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1 **Corticosterone's roles in avian migration: assessment of three hypotheses**

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8

9 **Abstract**

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

24 displayed. We also found moderate support for the hypothesis that CORT helps maintain
25 sustained travel, as CORT is generally higher during periods of flight, though few studies have
26 tested this hypothesis. We provide recommendations for future studies that would help to further
27 resolve the role of CORT in migration.

28

29 **Keywords:** bird, body condition, departure, fat, flight, glucocorticoid, hyperphagia, migratory
30 restlessness, physiological preparation, stopover

31

32 **Introduction**

33

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

47 Glucocorticoids (cortisol and corticosterone, hereafter CORT; see Box 1 for background on
48 CORT physiology) are involved in processes central to migration, such as feeding, energy
49 storage, locomotion, and energy use (Carruth et al., 2002; Seeber et al., 2018; Ramenofsky,
50 2011). CORT plays a permissive role in hyperphagia and lipogenesis and has therefore been
51 suggested to be important for both migratory preparation and refueling at stopovers. CORT is
52 also involved in the regulation of locomotor activity (Angelier et al., 2007; Breuner et al., 1998;
53 Breuner and Hahn, 2003) and elevated CORT levels have been proposed to play a role in
54 migratory departure (Cornelius et al., 2013; Eikenaar et al., 2020b). Finally, CORT facilitates
55 energy mobilization via glycogenolysis, lipolysis, and gluconeogenesis (Sapolsky et al. 2000),
56 and elevated CORT levels have also been implicated as important mediators during periods of
57 sustained migratory travel.

58 Although CORT has often been considered to play an important role in the process of
59 migration, there has been no systematic assessment of CORT's role in migration. Based on the
60 published literature, we outline three main hypotheses for how CORT regulates seasonal
61 migration during the preparation, departure, and travel stages. Although these hypotheses derive
62 primarily from the literature on bird migration, these hypotheses and the hypothetico-deductive
63 framework we outline here may be applicable more broadly. For each hypothesis, we have
64 developed a set of logical predictions, which we evaluate based on a review of the avian
65 literature. We have focused on avian species as birds have been the best-studied model for
66 understanding endocrine regulation of migration generally, and the role of CORT specifically
67 (Cornelius et al., 2013; DeSimone et al., 2020; Ramenofsky, 2011; Ramenofsky et al., 2012;
68 Watts et al., 2018). We have included studies focusing on spring and autumn migrations, which
69 involve travel to breeding and overwintering sites, respectively. While spring and autumn

70 migrations may be similar in terms of distance traveled, they often differ in other notable ways,
71 such as speed of travel, conditions experienced en route, and physiological status (Bauer et al.
72 2019). Therefore, while we summarize findings from both seasons together, we do discuss
73 potential seasonal differences for each prediction and hypothesis.

74 The first hypothesis for CORT's role in migration is that it facilitates physiological
75 preparations for migration (hereafter called the Physiological Preparation Hypothesis; Figure 1).
76 As birds are preparing to depart from origin and stopover sites, they must focus on finding and
77 consuming food to build fuel reserves (i.e., fat) to power their next flight stage. Previous studies
78 have posited that CORT may facilitate these physiological preparations, as CORT has generally
79 been shown to stimulate feeding behavior (Kitaysky et al., 2001) and lipogenesis (Holberton,
80 1999; Landys et al., 2004b) across several different stages of the annual cycle. However, while
81 some studies have found support that CORT may help facilitate physiological preparations for
82 migration, other studies have found opposing evidence (reviewed in Ramenofsky, 2011). We
83 evaluated the Physiological Preparation Hypothesis by testing two main predictions: Prediction
84 1) CORT should be positively associated with feeding behavior, food intake, fattening, weight
85 gain, and improvement in body condition either at stopover or prior to departure at origin, and
86 Prediction 2) CORT levels should be elevated during the period of pre-departure preparation.

87 The second hypothesized role for CORT is that it stimulates departure from origin and
88 stopover sites (hereafter called the Departure Stimulation Hypothesis; Figure 1). CORT is a good
89 candidate for this function as CORT has been shown to have positive relationships with overall
90 activity levels (Breuner et al., 1998) and movement away from perturbations (Ramenofsky et al.,
91 2012). In general, studies have shown that diel CORT rhythms typically peak just before the
92 active period in most bird species (Breuner et al., 1999; Romero and Ramage-Healey, 2000). If

93 high baseline CORT levels generally stimulate a transition from resting to foraging and other
94 daily activities, then it could be expected that higher CORT levels might also facilitate initiation
95 of migratory flight bouts. Furthermore, inclement weather cues such as a drop in temperature can
96 elevate baseline CORT levels (de Bruijn and Romero, 2011), and studies have shown that wild
97 birds with heightened CORT levels are more likely to abandon their current breeding site and
98 translocate to lower altitudes (Breuner and Hahn, 2003). Therefore, as elevated CORT levels
99 may signal the need to move away from sub-optimal habitats, then heightened CORT levels
100 might also stimulate departure from origin and stopover sites (Figure 1). We evaluated the
101 Departure Stimulation Hypothesis by testing two predictions: Prediction 3) CORT should
102 increase immediately prior to departure in free-living birds, and Prediction 4) CORT should be
103 higher when migratory restlessness is displayed in captive birds.

104 The third hypothesized role of CORT is that it supports sustained migratory flight (hereafter
105 called the Flight Support Hypothesis; Figure 1). The process of flight is energetically expensive
106 and requires high mobilization rates of energy reserves, most notably lipids (McWilliams et al.,
107 2004). CORT is a likely mediator for this process, as CORT has been shown to facilitate
108 lipolysis, gluconeogenesis, and proteolysis (Sapolsky et al., 2000). We evaluated the Flight
109 Support Hypothesis by testing: Prediction 5) CORT should be higher during periods of flight
110 compared to periods of no flight.

111 To test these three, non-mutually exclusive hypotheses, we systematically searched the
112 literature to find published studies that measured baseline CORT in birds during and
113 immediately before seasonal migration. We have focused on studies that measured baseline
114 levels of CORT, as seasonal migration is not a stressor and is instead a regular and predictable
115 stage of the annual cycle (Romero, 2002; see Box 1 for background on baseline and stress-

116 induced CORT levels). We do recognize that stress-induced CORT levels may impact the
117 process of migration, for example by stimulating birds to leave stopovers during poor weather
118 conditions. However, such processes likely reflect an “emergency life history stage” that can
119 occur at any point in the annual cycle, rather than being specific to migration (Lattin et al., 2015;
120 Wingfield et al., 1998). This review should clarify our understanding of CORT’s role in seasonal
121 avian migration and provide a helpful framework for future studies.

122

123 **Approach to literature search**

124

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

131 We then excluded any studies that did not occur during the migratory or pre-migratory period.
132 For field studies, we limited our analyses to studies that collected data during times of year that
133 birds were preparing for or actively undergoing migration. For laboratory studies, we limited our
134 analyses to studies that occurred either i) under natural photoperiods that matched when birds
135 would prepare for or migrate in the wild, ii) during a transition from long-days to short-days that
136 would simulate autumn migration in that species, or iii) during a transition from short-days to
137 long-days that would simulate spring migration in that species. For laboratory studies, we also
138 excluded those that did not give birds ample time (2+ weeks) to habituate to captivity, unless the

139 study showed evidence of no captivity stress in recent, wild-caught birds (Eikenaar et al., 2019).
140 Though we note that prolonged captivity (Dickens et al., 2009) and repeated sampling (Cyr and
141 Romero, 2009) can influence circulating CORT levels.

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

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

160 In general, our predictive framework required that studies had some sort of control
161 comparison, either via a control group or through repeated measures of the same individuals. We

162 therefore excluded field studies that only had population-level, rather than individual-level,
163 comparisons across time (Reneerkens et al., 2002) or across sites (O'Reilly and Wingfield,
164 1995), as these approaches did not allow direct tests of our predictions. We recognize that this
165 approach excludes a large amount of work examining endocrine mechanisms in avian migration-
166 notably, cross-sectional studies capturing birds near geographic barriers, and spring/autumn
167 comparisons. However, these studies are well reviewed in more general reviews and syntheses of
168 avian migration physiology (Cornelius et al., 2013; DeSimone et al., 2020; Ramenofsky, 2011;
169 Ramenofsky et al., 2012). We also note that our approach results in a heavy focus on
170 longitudinal studies, which skews our selection of studies towards captive studies (versus field),
171 and toward studies that use repeated blood sampling, which may affect circulating CORT levels
172 (Cyr and Romero, 2009). We provide more detail regarding the selection of articles for each
173 specific hypothesis and prediction, below.

174

175 **Hypothesis 1: Physiological Preparation Hypothesis**

176

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

179

180 The hypothesis that CORT promotes migration by stimulating physiological preparations
181 before departure leads to the prediction that CORT should be positively related with
182 physiological preparation. Physiological preparation for migration encompasses numerous
183 processes, including those involved in fat deposition, muscle hypertrophy, changes in organ size,
184 and blood composition (Berthold, 1996), and a variety of variables (e.g., feeding behavior,

185 changes in body fat and body mass) have been used to measure these processes. Here, we
186 categorized measures of physiological preparation into three main categories starting with those
187 that reflect behavioral process (feeding), then those that measure specific body tissues (measures
188 of fat and muscle), and finally integrative tissues measures (body mass). Explanations of the
189 different variables included in these three categories are detailed in Table 1. At origin sites,
190 physiological preparation can be examined over the few weeks prior to departure, but can also be
191 examined on a shorter, daily timescale. However, for stopover periods, which may be as short as
192 a few days, data are often limited to a daily timescale. To test this prediction, we searched for
193 studies in two main categories: i) those that used exogenous CORT, glucocorticoid agonists, or
194 glucocorticoid antagonists to manipulate CORT signaling and then assessed changes in one or
195 more measures of physiological preparation, and ii) those that examined naturally circulating
196 CORT levels and measures of physiological preparation in birds either transitioning into the
197 migratory state or caught during stopovers.

198 Because our prediction posits that CORT is stimulating or positively changing physiological
199 variables such as food intake or weight gain, in our second category (studies examining naturally
200 circulating levels of CORT) we restricted our analysis to studies that collected repeated measures
201 of physiological variables. Since data collected from a single time point provide no information
202 about whether and how a physiological variable is changing, use of repeated measures allows for
203 better assessment of whether birds were indeed preparing for eventual departure. The limited
204 utility of single time point correlations in this context is illustrated by findings that in refueling
205 Bar-tailed Godwits (*Limosa lapponica*), there is a positive relationship between baseline CORT
206 and body mass as well as a positive relationship between time since arrival and body mass
207 (Landys-Ciannelli et al., 2002); with these data one cannot identify whether birds with higher

208 CORT are undergoing greater increases in body mass or whether CORT levels became elevated
209 as birds achieve greater body mass (Eikenaar et al., 2013; Piersma et al., 2000).

210

211 *a) Studies manipulating glucocorticoid signaling*

212

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

231 as a true CORT antagonist or may be causing effects via antagonism of progesterone receptors
232 (Landys et al., 2006). In contrast to using a glucocorticoid antagonist, Holberton et al. (2007)
233 used a glucocorticoid receptor agonist (dexamethasone) on spring migratory Dark-eyed Juncos
234 (*Junco hyemalis*), and found that injections of dexamethasone caused no changes in food intake
235 compared to controls. Dolnik and Blyumental (1967) similarly found that feeding rates were not
236 elevated in autumnal pre-migratory Chaffinches (*Fringilla coelebs*) injected with hydrocortisone
237 compared to controls. Finally, in contrast to invasive methods that directly inject birds with
238 pharmacological compounds, Eikenaar (2017) increased CORT by feeding Northern Wheatears
239 (*Oenanthe oenanthe*) mealworms injected with CORT, and found that CORT-supplementation
240 did not significantly affect food intake in birds during a simulated autumnal migratory stopover
241 compared to controls. Overall, these studies provide very little support for the prediction that
242 CORT stimulates increased feeding in migratory birds.

243 Several studies have also tested the predictions that fattening and muscle hypertrophy are
244 positively related with CORT in migratory birds (Table 2a). The study that most supports this
245 prediction is Landys et al. (2004b), who found that while CORT implants increased fat
246 deposition in captive White-crowned Sparrows compared to controls, this fat increase was
247 negated with concurrent implantation of a CORT antagonistic (RU486). Other studies, in
248 contrast, do not support this prediction, as both Holberton et al. (2007) and Eikenaar (2017) did
249 not find that exogenous glucocorticoids increased fattening in spring migratory Dark-eyed
250 Juncos and autumnal migratory Northern Wheatears, respectively. Specifically, Holberton et al.
251 (2007) found that repeated injections of a glucocorticoid agonist (dexamethasone) did not affect
252 fat score and actually decreased body mass, while Eikenaar (2017) found that non-invasive
253 CORT supplementation failed to affect non-lean body mass. Together, these studies provide

254 mixed support for the prediction that CORT stimulates fattening and weight gain in migratory
255 birds.

256

257 *b) Studies examining naturally circulating CORT in captive birds*

258

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

269 Similar to studies on feeding, there is little support for the prediction that CORT is positively
270 related with rates of fattening and muscle hypertrophy (Table 2a). When Holberton et al. (2008)
271 transitioned captive Dark-eyed Juncos from short- to long-day photoperiods to stimulate the
272 transition to a spring migratory state, they found an increase in both subcutaneous fat stores and
273 baseline CORT. However, CORT was not highest during the time periods of greatest fat
274 deposition and was instead highest when birds had reached their peak fat score. This pattern
275 would be more consistent with a role for CORT in maintaining peak levels of fat deposits and/or
276 with CORT responding to changes in fat stores and elevated CORT levels reflecting

277 positive/enhanced body condition (Eikenaar et al., 2013; Piersma et al., 2000). In a study of
278 captive Dark-eyed Juncos, Fudickar et al. (2016) found no relationship between changes in
279 fattening and CORT as birds exposed to natural photoperiods increased fat scores but did not
280 change baseline CORT levels during the spring migratory preparation period. Also inconsistent
281 with the prediction are the findings of Wingfield et al. (1996), who examined captive Gambel's
282 White-crowned sparrows during the spring pre-migratory period and found that while birds had
283 significantly greater fat stores after photostimulation, changes in circulating CORT levels did not
284 parallel these changes in fat. Ramenofsky et al. (2017) also examined captive Gambel's White-
285 crowned Sparrows during the spring migratory preparation period, and also found an increase in
286 fat score and flight muscle profile but no change in baseline CORT levels. Robart et al. (2019)
287 also looked at flight muscle and fat changes during the spring migratory preparation period in
288 captive Pine Siskins (*Pinus spinus*), but found no changes in baseline CORT over time whereas
289 fat deposits and flight muscle size increased. Overall, these studies provide little support for the
290 prediction that elevated CORT enhances fat deposition and flight muscle hypertrophy in
291 migratory birds preparing for departure.

292 While some studies assess fat stores through visual scoring, other studies have quantified fat
293 stores as "fuel load" by calculating the ratio of total body mass to lean body mass (Table 1)
294 (Schmaljohann and Naef-Daenzer, 2011). Studies assessing changes in fuel load (i.e., fuel
295 deposition rate) have been conducted in the context of migratory stopovers, and the majority
296 have not found the predicted relationships with baseline CORT (Table 2a). In captive Northern
297 Wheatears, fuel deposition rate is negatively correlated with baseline CORT levels during both
298 autumnal (Eikenaar et al., 2014a) and spring (Eikenaar et al., 2013) stopovers, the opposite
299 pattern of the prediction. An additional study in Northern Wheatears that examined changes in

300 fecal glucocorticoid metabolites (fGCM) and fuel deposition rate across two nights during a
301 spring stopover found a positive relationship between these variables (Eikenaar et al. 2018b),
302 which the authors interpreted as consistent with CORT levels increasing in response to changing
303 fuel load, rather than stimulating fueling.

304 Studies examining body mass and baseline CORT relationships have found mixed support
305 for the prediction that CORT facilitates migratory preparation (Table 2a). Results from Henshaw
306 et al. (2009) do not support this prediction, as Thrush Nightingales exposed to magnetic fields
307 simulating a stopover site at the onset of autumn migration increased in weight but did not
308 change baseline CORT levels. Both Robart et al. (2019) and Ramenofsky et al. (2017) also saw
309 increases in body mass but no changes in baseline CORT levels in captive Pine Siskins and
310 White-crowned Sparrows entering the spring migratory condition, respectively. In contrast, four
311 studies using captive birds have found that both baseline CORT and body mass increase during
312 the transition into a migratory state (Yellow-rumped Warblers (*Setophaga coronata*), Holberton,
313 1999; Dark-eyed Juncos, Holberton et al., 2008; Red Knots, Landys et al. 2004a and Piersma et
314 al., 2000). However, in all four of these studies, CORT levels were not highest during the periods
315 of most rapid weight gain and were instead highest when birds had reached their peak body
316 mass. This pattern is exemplified in the study by Piersma et al. (2000), who specifically analyzed
317 how CORT varied with rate of body mass change, and found that CORT levels peaked when
318 body mass was stable and high. If, as these findings suggest, CORT is not stimulating increases
319 in body mass, then the positive relationship between body mass and baseline CORT observed in
320 many studies could arise either because (i) high body mass stimulates CORT secretion, and
321 therefore CORT may be an indicator of energetic readiness for departure, or (ii) both CORT and
322 body mass increase as departure nears, but these two variables are not directly related with each

323 other. Either of these relationships could occur under Hypothesis 2 (that CORT stimulates
324 migratory departure).

325

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

327

328 Although the period of “pre-departure preparation” is difficult to define, we focused on
329 studies that sampled birds during stopovers or in what was expected to be within 3 weeks of
330 migratory departure from breeding or wintering grounds. We did not include studies that
331 measured circulating CORT levels during different stages of the annual cycle more than several
332 weeks apart, as such studies do not allow us to distinguish between changes in CORT occurring
333 around migration and changes in CORT related to other transitions across the annual cycle. For
334 example, many studies measure CORT during winter, spring migration, and early breeding.
335 Because CORT levels typically increase during breeding (Romero, 2002), it is difficult to
336 determine whether an increase in CORT during spring migration is caused by the anticipation of
337 breeding or is instead related to migratory processes. The same may be true of autumn migration,
338 as studies usually measure CORT during molt, autumn migration, and winter. Because CORT
339 levels are typically at their nadir during molt (Romero, 2002), higher levels during autumn
340 migration may represent a return to unsuppressed levels, rather than processes related to
341 migration. Thus, to avoid confounding changes in CORT driven by these other stages of the
342 annual cycle, we focused on the period of pre-migratory preparation within a few weeks of
343 departure. We also included studies that examined migratory birds during stopovers, as birds
344 refuel and make physiological preparations for migratory flight during this time period. Studies
345 generally fell into two categories: a) those that compared migratory and non-migratory

346 individuals of a single species (i.e., partial migrants) living under the same environmental
347 conditions during the pre-migratory or stopover stages, and b) those that took repeated samples
348 from a migratory population over the pre-migratory or stopover period. For studies that took
349 repeated CORT samples, we compared CORT measures before and during the timepoints when
350 birds began increasing food intake, fattening, or gaining body weight, with the prediction that
351 CORT should be elevated during the period of physiological preparation compared to earlier
352 timepoints where body condition remained constant. For studies comparing migrants and non-
353 migrants within a species, we note that differences between migrants and non-migrants in the
354 timing of life history transitions (e.g., transition into the breeding state in non-migrants, while
355 migrants are preparing for spring migration), may affect baseline CORT comparisons between
356 these groups and must be considered when interpreting results.

357

358 *a) Studies comparing migratory and non-migratory individuals*

359

360 For species that contain both migratory and sedentary strategies (either at the subspecies or
361 individual level), elevated CORT levels during the pre-departure period could be indicated by
362 migratory individuals having higher baseline CORT levels than non-migratory individuals at this
363 time when living under the same environmental conditions (Table 2b). This prediction is
364 supported by a study examining Blue tits (*Cyanistes caeruleus*), which found that migratory
365 females had higher fecal glucocorticoid metabolite levels than resident females during an
366 autumnal stopover (Nilsson and Sandell, 2009). We note, however, the direct comparisons
367 between resident and migrant Blue Tits in this study are complicated by the fact that migrants
368 and residents were captured at different sites, and Blue Tits are not a strongly migratory species.

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

389

390 *b) Repeated CORT sampling over the pre-departure period*

391

392 In contrast to studies comparing migratory and non-migratory individuals, there were fewer
393 studies that tested whether naturally circulating CORT is elevated over the pre-departure period
394 (Table 2b). One of the studies that supports this prediction found that captive Dark-eyed Juncos
395 shifted from short- to long-day photoperiods significantly increased circulating levels of baseline
396 CORT (Holberton et al., 2008). However, Robart et al. (2019) found an increase in migratory
397 restlessness and body condition but did not find an increase in baseline CORT levels in a captive
398 nomadic and irruptive migrant (Pine Siskins) during the spring pre-migratory period. Similarly,
399 Ramenofsky et al. (2017) also did not find an increase in baseline CORT across the spring pre-
400 migratory period in captive Gambel's White-crowned Sparrows. Conflicting findings from these
401 studies could be due to photoperiod manipulations, as Robart et al. (2019) and Ramenofsky et al.
402 (2017) used natural photoperiods, whereas Holberton et al. (2008) abruptly transitioned juncos
403 from 10.5L:13.5D to 18L:6D. Given that natural photoperiods are more representative of wild
404 conditions, we suggest that these studies are more relevant for testing predictions in migratory
405 birds.

406

407 *Synthesis of predictions for Physiological Preparation Hypothesis*

408

409 Looking across all studies, there is generally weak support for the hypothesis that CORT
410 stimulates physiological preparation for migration (Tables 2a-b). For the prediction that CORT is
411 positively related with feeding, fat/muscle, and body weight (Prediction 1), support was found in
412 two out of six pharmacological studies (33%) and five out of twelve (42%) studies of naturally
413 circulating CORT levels. However, as mentioned earlier, all of the studies that found positive
414 relationships between naturally circulating CORT and fat stores or body mass did not see peak

415 CORT levels during the time periods of most rapid gain, therefore casting doubt on whether
416 elevated CORT is a cause or consequence of fat and body mass increases. For the prediction that
417 CORT is elevated during the pre-departure period (Prediction 2), three out eight (38%) found
418 decisive evidence that CORT levels were higher in migratory vs. non-migratory individuals
419 during pre-migration or stopover, while one out of three (33%) repeated measures studies found
420 that CORT increased as birds entered the preparatory period.

421 Since we considered both spring and autumn migration in testing this hypothesis, differences
422 across studies in the results could reflect differences between these two migratory periods. For
423 Prediction 1, this does not appear to be the case, with no consistent difference between spring
424 and autumn migration. However, all studies of partial migrants (Prediction 2) that found
425 complete or mixed support for the prediction were carried out during the autumn (Eikenaar et al.,
426 2018a; Fudickar et al., 2013; Nilsson and Sandell, 2009; Schwabl et al., 1984), whereas all
427 studies that found no support for the prediction were carried out during the spring (Bauer et al.,
428 2016; Eikenaar et al., 2015; Fudickar et al., 2016; Ramenofsky et al., 2017). This could be
429 explained by several mutually exclusive hypotheses. First, CORT levels may indeed increase
430 during the time of migratory pre-departure, but perhaps this can only be distinguished during the
431 autumn as during the springtime residents are increasing CORT in anticipation of breeding
432 (Romero, 2002), therefore obscuring the difference in CORT levels between migrants and non-
433 migrants during spring but not autumn. Second, CORT levels may not notably increase during
434 the migratory pre-departure period, and lower CORT levels in residents vs. migrants during
435 autumn could be due to residents finishing their molt later than migrants. This could be a likely
436 explanation as CORT levels are typically very low during molt (Romero, 2002) and residents
437 have less pressure to finish their molt quickly (Helm and Gwinner, 2006). A third alternative

438 hypothesis is that spring and autumnal migration have different demands, and therefore CORT
439 levels may only significantly increase during autumnal preparation. We find this last hypothesis
440 the least likely, as studies generally indicate that the energetic demands of migration are greater
441 during spring compared to autumn (Bauer et al., 2019; Romero et al., 2017).

442 Another factor that could contribute to variation in results across studies could be differences
443 in physiological preparation between origin and stopover sites. The partial migrant studies that
444 supported Prediction 2 generally were conducted during stopovers rather than prior to migration.
445 For the pre-migratory time period, only one out of five (20%) studies found support for the
446 prediction that CORT is elevated during migratory preparation (Fudickar et al., 2013), and this
447 support did not quite reach statistical significance. In contrast, all studies conducted during
448 stopovers found either complete (Eikenaar et al., 2018a; Nilsson and Sandell, 2009) or mixed
449 (Eikenaar et al., 2015) support. Differences between stopovers and departure from
450 breeding/wintering grounds are likely linked with temporal constraints on sampling, as stopovers
451 are generally shorter in duration (e.g., lasting few days) than the pre-migratory period. As the
452 stopover period is an intense period of refueling, these studies may be more likely to sample
453 birds when fuel deposition rates are high and are thus more likely to detect differences between
454 migrants and residents. If this is the case, this would suggest that stopover studies may be
455 particularly well suited for testing the hypothesis that CORT stimulates physiological
456 preparations linked with migration. Alternatively, stopover studies are also more likely to sample
457 birds that have just arrived from a long-distance flight. If CORT is heightened during flight itself
458 (Hypothesis 3), then higher CORT levels in migrants vs. residents may represent this transition
459 from flight to refueling, rather than the predicted stimulatory actions of CORT on hyperphagia
460 and fattening. Finally, stopover studies are probably also more likely to sample birds closer to

461 departure, which therefore makes it difficult to distinguish whether data supports the hypothesis
462 that (1) CORT stimulates physiological preparation for migration or (2) CORT mediates
463 departure from origin and stopovers. Distinguishing among these alternatives would require data
464 collected on a relatively fine temporal scale and when stage within the pre-migratory or stopover
465 period can be determined, though we recognize the challenges involved in collecting such data.

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

476

477 **Hypothesis 2: Departure Stimulation Hypothesis**

478

479 *Prediction 3: In free-living birds, CORT should increase immediately prior to departure.*

480

481 Migratory departure decisions are driven by a combination of both initial predictive and
482 supplementary cues. For example, cues such as photoperiod and body condition must reach a
483 threshold for birds to be in a ‘window of readiness’ (Akesson et al., 2017), whereas

484 supplementary cues such as weather conditions are important for the actual decision to finally
485 depart (Akesson and Hedenstrom, 2000; Sjoberg et al., 2015). CORT could act as a mediator
486 between such departure cues and actual departure in both cases, with increased CORT during the
487 days prior to departure facilitating a ‘window of readiness’ and/or increased CORT during the
488 hours prior to departure actually enabling the decision to initiate migratory flight. To test
489 whether CORT increases during the days or hours prior to departure, we restricted our selection
490 of studies to those that used wild, free-living birds where individual departure date or time could
491 be determined (Table 3a). We did this because there is significant variation among individuals in
492 departure dates both at origin and stopovers (Stanley et al., 2012). Thus, individual-level data are
493 likely needed to evaluate relationships between CORT and departure date.

494 We found only four studies (Eikenaar et al., 2014a; Eikenaar et al. 2017; Eikenaar et al.,
495 2018a; Eikenaar et al. 2020b) that met our criteria (Table 3a); this is likely due to the difficulty
496 of accurately measuring the timing of departure in wild, free-living birds. From a ‘window of
497 readiness’ perspective, Eikenaar et al. (2014b; 2017) found CORT was negatively related with
498 days until departure in Northern Wheatears during an autumn stopover, meaning that CORT
499 tended to be higher in birds that departed soon after sampling. Similarly, Eikenaar et al. (2018a)
500 also found a non-significant trend between baseline CORT and departure time, where European
501 blackbirds that departed the night after sampling tended to have higher CORT levels than
502 blackbirds that stayed one or more nights at an autumn stopover site. Stronger relationships
503 between CORT and departure time have been found over shorter temporal periods, as three
504 studies by Eikenaar et al. (2017; 2018a; 2020b) found that birds departing immediately after
505 sunset had higher CORT levels than individuals that departed later at night. While these results
506 only approached significance in European blackbirds (Eikenaar et al., 2018a), studies in

507 Northern Wheatears (Eikenaar et al. 2017; 2020b) are more conclusive. During an autumn
508 stopover, departing Wheatears significantly increased baseline CORT from 5 h before sunset to
509 those 2 h before sunset, whereas no such increase was seen in Wheatears that prolonged their
510 stopover one or more nights (Eikenaar et al. 2020b). When examining actual departure time, both
511 Eikenaar et al. (2017) and Eikenaar et al. (2020b) found a strong correlation with CORT levels
512 tending to be higher the closer the bird was to departure.

513

514 *Prediction 4: In captive birds, CORT should be higher when migratory restlessness is displayed*

515

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

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

535

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

537

538 We found several studies that took repeated measures on captive birds as they transitioned
539 from a pre-migratory stage with no migratory restlessness, to a migratory stage with migratory
540 restlessness (Table 3b). Ramenofsky et al. (1999) found that under natural spring photoperiods,
541 afternoon and nighttime CORT levels were significantly higher in captive Dark-eyed Juncos
542 expressing migratory restlessness compared to those in a pre-migratory state. As juncos are
543 night-time migrants, these findings support the prediction that CORT levels increase over the
544 days leading up to consistent expression of migratory restlessness. These results are in contrast to
545 Mishra et al. (2017), who found that nighttime CORT levels did not significantly differ between
546 Blackheaded Buntings (*Emberiza melanocephala*) in spring pre-migratory (no migratory
547 restlessness expression) and migratory (migratory restlessness expression) states. Buntings,
548 however, were not exposed to natural light cycles and were instead gradually shifted from
549 8L:16D to 16:8D over a 1- or 4-week period, where birds shifted to long days over a 1-week
550 period were considered to be in the pre-migratory condition and birds shifted to long days over a
551 4-week period were considered to be in the migratory condition.

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

566 Partially migratory species, such as the European Blackbird, provide another way to test the
567 prediction that CORT differs between pre-migratory birds and migratory birds. Schwabl et al.
568 (1984) studied captive European Blackbirds exposed to natural autumn photoperiods and found
569 that in females, but not in males, individuals displaying migratory restlessness had higher
570 nighttime CORT levels than sedentary individuals during the migratory period. Therefore, this
571 provides mixed support for the prediction that CORT increases from the pre-migratory to
572 migratory period (Table 3b). It should be noted, however, that sedentary individuals do not
573 migrate, and therefore may not be representative of a pre-migratory state and instead could
574 represent a pre-breeding state.

575 Finally, the stopover period provides another way to examine how CORT changes as birds
576 transition into a period of consistent migratory restlessness expression (Table 3b). Schwabl et al.
577 (1991) exposed captive Garden Warblers (*Sylvia borin*) to a simulated autumnal stopover by
578 manipulating food availability. The authors found that nighttime CORT levels were significantly
579 lower during the simulated stopover (no migratory restlessness) compared to the period of time
580 where migratory restlessness was displayed, thus supporting the prediction that CORT increases
581 from a pre-migratory stage with no migratory restlessness to a migratory stage with migratory
582 restlessness (Schwabl et al., 1991).

583

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

585

586 There is also mixed evidence that CORT increases over the hours prior to display of
587 migratory restlessness (Table 3b). Using nocturnal migrants, some studies have found that CORT
588 levels are higher during the night (when migratory restlessness is expressed) than during the day.
589 Ramenofsky et al. (1999) found that in spring migratory Dark-eyed Juncos, CORT levels
590 increased significantly from early morning and early afternoon levels to the late afternoon and
591 night – which reflected an earlier rise in CORT compared to juncos sampled at other stages of
592 the annual cycle. Similarly, Landys et al. (2004c) examined captive White-crowned sparrows in
593 a spring migratory state and found that CORT levels increased from the morning to the evening
594 and peaked during the early night; this pattern contrasted with that of birds in a short-day
595 wintering state that did not elevate CORT in the early night. On the other hand, Mishra et al.
596 (2017) found mixed support for the prediction that CORT is higher during the hours of migratory
597 restlessness, as both pre-migratory (no migratory restlessness expression) and migratory

598 (migratory restlessness expression) Blackheaded Buntings had higher CORT levels during the
599 evening and night than during the morning and afternoon. Similarly, Schwabl et al. (1984) found
600 that male European Blackbirds had higher CORT levels during the late night than during the
601 afternoon, regardless of whether they displayed migratory restlessness. Furthermore, Schwabl et
602 al. (1984) found that for female blackbirds displaying migratory restlessness, CORT levels did
603 not significantly differ between the early afternoon and the late night, thus providing no support
604 for the prediction that CORT levels are higher during the hours of migratory restlessness
605 expression. Schwabl et al. (1991) also found no support for this prediction in captive Garden
606 Warblers, as birds displaying migratory restlessness had relatively consistent CORT levels
607 throughout the day, except for dawn CORT levels (when flight typically ends) being
608 significantly higher than early morning CORT levels.

609

610 *Synthesis of predictions for Departure Stimulation Hypothesis*

611

612 In summary, studies of both free-living and captive birds provide modest evidence that CORT
613 increases over the days prior to departure, but stronger evidence that CORT increases over the
614 hours prior to departure (Tables 3a-b). The three studies from free-living birds that measured
615 patterns across days found trends in support of the hypothesis. Four out of seven studies of
616 captive birds that examined patterns across days (57%: Schwabl et al. 1991; Ramenofsky et al.
617 1999; Holberton et al. 2007, 2008) found that CORT is lower during a period of migratory
618 preparation compared to periods where migratory restlessness is expressed. However, Schwabl et
619 al. (1984) found that this trend was true for females but not males, and two studies (29%: Mishra
620 et al. 2017; Robart et al. 2019) found no differences in CORT in the days before and after initial

621 display of migratory restlessness in captive birds. Conflicting findings among these captive
622 studies could be explained by the type of photoperiodic manipulations used in experiments. The
623 strongest support came from studies using abrupt, unnatural photoperiod changes (Schwabl et al.
624 1991; Holberton et al. 2007, 2008; but see Mishra et al. 2017), whereas studies using naturally
625 changing photoperiods were much more mixed in their support (strong- Ramenofsky et al., 1999;
626 mixed- Schwabl et al., 1984; and no support- Robart et al., 2019). For studies that examined
627 within-day patterns, all three studies of free-living birds supported the hypothesis, though one
628 did not reach statistical significance. Among the five diel studies in captive birds, two provide
629 strong support (40%: Landys et al. 2004c; Ramenofsky et al. 1999) and one provides mixed
630 support (20%: Mishra et al. 2017) for the prediction that CORT increases across the day as birds
631 transition into migratory restlessness, whereas the two remaining studies (40%: Schwabl et al.
632 1984, 1991) provide no support for this prediction (Table 3b). Mixed results from the
633 abovementioned studies could be due to differences in the time of day of when samples were
634 collected. Specifically, for the hypothesis that CORT helps facilitate migratory departure, we
635 would expect that CORT levels should peak close to the actual time of departure. However,
636 some studies did not take samples around this time, and instead included samples from later in
637 the night (e.g. Schwabl et al. 1984). Moreover, variation among individuals in the timing of
638 departure or expression of migratory restlessness (Muller et al., 2016) and correspondingly
639 variation in the timing of peak CORT (Eikenaar et al., 2017), could also make relationships
640 difficult to discern. Conflicting findings could also be due to species differences in non-
641 migratory diel CORT rhythms, although we find this unlikely as there is strong evidence that
642 CORT peaks at the beginning of the active phase across most bird species (Schwabl et al., 2016).
643 Finally, relationships between CORT and migratory restlessness expression could vary between

644 spring and autumn, as we found that spring studies provided either strong (Landys et al., 2004c;
645 Ramenofsky et al., 1999) or mixed (Mishra et al., 2017) support for the prediction that CORT
646 and migratory restlessness are positively related on a diel basis, whereas autumnal studies
647 (Schwabl et al. 1984, 1991) generally did not find support that this prediction.

648 The results we have summarized here highlight several avenues for future research
649 investigating the role of CORT in stimulating migratory departures. To overcome some of the
650 challenges in interpreting results of captive experiments that we have highlighted here, we
651 suggest that future captive studies use naturally changing photoperiods and carefully consider the
652 time of day at which sampling is done. For both captive and free-living studies, greater
653 examination of fine-scale temporal variation across the day, paired with behavioral data, could
654 be very informative. For example, many nocturnal migrants show a period of quiescence
655 immediately before departure (or before the expression of MR in captivity) (Agatsuma and
656 Ramenofsky, 2006; Morton, 1967; Schofield et al., 2018). This period may function in cue
657 integration necessary for departure decisions, orientation for flight, and transitioning of
658 metabolic processes (Cochran et al., 2004; Coverdill et al., 2008; Ramenofsky et al., 2008), but
659 few studies have examined patterns of CORT secretion on the timescale of the transition from
660 quiescence to departure or MR (but see Ramenofsky and Wingfield, 2017). More broadly, we
661 suggest that future studies examine both males and females, as the work of Schwabl et al. (1984)
662 demonstrates that relationships between CORT and migratory restlessness expression may differ
663 by sex, and explore differences between spring and autumn migration. While existing studies of
664 free-living birds have some limitations – they represent just two species, have small samples
665 sizes, and were carried out during autumn stopovers – they highlight the exciting possibilities for
666 generating these sorts of datasets in an effort to better understand the physiological mechanisms

667 underpinning departure decisions. Thus, to the extent that this approach could be applied to other
668 species, other seasons, and departures from both origin sites and stopovers, this offers a
669 promising approach for future researchers. Additionally, in systems for which migratory
670 departures can be measured, we also suggest that pharmacological manipulations of CORT could
671 be used to test for a direct, causal role in migratory departure. While pharmacological studies
672 have drawbacks such as potentially generating unrealistic hormone levels or binding affinities,
673 these studies are nevertheless a logical first step towards understanding the functional role of
674 hormones on migratory physiology and behavior.

675

676 **Hypothesis 3: Flight Support Hypothesis**

677

678 *Prediction 5: CORT levels should be elevated during flight*

679

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

690 migratory flight and compared CORT levels with those during stopover. In all cases, we only
691 included studies that caught and sampled birds within minutes of ending flight.

692 Studies in homing pigeons were the first to examine relationships between CORT and long-
693 distance flight. While homing pigeons are domesticated birds derived from a non-migratory
694 species (*Columba livia*), these studies can help to answer the important question of whether
695 CORT levels are elevated during long periods of flight. Haase et al. (1986) found racing pigeons
696 flying over 115 km had significantly elevated CORT compared to resting controls that
697 experienced the same handling and sampling procedures. In contrast, Viswanathan et al. (1987)
698 found that homing pigeons did not have significantly elevated CORT levels after a 48 km flight
699 compared to resting controls. These differences may be attributed to the distance flown, and we
700 suggest that flights over 115 km may be more representative of those taken by migrating birds.
701 Therefore, studies in homing pigeons provide some support for the prediction that CORT is high
702 during flight (Table 4), though inferences from pigeons are limited by the fact that these are not
703 migratory birds. Furthermore, if functions of CORT during flight are dependent on processes that
704 occur during seasonal migratory preparation, then homing pigeons may not provide useful data
705 for testing the hypothesis that CORT is elevated during long-distance migratory flight.

706 Wind tunnel studies provide mixed support for the prediction that circulating CORT levels are
707 elevated during long-distance flight (Table 4). Casagrande et al. (2020) provide the strongest
708 support for this prediction, as they found that immediately after European starlings (*Sturnus*
709 *vulgaris*) flew for 6 hours in a wind tunnel, birds had significantly higher plasma CORT levels
710 compared to 2 days after flight. This study found the same pattern during both the autumn and
711 spring migratory periods. Conversely, Jenni-Eiermann et al. (2009) found that baseline CORT
712 levels in Red knots were similar before and after 2 or 10 hours of flight in a wind tunnel. Finally,

713 Scollon et al. (2004) found that baseline CORT levels were similar between resting female
714 White-crowned sparrows and those that were flown in a wind tunnel for 20–150 min during late
715 spring. Flown birds did trend towards higher baseline CORT than resting birds, however, and a
716 low sample size of 12 birds that experienced different durations of flight may have further
717 obscured baseline CORT differences between groups.

718 To examine whether wild, free-living birds have elevated CORT during flight, field studies
719 have focused on catching birds out of flight at or near important stopover sites along migratory
720 flyways (Table 4). For example, Falsone et al. (2009) caught migrating European robins
721 (*Erithacus rubecula*) at an Alpine pass during autumn migration. Since robins are nocturnal
722 migrants, those captured at night were caught during migratory flight, whereas those caught
723 during the day were stopping over at the site. The authors found that robins caught during flight
724 (nighttime capture) had significantly higher baseline CORT compared to robins caught while
725 resting and refueling (daytime capture). While non-migrating birds also demonstrate higher
726 levels of baseline CORT during the night compared to the day (Remage-Healey and Romero,
727 2000; Rich and Romero, 2001), these levels tend to peak around dawn, before the active period
728 (Breuner et al., 1999; Landys et al., 2004c). As Falsone et al. (2009) found no effect of capture
729 time on nighttime baseline CORT levels, this suggests that the elevation in CORT levels
730 observed during night in migrating European robins are likely driven by the act of flight itself,
731 rather than by normal diel patterns of CORT secretion. In a study of Bar-tailed godwits, Landys-
732 Ciannelli et al. (2002) compared birds resting and refueling during the single stopover (in the
733 Wadden Sea) of their 9,000 km migratory journey with birds caught out of flight (using decoys
734 and song playback) at a small island ~60 km before godwits reach the Wadden Sea. Similar to
735 Falsone et al. (2009), Landys-Ciannelli et al. (2002) found that godwits caught out of flight had

736 significantly higher baseline CORT levels than those resting at the stopover site. A limitation of
737 this study is that godwits caught out of flight were near the end of a continuous 4,000 km flight
738 and thus weighed less and had less fat compared to stopover birds; thus differences in CORT
739 could be due to differences in body condition. However, this alternative explanation is unlikely
740 as the authors found no direct relationship between size-corrected body mass and baseline CORT
741 in godwits caught before stopover.

742

743 *Synthesis of prediction for Flight Support Hypothesis*

744

745 In summary, one homing pigeon study, one wind tunnel study, and two field studies (57% of
746 studies examined) support the prediction that baseline CORT is elevated during flight (Table 4).
747 Supportive and non-supportive studies were evenly distributed across autumn and spring;
748 therefore, it is unlikely that mixed findings can be attributed to seasonal differences. For wind
749 tunnel studies, mixed findings could be related with the amount of time that birds were flying.
750 Jenni-Eiermann et al. (2009) posited that high CORT levels may be important during the
751 beginning portion of flight when metabolism switches from predominately using carbohydrates
752 to lipids for fuel (Jenni-Eiermann et al., 2002); this may therefore explain why they saw a trend
753 towards elevated levels after 2 hours of flight but not after 10 hours of flight in Red knots.
754 Casagrande et al. (2020), however, saw elevated CORT levels after 6 hours of flight in starlings,
755 which is long after the metabolic switch to lipids. It is also possible that CORT's role in
756 promoting flight is temporally dynamic, and that levels may only be elevated during certain
757 stages or phases of flight. Stages that may be supported by elevated CORT could include the
758 metabolic switch from carbohydrate- to lipid-based fueling and the end of the flight period when

759 birds are close to exhausting energy stores. An elevation of CORT occurring at the end of flight
760 corresponds well with the studies described here, as both field studies found elevated levels of
761 baseline CORT, either in a long-hop migrant right before stopover (godwits, Landys-Ciannelli et
762 al. 2002) or in a short-hop migrant caught out of flight (European robins, Falsone et al. 2009).
763 Additionally, for wind tunnel studies, European starlings were flown for durations similar to
764 what they would experience in the wild (Perdeck, 1958, 1964), whereas Red Knots generally fly
765 for bouts much longer than 10 h (Piersma and Davidson, 1992), and White-crowned sparrows
766 did not fly continuously and instead were flown in several 20 min bouts. If CORT is only
767 elevated at the end of bird's typical, continuous flight bout, then this may explain why starlings
768 had elevated CORT levels after wind tunnel flight, but knots and sparrows did not. Finally, lack
769 of important cues, notably appropriate light-dark cycles, may impact flight behavior (Breuner et
770 al., 1999; Coverdill et al., 2008; Ramenofsky and Wingfield, 2017). Therefore, appropriate
771 ambient light levels and time of day may be required to see typical CORT-flight relationships
772 during migration.

773 For future wind tunnel studies, we specifically recommend that flight duration is manipulated
774 or that during a long flight bout the wind tunnel is periodically stopped so repeated CORT
775 measurements could be taken, as this may better examine how CORT changes over the different
776 metabolic stages of migratory flight. Another element that should be considered in future wind
777 tunnel studies is food availability and fuel loads in captive birds, as captive birds with ad libitum
778 food may carry fuel loads that are much higher than those in wild, migrating birds. Concurrent
779 measurements of CORT and metabolites indicative of protein and fat breakdown (e.g. uric acid
780 and triglycerides, Jenni-Eiermann et al. 2002) would also shed light on the potential role of
781 CORT in flight, as we would predict positive relationships between CORT and uric

782 acid/triglyceride levels under the hypothesis that CORT facilitates the metabolic demands of
783 flight. Another approach that could be useful for future field studies would be to capture and
784 sample birds at stopover sites after large water crossings, (e.g. Gulf of Mexico), as this allows
785 good estimates of flight distance before sampling (Agostini et al., 2015). With a variety of
786 stopover sites, and therefore known flight distances, further relationships between CORT and
787 flight duration can be examined.

788

789 **Conclusions**

790

791 CORT has long been proposed to be an important endocrine signal coordinating migration
792 (Cornelius et al., 2013; Ramenofsky, 2011; Ramenofsky et al., 2012). However, the specific role
793 of CORT in migration has been the subject of much debate. Here we reviewed the literature to
794 test predictions that follow from three broad hypothesized functions of CORT in migration: the
795 Physiological Preparation Hypothesis, the Departure Stimulation Hypothesis, and the Flight
796 Support Hypothesis. We identified 32 studies with data that addressed predictions for one or
797 more of the hypotheses. Data to test the Physiological Preparation Hypothesis were most
798 common, but support for this hypothesis was weak. Overall, less than half of studies examined
799 met the predictions of the hypothesis. The strongest support for this hypothesis came from
800 studies that examined changes in baseline CORT across the pre-departure period; however, it is
801 important to note that these findings could also support the Departure Stimulation Hypothesis.
802 While fewer studies addressed the Departure Stimulation and Flight Support Hypotheses, there
803 was more support for both of these hypotheses. Support for the Departure Stimulation
804 Hypothesis was mixed, but we gave greater weight to studies conducted in wild, free-living birds

805 – these studies generally met the predictions of the hypothesis. Though we note that existing
806 studies of wild, free-living birds tend to be limited by small sample sizes and a focus on
807 departure from stopovers. The Flight Support Hypothesis also had mixed support, with results
808 from free-living birds sampled during migratory flight providing the strongest support. However,
809 more studies are necessary to better test this hypothesis.

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

824

825 **Authors' Contributions**

826

827 CMB and HEW conceived and wrote the manuscript.

828

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832

833 **Declaration of competing interest**

834

835 The authors report no competing interests

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842

843 **References**

844

845 Agatsuma, R., Ramenofsky, M., 2006. Migratory behaviour of captive white-crowned
846 sparrows, *Zonotrichia leucophrys gambelii*, differs during autumn and spring migration.
847 Behaviour 143, 1219-1240.

848 Agostini, N., Panuccio, M., Pasquaretta, C., 2015. Morphology, flight performance, and water
849 crossing tendencies of Afro-Palearctic raptors during migration. Current Zoology 61, 951-
850 958.

851 Akesson, S., Hedenstrom, A., 2000. Wind selectivity of migratory flight departures in birds.
852 Behavioral Ecology and Sociobiology 47, 140-144.

853 Akesson, S., Ilieva, M., Karagicheva, J., Rakhimberdiev, E., Tomotani, B., Helm, B., 2017.
854 Timing avian long-distance migration: from internal clock mechanisms to global flights.
855 Philosophical Transactions of the Royal Society B-Biological Sciences 372, 16.

856 Alerstam, T., Hedenstrom, A., Akesson, S., 2003. Long-distance migration: evolution and
857 determinants. Oikos 103, 247-260.

858 Angelier, F., Clement-Chastel, C., Gabrielsen, G.W., Chastel, O., 2007. Corticosterone and
859 time-activity budget: An experiment with Black-legged kittiwakes. Hormones and Behavior
860 52, 482-491.

861 Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of Corticosterone with
862 Feeding, Activity and Metabolism in Passerine Birds. Ornis Scandinavica 23, 355-365.

863 Bailleul, F., Lesage, V., Power, M., Doidge, D.W., Hammill, M.O., 2012. Migration phenology
864 of beluga whales in a changing Arctic. Clim. Res. 53, 169-178.

865 Bauer, C.M., Graham, J.L., Greives, T.J., 2019. Corticosterone negative feedback is weaker
866 during spring vs. autumn migration in a songbird (*Junco hyemalis*). General and
867 Comparative Endocrinology 280, 36-42.

868 Bauer, C.M., Needham, K.B., Le, C.N., Stewart, E.C., Graham, J.L., Ketterson, E.D., Greives,
869 T.J., 2016. Hypothalamic-pituitary-adrenal axis activity is not elevated in a songbird (*Junco*
870 *hyemalis*) preparing for migration. General and Comparative Endocrinology 232, 60-66.

871 Berthold, P., 1996. Control of Bird Migration. Chapman and Hall, London.

872 Berthold, P., Fiedler, W., Querner, U., 2000. Die Zugunruhe bei Vögeln — eine Darstellung
873 nach Videoaufnahmen bei Infrarotlichtbeleuchtung. Journal für Ornithologie 141, 285-299.

874 Berthold, P., Querner, U., 1988. Was Zugunruhe wirklich ist — eine quantitative Bestimmung
875 mit Hilfe von VideoAufnahmen bei Infrarotlichtbeleuchtung. *Journal für Ornithologie* 129,
876 372-375.

877 Boel, M., Aarestrup, K., Baktoft, H., Larsen, T., Madsen, S.S., Malte, H., Skov, C., Svendsen,
878 J.C., Koed, A., 2014. The Physiological Basis of the Migration Continuum in Brown Trout (
879 *Salmo trutta*). *Physiological and Biochemical Zoology* 87, 334-345.

880 Breuner, C.W., Greenberg, A.L., Wingfield, J.C., 1998. Noninvasive corticosterone treatment
881 rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys*
882 *gambelii*). *General and comparative endocrinology* 111, 386-394.

883 Breuner, C.W., Hahn, T.P., 2003. Integrating stress physiology, environmental change, and
884 behavior in free-living sparrows. *Hormones and behavior* 43, 115-123.

885 Breuner, C.W., Wingfield, J.C., 2000. Rapid Behavioral Response to Corticosterone Varies
886 with Photoperiod and Dose. *Hormones and Behavior* 37, 23-30.

887 Breuner, C.W., Wingfield, J.C., Romero, L.M., 1999. Diel rhythms of basal and stress-induced
888 corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *Journal of*
889 *Experimental Zoology* 284, 334-342.

890 Brodersen, J., Nilsson, P.A., Hansson, L.A., Skov, C., Bronmark, C., 2008. Condition-
891 dependent individual decision-making determines cyprinid partial migration. *Ecology* 89,
892 1195-1200.

893 Carruth, L.L., Jones, R.E., Norris, D.O., 2002. Cortisol and pacific salmon: A new look at the
894 role of stress hormones in olfaction and home-stream migration. *Integrative and*
895 *Comparative Biology* 42, 574-581.

896 Casagrande, S., DeMoranville, K.J., Trost, L., Pierce, B., Bryla, A., Dzialo, M., Sadowska,
897 E.T., Bauchinger, U., McWilliams, S.R., 2020. Dietary antioxidants attenuate the endocrine
898 stress response during long-duration flight of a migratory bird. *Proceedings of the Royal
899 Society B-Biological Sciences* 287.

900 Cochran, W.W., Mouritsen, H., Wikelski, M., 2004. Migrating songbirds recalibrate their
901 magnetic compass daily from twilight cues. *Science* 304, 405-408.

902 Cornelius, J.M., Boswell, T., Jenni-Eiermann, S., Breuner, C.W., Ramenofsky, M., 2013.
903 Contributions of endocrinology to the migration life history of birds. *General and
904 Comparative Endocrinology* 190, 47-60.

905 Coverdill, A.J., Bentley, G.E., Ramenofsky, M., 2008. Circadian and masking control of
906 migratory restlessness in Gambel's white-crowned sparrow (*Zonotrichia leucophrys
907 gambelii*). *Journal of Biological Rhythms* 23, 59-68.

908 Cyr, N.E., Romero, L.M., 2009. Identifying hormonal habituation in field studies of stress.
909 *General and Comparative Endocrinology* 161, 295-303.

910 de Bruijn, R., Romero, L.M., 2011. Behavioral and physiological responses of wild-caught
911 European starlings (*Sturnus vulgaris*) to a minor, rapid change in ambient temperature.
912 *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 160, 260-
913 266.

914 De Kloet, E.R., Veldhuis, H.D., Wagenaars, J.L., Bergink, E.W., 1984. Relative binding-
915 affinity of steroids for the corticosterone receptor system in rat hippocampus. *J. Steroid
916 Biochem. Mol. Biol.* 21, 173-178.

917 DeSimone, J.G., Ramirez, M.G., Elowe, C.R., Griego, M.S., Breuner, C.W., Gerson, A.R.,
918 2020. Developing a Stopover-CORT hypothesis: Corticosterone predicts body composition

919 and refueling rate in Gray Catbirds during migratory stopover. *Hormones and Behavior* 124,
920 10.

921 Dickens, M.J., Earle, K.A., Romero, L.M., 2009. Initial transference of wild birds to captivity
922 alters stress physiology. *General and comparative endocrinology* 160, 76-83.

923 Dolnik, V.R., Blyumental, T.I., 1967. Autumnal premigratory and migratory periods in
924 chaffinch (*Fringilla coelebs coelebs*) and some other temperatre-zone passerine birds.
925 *Condor* 69, 435-468.

926 Eikenaar, C., 2017. Endocrine regulation of fueling by hyperphagia in migratory birds. *J.*
927 *Comp. Physiol. A -Neuroethol. Sens. Neural Behav. Physiol.* 203, 439-445.

928 Eikenaar, C., Bairlein, F., Stowe, M., Jenni-Eiermann, S., 2014a. Corticosterone, food intake
929 and refueling in a long-distance migrant. *Hormones and Behavior* 65, 480-487.

930 Eikenaar, C., Ballstaedt, E., Hessler, S., Klinner, T., Muller, F., Schmaljohann, H., 2018a.
931 Cues, corticosterone and departure decisions in a partial migrant. *General and Comparative*
932 *Endocrinology* 261, 59-66.

933 Eikenaar, C., Fritsch, A., Bairlein, F., 2013. Corticosterone and migratory fueling in Northern
934 wheatears facing different barrier crossings. *General and Comparative Endocrinology* 186,
935 181-186.

936 Eikenaar, C., Hessler, S., Fischer, S., Bairlein, F., 2019. An exception to the rule: Captivity
937 does not stress wild migrating northern wheatears. *General and Comparative Endocrinology*
938 275, 25-29.

939 Eikenaar, C., Hessler, S., Hegemann, A., 2020a. Migrating birds rapidly increase constitutive
940 immune function during stopover. *R. Soc. Open Sci.* 7, 7.

941 Eikenaar, C., Klinner, T., Stowe, M., 2014b. Corticosterone predicts nocturnal restlessness in a
942 long-distance migrant. *Hormones and Behavior* 66, 324-329.

943 Eikenaar, C., Müller, F., Klinner, T., Bairlein, F., 2015. Baseline corticosterone levels are
944 higher in migrating than sedentary common blackbirds in autumn, but not in spring. *General
945 and Comparative Endocrinology*.

946 Eikenaar, C., Müller, F., Leutgeb, C., Hessler, S., Lebus, K., Taylor, P.D., Schmaljohann, H.,
947 2017. Corticosterone and timing of migratory departure in a songbird. *Proceedings of the
948 Royal Society B-Biological Sciences* 284.

949 Eikenaar, C., Müller, F., Ruppel, G., Stowe, M., 2018b. Endocrine regulation of migratory
950 departure from stopover: Evidence from a longitudinal migratory restlessness study on
951 northern wheatears. *Hormones and Behavior* 99, 9-13.

952 Eikenaar, C., Schafer, J., Hessler, S., Packmor, F., Schmaljohann, H., 2020b. Diel variation in
953 corticosterone and departure decision making in migrating birds. *Hormones and Behavior*
954 122, 6.

955 Falsone, K., Jenni-Eiermann, S., Jenni, L., 2009. Corticosterone in migrating songbirds during
956 endurance flight. *Hormones and Behavior* 56, 548-556.

957 Fanson, K.V., Nemeth, Z., Ramenofsky, M., Wingfield, J.C., Buchanan, K.L., 2017. Inter-
958 laboratory variation in corticosterone measurement: Implications for comparative ecological
959 and evolutionary studies. *Methods Ecol. Evol.* 8, 1745-1754.

960 Fudickar, A.M., Greives, T.J., Atwell, J.W., Stricker, C.A., Ketterson, E.D., 2016.
961 Reproductive Allochrony in Seasonally Sympatric Populations Maintained by Differential
962 Response to Photoperiod: Implications for Population Divergence and Response to Climate
963 Change. *American Naturalist* 187, 436-446.

964 Fudickar, A.M., Schmidt, A., Hau, M., Quetting, M., Partecke, J., 2013. Female-biased obligate
965 strategies in a partially migratory population. *Journal of Animal Ecology* 82, 863-871.

966 Fusani, L., 2008. Endocrinology in field studies: Problems and solutions for the experimental
967 design. *General and Comparative Endocrinology* 157, 249-253.

968 Goymann, W., 2012. On the use of non-invasive hormone research in uncontrolled, natural
969 environments: the problem with sex, diet, metabolic rate and the individual. *Methods Ecol.*
970 *Evol.* 3, 757-765.

971 Haase, E., Rees, A., Harvey, S., 1986. Flight stimulates adrenocortical activity in pigeons
972 (*Columa livia*). *General and Comparative Endocrinology* 61, 424-427.

973 Helm, B., Gwinner, E., 2006. Timing of molt as a buffer in the avian annual cycle. *Acta*
974 *Zoologica Sinica* 52, 703-706.

975 Henshaw, I., Fransson, T., Jakobsson, S., Jenni-Eiermann, S., Kullberg, C., 2009. Information
976 from the geomagnetic field triggers a reduced adrenocortical response in a migratory bird.
977 *Journal of Experimental Biology* 212, 2902-2907.

978 Hinch, S.G., Rand, P.S., 1998. Swim speeds and energy use of upriver-migrating sockeye
979 salmon (*Oncorhynchus nerka*): role of local environment and fish characteristics. *Can. J.*
980 *Fish. Aquat. Sci.* 55, 1821-1831.

981 Holberton, R.L., 1999. Changes in patterns of corticosterone secretion concurrent with
982 migratory fattening in a neotropical migratory bird. *General and Comparative*
983 *Endocrinology* 116, 49-58.

984 Holberton, R.L., Boswell, T., Hunter, M.J., 2008. Circulating prolactin and corticosterone
985 concentrations during the development of migratory condition in the Dark-eyed Junco,
986 *Junco hyemalis*. *General and Comparative Endocrinology* 155, 641-649.

987 Holberton, R.L., Wilson, C.M., Hunter, M.J., Cash, W.B., Sims, C.G., 2007. The role of
988 corticosterone in supporting migratory lipogenesis in the dark-eyed junco, *Junco hyemalis*:
989 A model for central and peripheral regulation. *Physiological and Biochemical Zoology* 80,
990 125-137.

991 Jenni-Eiermann, S., Hasselquist, D., Lindstrom, A., Koolhaas, A., Piersma, T., 2009. Are birds
992 stressed during long-term flights? A wind-tunnel study on circulating corticosterone in the
993 red knot. *General and Comparative Endocrinology* 164, 101-106.

994 Jenni-Eiermann, S., Jenni, L., 1991. Metabolic responses to flight and fasting in night-
995 migrating passerines. *Journal of Comparative Physiology B-Biochemical Systemic and*
996 *Environmental Physiology* 161, 465-474.

997 Jenni-Eiermann, S., Jenni, L., Kvist, A., Lindstrom, A., Piersma, T., Visser, G.H., 2002. Fuel
998 use and metabolic response to endurance exercise: a wind tunnel study of a long-distance
999 migrant shorebird. *Journal of Experimental Biology* 205, 2453-2460.

1000 Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 2001. Corticosterone facilitates begging and affects
1001 resource allocation in the black-legged kittiwake. *Behavioral Ecology* 12, 619-625.

1002 Landys, M.M., Piersma, T., Ramenofsky, M., Wingfield, J.C., 2004a. Role of the low-affinity
1003 glucocorticoid receptor in the regulation of behavior and energy metabolism in the
1004 migratory red knot *Calidris canutus islandica*. *Physiological and Biochemical Zoology* 77,
1005 658-668.

1006 Landys, M.M., Ramenofsky, M., Guglielmo, C.G., Wingfield, J.C., 2004b. The low-affinity
1007 glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's
1008 white-crowned sparrow *Zonotrichia leucophrys gambelii*. *Journal of Experimental Biology*
1009 207, 143-154.

1010 Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a
1011 seasonal baseline as compared to stress-related levels in the regulation of periodic life
1012 processes. *General and comparative endocrinology* 148, 132-149.

1013 Landys, M.M., Wingfield, J.C., Ramenofsky, M., 2004c. Plasma corticosterone increases
1014 during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys*
1015 *gambelli*. *Hormones and Behavior* 46, 574-581.

1016 Landys-Ciannelli, M.M., Ramenofsky, M., Piersma, T., Jukema, J., Wingfield, J.C., Castricum
1017 Ringing, G., 2002. Baseline and stress-induced plasma corticosterone during long-distance
1018 migration in the bar-tailed godwit, *Limosa lapponica*. *Physiological and Biochemical*
1019 *Zoology* 75, 101-110.

1020 Lattin, C.R., Breuner, C.W., Romero, L.M., 2015. Does corticosterone regulate the onset of
1021 breeding in free-living birds?: The CORT-Flexibility Hypothesis and six potential
1022 mechanisms for priming corticosteroid function. *Hormones and Behavior*.

1023 Linscott, J.A., Senner, N.R., 2021. Beyond refueling: Investigating the diversity of functions of
1024 migratory stopover events. *Ornithological Applications* 123.

1025 Löhmus, M., Sundstrom, F., Moore, F.R., 2006. Non-invasive corticosterone treatment changes
1026 foraging intensity in red-eyed vireos *Vireo olivaceus*. *Journal of Avian Biology* 37, 523-526.

1027 McWilliams, S.R., Guglielmo, C., Pierce, B., Klaassen, M., 2004. Flying, fasting, and feeding
1028 in birds during migration: a nutritional and physiological ecology perspective. *Journal of*
1029 *Avian Biology* 35, 377-393.

1030 Mishra, I., Singh, D., Kumar, V., 2017. Daily levels and rhythm in circulating corticosterone
1031 and insulin are altered with photostimulated seasonal states in night-migratory blackheaded
1032 buntings. *Hormones and Behavior* 94, 114-123.

1033 Morton, M.L., 1967. Diurnal feeding patterns in white-crowned sparrows, *Zonotrichia*
1034 *leucophrys gambelii*. Condor 69, 491-&.

1035 Muller, F., Taylor, P.D., Sjoberg, S., Muheim, R., Tsvey, A., Mackenzie, S.A., Schmaljohann,
1036 H., 2016. Towards a conceptual framework for explaining variation in nocturnal departure
1037 time of songbird migrants. Movement Ecology 4, 1-12.

1038 Nilsson, A.L.K., Sandell, M.I., 2009. Stress hormone dynamics: an adaptation to migration?
1039 Biology Letters 5, 480-483.

1040 O'Reilly, K.M., Wingfield, J.C., 1995. Spring and autumn migration in Arctic shorebirds- same
1041 distance, different strategies. American Zoologist 35, 222-233.

1042 Perdeck, A.C., 1958. Two types of orientation in migrating starlings, *Sturnus vulgaris* L., and
1043 chaffinches, *Fringilla coelebs* L., as revealed by displacement experiment. Ardea 46, 1-37.

1044 Perdeck, A.C., 1964. An experiment on the ending of autumn migration in starlings. Ardea 52,
1045 133-139.

1046 Piersma, T., Davidson, N., 1992. The migrations and annual cycles of five subspecies of knots
1047 in perspective. Wader Study Group Bull. 64, 187-197.

1048 Piersma, T., Reneerkens, J., Ramenofsky, M., 2000. Baseline corticosterone peaks in
1049 shorebirds with maximal energy stores for migration: A general preparatory mechanism for
1050 rapid behavioral and metabolic transitions? General and Comparative Endocrinology 120,
1051 118-126.

1052 Pradhan, D.S., Van Ness, R., Jalabert, C., Hamden, J.E., Austin, S.H., Soma, K.K.,
1053 Ramenofsky, M., Schlinger, B.A., 2019. Phenotypic flexibility of glucocorticoid signaling in
1054 skeletal muscles of a songbird preparing to migrate. Hormones and Behavior 116, 104586.

1055 Ramenofsky, M., 2011. Hormones in Migration and Reproductive Cycles of Birds, in: Norris,
1056 D.O., Lopez, K.H. (Eds.), *Hormones and Reproduction of Vertebrates, Vol 4: Birds*.
1057 Elsevier Academic Press Inc, San Diego, pp. 205-237.

1058 Ramenofsky, M., Agatsuma, R., Ramfar, T., 2008. Environmental conditions affect the
1059 behavior of captive, migratory white-crowned sparrows. *Condor* 110, 658-671.

1060 Ramenofsky, M., Campion, A.W., Perez, J.H., Krause, J.S., Nemeth, Z., 2017. Behavioral and
1061 physiological traits of migrant and resident whitecrowned sparrows: a common garden
1062 approach. *Journal of Experimental Biology* 220, 1330-1340.

1063 Ramenofsky, M., Cornelius, J.M., Helm, B., 2012. Physiological and behavioral responses of
1064 migrants to environmental cues. *Journal of Ornithology* 153, S181-S191.

1065 Ramenofsky, M., Savard, R., Greenwood, M.R.C., 1999. Seasonal and diel transitions in
1066 physiology and behavior in the migratory dark-eyed junco. *Comparative Biochemistry and*
1067 *Physiology a-Molecular and Integrative Physiology* 122, 385-397.

1068 Ramenofsky, M., Wingfield, J.C., 2007. Regulation of migration. *Bioscience* 57, 135-143.

1069 Ramenofsky, M., Wingfield, J.C., 2017. Regulation of complex behavioural transitions:
1070 migration to breeding. *Animal Behaviour* 124, 299-306.

1071 Remage-Healey, L., Romero, L.M., 2000. Daily and seasonal variation in response to stress in
1072 captive starlings (*Sturnus vulgaris*): Glucose. *General and Comparative Endocrinology* 119,
1073 60-68.

1074 Reneerkens, J., Morrison, R.I.G., Ramenofsky, M., Piersma, T., Wingfield, J.C., 2002. Baseline
1075 and stress-induced levels of corticosterone during different life cycle substages in a
1076 shorebird on the high arctic breeding grounds. *Physiological and Biochemical Zoology* 75,
1077 200-208.

1078 Rensel, M.A., Schlinger, B.A., 2016. Determinants and significance of corticosterone
1079 regulation in the songbird brain. *Gen Comp Endocrinol* 227, 136-142.

1080 Rich, E.L., Romero, L.M., 2001. Daily and photoperiod variations of basal and stress-induced
1081 corticosterone concentrations in house sparrows (*Passer domesticus*). *Journal of*
1082 *Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 171, 543-
1083 547.

1084 Robart, A.R., Morado, M.I., Watts, H.E., 2019. Declining food availability, corticosterone, and
1085 migratory response in a nomadic, irruptive migrant. *Hormones and Behavior* 110, 56-67.

1086 Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living
1087 vertebrates. *General and comparative endocrinology* 128, 1-24.

1088 Romero, L.M., Bauer, C.M., de Bruijn, R., Lattin, C.R., 2017. Seasonal Rhythms, in: Fink, G.
1089 (Ed.), *Stress: Neuroendocrinology and Neurobiology*. Academic Press, Oxford, UK.

1090 Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is
1091 under 3 min good enough? *Comparative Biochemistry and Physiology A-Molecular &*
1092 *Integrative Physiology* 140, 73-79.

1093 Romero, L.M., Ramage-Healey, L., 2000. Daily and seasonal variation in response to stress in
1094 captive starlings (*Sturnus vulgaris*): Corticosterone. *General and comparative endocrinology*
1095 119, 52-59.

1096 Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress
1097 responses? Integrating permissive, suppressive, stimulatory, and preparative actions.
1098 *Endocrine reviews* 21, 55-89.

1099 Sauman, I., Briscoe, A.D., Zhu, H.S., Shi, D.D., Froy, O., Stalleicken, J., Yuan, Q., Casselman,
1100 A., Reppert, S.M., 2005. Connecting the navigational clock to sun compass input in
1101 monarch butterfly brain. *Neuron* 46, 457-467.

1102 Sawyer, H., Kauffman, M.J., 2011. Stopover ecology of a migratory ungulate. *Journal of*
1103 *Animal Ecology* 80, 1078-1087.

1104 Schmaljohann, H., Naef-Daenzer, B., 2011. Body condition and wind support initiate the shift
1105 of migratory direction and timing of nocturnal departure in a songbird. *Journal of Animal*
1106 *Ecology* 80, 1115-1122.

1107 Schofield, L.N., Deppe, J.L., Diehl, R.H., Ward, M.P., Bolus, R.T., Zenzal, T.J., Smolinsky, J.,
1108 Moore, F.R., 2018. Occurrence of quiescence in free-ranging migratory songbirds.
1109 *Behavioral Ecology and Sociobiology* 72.

1110 Schwabl, H., Bairlein, F., Gwinner, E., 1991. Basal and stress-induced corticosterone levels of
1111 Garden warblers, *Sylvia borin*, during migration *Journal of Comparative Physiology B-*
1112 *Biochemical Systemic and Environmental Physiology* 161, 576-580.

1113 Schwabl, H., Wingfield, J.C., Farner, D.S., 1984. Endocrine correlates of autumnal behavior in
1114 sedentary and migratory individuals of a partially migratory population of the European
1115 blackbird (*Turdus merula*). *Auk* 101, 499-507.

1116 Schwabl, P., Bonaccorso, E., Goymann, W., 2016. Diurnal variation in corticosterone release
1117 among wild tropical forest birds. *Frontiers in Zoology* 13, 11.

1118 Scollon, E.J., Carr, J.A., Cobb, G.R., 2004. The effect of flight, fasting and p,p'-DDT on
1119 thyroid hormones and corticosterone in Gambel's white-crowned sparrow, *Zonotrichia*
1120 *leucophrys gambelli*. *Comparative Biochemistry and Physiology C-Toxicology &*
1121 *Pharmacology* 137, 179-189.

1122 Seeber, P.A., Quintard, B., Sicks, F., Dehnhard, M., Greenwood, A.D., Franz, M., 2018.
1123 Environmental stressors may cause equine herpesvirus reactivation in captive Grevy's zebras
1124 (*Equus grevyi*). *PeerJ* 6, 18.

1125 Sjoberg, S., Alerstam, T., Aring;esson, S., Schulz, A., Weidauer, A., Coppack, T., Muheim,
1126 R., 2015. Weather and fuel reserves determine departure and flight decisions in passerines
1127 migrating across the Baltic Sea. *Animal Behaviour* 104, 59-68.

1128 Stanley, C.Q., MacPherson, M., Fraser, K.C., McKinnon, E.A., Stutchbury, B.J.M., 2012.
1129 Repeat Tracking of Individual Songbirds Reveals Consistent Migration Timing but
1130 Flexibility in Route. *Plos One* 7, 6.

1131 Viswanathan, M., John, T.M., George, J.C., Etches, R.J., 1987. Flight effects on plasma-
1132 glucose, lactate, catecholamines, and corticosterone in homing pigeons. *Hormone and*
1133 *Metabolic Research* 19, 400-402.

1134 Watts, H.E., Cornelius, J.M., Fudickar, A.M., Perez, J., Ramenofsky, M., 2018. Understanding
1135 variation in migratory movements: A mechanistic approach. *General and Comparative*
1136 *Endocrinology* 256, 112-122.

1137 Wikelski, M., Tarlow, E.M., Raim, A., Diehl, R.H., Larkin, R.P., Visser, G.H., 2003. Costs of
1138 migration in free-flying songbirds. *Nature* 423, 704-704.

1139 Wingfield, J.C., 2018. *Environmental Endocrinology: Insights into the Diversity of Regulatory*
1140 *Mechanisms in Life Cycles*. *Integr Comp Biol* 58, 790-799.

1141 Wingfield, J.C., Hahn, T.P., Wada, M., Astheimer, L.B., Schoech, S., 1996. Interrelationship of
1142 day length and temperature on the control of gonadal development, body mass, and fat score
1143 in white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *General and Comparative*
1144 *Endocrinology* 101, 242-255.

1145 Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M.,
1146 Richardson, R.D., 1998. Ecological bases of hormone-behavior interactions: The
1147 "emergency life history stage". *American Zoologist* 38, 191-206.

1148 Wyckoff, T.B., Sawyer, H., Albeke, S.E., Garman, S.L., Kauffman, M.J., 2018. Evaluating the
1149 influence of energy and residential development on the migratory behavior of mule deer.
1150 *Ecosphere* 9, e02113.

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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 adrenocorticotrophic 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.

1154 **Figure and Table Captions**

1155

1156 **Figure 1.** The migratory life cycle stage includes a number of substages during which CORT
1157 may be an important regulatory signal. Many birds make physiological preparations (e.g., fat
1158 deposition, flight muscle hypertrophy) for migration. CORT is hypothesized to stimulate these
1159 preparations (H1). Following preparation, CORT has been hypothesized to be involved in
1160 stimulating departure (H2); here CORT could operate on the scale of days to signal a window of
1161 readiness to depart or on the scale of hours to determine the precise timing of departure. Once
1162 birds depart there is a period of active flight. CORT has been hypothesized to support this
1163 metabolically demanding period (H3). In many species, migration involves stopovers en route in
1164 which birds stop to refuel before departing for another bout of flight. CORT may be involved in
1165 stimulating preparations that are part of refueling (H1) and departures (H2) during stopovers as
1166 well.

1167 **Table 1.** Explanation of different terms used to describe physiological preparation in migratory
1168 birds. We grouped variables into three main categories: feeding, tissue-specific measures, and
1169 integrative tissue measures. Tissue-specific measures assessed either fat (fat score and fuel load)
1170 or muscle.

1171 **Table 2a.** Summary of studies examining the Physiological Preparation Hypothesis, under
1172 Prediction 1 that corticosterone (CORT) is positively associated with feeding (either feeding
1173 behavior or food intake rates), fat (fat score, fuel load, or fuel deposition rate) increase, and body
1174 mass increase during preparation for migration. Studies tested this prediction via a)
1175 pharmacological manipulation of CORT signaling or b) examination of naturally circulating

1176 CORT levels. Studies using hormone manipulations gave birds either exogenous CORT, RU486
1177 (a glucocorticoid receptor antagonist), or dexamethasone (DEX, a glucocorticoid receptor
1178 agonist).

1179

1180 **Table 2b.** Summary of studies examining the Physiological Preparation Hypothesis, under
1181 Prediction 2 that corticosterone (CORT) increases during the period of pre-departure preparation.
1182 Studies tested this prediction by either a) comparing migratory and non-migratory individuals
1183 within the same species or b) taking repeated CORT measures over the period of migratory
1184 preparation in either free-living (“field”) or captive (“lab”) birds.

1185

1186 **Table 3a.** Summary of studies examining the Departure Stimulation Hypothesis, under
1187 Prediction 3 that corticosterone (CORT) increases prior to migratory departure in free-living
1188 birds. Studies fell into two temporal categories: a) those that examined whether CORT increases
1189 over the days prior to departure, and b) those that examined whether CORT increases over the
1190 hours prior to departure.

1191

1192 **Table 3b.** Summary of studies examining the Departure Stimulation Hypothesis, under
1193 Prediction 4 that corticosterone (CORT) is elevated in association with the expression of
1194 migratory restlessness (migratory restlessness) in captive birds. Studies fell into two temporal
1195 categories: a) those that examined whether CORT increases as birds transition from a pre-
1196 migratory state with no migratory restlessness to a migratory state with migratory restlessness,
1197 and b) those that examined whether CORT increases over the hours leading up to migratory
1198 restlessness expression. Unnatural photoperiods included those that kept birds on constant,

1199 unchanging photoperiods for long periods of time, or those that made abrupt transitions from
1200 short to long days or vice versa.

1201

1202 **Table 4.** Summary of studies examining the Flight Support Hypothesis, under Prediction 5 that
1203 corticosterone (CORT) levels are elevated during flight. In all studies, CORT was measured
1204 during the time of day each species normally migrates in the wild. Flight distance represents
1205 whether the examined flight bout was generally typical or shorter than normal for each species.

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

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?
<i>a) Studies using hormone manipulations</i>					
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 & 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
<i>b) Studies measuring naturally circulating CORT</i>					
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

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?
<i>a) Migrant vs. non-migrant studies</i>						
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
<i>b) Repeated measures studies</i>						
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

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. 2014a	<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. 2020	<i>Oenanthe oenanthe</i>	Hours	Stopover	Autumn	Yes
Eikenaar et al. 2018a	<i>Turdus merula</i>	Hours	Stopover	Autumn	Yes ^a

^a Statistically non-significant trend

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?
<i>a) Across days</i>						
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
<i>b) Diel</i>						
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

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