



Endocrine regulation of migratory departure from stopover: Evidence from a longitudinal migratory restlessness study on northern wheatears

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ABSTRACT

Most migrating birds make stopovers to replenish fuel stores. The decision to resume migration from stopover to a large extent shapes the temporal organization of migration. This decision is known to be shaped by a suite of intrinsic and extrinsic factors such as the bird's fuel stores and current weather conditions. However, how departures from stopover are physiologically regulated is largely unknown. We here present data that strongly indicate that corticosterone, a hormone with a stimulatory effect on locomotion, acts as a mediator between fuel stores and departure from stopover. In migrating northern wheatears (*Oenanthe oenanthe*) temporarily caged at stopover, we observed a positive relationship between the change in fuel stores and the concurrent change in glucocorticoid metabolite (GCM) levels measured in the birds' droppings. We also found a positive relationship between the change in GCM levels and the change in the intensity of nocturnal migratory restlessness. As in northern wheatears nocturnal migratory restlessness is an accurate proxy for stopover departure likelihood, our results indicate that corticosterone mediates between fuel stores and the decision to resume migration. Our unique longitudinal study represents a considerable advance in our understanding of the endocrine regulation of avian migration.

1. Introduction

Most songbird migrants travel between their breeding and wintering grounds following a stop-and-go strategy with alternating cycles of migratory movement (flight) and fuel accumulation (migratory fueling) during stopover. Stopover departure decisions, i.e. decisions when to leave a stopover site and resume migration, to a large extent determine the speed of migration and thereby arrival time at the breeding and wintering grounds (Schmaljohann and Both, 2017). Stopover departure decisions are known to be shaped by cues from innate rhythms (time within the migration season), intrinsic factors (e.g. fuel stores), and extrinsic factors (e.g. wind conditions) (Chernetsov, 2012; Jenni and Schaub, 2003), but how these cues are translated into actual departure is not very well known. Corticosterone, the main glucocorticoid hormone in birds, is thought to serve metabolic functions and to be involved in the uptake, storage and release of energy, and is known to have a stimulatory effect on locomotor activity (Landys et al., 2006, and references therein). Comparative and experimental work has indicated that circulating levels of corticosterone reflect an animal's energetic needs, both immediate, unforeseen demands and predictable demands occurring on a larger temporal scale, i.e. on a seasonal basis (e.g. Hau

et al., 2010; Jimeno et al., 2017; Romero, 2002). Avian migration is a life-history stage characterized by predictable high energy demands and extreme locomotor activity, rendering corticosterone a promising candidate for the regulation of migratory behavior. Löhms et al. (2006) found that exogenous corticosterone increased the frequency of visits to the food bowl in migrants temporarily caged at a stopover site, however, it was unknown if food intake increased or whether this observation was attributable to corticosterone stimulating locomotor activity. Currently, it seems that corticosterone's role in migratory fueling is merely permissive (Holberton et al., 2007; Landys et al., 2004), and that it does not stimulate fueling (Eikenaar, 2017). Rather, corticosterone may play a role in the regulation of departure behavior, and could even act as a mediator between departure cues and actual departure; corticosterone, at baseline or moderately elevated levels, has been linked to both the strength of intrinsic departure cues and to (proxies for) stopover departure likelihood. First, positive associations have been found between corticosterone level and migrants' fuel stores (e.g. Landys-Cianelli et al., 2002; Piersma et al., 2000) and time within the migration season (Falsone et al., 2009). Second, in passerine migrants, caught and temporarily caged at stopover, corticosterone levels were positively correlated with nocturnal locomotor activity (Eikenaar

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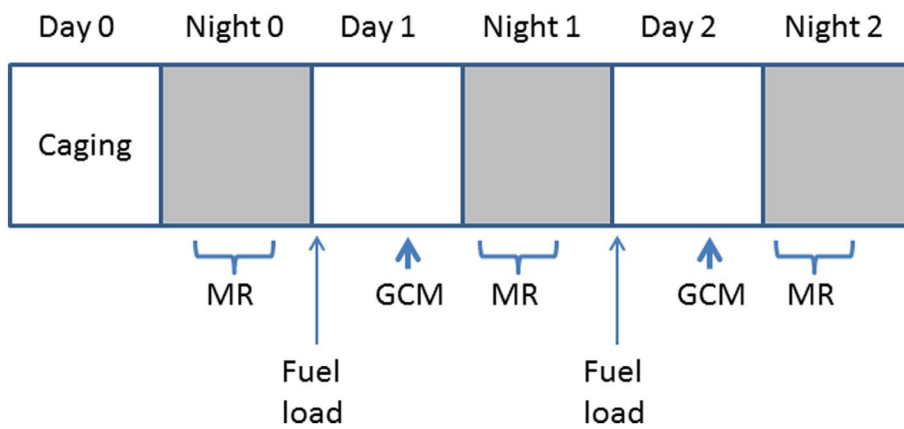


Fig. 1. Schematic representation of the experiment. Within-individual changes in the parameters of interest were calculated by subtracting the values measured on day 1 or night 1 from the values measured on day 2 or night 2. GCM: glucocorticoid metabolite sampling; MR: migratory restlessness measurements.

et al., 2014a; Löhmus et al., 2003), which may be indicative of stopover departure. Recently, a study on northern wheatears (*Oenanthe oenanthe*, wheatear hereafter), a long-distance migrant, integrated these fragmented observations. Eikenaar et al. (2017) showed that in wheatears caught at stopover during autumn, corticosterone levels increase with stronger departure cues, i.e. with the progress of the migration season, with more wind support, and tend to increase with larger fuel stores. Corticosterone in its turn appears to stimulate departure, as individuals with relatively high corticosterone levels had both higher departure likelihood and departed earlier within the night than individuals with relatively low corticosterone levels (Eikenaar et al., 2017). The mediating role of corticosterone may be ubiquitous among migrating birds; the patterns described for wheatears in Eikenaar et al. (2017) are virtually identical in common blackbirds (*Turdus merula*), a species with a migration strategy very different from that of wheatears (Eikenaar et al., under review). High corticosterone in migrants ready to depart is thought to have at least two functions: it probably regulates the release of the large amount of energy that the birds will soon need when they are flying (Landys-Cianelli et al., 2002; Piersma et al., 2000), and it likely has a direct effect on departure through corticosterone's stimulatory effect on locomotion (Landys et al., 2006, and references therein). The fact that corticosterone levels explain almost half of the variation in migrating wheatears' nocturnal departure time (Eikenaar et al., 2017) provides (circumstantial) evidence for the second function; energy requirements per time unit of flight unlikely differ between birds departing early or late in the night, hence, corticosterone does more than facilitating the release of energy from the fuel stores.

In most avian species, migrants travel individually, i.e. each bird makes its own decision when to depart from a stopover site to resume migration. Hence, when studying the physiological mechanism(s) regulating migratory decision making, longitudinal data are far more informative than cross-sectional data. It also means that if corticosterone indeed mediates between departure cues and departure behavior, then also *within an individual* migrant, corticosterone level should increase with stronger departure cues, and this increase should subsequently increase the individual's motivation to depart. In the current study we investigated this by measuring within individual changes in fuel stores (an intrinsic departure cue), corticosterone metabolite levels, and departure likelihood. To be able to collect these data, we temporarily caged migrating wheatears caught at a stopover site in spring. To avoid repeated blood-sampling within a short time period and to be able to measure several individuals simultaneously, we measured glucocorticoid metabolite (GCM) levels in the birds' excreta, a method which we have validated for wheatears and successfully used before (Eikenaar et al., 2014a, 2014b). We measured the birds' intensity of nocturnal migratory restlessness, which in wheatears is a good proxy for an individual's departure likelihood in the field (Eikenaar et al., 2014c). We expected that within-individual changes in fuel stores are predictive of changes in GCM levels, and that changes in GCM levels are predictive of

changes in the intensity of migratory restlessness.

2. Methods

2.1. Experimental procedures

The study was conducted on Helgoland (54°11' N, 07°55' E), a small island ca. 50 km off the German North Sea coastline. From 10 to 25 April 2016, migrating wheatears were caught using mealworm-baited spring traps. Upon trapping, birds were ringed, sexed on plumage following Svensson (1992), and wing length (maximum chord) was measured to the nearest 0.5 mm. Wing length was used to calculate lean body mass (LBM), employing a linear regression based on 220 'lean' wheatears caught on Helgoland in earlier studies: $LBM [g] = 0.29 \text{ g mm}^{-1} \times \text{wing length [mm]} - 6.85 \text{ g}$ (linear regression: $n = 220$, $F_{1,218} = 95.07$, $adj-R^2 = 0.30$, $p < 0.0001$, after Schmaljohann and Naef-Daenzer (2011)). Approx. 1–3 h after trapping, 26 birds (14 males and 12 females) were put in individual cages (40 × 40 × 30 cm) set up in two indoor rooms with artificial lighting. Upon caging, each bird received a food tray with 40 g of mealworms (*Tenebrio monitor*). Birds were held in captivity for three nights (Fig. 1), after which they were released. The lights in the rooms were switched on at local sunrise and again switched off at local sunset. The temperature in the rooms was held constant at approx. 20 °C, and birds had ad libitum access to water. Each morning, immediately after lights on, each bird was weighed to the nearest 0.1 g. The morning body mass was, together with LBM, used to calculate the bird's fuel stores at that moment: $\text{fuel load} = (\text{body mass [g]} - LBM [g]) / LBM [g]$. Fuel load thus represents the amount of fuel a bird carries relative to its lean body mass. Recent measurements of birds' fat stores by quantitative magnetic resonance indicated that wheatears temporarily caged on Helgoland can deposit significant amounts of fat (> 1 g at a LBM of approx. 22 g) within a single day (T. Klinner, unpublished data). This shows that the changes in fuel load that we report in the current study reflect changes in fat stores (and not water gain/loss). Immediately after being weighed each bird received a food tray with 40 g of mealworms. Food was removed 1 h before lights off and weighed to determine daily food intake.

For the measurement of GCM levels, in the first and second full day in captivity, birds' droppings were collected on paper sheets placed on the cage bottom. Droppings were collected during a 30 min period starting 8 h after lights on. As 30 min lies well below wheatears' gut passage time (Franz Bairlein, unpublished data, Eikenaar et al., 2014b), any stress resulting from the placing of the paper sheets could not have been detectable in our samples. For each bird, all droppings collected during a 30 min period were put in a sealable 2 ml eppendorf tube, weighed and homogenized, and frozen at –20 °C until later processing (see below).

Nocturnal migratory restlessness was recorded automatically with motion-sensitive microphones, attached to the right wall of each cage.

Each time a bird moved, this generated an impulse that was transmitted to a recording device. To avoid the recording of occasional non-migratory activity, we set a threshold of three impulses per second before it was recorded as an activity count (Maggini and Bairlein, 2010). Intensity of migratory restlessness was defined as the number of 15 min periods between 1 and 5.5 h after lights off, during which a bird showed at least five activity counts, as this proved to be a good approximation for the motivation of free-flying wheatears to resume migration (Eikenaar et al., 2014c).

All procedures were approved by the Ministry for Agriculture the Environment and rural Areas, Schleswig-Holstein, Germany. Ethics number: V 312-7224.123-11.

2.2. GCM assay

Homogenized samples were thawed at room temperature. To extract GCMs, 0.05 g excreta were transferred to new tubes and 0.5 ml 60% methanol was added (Palme et al., 2013). For samples < 0.05 g proportionally less methanol was added. Tubes were shaken for 1 min at maximum speed on a hand-vortex followed by 15 min shaking on a multi-vortex at 400 rpm. Tubes were centrifuged for 1 min and 100 μ l of the supernatant was transferred to new tubes, which were subsequently placed in a drying oven at 50 °C until the samples were dried down. Sample extracts were then shipped to Vienna (University of Veterinary Medicine) where samples were re-dissolved in 100 μ l of 60% methanol and diluted 1:5 in assay buffer. Samples were assayed using antibodies against 4-pregnene-17 α ,21-diol-3,11,20-trione-21-HS bound to bovine serum albumin (Stöwe et al., 2013). This assay has previously been validated for male and female wheatears with an ACTH-challenge (Eikenaar et al., 2014b). Samples were assayed in duplicate on three microplates with an intra-assay variation of 4.2%, and an inter-assay variation of 5.20% and 3.33% for the high and the low level pool, respectively. The samples of a given bird were always assayed on the same microplate.

2.3. Data analysis

We performed 2 sets of general linear models. In the first set, the change in GCM level from day 1 to day 2 (Fig. 1) was the dependent variable and the change in fuel load from day 1 to day 2 (Fig. 1) the explanatory variable. In the second set, the change in migratory restlessness from night 1 to night 2 (Fig. 1) was the dependent variable and the change in GCM level from day 1 to day 2 (Fig. 1) the explanatory variable. As in wheatears, refueling rate and associated stopover departure decisions may differ between the sexes (Dierschke et al., 2005; Schmaljohann et al., 2015), sex and the interaction between sex and the other explanatory variable were entered into the models. Model selection was done using stepwise backward elimination of non-significant terms ($p > 0.05$) in order of least significance. Cross sectional relationships (bivariate correlations) between fuel load and GCM level and between GCM level and migratory restlessness are shown in the Supplement.

3. Results and discussion

Daily food intake did not differ between day 1 and 2 (Paired-samples t -test: $t = -0.36$, $p = 0.27$, $n = 25$). With the exception of one individual, all birds accumulated fuel from the first to the second morning in captivity. The within-individual change in fuel load predicted the concurrent within-individual change in GCM levels (Table 1, Fig. 2). In its turn, the within-individual change in GCM levels from the first to second full day in captivity (day 1 to day 2, see Fig. 1) predicted the within-individual change in the intensity of migratory restlessness from night 1 to night 2 (Table 1, Fig. 3). Sex did not explain variation in the change of GCM level or migratory restlessness (Table 1).

In wheatears experimental moderate elevation of corticosterone

Table 1

Model summaries examining the changes in GCM level (top model) and changes in migratory restlessness (bottom model) in relation to changes in fuel load and changes in GCM level, respectively, and sex. Variable statistics are given as in the step prior to removal from the model. The final model is in bold. In all models $df = 1$.

Model	Variable	B \pm SE	t	p
GCM level (n = 26)	Fuel load	4171 \pm 1602	2.60	0.016
	Sex	-195 \pm 178	-1.10	0.29
	Sex * fuel load	447 \pm 3701	0.12	0.91
Migratory restlessness (n = 26)	GCM level	0.004 \pm 0.002	2.32	0.029
	Sex	0.34 \pm 1.60	0.21	0.84
	Sex * GCM level	-0.002 \pm 0.003	-0.47	0.64

Reference category for sex was female.

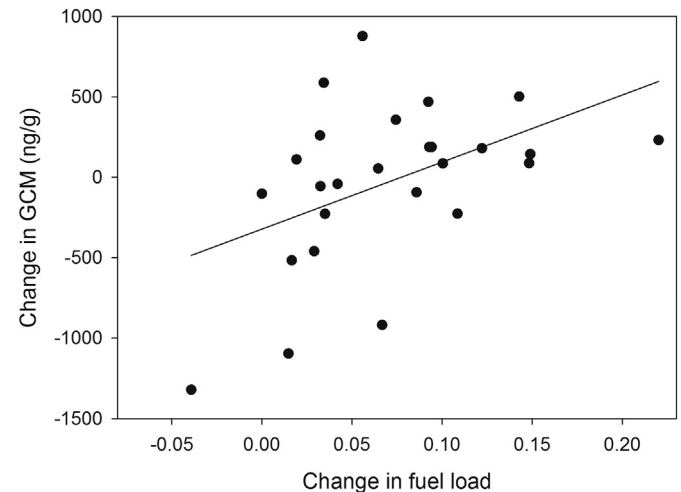


Fig. 2. The change in dropping glucocorticoid metabolite (GCM) levels from the first to the second full day in captivity in relation to the concurrent change in fuel load. N = 26.

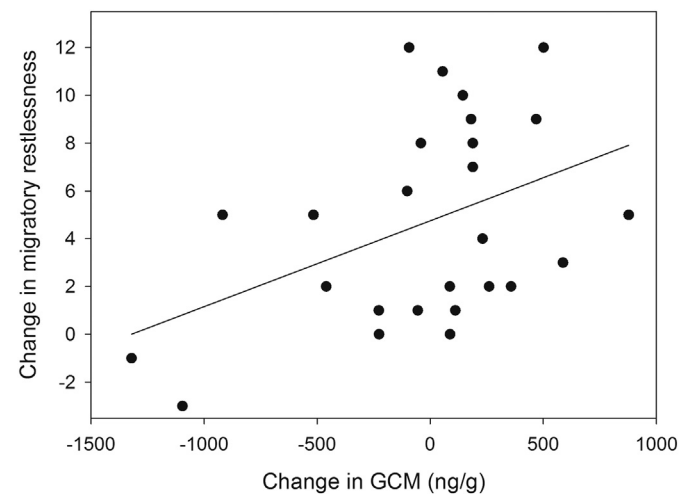


Fig. 3. The change in migratory restlessness from night 1 to night 2 (see Fig. 1) in relation to the change in dropping glucocorticoid metabolite (GCM) levels in the preceding days. N = 26.

level does not affect food intake and fuel accumulation (Eikenaar, 2017). Hence, the relationship between fuel load and GCM levels is very likely explained by fuel stores affecting corticosterone secretion and not vice versa. Also, in the current study the change in GCM levels from day 1 to day 2 was not correlated with the change in migratory restlessness from night 0 to night 1 (Spearman's $\rho = -0.21$, $p = 0.30$, $n = 26$), i.e. changes in locomotor activity did not predict changes in GCM levels. Hence, the changes in GCM levels likely caused the changes in

restlessness we observed, which matches the general finding that corticosterone stimulates locomotor activity in a variety of taxa (reptiles, e.g. Cash and Holberton, 1999; birds, e.g. Breuner et al., 1998; mammals, e.g. Mirza et al., 2016). In Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) injections with RU486, an antagonist to the low-affinity glucocorticoid receptor (GR), did not affect nocturnal locomotor activity (Landys et al., 2004). As corticosterone administration can have rapid effects on locomotor activity (e.g. Breuner et al., 1998), Landys et al. (2004) suggested that corticosterone does not regulate migratory restlessness through the GR, but perhaps through a non-genomic membrane-associated receptor. The molecular mechanisms of corticosterone's regulation of locomotor activity are unclear, but perhaps the neurosteroid 7 α -hydroxypregnenolone (7 α -OH PREG) is involved. 7 α -OH PREG has a stimulatory effect on locomotion in a variety of vertebrates, including Japanese quail (*Coturnix japonica*), and in newts, corticosterone at stress related levels stimulates 7 α -OH PREG synthesis (Tsutsui et al., 2017). Whether the same mechanism operates at (moderately elevated) baseline levels of corticosterone and in birds is currently unknown.

Irrespective of the exact molecular mechanism responsible for corticosterone's stimulatory effect on locomotion, the results from our unique longitudinal study very strongly indicate that corticosterone mediates between fuel stores and the expression of migratory restlessness, and thus stopover departure likelihood (Eikenaar et al., 2014c). Unexpectedly though, part of the birds did not increase their GCM levels even though they were refueling. Also unanticipated, some birds increased their migratory restlessness without an increase in GCM levels. The patterns depicted in Figs. 2 and 3 thus are slightly shifted from what we expected. These shifts are perhaps explained by the birds being initially stressed by caging. Undoubtedly, birds are stressed when taken from the wild into captivity, which is apparent from their high corticosterone levels, sometimes accompanied by body mass loss after caging (e.g. Adams et al., 2011; Angelier et al., 2017; Lattin et al., 2017; Wingfield et al., 1982). Some species acclimatize to being caged within a few days and recover body mass and decrease corticosterone levels (e.g. white-crowned sparrow, Wingfield et al., 1982), whereas in other species, caging stress is more chronic and can last several weeks (e.g. house sparrow (*Passer domesticus*), Lattin et al., 2017). Migrating wheatears appear to adapt exceptionally well to caging and most individuals immediately start to feed and increase their body mass (Corman et al., 2014; Eikenaar et al., 2014c; this study). Additionally, confinement does not seem to stress wheatears very much; five birds sampled after 5, 6, and 7 days of caging had a mean and SD corticosterone level of 3.10 ng/ml \pm 1.82 (C. Eikenaar, unpublished data), which is similar to that measured in free-flying wheatears (e.g. Eikenaar et al., 2013). Of course it is still possible that in the first few days of captivity wheatears are stressed and have higher circulating corticosterone levels. If, while being caged, the birds' stress gradually decreased due to familiarization, this would have resulted in a decrease of corticosterone levels from the first to second full day of caging. In other words, the increase in GCM level as a consequence of refueling is less apparent than expected because, while refueling, the birds acclimatize and the corticosterone signal from caging stress decreases. More structural detailed measurements of corticosterone levels in temporarily caged wheatears are required to determine whether wheatears are indeed an exception to the general pattern of high corticosterone secretion in caged wild birds.

To summarize, our longitudinal study on wheatears provides further support for the tentative conclusion from a previous cross-sectional study on this species stating that corticosterone mediates between departure cues and stopover departure likelihood (Eikenaar et al., 2017). This represents a considerable step forward in our understanding of the endocrine regulation of avian migration. Questions that now need answering are, for example, how corticosterone is upregulated when fuel stores increase. Also, corticosterone unlikely is the only hormone regulating stopover departure decisions. Previous work has indicated that

hormones such as melatonin and ghrelin may be part of the complex regulatory mechanism that underlies the temporal organization of migration (e.g. Fusani et al., 2013; Goymann et al., 2017). How these hormones together orchestrate one of the world's most spectacular and visible natural phenomena is a question that will require collective and integrative studies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yhbeh.2018.01.008>.

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