



Review article

Corticosterone's roles in avian migration: Assessment of three hypotheses

Carolyn M. Bauer^{a,*}, Heather E. Watts^{b,c}^a Department of Biology, Swarthmore College, Swarthmore, PA, USA^b School of Biological Sciences, Washington State University, Pullman, WA, USA^c Center for Reproductive Biology, Washington State University, Pullman, WA, USA

ARTICLE INFO

Keywords:

Bird
 Body condition
 Departure
 Fat
 Flight
 Glucocorticoid
 Hyperphagia
 Migratory restlessness
 Physiological preparation
 Stopover

ABSTRACT

While corticosterone (CORT) is often suggested to be an important hormone regulating processes necessary for avian migration, there has been no systematic assessment of CORT's role in migration. Prior to migration, birds increase fat stores and flight muscle size to prepare for the high energetic costs associated with long-distance flight. After attaining sufficient energetic stores, birds then make the actual decision to depart from their origin site. Once en route birds alternate between periods of flight and stopovers, during which they rest and refuel for their next bouts of endurance flight. Here, we evaluate three non-mutually exclusive hypotheses that have been proposed in the literature for CORT's role in migration. (1) CORT facilitates physiological preparations for migration [e.g. hyperphagia, fattening, and flight muscle hypertrophy]. (2) CORT stimulates departure from origin or stopover sites. (3) CORT supports sustained migratory travel. After examining the literature to test predictions stemming from each of these three hypotheses, we found weak support for a role of CORT in physiological preparation for migration. However, we found moderate support for a role of CORT in stimulating departures, as CORT increases immediately prior to departure and is higher when migratory restlessness is displayed. We also found moderate support for the hypothesis that CORT helps maintain sustained travel, as CORT is generally higher during periods of flight, though few studies have tested this hypothesis. We provide recommendations for future studies that would help to further resolve the role of CORT in migration.

1. Introduction

Migration is a challenging feat for animals. Prior to departure from breeding or overwintering sites, hereafter referred to as 'origin' locations, many animals expend significant energy and time to build energetic reserves (Brodersen et al., 2008; Ramenofsky and Wingfield, 2007), a process they will often need to repeat during stopovers – periods when travel temporarily stops to allow for recovery and refueling (Eikenaar et al., 2020a; Linscott and Senner, 2021) – along their migratory route (Sawyer and Kauffman, 2011; Alerstam et al., 2003). Once physiological preparation is complete, determining appropriate times to depart from origin and stopover sites are critical decisions (Bailleul et al., 2012; Sauman et al., 2005; Eikenaar et al., 2013), as animals must balance factors such as current energetic state, weather conditions, food and shelter availability at their current site, and the risk of continuing travel through unfamiliar terrain. Finally, the process of migrating, whether via walking, swimming, or flying, is also energetically demanding (Wyckoff et al., 2018; Hinch and Rand, 1998; Wikelski et al., 2003) and may require migrating animals to maintain high

metabolic rates for long periods of time.

Glucocorticoids (cortisol and corticosterone, hereafter CORT; see Box 1 for background on CORT physiology) are involved in processes central to migration, such as feeding, energy storage, locomotion, and energy use (Carruth et al., 2002; Seeber et al., 2018; Ramenofsky, 2011). CORT plays a permissive role in hyperphagia and lipogenesis and has therefore been suggested to be important for both migratory preparation and refueling at stopovers. CORT is also involved in the regulation of locomotor activity (Angelier et al., 2007; Breuner et al., 1998; Breuner and Hahn, 2003) and elevated CORT levels have been proposed to play a role in migratory departure (Cornelius et al., 2013; Eikenaar et al., 2020b). Finally, CORT facilitates energy mobilization via glycogenolysis, lipolysis, and gluconeogenesis (Sapolsky et al., 2000), and elevated CORT levels have also been implicated as important mediators during periods of sustained migratory travel.

Although CORT has often been considered to play an important role in the process of migration, there has been no systematic assessment of CORT's role in migration. Based on the published literature, we outline three main hypotheses for how CORT regulates seasonal migration

* Corresponding author.

E-mail address: cbauer1@swarthmore.edu (C.M. Bauer).<https://doi.org/10.1016/j.yhbeh.2021.105033>

Received 11 January 2021; Received in revised form 30 June 2021; Accepted 3 July 2021

Available online 14 July 2021

0018-506X/© 2021 Elsevier Inc. All rights reserved.

during the preparation, departure, and travel stages. Although these hypotheses derive primarily from the literature on bird migration, these hypotheses and the hypothetico-deductive framework we outline here may be applicable more broadly. For each hypothesis, we have developed a set of logical predictions, which we evaluate based on a review of the avian literature. We have focused on avian species as birds have been the best-studied model for understanding endocrine regulation of migration generally, and the role of CORT specifically (Cornelius et al., 2013; DeSimone et al., 2020; Ramenofsky, 2011; Ramenofsky et al., 2012; Watts et al., 2018). We have included studies focusing on spring and autumn migrations, which involve travel to breeding and overwintering sites, respectively. While spring and autumn migrations may be similar in terms of distance traveled, they often differ in other notable ways, such as speed of travel, conditions experienced en route, and physiological status (Bauer et al., 2019). Therefore, while we summarize findings from both seasons together, we do discuss potential seasonal differences for each prediction and hypothesis.

The first hypothesis for CORT's role in migration is that it facilitates physiological preparations for migration (hereafter called the Physiological Preparation Hypothesis; Fig. 1). As birds are preparing to depart from origin and stopover sites, they must focus on finding and consuming food to build fuel reserves (i.e., fat) to power their next flight stage. Previous studies have posited that CORT may facilitate these physiological preparations, as CORT has generally been shown to stimulate feeding behavior (Kitaysky et al., 2001) and lipogenesis (Holberton, 1999; Landys et al., 2004b) across several different stages of the annual cycle. However, while some studies have found support that CORT may help facilitate physiological preparations for migration, other studies have found opposing evidence (reviewed in Ramenofsky, 2011). We evaluated the Physiological Preparation Hypothesis by testing two main predictions: Prediction 1) CORT should be positively associated with feeding behavior, food intake, fattening, weight gain, and improvement in body condition either at stopover or prior to departure at origin, and Prediction 2) CORT levels should be elevated during the period of pre-departure preparation.

The second hypothesized role for CORT is that it stimulates departure from origin and stopover sites (hereafter called the Departure Stimulation Hypothesis; Fig. 1). CORT is a good candidate for this function as CORT has been shown to have positive relationships with overall activity levels (Breuner et al., 1998) and movement away from perturbations (Ramenofsky et al., 2012). In general, studies have shown that diel CORT rhythms typically peak just before the active period in most bird species (Breuner et al., 1999; Romero and Remage-Healey, 2000). If high baseline CORT levels generally stimulate a transition from resting to foraging and other daily activities, then it could be expected that higher CORT levels might also facilitate initiation of

migratory flight bouts. Furthermore, inclement weather cues such as a drop in temperature can elevate baseline CORT levels (de Bruijn and Romero, 2011), and studies have shown that wild birds with heightened CORT levels are more likely to abandon their current breeding site and translocate to lower altitudes (Breuner and Hahn, 2003). Therefore, as elevated CORT levels may signal the need to move away from sub-optimal habitats, then heightened CORT levels might also stimulate departure from origin and stopover sites (Fig. 1). We evaluated the Departure Stimulation Hypothesis by testing two predictions: Prediction 3) CORT should increase immediately prior to departure in free-living birds, and Prediction 4) CORT should be higher when migratory restlessness is displayed in captive birds.

The third hypothesized role of CORT is that it supports sustained migratory flight (hereafter called the Flight Support Hypothesis; Fig. 1). The process of flight is energetically expensive and requires high mobilization rates of energy reserves, most notably lipids (McWilliams et al., 2004). CORT is a likely mediator for this process, as CORT has been shown to facilitate lipolysis, gluconeogenesis, and proteolysis (Sapolsky et al., 2000). We evaluated the Flight Support Hypothesis by testing: Prediction 5) CORT should be higher during periods of flight compared to periods of no flight.

To test these three, non-mutually exclusive hypotheses, we systematically searched the literature to find published studies that measured baseline CORT in birds during and immediately before seasonal migration. We have focused on studies that measured baseline levels of CORT, as seasonal migration is not a stressor and is instead a regular and predictable stage of the annual cycle (Romero, 2002; see Box 1 for background on baseline and stress-induced CORT levels). We do recognize that stress-induced CORT levels may impact the process of migration, for example by stimulating birds to leave stopovers during poor weather conditions. However, such processes likely reflect an "emergency life history stage" that can occur at any point in the annual cycle, rather than being specific to migration (Lattin et al., 2016; Wingfield et al., 1998). This review should clarify our understanding of CORT's role in seasonal avian migration and provide a helpful framework for future studies.

2. Approach to literature search

We used major databases including Web of Science, SCOPUS, and Google Scholar to perform our literature search. Searches included combinations of the following terms: "corticosterone", "glucocorticoid*", "migrat*", and "avian OR bird*". After completing these searches, we checked the references of each preliminary article as well as all papers that cited each preliminary article in an effort to find papers that did not show up in our initial database searches.

We then excluded any studies that did not occur during the migratory

Box 1

Overview of CORT physiology

Secretion of CORT is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Release of corticotropin-releasing factor (CRF) from the hypothalamus stimulates secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn stimulates release of CORT from the adrenal glands into the bloodstream. HPA-axis activity is regulated in part by negative feedback at multiple levels of the HPA-axis, with elevated CORT levels leading to suppression of further CRF and ACTH release. As CORT circulates it exerts effects on target tissues by binding to two different receptor types: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). MR has high-affinity for CORT and is activated when CORT levels are low. On the other hand, GR is a low-affinity receptor that is activated primarily when CORT levels are high. The availability of CORT to activate MR and GR receptors can be further modulated by carrier proteins (corticosterone-binding globulin [CBG]) and conversion enzymes (11 β hydroxysteroid dehydrogenase [HSD] type 1 and type 2), as CORT bound to CBG cannot activate receptors, and conversion enzymes can convert CORT to its inactive form (11 β -HSD Type 2) or vice versa (11 β -HSD Type 1).

Unperturbed animals generally display relatively low levels of CORT (referred to as baseline levels), which are important in supporting basic physiological processes (Sapolsky et al., 2000). At these baseline levels, CORT likely has substantial binding only to MR. Within a few minutes of exposure to unpredictable and/or noxious stimuli (i.e. stressors), however, circulating CORT levels rapidly elevate and can be termed "stress-induced levels." At these stress-induced levels, CORT likely generates different physiological and behavioral effects via binding to GR.

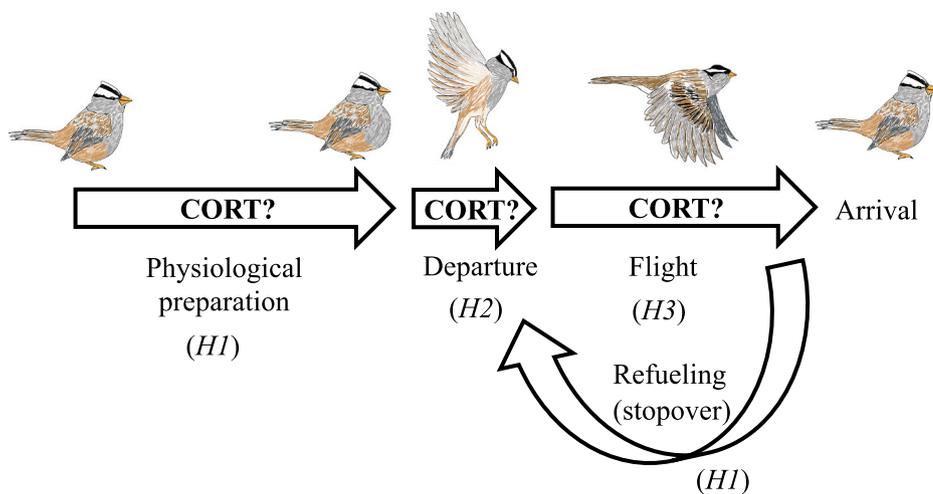


Fig. 1. The migratory life cycle stage includes a number of substages during which CORT may be an important regulatory signal. Many birds make physiological preparations (e.g., fat deposition, flight muscle hypertrophy) for migration. CORT is hypothesized to stimulate these preparations (H1). Following preparation, CORT has been hypothesized to be involved in stimulating departure (H2); here CORT could operate on the scale of days to signal a window of readiness to depart or on the scale of hours to determine the precise timing of departure. Once birds depart there is a period of active flight. CORT has been hypothesized to support this metabolically demanding period (H3). In many species, migration involves stopovers en route in which birds stop to refuel before departing for another bout of flight. CORT may be involved in stimulating preparations that are part of refueling (H1) and departures (H2) during stopovers as well.

or pre-migratory period. For field studies, we limited our analyses to studies that collected data during times of year that birds were preparing for or actively undergoing migration. For laboratory studies, we limited our analyses to studies that occurred either i) under natural photoperiods that matched when birds would prepare for or migrate in the wild, ii) during a transition from long-days to short-days that would simulate autumn migration in that species, or iii) during a transition from short-days to long-days that would simulate spring migration in that species. For laboratory studies, we also excluded those that did not give birds ample time (2+ weeks) to habituate to captivity, unless the study showed evidence of no captivity stress in recent, wild-caught birds (Eikenaar et al., 2019). Though we note that prolonged captivity (Dickens et al., 2009) and repeated sampling (Cyr and Romero, 2009) can influence circulating CORT levels.

Regardless of setting (field or captivity), only studies that measured baseline CORT levels were included, with the exception of studies that experimentally manipulated CORT signaling via administration of exogenous CORT, glucocorticoid agonists, or glucocorticoid antagonists. Included studies needed to measure circulating plasma levels of CORT within 3 min of stress exposure (Romero and Reed, 2005), or needed to measure CORT metabolites within a timeframe where metabolites would represent circulating CORT levels in an unperturbed state. We do recognize, however, that confounding factors such as diet, sex, and season may impact the extent to which CORT metabolites levels reflect circulating CORT levels (Goymann, 2012). We also avoided comparing CORT values between studies, as Fanson et al. (2017) have shown high inter-lab variation in CORT measurement through radio- or enzyme-immunoassays.

CORT has also been proposed to be an important mediator of escape movements, including fugitive migrations, that occur as part of an emergency life history stage when circulating CORT levels are elevated to stress-induced levels (Wingfield et al., 1998). However, the cues stimulating these movements and the role of CORT may differ substantially from what occurs during more predictable seasonal migrations (Watts et al., 2018). Therefore, we did not include studies of fugitive migration in this review. But we note that fugitive migrations are another context in which more work is needed in order to understand the role(s) of CORT in regulating migratory responses more broadly.

In general, our predictive framework required that studies had some sort of control comparison, either via a control group or through repeated measures of the same individuals. We therefore excluded field studies that only had population-level, rather than individual-level, comparisons across time (Reneerkens et al., 2002) or across sites (O'Reilly and Wingfield, 1995), as these approaches did not allow direct tests of our predictions. We recognize that this approach excludes a large amount of work examining endocrine mechanisms in avian migration-

notably, cross-sectional studies capturing birds near geographic barriers, and spring/autumn comparisons. However, these studies are well reviewed in more general reviews and syntheses of avian migration physiology (Cornelius et al., 2013; DeSimone et al., 2020; Ramenofsky, 2011; Ramenofsky et al., 2012). We also note that our approach results in a heavy focus on longitudinal studies, which skews our selection of studies towards captive studies (versus field), and towards studies that use repeated blood sampling, which may affect circulating CORT levels (Cyr and Romero, 2009). We provide more detail regarding the selection of articles for each specific hypothesis and prediction, below.

3. Hypothesis 1: Physiological Preparation Hypothesis

3.1. Prediction 1: CORT should be positively associated with feeding, fattening, muscle hypertrophy, and body mass gain either at stopover or prior to departure at origin

The hypothesis that CORT promotes migration by stimulating physiological preparations before departure leads to the prediction that CORT should be positively related with physiological preparation. Physiological preparation for migration encompasses numerous processes, including those involved in fat deposition, muscle hypertrophy, changes in organ size, and blood composition (Berthold, 1996), and a variety of variables (e.g., feeding behavior, changes in body fat and body mass) have been used to measure these processes. Here, we categorized measures of physiological preparation into three main categories starting with those that reflect behavioral processes (feeding), then those that measure specific body tissues (measures of fat and muscle), and finally integrative tissues measures (body mass). Explanations of the different variables included in these three categories are detailed in Table 1. At origin sites, physiological preparation can be examined over the few weeks prior to departure, but can also be examined on a shorter, daily timescale. However, for stopover periods, which may be as short as a few days, data are often limited to a daily timescale. To test this prediction, we searched for studies in two main categories: i) those that used exogenous CORT, glucocorticoid agonists, or glucocorticoid antagonists to manipulate CORT signaling and then assessed changes in one or more measures of physiological preparation, and ii) those that examined naturally circulating CORT levels and measures of physiological preparation in birds either transitioning into the migratory state or caught during stopovers.

Because our prediction posits that CORT is stimulating or positively changing physiological variables such as food intake or weight gain, in our second category (studies examining naturally circulating levels of CORT) we restricted our analysis to studies that collected repeated measures of physiological variables. Since data collected from a single

Table 1

Explanation of different terms used to describe physiological preparation in migratory birds. We grouped variables into three categories: feeding, tissue-specific measures, and integrative tissue measures. Tissue-specific measures assessed either fat (fat score and fuel load) or muscle.

Category	Response variable	Definition	Synonyms, related terms
Feeding	Feeding behavior	Number of trips to the food dish over time	Hyperphagia
	Food intake	Amount of food consumed over time	Hyperphagia
Tissue-specific measures	Fat score	Visual estimation of subcutaneous abdominal and/or furcular fat stores, usually scored on low-high scale of 0–5	Fattening, fat deposits, fat stores
	Fuel load	Calculated as [(body mass – lean body mass) / lean body mass]. Lean body mass determined from species-specific structural measurements.	Non-lean body mass, fuel deposition rate (change in fuel load over time)
	Muscle score	Visual estimation of pectoralis muscle size, usually scored on low-high scale of 0–3	Muscle hypertrophy, flight muscle profile, flight muscle size
Integrative tissue measures	Body mass	Body mass, or body weight	Weight gain

time point provide no information about whether and how a physiological variable is changing, use of repeated measures allows for better assessment of whether birds were indeed preparing for eventual departure. The limited utility of single time point correlations in this context is illustrated by findings that in refueling Bar-tailed Godwits (*Limosa lapponica*), there is a positive relationship between baseline CORT and body mass as well as a positive relationship between time

since arrival and body mass (Landys-Ciannelli et al., 2002); with these data one cannot identify whether birds with higher CORT are undergoing greater increases in body mass or whether CORT levels became elevated as birds achieve greater body mass (Eikenaar et al., 2013; Piersma et al., 2000).

a) Studies manipulating glucocorticoid signaling

Experimental manipulations testing relationships between CORT and feeding have found mixed, but generally weak, support for the prediction that elevated CORT levels are associated with higher rates of feeding (Table 2a). The study with the strongest support for this prediction is Löhmus et al. (2006), which found that captive Red-eyed Vireos (*Vireo olivaceus*) that voluntarily consumed a CORT-injected mealworm during autumn migration made more trips to the food dish compared to control birds. However, we note that food dish visitation rates do not necessarily reflect actual food intake; CORT administration may increase overall activity rates, which could cause birds to more frequently be in proximity to the food dish. Indeed, Landys et al. (2004a) found that injections of a glucocorticoid antagonist (RU486, which blocks binding of CORT at the glucocorticoid receptor), decreased feeding behavior in captive Red Knots (*Calidris canutus*) during autumn migration but did not significantly affect rates of food intake. On the other hand, Landys et al. (2004b) found that compared to control birds, RU486 implants decreased food intake in spring migratory White-crowned Sparrows (*Zonotrichia leucophrys*) as would be expected under the prediction that CORT promotes food intake. However, interpretation of this study is complicated by the fact that CORT concentrations were lower in RU486-treated birds compared to controls, which is opposite of what should happen as antagonism of glucocorticoid receptors should also reduce negative feedback and lead to elevated CORT levels. This unexpected outcome therefore casts doubt on whether RU486 acted as a true CORT antagonist or may be causing effects via antagonism of progesterone receptors (Landys et al., 2006). In contrast to using a

Table 2a

Summary of studies examining the Physiological Preparation Hypothesis, under Prediction 1 that corticosterone (CORT) is positively associated with feeding (either feeding behavior or food intake rates), fat (fat score, fuel load, or fuel deposition rate) increase, and body mass increase during preparation for migration. Studies tested this prediction via a) pharmacological manipulation of CORT signaling or b) examination of naturally circulating CORT levels. Studies using hormone manipulations gave birds either exogenous CORT, RU486 (a glucocorticoid receptor antagonist), or dexamethasone (DEX, a glucocorticoid receptor agonist).

Reference	Species	Response variable	Hormone manipulation	Season	Supports prediction?
a) Studies using hormone manipulations					
Löhmus et al., 2006	<i>Vireo olivaceus</i>	Feeding behavior	CORT	Autumn	Yes
Landys et al., 2004b	<i>Zonotrichia leucophrys</i>	Food intake	RU486	Spring	Yes
Dolnik and Blyumental, 1967	<i>Fringilla coelebs</i>	Feeding behavior	CORT	Autumn	No
Landys et al., 2004a	<i>Calidris canutus</i>	Food intake	RU486	Autumn	No
Holberton et al., 2007	<i>Junco hyemalis</i>	Food intake	DEX	Spring	No
Eikenaar, 2017	<i>Oenanthe oenanthe</i>	Food intake	CORT	Autumn	No
Landys et al., 2004b	<i>Zonotrichia leucophrys</i>	Fat score	RU486	Spring	Yes
Holberton et al., 2007	<i>Junco hyemalis</i>	Fat score	DEX	Spring	No
Eikenaar, 2017	<i>Oenanthe oenanthe</i>	Fuel load	CORT	Autumn	No
Holberton et al., 2007	<i>Junco hyemalis</i>	Body mass	DEX	Spring	No
b) Studies measuring naturally circulating CORT					
Henshaw et al., 2009	<i>Luscinia luscinia</i>	Food intake		Autumn	No
Holberton et al., 2008	<i>Junco hyemalis</i>	Fat score		Spring	Yes
Fudickar et al., 2016	<i>Junco hyemalis</i>	Fat score		Spring	No
Wingfield et al., 1996	<i>Zonotrichia leucophrys</i>	Fat score		Spring	No
Ramenofsky et al., 2017	<i>Zonotrichia leucophrys</i>	Fat and muscle score		Spring	No
Robart et al., 2019	<i>Spinus pinus</i>	Fat and muscle score		Spring	No
Eikenaar et al., 2018b	<i>Oenanthe oenanthe</i>	Fuel load		Spring	Yes
Eikenaar et al., 2014a	<i>Oenanthe oenanthe</i>	Fuel deposition rate		Autumn	No
Eikenaar et al., 2013	<i>Oenanthe oenanthe</i>	Fuel deposition rate		Spring	No
Holberton, 1999	<i>Setophaga coronata</i>	Body mass		Spring	Yes
Holberton et al., 2008	<i>Junco hyemalis</i>	Body mass		Spring	Yes
Piersma et al., 2000	<i>Calidris canutus</i>	Body mass		Spring	Yes
Landys et al., 2004a	<i>Calidris canutus</i>	Body mass		Autumn	Yes
Robart et al., 2019	<i>Spinus pinus</i>	Body mass		Spring	No
Ramenofsky et al., 2017	<i>Zonotrichia leucophrys</i>	Body mass		Spring	No
Henshaw et al., 2009	<i>Luscinia luscinia</i>	Body mass		Autumn	No

glucocorticoid antagonist, [Holberton et al. \(2007\)](#) used a glucocorticoid receptor agonist (dexamethasone) on spring migratory Dark-eyed Juncos (*Junco hyemalis*), and found that injections of dexamethasone caused no changes in food intake compared to controls. [Dolnik and Blyumental \(1967\)](#) similarly found that feeding rates were not elevated in autumnal pre-migratory Chaffinches (*Fringilla coelebs*) injected with hydrocortisone compared to controls. Finally, in contrast to invasive methods that directly inject birds with pharmacological compounds, [Eikenaar \(2017\)](#) increased CORT by feeding Northern Wheatears (*Oenanthe oenanthe*) mealworms injected with CORT, and found that CORT-supplementation did not significantly affect food intake in birds during a simulated autumnal migratory stopover compared to controls. Overall, these studies provide very little support for the prediction that CORT stimulates increased feeding in migratory birds.

Several studies have also tested the predictions that fattening and muscle hypertrophy are positively related with CORT in migratory birds ([Table 2a](#)). The study that most supports this prediction is [Landys et al. \(2004b\)](#), who found that while CORT implants increased fat deposition in captive White-crowned Sparrows compared to controls, this fat increase was negated with concurrent implantation of a CORT antagonist (RU486). Other studies, in contrast, do not support this prediction, as both [Holberton et al. \(2007\)](#) and [Eikenaar \(2017\)](#) did not find that exogenous glucocorticoids increased fattening in spring migratory Dark-eyed Juncos and autumnal migratory Northern Wheatears, respectively. Specifically, [Holberton et al. \(2007\)](#) found that repeated injections of a glucocorticoid agonist (dexamethasone) did not affect fat score and actually decreased body mass, while [Eikenaar \(2017\)](#) found that non-invasive CORT supplementation failed to affect non-lean body mass. Together, these studies provide mixed support for the prediction that CORT stimulates fattening and weight gain in migratory birds.

b) Studies examining naturally circulating CORT in captive birds

Several studies have examined circulating CORT levels in captive birds during the pre-migratory and migratory periods ([Table 2a](#)). Many of these studies involve long-term housing of birds and use photoperiodic manipulations to transition birds into spring or autumnal migratory states. Other studies are shorter-term, and usually involve capturing birds during stopovers and housing them for several days. We only found one study investigating feeding and CORT levels during avian migration ([Table 2a](#)). [Henshaw et al. \(2009\)](#) found that exposing Thrush Nightingales (*Luscinia luscinia*) to magnetic fields simulating a stopover location at the onset of autumn migration caused an increase in food intake over time but no change in baseline CORT. Therefore, this study does not support the prediction that elevated CORT facilitates hyperphagia during stopovers.

Similar to studies on feeding, there is little support for the prediction that CORT is positively related with rates of fattening and muscle hypertrophy ([Table 2a](#)). When [Holberton et al. \(2008\)](#) transitioned captive Dark-eyed Juncos from short- to long-day photoperiods to stimulate the transition to a spring migratory state, they found an increase in both subcutaneous fat stores and baseline CORT. However, CORT was not highest during the time periods of greatest fat deposition and was instead highest when birds had reached their peak fat score. This pattern would be more consistent with a role for CORT in maintaining peak levels of fat deposits and/or with CORT responding to changes in fat stores and elevated CORT levels reflecting positive/enhanced body condition ([Eikenaar et al., 2013](#); [Piersma et al., 2000](#)). In a study of captive Dark-eyed Juncos, [Fudickar et al. \(2016\)](#) found no relationship between changes in fattening and CORT as birds exposed to natural photoperiods increased fat scores but did not change baseline CORT levels during the spring migratory preparation period. Also inconsistent with the prediction are the findings of [Wingfield et al. \(1996\)](#), who examined captive Gambel's White-crowned sparrows during the spring pre-migratory period and found that while birds had significantly greater fat stores after photostimulation, changes in circulating CORT

levels did not parallel these changes in fat. [Ramenofsky et al. \(2017\)](#) also examined captive Gambel's White-crowned Sparrows during the spring migratory preparation period, and also found an increase in fat score and flight muscle profile but no change in baseline CORT levels. [Robart et al. \(2019\)](#) also looked at flight muscle and fat changes during the spring migratory preparation period in captive Pine Siskins (*Pinus spinus*), but found no changes in baseline CORT over time whereas fat deposits and flight muscle size increased. Overall, these studies provide little support for the prediction that elevated CORT enhances fat deposition and flight muscle hypertrophy in migratory birds preparing for departure.

While some studies assess fat stores through visual scoring, other studies have quantified fat stores as “fuel load” by calculating the ratio of total body mass to lean body mass ([Table 1](#)) ([Schmaljohann and Naef-Daenzer, 2011](#)). Studies assessing changes in fuel load (i.e., fuel deposition rate) have been conducted in the context of migratory stopovers, and the majority have not found the predicted relationships with baseline CORT ([Table 2a](#)). In captive Northern Wheatears, fuel deposition rate is negatively correlated with baseline CORT levels during both autumnal ([Eikenaar et al., 2014a](#)) and spring ([Eikenaar et al., 2013](#)) stopovers, the opposite pattern of the prediction. An additional study in Northern Wheatears that examined changes in fecal glucocorticoid metabolites (fGCM) and fuel deposition rate across two nights during a spring stopover found a positive relationship between these variables ([Eikenaar et al., 2018b](#)), which the authors interpreted as consistent with CORT levels increasing in response to changing fuel load, rather than stimulating fueling.

Studies examining body mass and baseline CORT relationships have found mixed support for the prediction that CORT facilitates migratory preparation ([Table 2a](#)). Results from [Henshaw et al. \(2009\)](#) do not support this prediction, as Thrush Nightingales exposed to magnetic fields simulating a stopover site at the onset of autumn migration increased in weight but did not change baseline CORT levels. Both [Robart et al. \(2019\)](#) and [Ramenofsky et al. \(2017\)](#) also saw increases in body mass but no changes in baseline CORT levels in captive Pine Siskins and White-crowned Sparrows entering the spring migratory condition, respectively. In contrast, four studies using captive birds have found that both baseline CORT and body mass increase during the transition into a migratory state (Yellow-rumped Warblers (*Setophaga coronata*), [Holberton, 1999](#); Dark-eyed Juncos, [Holberton et al., 2008](#); Red Knots, [Landys et al., 2004a](#) and [Piersma et al., 2000](#)). However, in all four of these studies, CORT levels were not highest during the periods of most rapid weight gain and were instead highest when birds had reached their peak body mass. This pattern is exemplified in the study by [Piersma et al. \(2000\)](#), who specifically analyzed how CORT varied with rate of body mass change, and found that CORT levels peaked when body mass was stable and high. If, as these findings suggest, CORT is not stimulating increases in body mass, then the positive relationship between body mass and baseline CORT observed in many studies could arise either because (i) high body mass stimulates CORT secretion, and therefore CORT may be an indicator of energetic readiness for departure, or (ii) both CORT and body mass increase as departure nears, but these two variables are not directly related with each other. Either of these relationships could occur under Hypothesis 2 (that CORT stimulates migratory departure).

3.2. Prediction 2: CORT levels should be elevated during the period of pre-departure preparation

Although the period of “pre-departure preparation” is difficult to define, we focused on studies that sampled birds during stopovers or in what was expected to be within 3 weeks of migratory departure from breeding or wintering grounds. We did not include studies that measured circulating CORT levels during different stages of the annual cycle more than several weeks apart, as such studies do not allow us to distinguish between changes in CORT occurring around migration and changes in CORT related to other transitions across the annual cycle. For

example, many studies measure CORT during winter, spring migration, and early breeding. Because CORT levels typically increase during breeding (Romero, 2002), it is difficult to determine whether an increase in CORT during spring migration is caused by the anticipation of breeding or is instead related to migratory processes. The same may be true of autumn migration, as studies usually measure CORT during molt, autumn migration, and winter. Because CORT levels are typically at their nadir during molt (Romero, 2002), higher levels during autumn migration may represent a return to unsuppressed levels, rather than processes related to migration. Thus, to avoid confounding changes in CORT driven by these other stages of the annual cycle, we focused on the period of pre-migratory preparation within a few weeks of departure. We also included studies that examined migratory birds during stopovers, as birds refuel and make physiological preparations for migratory flight during this time period. Studies generally fell into two categories: a) those that compared migratory and non-migratory individuals of a single species (i.e., partial migrants) living under the same environmental conditions during the pre-migratory or stopover stages, and b) those that took repeated samples from a migratory population over the pre-migratory or stopover period. For studies that took repeated CORT samples, we compared CORT measures before and during the timepoints when birds began increasing food intake, fattening, or gaining body weight, with the prediction that CORT should be elevated during the period of physiological preparation compared to earlier timepoints where body condition remained constant. For studies comparing migrants and non-migrants within a species, we note that differences between migrants and non-migrants in the timing of life history transitions (e.g., transition into the breeding state in non-migrants, while migrants are preparing for spring migration), may affect baseline CORT comparisons between these groups and must be considered when interpreting results.

a) Studies comparing migratory and non-migratory individuals

For species that contain both migratory and sedentary strategies (either at the subspecies or individual level), elevated CORT levels during the pre-departure period could be indicated by migratory individuals having higher baseline CORT levels than non-migratory individuals at this time when living under the same environmental conditions (Table 2b). This prediction is supported by a study examining Blue tits (*Cyanistes caeruleus*), which found that migratory females had higher fecal glucocorticoid metabolite levels than resident females during an autumnal stopover (Nilsson and Sandell, 2009). We note, however, the direct comparisons between resident and migrant Blue Tits

in this study are complicated by the fact that migrants and residents were captured at different sites, and Blue Tits are not a strongly migratory species. Similarly, four different studies in European blackbirds (*Turdus merula*) also provide mixed support. Eikenaar et al. (2015, 2018a) found that baseline CORT levels were slightly higher in migratory versus non-migratory blackbirds during autumn, but not spring, stopovers (Eikenaar et al., 2015). Fudickar et al. (2013) also found that migratory blackbirds had slightly higher CORT levels than resident blackbirds during the autumnal pre-migratory period. But, in another study during the autumn pre-migratory period, Schwabl et al. (1984) found that while baseline CORT was significantly higher in migratory versus non-migratory first-year males, it did not differ with migratory status in adult males, adult females, or first-year females. Furthermore, in captive individuals, Schwabl et al. (1984) found that while migratory female blackbirds had higher circulating baseline CORT levels than non-migratory females during the pre-migratory period, the opposite was true in males. While European Blackbird studies provide mixed support that CORT may be facilitating processes necessary for migratory preparation, studies in other species provide no support for this prediction. Ramenofsky et al. (2017) found no differences in baseline CORT levels between migratory (Gambel's, *Zonotrichia leucophrys gambelli*) and resident (Nuttall's, *Z. l. nuttalli*) White-crowned Sparrows during the period leading up to spring migratory restlessness when housed in captivity under common garden conditions. Furthermore, both field (Bauer et al., 2016) and laboratory common garden (Fudickar et al., 2016) studies in Dark-eyed Juncos found that resident Dark-eyed Juncos had significantly higher baseline CORT levels than migrant individuals during the spring pre-migratory period – a pattern that is in direct contrast to the original prediction.

b) Repeated CORT sampling over the pre-departure period

In contrast to studies comparing migratory and non-migratory individuals, there were fewer studies that tested whether naturally circulating CORT is elevated over the pre-departure period (Table 2b). One of the studies that supports this prediction found that captive Dark-eyed Juncos shifted from short- to long-day photoperiods significantly increased circulating levels of baseline CORT (Holberton et al., 2008). However, Robart et al. (2019) found an increase in migratory restlessness and body condition but did not find an increase in baseline CORT levels in a captive nomadic and irruptive migrant (Pine Siskins) during the spring pre-migratory period. Similarly, Ramenofsky et al. (2017) also did not find an increase in baseline CORT across the spring pre-migratory period in captive Gambel's White-crowned Sparrows.

Table 2b

Summary of studies examining the Physiological Preparation Hypothesis, under Prediction 2 that corticosterone (CORT) increases during the period of pre-departure preparation. Studies tested this prediction by either a) comparing migratory and non-migratory individuals within the same species or b) taking repeated CORT measures over the period of migratory preparation in either free-living ("field") or captive ("lab") birds.

Reference	Species	Field/lab	Photoperiod	Migratory stage	Season	Supports prediction?
a) Migrant vs. non-migrant studies						
Nilsson and Sandell, 2009	<i>Cyanistes caeruleus</i>	Field	Natural	Stopover	Autumn	Yes
Fudickar et al., 2013	<i>Turdus merula</i>	Field	Natural	Origin	Autumn	Yes
Eikenaar et al., 2018a	<i>Turdus merula</i>	Field	Natural	Stopover	Autumn	Yes
Schwabl et al., 1984	<i>Turdus merula</i>	Field	Natural	Origin	Autumn	Mixed ^a
Eikenaar et al., 2015	<i>Turdus merula</i>	Field	Natural	Stopover	Autumn	Yes
					Spring	No
Ramenofsky et al., 2017	<i>Zonotrichia leucophrys</i>	Lab	Natural	Origin	Spring	No
Bauer et al., 2016	<i>Junco hyemalis</i>	Field	Natural	Origin	Spring	No
Fudickar et al., 2016	<i>Junco hyemalis</i>	Lab	Natural	Origin	Spring	No
b) Repeated measures studies						
Holberton et al., 2008	<i>Junco hyemalis</i>	Lab	Unnatural ^b	Origin	Spring	Yes
Robart et al., 2019	<i>Spinus pinus</i>	Lab	Natural	Origin	Spring	No
Ramenofsky et al., 2017	<i>Zonotrichia leucophrys</i>	Lab	Natural	Origin	Spring	No

^a CORT was significantly higher in wild migrants vs. non-migrants in first-year males, but not in first-year females or older males and females, and CORT was significantly higher in captive migrant vs. non-migrant females, but was lower in captive migrant vs. non-migrant males.

^b Transitioned abruptly from 10.5L:13.5D to 18L:6D.

Conflicting findings from these studies could be due to photoperiod manipulations, as Robart et al. (2019) and Ramenofsky et al. (2017) used natural photoperiods, whereas Holberton et al. (2008) abruptly transitioned juncos from 10.5L:13.5D to 18L:6D. Given that natural photoperiods are more representative of wild conditions, we suggest that these studies are more relevant for testing predictions in migratory birds.

3.3. Synthesis of predictions for Physiological Preparation Hypothesis

Looking across all studies, there is generally weak support for the hypothesis that CORT stimulates physiological preparation for migration (Tables 2a–2b). For the prediction that CORT is positively related with feeding, fat/muscle, and body weight (Prediction 1), support was found in two out of six pharmacological studies (33%) and five out of twelve (42%) studies of naturally circulating CORT levels. However, as mentioned earlier, all of the studies that found positive relationships between naturally circulating CORT and fat stores or body mass did not see peak CORT levels during the time periods of most rapid gain, therefore casting doubt on whether elevated CORT is a cause or consequence of fat and body mass increases. For the prediction that CORT is elevated during the pre-departure period (Prediction 2), three out of eight (38%) found decisive evidence that CORT levels were higher in migratory vs. non-migratory individuals during pre-migration or stopover, while one out of three (33%) repeated measures studies found that CORT increased as birds entered the preparatory period.

Since we considered both spring and autumn migration in testing this hypothesis, differences across studies in the results could reflect differences between these two migratory periods. For Prediction 1, this does not appear to be the case, with no consistent difference between spring and autumn migration. However, all studies of partial migrants (Prediction 2) that found complete or mixed support for the prediction were carried out during the autumn (Eikenaar et al., 2018a; Fudickar et al., 2013; Nilsson and Sandell, 2009; Schwabl et al., 1984), whereas all studies that found no support for the prediction were carried out during the spring (Bauer et al., 2016; Eikenaar et al., 2015; Fudickar et al., 2016; Ramenofsky et al., 2017). This could be explained by several mutually exclusive hypotheses. First, CORT levels may indeed increase during the time of migratory pre-departure, but perhaps this can only be distinguished during the autumn as during the springtime residents are increasing CORT in anticipation of breeding (Romero, 2002), therefore obscuring the difference in CORT levels between migrants and non-migrants during spring but not autumn. Second, CORT levels may not notably increase during the migratory pre-departure period, and lower CORT levels in residents vs. migrants during autumn could be due to residents finishing their molt later than migrants. This could be a likely explanation as CORT levels are typically very low during molt (Romero, 2002) and residents have less pressure to finish their molt quickly (Helm and Gwinner, 2006). A third alternative hypothesis is that spring and autumn migration have different demands, and therefore CORT levels may only significantly increase during autumnal preparation. We find this last hypothesis the least likely, as studies generally indicate that the energetic demands of migration are greater during spring compared to autumn (Bauer et al., 2019; Romero et al., 2017).

Another factor that could contribute to variation in results across studies could be differences in physiological preparation between origin and stopover sites. The partial migrant studies that supported Prediction 2 generally were conducted during stopovers rather than prior to migration. For the pre-migratory time period, only one out of five (20%) studies found support for the prediction that CORT is elevated during migratory preparation (Fudickar et al., 2013), and this support did not quite reach statistical significance. In contrast, all studies conducted during stopovers found either complete (Eikenaar et al., 2018a; Nilsson and Sandell, 2009) or mixed (Eikenaar et al., 2015) support. Differences between stopovers and departure from breeding/wintering grounds are likely linked with temporal constraints on sampling, as stopovers are

generally shorter in duration (e.g., lasting few days) than the pre-migratory period. As the stopover period is an intense period of refueling, these studies may be more likely to sample birds when fuel deposition rates are high and are thus more likely to detect differences between migrants and residents. If this is the case, this would suggest that stopover studies may be particularly well suited for testing the hypothesis that CORT stimulates physiological preparations linked with migration. Alternatively, stopover studies are also more likely to sample birds that have just arrived from a long-distance flight. If CORT is heightened during flight itself (Hypothesis 3), then higher CORT levels in migrants vs. residents may represent this transition from flight to refueling, rather than the predicted stimulatory actions of CORT on hyperphagia and fattening. Finally, stopover studies are probably also more likely to sample birds closer to departure, which therefore makes it difficult to distinguish whether data supports the hypothesis that (1) CORT stimulates physiological preparation for migration or (2) CORT mediates departure from origin and stopovers. Distinguishing among these alternatives would require data collected on a relatively fine temporal scale and when stage within the pre-migratory or stopover period can be determined, though we recognize the challenges involved in collecting such data.

Finally, we also recognize that while hormone manipulations can be an excellent way to directly test physiological and behavioral effects of a specific hormone, oftentimes these techniques result in unrealistic hormone levels (Astheimer et al., 1992), different receptor binding affinities (e.g. dexamethasone, De Kloet et al., 1984), or unintended endocrine changes such as binding to non-target hormone receptors (e.g. RU486, Landys et al., 2006). For example, many hormone implants cause circulating hormones to reach suprphysiological levels, oftentimes above natural stress-induced levels (Fusani, 2008; Landys et al., 2006). As the assumed stimulatory effects of CORT on migratory preparation should often be occurring at levels well below stress-induced levels, this highlights the need to use caution in interpreting results from pharmacological manipulations that are outside the relevant range.

4. Hypothesis 2: Departure Stimulation Hypothesis

4.1. Prediction 3: in free-living birds, CORT should increase immediately prior to departure

Migratory departure decisions are driven by a combination of both initial predictive and supplementary cues. For example, cues such as photoperiod and body condition must reach a threshold for birds to be in a ‘window of readiness’ (Akesson et al., 2017), whereas supplementary cues such as weather conditions are important for the actual decision to finally depart (Akesson and Hedenstrom, 2000; Sjöberg et al., 2015). CORT could act as a mediator between such departure cues and actual departure in both cases, with increased CORT during the days prior to departure facilitating a ‘window of readiness’ and/or increased CORT during the hours prior to departure actually enabling the decision to initiate migratory flight. To test whether CORT increases during the days or hours prior to departure, we restricted our selection of studies to those that used wild, free-living birds where individual departure date or time could be determined (Table 3a). We did this because there is significant variation among individuals in departure dates both at origin and stopovers (Stanley et al., 2012). Thus, individual-level data are likely needed to evaluate relationships between CORT and departure date.

We found only four studies (Eikenaar et al., 2014b; Eikenaar et al., 2017; Eikenaar et al., 2018a; Eikenaar et al., 2020b) that met our criteria (Table 3a); this is likely due to the difficulty of accurately measuring the timing of departure in wild, free-living birds. From a ‘window of readiness’ perspective, Eikenaar et al. (2014b, 2017) found CORT was negatively related with days until departure in Northern Wheatears during an autumn stopover, meaning that CORT tended to be higher in birds that departed soon after sampling. Similarly, Eikenaar et al. (2018a) also found a non-significant trend between baseline CORT and

Table 3a

Summary of studies examining the Departure Stimulation Hypothesis, under Prediction 3 that corticosterone (CORT) increases prior to migratory departure in free-living birds. Studies fell into two temporal categories: a) those that examined whether CORT increases over the days prior to departure, and b) those that examined whether CORT increases over the hours prior to departure.

Reference	Species	Temporal period	Migratory stage	Season	Supports prediction?
Eikenaar et al., 2014b	<i>Oenanthe oenanthe</i>	Days	Stopover	Autumn	Yes ^a
Eikenaar et al., 2017	<i>Oenanthe oenanthe</i>	Days	Stopover	Autumn	Yes
Eikenaar et al., 2018a	<i>Turdus merula</i>	Days	Stopover	Autumn	Yes ^a
Eikenaar et al., 2017	<i>Oenanthe oenanthe</i>	Hours	Stopover	Autumn	Yes
Eikenaar et al., 2020b	<i>Oenanthe oenanthe</i>	Days	Stopover	Autumn	Yes
Eikenaar et al., 2018a	<i>Turdus merula</i>	Hours	Stopover	Autumn	Yes ^a

^a Statistically non-significant trend.

departure time, where European blackbirds that departed the night after sampling tended to have higher CORT levels than blackbirds that stayed one or more nights at an autumn stopover site. Stronger relationships between CORT and departure time have been found over shorter temporal periods, as three studies by Eikenaar et al. (2017, 2018a, 2020b) found that birds departing immediately after sunset had higher CORT levels than individuals that departed later at night. While these results only approached significance in European blackbirds (Eikenaar et al., 2018a), studies in Northern Wheatears (Eikenaar et al., 2017; Eikenaar et al., 2020b) are more conclusive. During an autumn stopover, departing Wheatears significantly increased baseline CORT from 5 h before sunset to those 2 h before sunset, whereas no such increase was seen in Wheatears that prolonged their stopover one or more nights (Eikenaar et al., 2020b). When examining actual departure time, both Eikenaar et al. (2017) and Eikenaar et al. (2020b) found a strong correlation with CORT levels tending to be higher the closer the bird was to departure.

4.2. Prediction 4: in captive birds, CORT should be higher when migratory restlessness is displayed

Under captive conditions, many birds begin displaying migratory restlessness as they enter the migratory life cycle stage. Perch hopping, take-off flights, and wing flapping are all behaviors indicative of migratory restlessness, and are especially noticeable in nocturnal migrants as these behaviors displace normal resting and sleeping (Agatsuma and Ramenofsky, 2006; Berthold et al., 2000; Berthold and Querner, 1988). The expression of migratory restlessness has generally been interpreted as a readiness to depart (Berthold, 1996; Eikenaar et al., 2014b). Therefore, the hypothesis that CORT stimulates migratory departure predicts that in captive birds, CORT levels will be elevated in association with migratory restlessness as birds transition into a migratory state. We found that relevant research was conducted on two different temporal scales. a) Some studies examined how CORT changed over several days, typically from a pre-migratory state with no migratory restlessness to a migratory state with consistent migratory restlessness, and b) other studies examined how CORT changed within a day as birds neared their typical departure hour, thus examining a transition from normal, daily activities to display of migratory restlessness. With respect to these temporal scales, the hypothesis predicts that a) CORT

levels will be higher during the stage where migratory restlessness is expressed, compared to pre-migratory stages (at either origin or stopover) where migratory restlessness is not expressed, and that b) in birds expressing migratory restlessness, CORT levels will be higher immediately before and during the hours of migratory restlessness expression compared to times of days when migratory restlessness is not expressed.

a) Comparisons of CORT across days between birds in pre-migratory and migratory states

We found several studies that took repeated measures on captive birds as they transitioned from a pre-migratory stage with no migratory restlessness, to a migratory stage with migratory restlessness (Table 3b). Ramenofsky et al. (1999) found that under natural spring photoperiods, afternoon and nighttime CORT levels were significantly higher in captive Dark-eyed Juncos expressing migratory restlessness compared to those in a pre-migratory state. As juncos are night-time migrants, these findings support the prediction that CORT levels increase over the days leading up to consistent expression of migratory restlessness. These results are in contrast to Mishra et al. (2017), who found that nighttime CORT levels did not significantly differ between Blackheaded Buntings (*Emberiza melanocephala*) in spring pre-migratory (no migratory restlessness expression) and migratory (migratory restlessness expression) states. Buntings, however, were not exposed to natural light cycles and were instead gradually shifted from 8L:16D to 16L:8D over a 1- or 4-week period, where birds shifted to long days over a 1-week period were considered to be in the pre-migratory condition and birds shifted to long days over a 4-week period were considered to be in the migratory condition.

While both Ramenofsky et al. (1999) and Mishra et al. (2017) measured baseline CORT a few hours before or during migratory restlessness expression, other repeated measures studies (Holberton et al., 2008; Holberton et al., 2007; Robart et al., 2019) measured CORT in the morning, generally a few hours after migratory restlessness expression ceased (Table 3b). Holberton et al. (2007, 2008) found support for the prediction that CORT positively relates with migratory restlessness, as both baseline CORT and migratory restlessness increased in concert as captive Dark-eyed Juncos transitioned from a spring pre-migratory state with no migratory restlessness to a migratory state with migratory restlessness. This contrasts with the findings of Robart et al. (2019), however, as they found that captive Pine Siskin baseline CORT levels did not change as birds transitioned into a migratory restlessness state under spring conditions. Under the hypothesis that CORT levels positively relate with migratory restlessness, we give less weight to these studies than those that measured CORT within a few hours of rather than during migratory restlessness expression (Mishra et al., 2017; Ramenofsky et al., 1999), as CORT generally shows strong diel rhythms (Breuner and Wingfield, 2000).

Partially migratory species, such as the European Blackbird, provide another way to test the prediction that CORT differs between pre-migratory birds and migratory birds. Schwabl et al. (1984) studied captive European Blackbirds exposed to natural autumn photoperiods and found that in females, but not in males, individuals displaying migratory restlessness had higher nighttime CORT levels than sedentary individuals during the migratory period. Therefore, this provides mixed support for the prediction that CORT increases from the pre-migratory to migratory period (Table 3b). It should be noted, however, that sedentary individuals do not migrate, and therefore may not be representative of a pre-migratory state and instead could represent a pre-breeding state.

Finally, the stopover period provides another way to examine how CORT changes as birds transition into a period of consistent migratory restlessness expression (Table 3b). Schwabl et al. (1991) exposed captive Garden Warblers (*Sylvia borin*) to a simulated autumnal stopover by manipulating food availability. The authors found that nighttime CORT levels were significantly lower during the simulated stopover (no migratory restlessness) compared to the period of time where migratory

Table 3b

Summary of studies examining the Departure Stimulation Hypothesis, under Prediction 4 that corticosterone (CORT) is elevated in association with the expression of migratory restlessness (MR) in captive birds. Studies fell into two temporal categories: a) those that examined whether CORT increases as birds transition from a pre-migratory state with no MR to a migratory state with MR, and b) those that examined whether CORT increases over the hours leading up to MR expression. Unnatural photoperiods included those that kept birds on constant, unchanging photoperiods for long periods of time, or those that made abrupt transitions from short to long days or vice versa.

Reference	Species	When CORT measured	Photoperiod	Migratory stage	Season	Supports prediction?
a) Across days						
Ramenofsky et al., 1999	<i>Junco hyemalis</i>	Night	Natural	Origin	Spring	Yes
Schwabl et al., 1984	<i>Turdus merula</i>	Night	Natural	Origin	Autumn	Mixed ^a
Mishra et al., 2017	<i>Emberiza melanocephala</i>	Night	Unnatural	Origin	Spring	No
Schwabl et al., 1991	<i>Sylvia borin</i>	Night	Unnatural	Stopover	Autumn	Yes
Robart et al., 2019	<i>Spinus pinus</i>	Morning	Natural	Origin	Spring	No
Holberton et al., 2008	<i>Junco hyemalis</i>	Morning	Unnatural	Origin	Spring	Yes
Holberton et al., 2007	<i>Junco hyemalis</i>	Morning	Unnatural	Origin	Spring	Yes
b) Diel						
Ramenofsky et al., 1999	<i>Junco hyemalis</i>	Multiple	Natural	Origin	Spring	Yes
Landys et al., 2004c	<i>Zonotrichia leucophrys</i>	Multiple	Unnatural	Origin	Spring	Yes
Mishra et al., 2017	<i>Emberiza melanocephala</i>	Multiple	Unnatural	Origin	Spring	Mixed ^b
Schwabl et al., 1984	<i>Turdus merula</i>	Multiple	Natural	Origin	Autumn	No
Schwabl et al., 1991	<i>Sylvia borin</i>	Multiple	Unnatural	Stopover	Autumn	No

^a CORT was significantly higher in migrants vs. non-migrants in females, but not in males.

^b Pre-migratory buntings with no MR also showed higher nighttime than daytime CORT levels.

restlessness was displayed, thus supporting the prediction that CORT increases from a pre-migratory stage with no migratory restlessness to a migratory stage with migratory restlessness (Schwabl et al., 1991).

b) Diel CORT levels associated with the expression of migratory restlessness

There is also mixed evidence that CORT increases over the hours prior to display of migratory restlessness (Table 3b). Using nocturnal migrants, some studies have found that CORT levels are higher during the night (when migratory restlessness is expressed) than during the day. Ramenofsky et al. (1999) found that in spring migratory Dark-eyed Juncos, CORT levels increased significantly from early morning and early afternoon levels to the late afternoon and night – which reflected an earlier rise in CORT compared to juncos sampled at other stages of the annual cycle. Similarly, Landys et al. (2004c) examined captive White-crowned sparrows in a spring migratory state and found that CORT levels increased from the morning to the evening and peaked during the early night; this pattern contrasted with that of birds in a short-day wintering state that did not elevate CORT in the early night. On the other hand, Mishra et al. (2017) found mixed support for the prediction that CORT is higher during the hours of migratory restlessness, as both pre-migratory (no migratory restlessness expression) and migratory (migratory restlessness expression) Blackheaded Buntings had higher CORT levels during the evening and night than during the morning and afternoon. Similarly, Schwabl et al. (1984) found that male European Blackbirds had higher CORT levels during the late night than during the afternoon, regardless of whether they displayed migratory restlessness. Furthermore, Schwabl et al. (1984) found that for female blackbirds displaying migratory restlessness, CORT levels did not significantly differ between the early afternoon and the late night, thus providing no support for the prediction that CORT levels are higher during the hours of migratory restlessness expression. Schwabl et al. (1991) also found no support for this prediction in captive Garden Warblers, as birds displaying migratory restlessness had relatively consistent CORT levels throughout the day, except for dawn CORT levels (when flight typically ends) being significantly higher than early morning CORT levels.

4.3. Synthesis of predictions for Departure Stimulation Hypothesis

In summary, studies of both free-living and captive birds provide modest evidence that CORT increases over the days prior to departure, but stronger evidence that CORT increases over the hours prior to departure (Tables 3a–3b). The four studies from free-living birds that

measured patterns across days found trends in support of the hypothesis. Four out of seven studies of captive birds that examined patterns across days (57%: Schwabl et al., 1991; Ramenofsky et al., 1999; Holberton et al., 2007, 2008) found that CORT is lower during a period of migratory preparation compared to periods where migratory restlessness is expressed. However, Schwabl et al. (1984) found that this trend was true for females but not males, and two studies (29%: Mishra et al., 2017; Robart et al., 2019) found no differences in CORT in the days before and after initial display of migratory restlessness in captive birds. Conflicting findings among these captive studies could be explained by the type of photoperiodic manipulations used in experiments. The strongest support came from studies using abrupt, unnatural photoperiod changes (Schwabl et al., 1991; Holberton et al., 2007, 2008; but see Mishra et al., 2017), whereas studies using naturally changing photoperiods were much more mixed in their support (strong- Ramenofsky et al., 1999; mixed- Schwabl et al., 1984; and no support- Robart et al., 2019). For studies that examined within-day patterns, all four studies of free-living birds supported the hypothesis, though one did not reach statistical significance. Among the five diel studies in captive birds, two provide strong support (40%: Landys et al., 2004c; Ramenofsky et al., 1999) and one provides mixed support (20%: Mishra et al., 2017) for the prediction that CORT increases across the day as birds transition into migratory restlessness, whereas the two remaining studies (40%: Schwabl et al., 1984, 1991) provide no support for this prediction (Table 3b). Mixed results from the abovementioned studies could be due to differences in the time of day of when samples were collected. Specifically, for the hypothesis that CORT helps facilitate migratory departure, we would expect that CORT levels should peak close to the actual time of departure. However, some studies did not take samples around this time, and instead included samples from later in the night (e.g. Schwabl et al., 1984). Moreover, variation among individuals in the timing of departure or expression of migratory restlessness (Muller et al., 2016) and correspondingly variation in the timing of peak CORT (Eikenaar et al., 2017), could also make relationships difficult to discern. Conflicting findings could also be due to species differences in non-migratory diel CORT rhythms, although we find this unlikely as there is strong evidence that CORT peaks at the beginning of the active phase across most bird species (Schwabl et al., 2016). Finally, relationships between CORT and migratory restlessness expression could vary between spring and autumn, as we found that spring studies provided either strong (Landys et al., 2004c; Ramenofsky et al., 1999) or mixed (Mishra et al., 2017) support for the prediction that CORT and migratory restlessness are positively related on a diel basis, whereas autumnal studies (Schwabl

et al., 1984, 1991) generally did not find support for this prediction.

The results we have summarized here highlight several avenues for future research investigating the role of CORT in stimulating migratory departures. To overcome some of the challenges in interpreting results of captive experiments that we have highlighted here, we suggest that future captive studies use naturally changing photoperiods and carefully consider the time of day at which sampling is done. For both captive and free-living studies, greater examination of fine-scale temporal variation across the day, paired with behavioral data, could be very informative. For example, many nocturnal migrants show a period of quiescence immediately before departure (or before the expression of MR in captivity) (Agatsuma and Ramenofsky, 2006; Morton, 1967; Schofield et al., 2018). This period may function in cue integration necessary for departure decisions, orientation for flight, and transitioning of metabolic processes (Cochran et al., 2004; Coverdill et al., 2008; Ramenofsky et al., 2008), but few studies have examined patterns of CORT secretion on the timescale of the transition from quiescence to departure or MR (but see Ramenofsky and Wingfield, 2017). More broadly, we suggest that future studies examine both males and females, as the work of Schwabl et al. (1984) demonstrates that relationships between CORT and migratory restlessness expression may differ by sex, and explore differences between spring and autumn migration. While existing studies of free-living birds have some limitations – they represent just two species, have small sample sizes, and were carried out during autumn stopovers – they highlight the exciting possibilities for generating these sorts of datasets in an effort to better understand the physiological mechanisms underpinning departure decisions. Thus, to the extent that this approach could be applied to other species, other seasons, and departures from both origin sites and stopovers, this offers a promising approach for future researchers. Additionally, in systems for which migratory departures can be measured, we also suggest that pharmacological manipulations of CORT could be used to test for a direct, causal role in migratory departure. While pharmacological studies have drawbacks such as potentially generating unrealistic hormone levels or binding affinities, these studies are nevertheless a logical first step towards understanding the functional role of hormones on migratory physiology and behavior.

5. Hypothesis 3: Flight Support Hypothesis

5.1. Prediction 5: CORT levels should be elevated during flight

Given CORT's well-known effects on mobilizing energy, it has long been posited that elevated CORT levels facilitate high metabolic rates during long periods of flight (Jenni-Eiermann and Jenni, 1991; Jenni-Eiermann et al., 2002). Glucocorticoids have been shown to stimulate and increase glycogenolysis, gluconeogenesis, lipolysis, proteolysis, and resistance to insulin (Sapolsky et al., 2000), all important processes that increase circulating levels of glucose and other energy molecules for working muscle cells. Evidence of increased circulating CORT levels during migration has been scant, however, given the difficulty of capturing birds during flight in order to measure CORT. The studies we found generally fell into three categories: a) those that used domesticated, non-migratory, homing pigeons as subjects, b) those that examined wild or wild-derived birds flying in a wind tunnel, and c) those that captured wild birds out of migratory flight and compared CORT levels with those during stopover. In all cases, we only included studies that caught and sampled birds within minutes of ending flight.

Studies in homing pigeons were the first to examine relationships between CORT and long-distance flight. While homing pigeons are domesticated birds derived from a non-migratory species (*Columba livia*), these studies can help to answer the important question of whether CORT levels are elevated during long periods of flight. Haase et al. (1986) found racing pigeons flying over 115 km had significantly elevated CORT compared to resting controls that experienced the same handling and sampling procedures. In contrast, Viswanathan et al.

(1987) found that homing pigeons did not have significantly elevated CORT levels after a 48 km flight compared to resting controls. These differences may be attributed to the distance flown, and we suggest that flights over 115 km may be more representative of those taken by migrating birds. Therefore, studies in homing pigeons provide some support for the prediction that CORT is high during flight (Table 4), though inferences from pigeons are limited by the fact that these are not migratory birds. Furthermore, if functions of CORT during flight are dependent on processes that occur during seasonal migratory preparation, then homing pigeons may not provide useful data for testing the hypothesis that CORT is elevated during long-distance migratory flight.

Wind tunnel studies provide mixed support for the prediction that circulating CORT levels are elevated during long-distance flight (Table 4). Casagrande et al. (2020) provide the strongest support for this prediction, as they found that immediately after European starlings (*Sturnus vulgaris*) flew for 6 h in a wind tunnel, birds had significantly higher plasma CORT levels compared to 2 days after flight. This study found the same pattern during both the autumn and spring migratory periods. Conversely, Jenni-Eiermann et al. (2009) found that baseline CORT levels in Red knots were similar before and after 2 or 10 h of flight in a wind tunnel. Finally, Scollon et al. (2004) found that baseline CORT levels were similar between resting female White-crowned sparrows and those that were flown in a wind tunnel for 20–150 min during late spring. Flown birds did trend towards higher baseline CORT than resting birds, however, and a low sample size of 12 birds that experienced different durations of flight may have further obscured baseline CORT differences between groups.

To examine whether wild, free-living birds have elevated CORT during flight, field studies have focused on catching birds out of flight at or near important stopover sites along migratory flyways (Table 4). For example, Falsone et al. (2009) caught migrating European robins (*Erithacus rubecula*) at an Alpine pass during autumn migration. Since robins are nocturnal migrants, those captured at night were caught during migratory flight, whereas those caught during the day were stopping over at the site. The authors found that robins caught during flight (nighttime capture) had significantly higher baseline CORT compared to robins caught while resting and refueling (daytime capture). While non-migrating birds also demonstrate higher levels of baseline CORT during the night compared to the day (Remage-Healey and Romero, 2000; Rich and Romero, 2001), these levels tend to peak around dawn, before the active period (Breuner et al., 1999; Landys et al., 2004c). As Falsone et al. (2009) found no effect of capture time on nighttime baseline CORT

Table 4

Summary of studies examining the Flight Support Hypothesis, under Prediction 5 that corticosterone (CORT) levels are elevated during flight. In all studies, CORT was measured during the time of day each species normally migrates in the wild. Flight distance represents whether the examined flight bout was generally typical or shorter than normal for each species.

Reference	Species	Season	Flight distance	Supports prediction?
Haase et al., 1986	<i>Columba livia</i>		Typical ^a	Yes
Viswanathan et al., 1987	<i>Columba livia</i>		Short ^a	No
Casagrande et al., 2020	<i>Sturnus vulgaris</i>	Autumn/ Spring	Typical	Yes
Jenni-Eiermann et al., 2009	<i>Calidris canutus</i>	Autumn	Short	No
Scollon et al., 2004	<i>Zonotrichia leucophrys</i>	Spring	Short ^b	No
Falsone et al., 2009	<i>Erithacus rubecula</i>	Autumn	Typical	Yes
Landys-Ciannelli et al., 2002	<i>Limosa lapponica</i>	Spring	Typical	Yes

^a *C. livia* is not migratory, so we assigned the longer flight distance of 115 km as typical, and the shorter flight distance of 48 km as short.

^b *Z. leucophrys* was also flown in several 20 min sessions, which is also atypical of a normal migratory flight bout.

levels, this suggests that the elevation in CORT levels observed during night in migrating European robins are likely driven by the act of flight itself, rather than by normal diel patterns of CORT secretion. In a study of Bar-tailed godwits, Landys-Ciannelli et al. (2002) compared birds resting and refueling during the single stopover (in the Wadden Sea) of their 9000 km migratory journey with birds caught out of flight (using decoys and song playback) at a small island ~60 km before godwits reach the Wadden Sea. Similar to Falsone et al. (2009), Landys-Ciannelli et al. (2002) found that godwits caught out of flight had significantly higher baseline CORT levels than those resting at the stopover site. A limitation of this study is that godwits caught out of flight were near the end of a continuous 4000 km flight and thus weighed less and had less fat compared to stopover birds; thus differences in CORT could be due to differences in body condition. However, this alternative explanation is unlikely as the authors found no direct relationship between size-corrected body mass and baseline CORT in godwits caught before stopover.

5.2. Synthesis of prediction for Flight Support Hypothesis

In summary, one homing pigeon study, one wind tunnel study, and two field studies (57% of studies examined) support the prediction that baseline CORT is elevated during flight (Table 4). Supportive and non-supportive studies were evenly distributed across autumn and spring; therefore, it is unlikely that mixed findings can be attributed to seasonal differences. For wind tunnel studies, mixed findings could be related with the amount of time that birds were flying. Jenni-Eiermann et al. (2009) posited that high CORT levels may be important during the beginning portion of flight when metabolism switches from predominantly using carbohydrates to lipids for fuel (Jenni-Eiermann et al., 2002); this may therefore explain why they saw a trend towards elevated levels after 2 h of flight but not after 10 h of flight in Red knots. Casagrande et al. (2020), however, saw elevated CORT levels after 6 h of flight in starlings, which is long after the metabolic switch to lipids. It is also possible that CORT's role in promoting flight is temporally dynamic, and that levels may only be elevated during certain stages or phases of flight. Stages that may be supported by elevated CORT could include the metabolic switch from carbohydrate- to lipid-based fueling and the end of the flight period when birds are close to exhausting energy stores. An elevation of CORT occurring at the end of flight corresponds well with the studies described here, as both field studies found elevated levels of baseline CORT, either in a long-hop migrant right before stopover (godwits, Landys-Ciannelli et al., 2002) or in a short-hop migrant caught out of flight (European robins, Falsone et al., 2009). Additionally, for wind tunnel studies, European starlings were flown for durations similar to what they would experience in the wild (Perdeck, 1958, 1964), whereas Red Knots generally fly for bouts much longer than 10 h (Piersma and Davidson, 1992), and White-crowned sparrows did not fly continuously and instead were flown in several 20 min bouts. If CORT is only elevated at the end of bird's typical, continuous flight bout, then this may explain why starlings had elevated CORT levels after wind tunnel flight, but knots and sparrows did not. Finally, lack of important cues, notably appropriate light-dark cycles, may impact flight behavior (Breuner et al., 1999; Coverdill et al., 2008; Ramenofsky and Wingfield, 2017). Therefore, appropriate ambient light levels and time of day may be required to see typical CORT-flight relationships during migration.

For future wind tunnel studies, we specifically recommend that flight duration is manipulated or that during a long flight bout the wind tunnel is periodically stopped so repeated CORT measurements could be taken, as this may better examine how CORT changes over the different metabolic stages of migratory flight. Another element that should be considered in future wind tunnel studies is food availability and fuel loads in captive birds, as captive birds with ad libitum food may carry fuel loads that are much higher than those in wild, migrating birds. Concurrent measurements of CORT and metabolites indicative of protein and fat breakdown (e.g. uric acid and triglycerides, Jenni-Eiermann

et al., 2002) would also shed light on the potential role of CORT in flight, as we would predict positive relationships between CORT and uric acid/triglyceride levels under the hypothesis that CORT facilitates the metabolic demands of flight. Another approach that could be useful for future field studies would be to capture and sample birds at stopover sites after large water crossings, (e.g. Gulf of Mexico), as this allows good estimates of flight distance before sampling (Agostini et al., 2015). With a variety of stopover sites, and therefore known flight distances, further relationships between CORT and flight duration can be examined.

6. Conclusions

CORT has long been proposed to be an important endocrine signal coordinating migration (Cornelius et al., 2013; Ramenofsky, 2011; Ramenofsky et al., 2012). However, the specific role of CORT in migration has been the subject of much debate. Here we reviewed the literature to test predictions that follow from three broad hypothesized functions of CORT in migration: the Physiological Preparation Hypothesis, the Departure Stimulation Hypothesis, and the Flight Support Hypothesis. We identified 37 studies with data that addressed predictions for one or more of the hypotheses. Data to test the Physiological Preparation Hypothesis were most common, but support for this hypothesis was weak. Overall, less than half of studies examined met the predictions of the hypothesis. The strongest support for this hypothesis came from studies that examined changes in baseline CORT across the pre-departure period; however, it is important to note that these findings could also support the Departure Stimulation Hypothesis. While fewer studies addressed the Departure Stimulation and Flight Support Hypotheses, there was more support for both of these hypotheses. Support for the Departure Stimulation Hypothesis was mixed, but we gave greater weight to studies conducted in wild, free-living birds – these studies generally met the predictions of the hypothesis. Though we note that existing studies of wild, free-living birds tend to be limited by small sample sizes and a focus on departure from stopovers. The Flight Support Hypothesis also had mixed support, with results from free-living birds sampled during migratory flight providing the strongest support. However, more studies are necessary to better test this hypothesis.

We hope that the hypotheses and predictions laid out here provide a clear framework that can be used in future studies to effectively and directly test the role(s) of CORT in migration. Moreover, although we have focused here on the role of changes in circulating CORT in supporting migration, changes in other elements of CORT signaling pathways could also be important in stimulating or supporting migratory behavior and physiology. For example, changes in mineralocorticoid and glucocorticoid receptors, corticosteroid-binding globulin, and 11 β hydroxysteroid dehydrogenase (11 β -HSD) type 1 and type 2 enzymes, could act to alter signaling, even in the absence of changes in circulating CORT levels (Pradhan et al., 2019; Rensel and Schlinger, 2016; Wingfield, 2018). These other mechanisms have received much less attention in the context of migration, but the framework of hypotheses and predictions laid out here could be expanded to include them. A better understanding of the role of CORT signaling in avian migration and an application of this approach in other taxonomic groups will likely further our knowledge of the behavioral and physiological roles that glucocorticoids play across animal systems.

Funding

HEW was supported by funding from NSF (IOS-1756976 and IOS-1755245).

CRedit authorship contribution statement

Carolyn M. Bauer: Conceptualization, Writing – original draft.
Heather E. Watts: Conceptualization, Writing – original draft.

Declaration of competing interest

The authors report no competing interests.

Acknowledgements

We thank M. Ramenofsky, T. Greives, and L.M. Romero for helpful discussion of these ideas. We also thank two anonymous reviewers and B. Helm for thoughtful comments on earlier drafts of this manuscript.

References

- Agatsuma, R., Ramenofsky, M., 2006. Migratory behaviour of captive white-crowned sparrows, *Zonotrichia leucophrys gambelii*, differs during autumn and spring migration. *Behaviour* 143, 1219–1240.
- Agostini, N., Panuccio, M., Pasquaretta, C., 2015. Morphology, flight performance, and water crossing tendencies of Afro-Paleartic raptors during migration. *Current Zoology* 61, 951–958.
- Akesson, S., Hedenstrom, A., 2000. Wind selectivity of migratory flight departures in birds. *Behav. Ecol. Sociobiol.* 47, 140–144.
- Akesson, S., Ilieva, M., Karagicheva, J., Rakhimberdiev, E., Tomotani, B., Helm, B., 2017. Timing avian long-distance migration: from internal clock mechanisms to global flights. *Philosophical Transactions of the Royal Society B-Biological Sciences* 372, 16.
- Alerstam, T., Hedenstrom, A., Akesson, S., 2003. Long-distance migration: evolution and determinants. *Oikos* 103, 247–260.
- Angelier, F., Clement-Chastel, C., Gabrielsen, G.W., Chastel, O., 2007. Corticosterone and time-activity budget: an experiment with Black-legged kittiwakes. *Horm. Behav.* 52, 482–491.
- Asheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scand.* 23, 355–365.
- Bailleul, F., Lesage, V., Power, M., Doidge, D.W., Hammill, M.O., 2012. Migration phenology of beluga whales in a changing Arctic. *Clim. Res.* 53, 169–178.
- Bauer, C.M., Needham, K.B., Le, C.N., Stewart, E.C., Graham, J.L., Ketterson, E.D., Greives, T.J., 2016. Hypothalamic-pituitary-adrenal axis activity is not elevated in a songbird (*Junco hyemalis*) preparing for migration. *Gen. Comp. Endocrinol.* 232, 60–66.
- Bauer, C.M., Graham, J.L., Greives, T.J., 2019. Corticosterone negative feedback is weaker during spring vs. autumn migration in a songbird (*Junco hyemalis*). *Gen. Comp. Endocrinol.* 280, 36–42.
- Berthold, P., 1996. *Control of Bird Migration*. Chapman and Hall, London.
- Berthold, P., Querner, U., 1988. Was Zugenruhe wirklich ist — eine quantitative Bestimmung mit Hilfe von Videoaufnahmen bei Infrarotlichtbeleuchtung. *J. Ornithol.* 129, 372–375.
- Berthold, P., Fiedler, W., Querner, U., 2000. Die Zugenruhe bei Vögeln — eine Darstellung nach Videoaufnahmen bei Infrarotlichtbeleuchtung. *J. Ornithol.* 141, 285–299.
- Breuner, C.W., Hahn, T.P., 2003. Integrating stress physiology, environmental change, and behavior in free-living sparrows. *Horm. Behav.* 43, 115–123.
- Breuner, C.W., Wingfield, J.C., 2000. Rapid behavioral response to corticosterone varies with photoperiod and dose. *Horm. Behav.* 37, 23–30.
- Breuner, C.W., Greenberg, A.L., Wingfield, J.C., 1998. Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Gen. Comp. Endocrinol.* 111, 386–394.
- Breuner, C.W., Wingfield, J.C., Romero, L.M., 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *J. Exp. Zool.* 284, 334–342.
- Brodersen, J., Nilsson, P.A., Hansson, L.A., Skov, C., Bronmark, C., 2008. Condition-dependent individual decision-making determines cyprinid partial migration. *Ecology* 89, 1195–1200.
- Carruth, L.L., Jones, R.E., Norris, D.O., 2002. Cortisol and pacific salmon: a new look at the role of stress hormones in olfaction and home-stream migration. *Integr. Comp. Biol.* 42, 574–581.
- Casagrande, S., DeMoranville, K.J., Trost, L., Pierce, B., Bryla, A., Dzialo, M., Sadowska, E.T., Bauchinger, U., McWilliams, S.R., 2020. Dietary antioxidants attenuate the endocrine stress response during long-duration flight of a migratory bird. *Proceedings of the Royal Society B-Biological Sciences* 287.
- Cochran, W.W., Mouritsen, H., Wikelski, M., 2004. Migrating songbirds recalibrate their magnetic compass daily from twilight cues. *Science* 304, 405–408.
- Cornelius, J.M., Boswell, T., Jenni-Eiermann, S., Breuner, C.W., Ramenofsky, M., 2013. Contributions of endocrinology to the migration life history of birds. *Gen. Comp. Endocrinol.* 190, 47–60.
- Coverdill, A.J., Bentley, G.E., Ramenofsky, M., 2008. Circadian and masking control of migratory restlessness in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *J. Biol. Rhythms.* 23, 59–68.
- Cyr, N.E., Romero, L.M., 2009. Identifying hormonal habituation in field studies of stress. *Gen. Comp. Endocrinol.* 161, 295–303.
- de Bruijn, R., Romero, L.M., 2011. Behavioral and physiological responses of wild-caught European starlings (*Sturnus vulgaris*) to a minor, rapid change in ambient temperature. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* 160, 260–266.
- De Kloet, E.R., Veldhuis, H.D., Wagenaars, J.L., Bergink, E.W., 1984. Relative binding-affinity of steroids for the corticosterone receptor system in rat hippocampus. *J. Steroid Biochem. Mol. Biol.* 21, 173–178.
- DeSimone, J.G., Ramirez, M.G., Elowe, C.R., Griego, M.S., Breuner, C.W., Gerson, A.R., 2020. Developing a Stopover-CORT hypothesis: corticosterone predicts body composition and refueling rate in Gray Catbirds during migratory stopover. *Horm. Behav.* 124, 10.
- Dickens, M.J., Earle, K.A., Romero, L.M., 2009. Initial transference of wild birds to captivity alters stress physiology. *Gen. Comp. Endocrinol.* 160, 76–83.
- Dolnik, V.R., Blyumental, T.I., 1967. Autumnal premigratory and migratory periods in chaffinch (*Fringilla coelebs coelebs*) and some other temperate-zone passerine birds. *Condor* 69, 435–468.
- Eikeenaar, C., 2017. Endocrine regulation of fueling by hyperphagia in migratory birds. *J. Comp. Physiol. A - Neuroethol. Sens. Neural Behav. Physiol.* 203, 439–445.
- Eikeenaar, C., Fritzsche, A., Bairlein, F., 2013. Corticosterone and migratory fueling in Northern wheatears facing different barrier crossings. *Gen. Comp. Endocrinol.* 186, 181–186.
- Eikeenaar, C., Bairlein, F., Stowe, M., Jenni-Eiermann, S., 2014a. Corticosterone, food intake and refueling in a long-distance migrant. *Horm. Behav.* 65, 480–487.
- Eikeenaar, C., Klinner, T., Stowe, M., 2014b. Corticosterone predicts nocturnal restlessness in a long-distance migrant. *Horm. Behav.* 66, 324–329.
- Eikeenaar, C., Müller, F., Klinner, T., Bairlein, F., 2015. Baseline corticosterone levels are higher in migrating than sedentary common blackbirds in autumn, but not in spring. *Gen. Comp. Endocrinol.* 224, 121–125.
- Eikeenaar, C., Müller, F., Leutgeb, C., Hessler, S., Lebus, K., Taylor, P.D., Schmaljohann, H., 2017. Corticosterone and timing of migratory departure in a songbird. *Proceedings of the Royal Society B-Biological Sciences* 284.
- Eikeenaar, C., Ballstaedt, E., Hessler, S., Klinner, T., Müller, F., Schmaljohann, H., 2018a. Cues, corticosterone and departure decisions in a partial migrant. *Gen. Comp. Endocrinol.* 261, 59–66.
- Eikeenaar, C., Müller, F., Ruppel, G., Stowe, M., 2018b. Endocrine regulation of migratory departure from stopover: evidence from a longitudinal migratory restlessness study on northern wheatears. *Horm. Behav.* 99, 9–13.
- Eikeenaar, C., Hessler, S., Fischer, S., Bairlein, F., 2019. An exception to the rule: captivity does not stress wild migrating northern wheatears. *Gen. Comp. Endocrinol.* 275, 25–29.
- Eikeenaar, C., Hessler, S., Hegemann, A., 2020a. Migrating birds rapidly increase constitutive immune function during stopover. *R. Soc. Open Sci.* 7, 7.
- Eikeenaar, C., Schafer, J., Hessler, S., Packmor, F., Schmaljohann, H., 2020b. Diel variation in corticosterone and departure decision making in migrating birds. *Horm. Behav.* 122 (6).
- Falsone, K., Jenni-Eiermann, S., Jenni, L., 2009. Corticosterone in migrating songbirds during endurance flight. *Horm. Behav.* 56, 548–556.
- Fanson, K.V., Nemeth, Z., Ramenofsky, M., Wingfield, J.C., Buchanan, K.L., 2017. Inter-laboratory variation in corticosterone measurement: implications for comparative ecological and evolutionary studies. *Methods Ecol. Evol.* 8, 1745–1754.
- Fudickar, A.M., Schmidt, A., Hau, M., Quetting, M., Partecke, J., 2013. Female-biased obligate strategies in a partially migratory population. *J. Anim. Ecol.* 82, 863–871.
- Fudickar, A.M., Greives, T.J., Atwell, J.W., Stricker, C.A., Ketterson, E.D., 2016. Reproductive allochryony in seasonally sympatric populations maintained by differential response to photoperiod: implications for population divergence and response to climate change. *Am. Nat.* 187, 436–446.
- Fusani, L., 2008. Endocrinology in field studies: problems and solutions for the experimental design. *Gen. Comp. Endocrinol.* 157, 249–253.
- Goyman, W., 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. *Methods Ecol. Evol.* 3, 757–765.
- Haase, E., Rees, A., Harvey, S., 1986. Flight stimulates adrenocortical activity in pigeons (*Columa livia*). *Gen. Comp. Endocrinol.* 61, 424–427.
- Helm, B., Gwinner, E., 2006. Timing of molt as a buffer in the avian annual cycle. *Acta Zool. Sin.* 52, 703–706.
- Henshaw, I., Fransson, T., Jakobsson, S., Jenni-Eiermann, S., Kullberg, C., 2009. Information from the geomagnetic field triggers a reduced adrenocortical response in a migratory bird. *J. Exp. Biol.* 212, 2902–2907.
- Hinch, S.G., Rand, P.S., 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): role of local environment and fish characteristics. *Can. J. Fish. Aquat. Sci.* 55, 1821–1831.
- Holberton, R.L., 1999. Changes in patterns of corticosterone secretion concurrent with migratory fattening in a neotropical migratory bird. *Gen. Comp. Endocrinol.* 116, 49–58.
- Holberton, R.L., Wilson, C.M., Hunter, M.J., Cash, W.B., Sims, C.G., 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.
- Holberton, R.L., Boswell, T., Hunter, M.J., 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.
- Jenni-Eiermann, S., Jenni, L., 1991. Metabolic responses to flight and fasting in night-migrating passerines. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 161, 465–474.
- Jenni-Eiermann, S., Jenni, L., Kvist, A., Lindstrom, A., Piersma, T., Visser, G.H., 2002. Fuel use and metabolic response to endurance exercise: a wind tunnel study of a long-distance migrant shorebird. *J. Exp. Biol.* 205, 2453–2460.
- Jenni-Eiermann, S., Hasselquist, D., Lindstrom, A., Koolhaas, A., Piersma, T., 2009. Are birds stressed during long-term flights? A wind-tunnel study on circulating corticosterone in the red knot. *Gen. Comp. Endocrinol.* 164, 101–106.

- Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 2001. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behav. Ecol.* 12, 619–625.
- Landys, M.M., Piersma, T., s, Wingfield, J.C., 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, M.M., Ramenofsky, M., Guglielmo, C.G., Wingfield, J.C., 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, M.M., Wingfield, J.C., Ramenofsky, M., 2004c. Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys gambelii*. *Horm. Behav.* 46, 574–581.
- Landys, M.M., Ramenofsky, M., Wingfield, J.C., 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, M.M., Ramenofsky, M., Piersma, T., Jukema, J., Wingfield, J.C., Castricum Ringing, G., 2002. Baseline and stress-induced plasma corticosterone during long-distance migration in the bar-tailed godwit, *Limosa lapponica*. *Physiol. Biochem. Zool.* 75, 101–110.
- Lattin, C.R., Breuner, C.W., Romero, L.M., 2016. Does corticosterone regulate the onset of breeding in free-living birds?: the CORT-flexibility hypothesis and six potential mechanisms for priming corticosteroid function. *Horm. Behav.* 78, 107–120.
- Linscott, J.A., Senner, N.R., 2021. Beyond refueling: investigating the diversity of functions of migratory stopover events. *Ornithological Applications* 123.
- Löhms, M., Sundstrom, F., Moore, F.R., 2006. Non-invasive corticosterone treatment changes foraging intensity in red-eyed vireos *Vireo olivaceus*. *J. Avian Biol.* 37, 523–526.
- McWilliams, S.R., Guglielmo, C., Pierce, B., Klaassen, M., 2004. Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *J. Avian Biol.* 35, 377–393.
- Mishra, I., Singh, D., Kumar, V., 2017. Daily levels and rhythm in circulating corticosterone and insulin are altered with photostimulated seasonal states in night-migratory blackheaded buntings. *Horm. Behav.* 94, 114–123.
- Morton, M.L., 1967. Diurnal feeding patterns in white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *Condor* 69 (491-4).
- Muller, F., Taylor, P.D., Sjoberg, S., Muheim, R., Tsvey, A., Mackenzie, S.A., Schmaljohann, H., 2016. Towards a conceptual framework for explaining variation in nocturnal departure time of songbird migrants. *Movement Ecology* 4, 1–12.
- Nilsson, A.L.K., Sandell, M.I., 2009. Stress hormone dynamics: an adaptation to migration? *Biol. Lett.* 5, 480–483.
- O'Reilly, K.M., Wingfield, J.C., 1995. Spring and autumn migration in Arctic shorebirds—same distance, different strategies. *Am. Zool.* 35, 222–233.
- Perdeck, A.C., 1958. Two types of orientation in migrating starlings, *Sturnus vulgaris* L., and chaffinches, *Fringilla coelebs* L., as revealed by displacement experiment. *Ardea* 46, 1–37.
- Perdeck, A.C., 1964. An experiment on the ending of autumn migration in starlings. *Ardea* 52, 133–139.
- Piersma, T., Davidson, N., 1992. The migrations and annual cycles of five subspecies of knots in perspective. *Wader Study Group Bull.* 64, 187–197.
- 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.
- Pradhan, D.S., Van Ness, R., Jalabert, C., Hamden, J.E., Austin, S.H., Soma, K.K., Ramenofsky, M., Schlinger, B.A., 2019. Phenotypic flexibility of glucocorticoid signaling in skeletal muscles of a songbird preparing to migrate. *Horm. Behav.* 116, 104586.
- Ramenofsky, M., 2011. Hormones in migration and reproductive cycles of birds. In: Norris, D.O., Lopez, K.H. (Eds.), *Hormones and Reproduction of Vertebrates, Birds*, vol. 4. Elsevier Academic Press Inc, San Diego, pp. 205–237.
- Ramenofsky, M., Wingfield, J.C., 2007. Regulation of migration. *Bioscience* 57, 135–143.
- Ramenofsky, M., Wingfield, J.C., 2017. Regulation of complex behavioural transitions: migration to breeding. *Anim. Behav.* 124, 299–306.
- Ramenofsky, M., Savard, R., Greenwood, M.R.C., 1999. Seasonal and diel transitions in physiology and behavior in the migratory dark-eyed junco. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology* 122, 385–397.
- Ramenofsky, M., Agatsuma, R., Ramfar, T., 2008. Environmental conditions affect the behavior of captive, migratory white-crowned sparrows. *Condor* 110, 658–671.
- Ramenofsky, M., Cornelius, J.M., Helm, B., 2012. Physiological and behavioral responses of migrants to environmental cues. *J. Ornithol.* 153, S181–S191.
- Ramenofsky, M., Campion, A.W., Perez, J.H., Krause, J.S., Nemeth, Z., 2017. Behavioral and physiological traits of migrant and resident white-crowned sparrows: a common garden approach. *J. Exp. Biol.* 220, 1330–1340.
- Remage-Healey, L., Romero, L.M., 2000. Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): glucose. *Gen. Comp. Endocrinol.* 119, 60–68.
- Reneerkens, J., Morrison, R.I.G., Ramenofsky, M., Piersma, T., Wingfield, J.C., 2002. Baseline and stress-induced levels of corticosterone during different life cycle substages in a shorebird on the high arctic breeding grounds. *Physiol. Biochem. Zool.* 75, 200–208.
- Rensel, M.A., Schlinger, B.A., 2016. Determinants and significance of corticosterone regulation in the songbird brain. *Gen. Comp. Endocrinol.* 227, 136–142.
- Rich, E.L., Romero, L.M., 2001. Daily and photoperiod variations of basal and stress-induced corticosterone concentrations in house sparrows (*Passer domesticus*). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 171, 543–547.
- Robart, A.R., Morado, M.I., Watts, H.E., 2019. Declining food availability, corticosterone, and migratory response in a nomadic, irruptive migrant. *Horm. Behav.* 110, 56–67.
- Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128, 1–24.
- Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* 140, 73–79.
- Romero, L.M., Remage-Healey, L., 2000. Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): Corticosterone. *Gen. Comp. Endocrinol.* 119, 52–59.
- Romero, L.M., Bauer, C.M., de Bruijn, R., Lattin, C.R., 2017. *Seasonal rhythms*, in: Fink, G. (Ed.), *Stress: Neuroendocrinology and Neurobiology*. Academic Press, Oxford, UK.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89.
- Sauman, I., Briscoe, A.D., Zhu, H.S., Shi, D.D., Froy, O., Stalleicken, J., Yuan, Q., Casselman, A., Reppert, S.M., 2005. Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron* 46, 457–467.
- Sawyer, H., Kauffman, M.J., 2011. Stopover ecology of a migratory ungulate. *J. Anim. Ecol.* 80, 1078–1087.
- Schmaljohann, H., Naef-Daenzer, B., 2011. Body condition and wind support initiate the shift of migratory direction and timing of nocturnal departure in a songbird. *J. Anim. Ecol.* 80, 1115–1122.
- Schofield, L.N., Deppe, J.L., Diehl, R.H., Ward, M.P., Bolus, R.T., Zenzal, T.J., Smolinsky, J., Moore, F.R., 2018. Occurrence of quiescence in free-ranging migratory songbirds. *Behav. Ecol. Sociobiol.* 72.
- Schwabl, H., Wingfield, J.C., Farner, D.S., 1984. Endocrine correlates of autumnal behavior in sedentary and migratory individuals of a partially migratory population of the European blackbird (*Turdus merula*). *Auk* 101, 499–507.
- Schwabl, H., Bairlein, F., Gwinner, E., 1991. Basal and stress-induced corticosterone levels of Garden warblers, *Sylvia borin*, during migration. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 161, 576–580.
- Schwabl, P., Bonaccorso, E., Goymann, W., 2016. Diurnal variation in corticosterone release among wild tropical forest birds. *Front. Zool.* 13, 11.
- Scollon, E.J., Carr, J.A., Cobb, G.R., 2004. The effect of flight, fasting and p,p'-DDT on thyroid hormones and corticosterone in Gambel's white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 137, 179–189.
- Seeber, P.A., Quintard, B., Sicks, F., Dehnhard, M., Greenwood, A.D., Franz, M., 2018. Environmental stressors may cause equine herpesvirus reactivation in captive Grevy's zebras (*Equus grevyi*). *PeerJ* 6, 18.
- Sjoberg, S., Alerstam, T., Aringesson, S., Schulz, A., Weidauer, A., Coppack, T., Muheim, R., 2015. Weather and fuel reserves determine departure and flight decisions in passerines migrating across the Baltic Sea. *Anim. Behav.* 104, 59–68.
- Stanley, C.Q., MacPherson, M., Fraser, K.C., McKinnon, E.A., Stutchbury, B.J.M., 2012. Repeat tracking of individual songbirds reveals consistent migration timing but flexibility in route. *PLoS One* 7, 6.
- Viswanathan, M., John, T.M., George, J.C., Etches, R.J., 1987. Flight effects on plasma-glucose, lactate, catecholamines, and corticosterone in homing pigeons. *Horm. Metab. Res.* 19, 400–402.
- Watts, H.E., Cornelius, J.M., Fudickar, A.M., Perez, J., Ramenofsky, M., 2018. Understanding variation in migratory movements: a mechanistic approach. *Gen. Comp. Endocrinol.* 256, 112–122.
- Wikelski, M., Tarlow, E.M., Raim, A., Diehl, R.H., Larkin, R.P., Visser, G.H., 2003. Costs of migration in free-flying songbirds. *Nature* 423, 704–704.
- Wingfield, J.C., 2018. Environmental endocrinology: insights into the diversity of regulatory mechanisms in life cycles. *Integr. Comp. Biol.* 58, 790–799.
- Wingfield, J.C., Hahn, T.P., Wada, M., Astheimer, L.B., Schoech, S., 1996. Interrelationship of day length and temperature on the control of gonadal development, body mass, and fat score in white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *Gen. Comp. Endocrinol.* 101, 242–255.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am. Zool.* 38, 191–206.
- Wyckoff, T.B., Sawyer, H., Albeke, S.E., Garman, S.L., Kauffman, M.J., 2018. Evaluating the influence of energy and residential development on the migratory behavior of mule deer. *Ecosphere* 9, e02113.