the same barn swallows in South Africa (Raja-aho et al. 2012). Because biotransformation of toxic pollutants is often associated with increased energy turnover (Parkinson and Ogilvie 2008), it may influence corticosterone regulation. Recent studies have found a positive association with toxic pollutants and elevated baseline corticosterone levels in birds (Verboven et al. 2010; Nordstad et al. 2012). In addition to seasonal changes, the

differences in corticosterone secretion before spring and autumn migration may also be due to year effects, e.g. food availability during nestling, fuelling or migration, as well as the exposure to toxic pollutants may vary substantially.

Before autumn migration barn swallows responded strongly to acute capture and handling stress by increasing the corticosterone level, which is typical for most bird species (Wingfield and Romero 2001). In contrast, before spring migration the adrenocortical response was clearly blunted, most likely due to already elevated baseline levels, since birds with high levels of baseline corticosterone had lower adrenocortical response. Autumn birds with greater relative body mass also seemed to suppress the secretion of corticosterone more than the light ones. During the migratory period, adrenocortical response can be reduced in order to protect skeletal muscles from the hormone's catabolic activity, which is what the second prediction of MMH proposes (Holberton et al. 1996; Holberton 1999; Jenni et al. 2000). Our barn swallows in migratory condition had a blunted corticosterone response to capture and handling stress compared to the clear response found in breeding barn swallows (Jenni-Eiermann et al. 2008). Adrenocortical stress response in birds can also be down-regulated in situations when high levels of the hormone may compromise growth (Sims and Holberton 2000), reproduction (Wingfield and Silverin 1986; Wingfield et al. 1994a, b; Astheimer et al. 1995), and moult (Sapolsky et al. 2000; Romero 2002; Cyr et al. 2008) or when birds are forced to forage in poor quality habitats (Marra and Holberton 1998).

Before spring migration barn swallows were lighter than before autumn migration. There may be several reasons for that. Barn swallows were possibly suffering from oxidative stress and they had high biotransformation activity (Rajahaaho et al. 2012), which may be associated with low body mass. During moult hormonal control also prevents fattening, which may decrease the risk for predation (e.g. Swaddle et al. 1999). Bijlsma and van den Brink (2005) suggest in their study of wintering barn swallows that “weight watching” is one possible way to reduce predation risk. Moulting barn swallows spend most of their active time in the air and may lower their body mass as a response to impaired flight performance (Keith et al. 1992; Cuthill and Houston 1997; van den Brink et al. 1997). Furthermore, the migration route northwards to the breeding grounds may not be that demanding at the beginning. There are no large ecological barriers to cross before the Sahara desert and thus no need for excessive fuelling in South Africa, which was reflected in relative low body masses in birds sampled in our study.

In conclusion, we found no clear associations with the baseline levels of corticosterone and the development of body condition before spring and autumn migration in the

barn swallow. Therefore, our data do not support the first prediction of the MMH. Nevertheless, high baseline levels of corticosterone in spring and high residual mass in autumn seemed to blunt the adrenocortical response lending support to the second prediction of the MMH.

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## References

- Altwegg R, Broms K, Erni B, Barnard P, Midgley GF, Underhill LG (2012) Novel methods reveal shifts in migration phenology of barn swallows in South Africa. *Proc R Soc Lond B* 279:1485–1490
- Ambrosini R, Møller AP, Saino N (2009) A quantitative measure of migratory connectivity. *J Theor Biol* 257:203–211
- Astheimer LB, Buttemer WA, Wingfield JC (1992) Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scand* 23:355–365
- Astheimer LB, Buttemer WA, Wingfield JC (1995) Seasonal and acute changes in adrenocortical stress response in an Arctic-breeding bird. *Horm Behav* 29:442–457
- Bijlsma RG, van den Brink B (2005) A barn swallow *Hirundo rustica* roost under attack: timing and risks in the presence of African hobbies *Falco cuvieri*. *Ardea* 93:37–48
- Breuner CW, Greenberg AL, Wingfield JC (1998) Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Gen Comp Endocrinol* 111:386–394
- Breuner CW, Hahn TP (2003) Integrating stress physiology, environmental change, and behavior in free-living sparrows. *Horm Behav* 43:115–123
- BWPi2.0 (2003–2006) Birds of the western Palearctic interactive (DVD). Bird Guides, Sheffield
- Cramp S (1988) The birds of the western palearctic, vol 5. Oxford University Press, Oxford
- Crawley MJ (1993) GLIM for ecologists. Blackwell, Oxford
- Cuthill IC, Houston AI (1997) Managing time and energy. In: Krebs JR, Davies NB (eds) Behavioural ecology: an evolutionary approach, 4th edn. Blackwell, Oxford, pp 97–120
- Cyr NE, Wikelski M, Romero LM (2008) Increased energy expenditure but decreased stress responsiveness during molt. *Physiol Biochem Zool* 81:452–462
- Dallman MF, La Fleur SE, Pecoraro NC, Gomez F, Houshyar H, Akana SF (2004) Minireview: glucocorticoids—food intake, abdominal obesity, and wealthy nations in 2004. *Endocrinology* 145:2633–2638
- Falsone K, Jenni-Eiermann S, Jenni L (2009) Corticosterone in migrating songbirds during endurance flight. *Horm Behav* 56:548–556

- Ginn HB, Melville DS (1983) Moulting in birds. BTO Guide 19. BTO, Hertfordshire
- Harvey S, Phillips JG, Rees A, Hall TR (1984) Stress and adrenal function. *J Exp Zool* 232:633–645
- Holberton RL (1999) Changes in patterns of corticosterone secretion concurrent with migratory fattening in a neotropical migratory bird. *Gen Comp Endocrinol* 116:49–58
- Holberton RL, Boswell T, Hunter MJ (2008) Circulating prolactin and corticosterone concentrations during the development of migratory condition in the dark-eyed junco, *Junco hyemalis*. *Gen Comp Endocrinol* 155:641–649
- Holberton RL, Parrish JD, Wingfield JC (1996) Modulation of the adrenocortical stress response in neotropical migrants during autumn migration. *Auk* 113:558–564
- Holberton RL, Wilson CM, Hunter MJ, Cash WB, Sims CG (2007) The role of corticosterone in supporting migratory lipogenesis in the dark-eyed junco, *Junco hyemalis*: a model for central and peripheral regulation. *Physiol Biochem Zool* 80:125–137
- Holmes WN, Phillips JG (1976) The adrenal cortex in birds. In: I. C-J, I. H (eds) General and comparative endocrinology of the adrenal cortex. Academic, New York, pp 293–420
- Jenni L, Jenni-Eiermann S, Spina F, Schwabl H (2000) Regulation of protein breakdown and adrenocortical response to stress in birds during migratory flight. *Am J Physiol Regul Integr Comp Physiol* 278:R1182–R1189
- Jenni L, Winkler R (1994) Moulting and ageing of European passerines. Academic, London
- Jenni-Eiermann S, Glaus E, Gruebler M, Schwabl H, Jenni L (2008) Glucocorticoid response to food availability in breeding barn swallows (*Hirundo rustica*). *Gen Comp Endocrinol* 155:558–565
- Keith S, Urban EK, Fry CH (1992) The birds of Africa, vol 4. Academic, London
- Landys MM, Piersma T, Ramenofsky M, Wingfield JC (2004a) Role of the low-affinity glucocorticoid receptor in the regulation of behavior and energy metabolism in the migratory red knot *Calidris canutus islandica*. *Physiol Biochem Zool* 77:658–668
- Landys MM, Ramenofsky M, Guglielmo CG, Wingfield JC (2004b) The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*. *J Exp Biol* 207:143–154
- Landys MM, Ramenofsky M, Wingfield JC (2006) Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen Comp Endocrinol* 148:132–149
- Landys-Ciannelli MM, Ramenofsky M, Piersma T, Jukema J, Wingfield JC, Castr Ring G (2002) Baseline and stress-induced plasma corticosterone during long-distance migration in the bartailed godwit, *Limosa lapponica*. *Physiol Biochem Zool* 75:101–110
- Löhms M, Sandberg R, Holberton RL, Moore FR (2003) Corticosterone levels in relation to migratory readiness in red-eyed vireos (*Vireo olivaceus*). *Behav Ecol Sociobiol* 54:233–239
- Löhms M, Sundström F, Moore FR (2006) Non-invasive corticosterone treatment changes foraging intensity in red-eyed vireos *Vireo olivaceus*. *J Avian Biol* 37:523–526
- Long JA, Holberton RL (2004) Corticosterone secretion, energetic condition, and a test of the migration modulation hypothesis in the hermit thrush (*Catharus guttatus*), a short-distance migrant. *Auk* 121:1094–1102
- Love OP, Chin EH, Wynne-Edwards KE, Williams TD (2005) Stress hormones: a link between maternal condition and sex-biased reproductive investment. *Am Nat* 166:751–766
- Marra PP, Holberton RL (1998) Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia* 116:284–292
- Nordstad T, Moec B, Bustnes JO, Bech C, Chastel O, Goutte A (2012) Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. *Environ Pollut* 164:219–226
- Norris DO (2007) Vertebrate endocrinology, 4th edn. Academic Press, San Diego
- Ormerod SJ (1989) The influence of weather on the body mass of migrating swallows *Hirundo rustica* in south Wales. *Ring Migr* 10:65–74
- Parkinson A, Ogilvie BW (2008) Biotransformation of xenobiotics. In: Klaassen CD (ed) Casarett and Dull's toxicology: the basic science of poisons. McGraw Hill, New York
- Piersma T, Ramenofsky M (1998) Long-term decreases of corticosterone in captive migrant shorebirds that maintain seasonal mass and moult cycles. *J Avian Biol* 29:97–104
- Piersma T, Reneerkens J, Ramenofsky M (2000) Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? *Gen Comp Endocrinol* 120:118–126
- Pilastro A, Magnani A (1997) Weather conditions and fat accumulation dynamics in pre-migratory roosting barn swallows *Hirundo rustica*. *J Avian Biol* 28:338–344
- Raja-aho S (2005) Fuelling for autumn migration of the barn swallow (*Hirundo rustica* L.). Master's thesis, University of Turku, Turku
- Raja-aho S, Kanerva M, Eeva T, Lehtikoinen E, Suorsa P, Gao K, Vosloo D, Nikinmaa M (2012) Seasonal variation in the regulation of redox state and some biotransformation enzyme activities in the barn swallow (*Hirundo rustica* L.). *Physiol Biochem Zool* 85:148–158
- Raja-aho S, Suorsa P, Vainio M, Nikinmaa M, Lehtikoinen E, Eeva T (2010) Body condition is associated with adrenocortical response in the barn swallow (*Hirundo rustica* L.) during early stages of autumn migration. *Oecologia* 163:323–332
- Romero LM (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen Comp Endocrinol* 128:1–24
- Romero LM, Ramenofsky M, Wingfield JC (1997) Season and migration alters the corticosterone response to capture and handling in an arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *Comp Biochem Physiol C* 116:171–177
- Romero LM, Reed JM (2005) Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp Biochem Physiol A* 140:73–79
- Romero LM, Soma KK, Wingfield JC (1998) Hypothalamic-pituitary-adrenal axis changes allow seasonal modulation of corticosterone in a bird. *Am J Physiol Regul Integr Comp Physiol* 274:R1338–R1344
- Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrin Rev* 21:55–89
- SAS (2008) SAS/STAT® 9.2 user's guide. SAS Institute, Cary
- Schaub M, Jenni L (2000) Body mass of six long-distance migrant passerine species along the autumn migration route. *J Ornithol* 141:441–460
- Sims CG, Holberton R (2000) Development of the corticosterone stress response in young mockingbirds (*Mimus polyglottos*). *Gen Comp Endocrinol* 119:193–201
- Smith GT, Wingfield JC, Veit RR (1994) Adrenocortical-response to stress in the common diving petrel, *Pelecanoides urinatrix*. *Physiol Zool* 67:526–537
- Steidl RJ, Thomas L (2001) Power analysis and experimental design. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Oxford University Press, New York, pp 14–36



- Svensson L (1992) Identification guide to European passerines. Fingraf, Södertälje
- Swaddle JP, Williams EV, Rayner JMV (1999) The effect of flight feather moult on escape take-off performance in starlings. *J Avian Biol* 30:351–358
- Szép T, Møller AP, Piper S, Nuttall R, Szabo ZD, Pap PL (2006) Searching for potential wintering and migration areas of a Danish barn swallow population in South Africa by correlating NDVI with survival estimates. *J Ornithol* 147:245–253
- Szép T, Møller AP, Piper S, Nuttall R, Szabo ZD, Pap PL (2007) Migratory connectivity in barn swallows and other hirundines. *J Ornithol* 148:257–260
- Tachibana T, Oikawa D, Takahashi H, Boswell T, Furuse M (2007) The anorexic effect of alpha-melanocyte-stimulating hormone is mediated by corticotrophin-releasing factor in chicks. *Comp Biochem Physiol A* 147:173–178
- Tøttrup AP, Klaassen RHG, Strandberg R, Thorup K, Kristensen MW, Jørgensen PS, Fox J, Afanasyev V, Rahbek C, Alerstam T (2012) The annual cycle of a trans-equatorial Eurasian-African passerine migrant: different spatio-temporal strategies for autumn and spring migration. *Proc R Soc Lond B* 279:1008–1016
- uMoya-NILU (2007) Air quality scoping study for the epsilon proposed substation, report for PBA International (SA) Ltd. Report No. uMN007-07. uMoya-NILU Consulting, Durban
- van den Brink B, Bijlsma RG, van der Have T (1997) European swallows *Hirundo rustica* in Botswana. WIWO-report no. 56. In: <http://www.wiwo.org>, Zeist
- Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP (2010) Adrenocortical function of arctic-breeding glaucous gulls in relation to persistent organic pollutants. *Gen Comp Endocrinol* 166:25–32
- Wingfield JC (1994) Modulation of the adrenocortical response to stress in birds. In: Davey KG, Peter RE, Tobe SS (eds) Perspectives in comparative endocrinology. National Research Council, Ontario, pp 520–528
- Wingfield JC, Deviche P, Sharbaugh S, Astheimer LB, Holberton R, Suydam R, Hunt K (1994a) Seasonal-changes of the adrenocortical responses to stress in redpolls, *Acanthis flammea*, in Alaska. *J Exp Zool* 270:372–380
- Wingfield JC, Hunt K, Breuner KD, Fowler GS, Freed L, Lepson J (1997) Environmental stress, field endocrinology, and conservation biology. In: Clemmons JR, Buchholz R (eds) Behavioral approaches to conservation in the wild. Cambridge University Press, Cambridge, pp 95–131
- Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD (1998) Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am Zool* 38:191–206
- Wingfield JC, Romero LM (2001) Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen BS, Goodman HM (eds) Handbook of Physiology. Section 7: The endocrine system, vol 4. Coping with environment: Neural and Endocrine Mechanisms. Oxford University Press, New York, pp 211–234
- Wingfield JC, Silverin B (1986) Effects of corticosterone on territorial behavior of free-living male song sparrows, *Melospiza melodia*. *Horm Behav* 20:405–417
- Wingfield JC, Smith JP, Farner DS (1982) Endocrine responses of white-crowned sparrows to environmental-stress. *Condor* 84:399–409
- Wingfield JC, Suydam R, Hunt K (1994b) The adrenocortical responses to stress in snow buntings (*Plectrophenax nivalis*) and Lapland longspurs (*Calcarius lapponicus*) at Barrow, Alaska. *Comp Biochem Physiol C* 108:299–306
- Yohannes E, Biebach H, Nikolaus G, Pearson DJ (2009a) Passerine migration strategies and body mass variation along geographic sectors across east Africa, the middle east and the Arabian Peninsula. *J Ornithol* 150:369–381
- Yohannes E, Biebach H, Nikolaus G, Pearson DJ (2009b) Migration speeds among eleven species of long-distance migrating passerines across Europe, the desert and eastern Africa. *J Ornithol* 40:126–134