Regulation of complex behavioral transitions: migration to breeding.

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42 **Abstract**

43 The evolution of migration combines the selective advantages of breeding with ephemeral
44 resources at high latitude or altitude with the benefits of overwintering at distant sites with
45 adequate resources to promote survival. Migration between sites involves changes in behaviour
46 especially transition from migration to nesting, or in autumn transition to wintering stage. We
47 focus here on vernal transition. Because migration and onset of nesting are mutually exclusive
48 events, transition from a social, hyperphagic and highly mobile state to territoriality and pair-
49 bonding involves complex behavioral changes. In arctic regions, weather in spring is
50 unpredictable adding another dimension of complexity to the migration/breeding transition.
51 Nonetheless, the brief arctic summer requires that songbirds arrive on the breeding grounds
52 while conditions may still be unfavorable thus delaying nesting until conditions ameliorate. This
53 requires flexibility and coordination of migration behavior to be able to leave if weather
54 deteriorates, or become territorial, attract a mate and begin nesting within hours when conditions
55 permit. In white-crowned sparrows, neuroendocrine regulation of reproductive development
56 progresses throughout vernal migration so that males and females are mature when they arrive on
57 the nesting grounds, but final ovarian maturation leading to ovulation and reproductive behaviors
58 is suppressed. What provides the “brake” to onset of nesting? We focus on the adrenocortical
59 response to acute stress that is greatly increased at arrival, especially in males, probably to enable
60 flexibility in behavioural responses to severe weather. More recent evidence suggests that a
61 hypothalamic peptide, gonadotropin-inhibiting hormone (GnIH) may provide the brake for onset
62 of nesting without suppressing development of a functional reproductive system. Experimental
63 investigations show that gene silencing of GnIH in the brain increases singing and reproductive

behaviors accompanying onset of nesting. Thus we suggest that corticosteroids and GnIH play important regulatory roles specifically at the transition from vernal migration to onset of nesting.

Introduction.

Avian species that breed in the Arctic are almost all migrants spending the winter at lower latitudes (Piersma, 1994; Pielou, 1994; Dingle, 2014). On their return in spring, they are frequently faced with extreme conditions including complete snow cover and temperatures well below freezing. Even in warmer springs when snow cover is almost gone the potential for further snowstorms covering patchy food resources is very great. Despite these unpredictable conditions, arriving migrants need to locate a nesting area, establish territories and form pair bonds as early as possible and then initiate nesting activities when conditions are conducive for breeding to ensure greatest reproductive success. However, they must also adjust their physiology and behavior to withstand severe weather and sudden food shortages (O'Reilly and Wingfield, 1995; Wingfield and Hunt, 2002; Wingfield et al., 2004). Because blizzards and sub-freezing weather can occur unpredictably at any time during the spring, summer and early autumn, migrant birds are faced with “trade-offs” of needing to nest and ensure reproductive success or respond to severe local conditions favoring self-preservation to the detriment of reproductive success. Such trade-offs require rapid and complex transitions of behavior and physiology, but the mechanisms underlying such mobile flexibility, even nomadism, followed by territorial and reproductive behavior are only beginning to be understood.

On the North Slope of Alaska (66-71°N), snow may melt from the tundra as early as the first week in May, but subsequent storms (and temperatures down to -11°C or lower) may inundate early migrants. In other years, snow and sub-freezing temperatures may persist into late

87 May and early June (Hahn et al., 1995). Further north, as the coastal plain nears the Arctic Ocean,
88 temperature fluctuations are less extreme possibly due to the ameliorating effects of the ocean
89 (Stieglitz et al., 2003). The breeding season of passerines at the northern limit of their range is
90 three-four weeks longer than at Toolik Lake Field Station about 160 Km to the south. In other
91 Arctic study areas (e.g. field sites at Thule in NW Greenland, 76°N), at the northern edge of
92 breeding grounds for songbirds, weather is even more severe with some of the most truncated
93 breeding seasons known (Wingfield et al., 2004). Taken together, there is growing evidence that
94 flexible hormone-behavior adaptations have evolved in arctic-breeding birds to cope with
95 unpredictable weather, patchy trophic resources and fluctuating numbers of predators that all
96 vary not only with location in the Arctic, but also from year to year.

97 As migrants approach breeding sites, flight paths become more dispersed (Emlen et al.,
98 1976), and birds frequently become locally nomadic searching for refugia with food patches and
99 cover (Gwinner and Czeschlik, 1977; Wingfield et al., 2004; Wingfield and Hunt, 2002;
100 Cornelius et al., 2013). This is especially true if severe weather conditions prevail. Nonetheless,
101 migrants must endure and eventually settle when habitats conducive to breeding become
102 available (Wingfield et al., 2004; Romero and Wingfield, 2015). Nomadic movements tend to be
103 prolonged in years when weather is bad with deep snow pack, but songbirds can quickly
104 transition from arrival to settlement, territory formation and pair bonding behavior when nesting
105 habitat becomes accessible. Not infrequently later snow storms can blanket established territories
106 and birds revert to nomadism once more to locate refugia and food patches (Wingfield et al.,
107 2004; Breuner and Hahn, 2003). This may happen in response to severe weather events even
108 after nesting has begun. Thus, the ability to switch back to nomadic movements, form loose
109 foraging flocks and turn off territoriality and breeding is crucial for these high latitude / altitude

birds to survive capricious conditions and to maximize reproductive success (Astheimer et al., 1995; Hahn et al., 1995).

How are these complex transitions of behavior and physiology accomplished? Firstly, birds migrate seasonally and arrive at destinations in loose groups. Moving in small flocks, may be important to coordinate communication, orientation, arrival and subsequent events. To quote Low (2014) “Birds that live on grain maximize food-finding and safety by usually roaming in groups, a habit that suits colonization (arrive alone and you can expect to die alone)”. The arctic spring is short and unpredictable and coordination is important leading up to settlement, territoriality and pair-bonding. As such there appears to be facultative overlap of the final sub-stages of migration with settlement and onset of breeding. Once settled and paired, commencement of nesting follows when resources are sufficient; however flexibility in timing at this juncture is reduced because incubation length and time to fledge young are more fixed (Wingfield et al., 2004; Ramenofsky and Wingfield, 2006; Wingfield, 2008). Here we will highlight complex transitions in behavior and how they might be controlled from vernal migration to onset of nesting in arctic breeding songbirds.

Migration toward the breeding grounds.

Gambel’s White-crowned Sparrow, *Zonotrichia leucophrys gambelii*, (GWCS) is a long-distance nocturnal migrant. It travels great distances between its arctic breeding range and over-wintering sites extending from the lower 48 states into Mexico. In captivity, GWCS exhibit migratory behavior and physiology characteristic of free-living birds that include hyperphagia, fattening, and displays of nocturnal locomotor activity termed migratory restlessness also called Zugunruhe, a representation of migratory flight in captive birds (Ramenofsky and Németh, 2014; Agatsuma and Ramenofsky, 2006; Ramenofsky et al., 2003). The endocrine control of vernal migration has

133 been the subject of research for over 60 years, but the coordination of preparations for migration
134 and stopover biology resulting in refueling for the next flight remains unclear. Testosterone and
135 prolactin are involved in the fattening process and glucocorticoids and thyroid hormones are
136 probably important for most vernal events (see Wingfield et al., 1990; Ramenofsky and
137 Wingfield, 2006; Ramenofsky et al., 2012; Perez et al., in press). Here we will focus on the
138 hormonal bases of flexibility during the transition from migration to breeding rather than
139 migration or reproduction specifically.

140 Next we review the evidence for a role of corticosterone during migratory flight. There is
141 extensive evidence that baseline plasma levels of corticosterone are involved in metabolism (e.g.
142 glucose and fatty acid management), foraging and locomotor activity associated with migration
143 and facultative movements (Sapolsky et al., 2000; Landys et al., 2006; Ramenofsky, 2011;
144 Romero and Wingfield, 2015). Consistent with these actions, elevated levels of plasma
145 corticosterone have been documented in a variety of long distance migrants when captured en
146 route during autumn, at departure from stopover sites in spring, or on arrival at the breeding
147 grounds (Landys-Ciannelli et al., 2002; Falsone et al., 2009; Reneerkens et al., 2002) as well as
148 in captive birds expressing nocturnal migratory restlessness (Ramenofsky et al., 2008). In captive
149 GWCS held on a long-day photoperiod (18Light:6Dark) a clear expression of a diel sequence of
150 behaviors is evident (Agatsuma and Ramenofsky, 2006). Throughout the daylight hours
151 (photophase), birds feed, rest and show general locomotor activity. During the final 2 to 3 hours
152 of the photophase, they enter a quiescent stage and remain completely still until onset of the dark
153 or scotophase at which time they commence migratory restlessness, including a bill-up position
154 in which the bill points skyward (Landys et al., 2004; Agatsuma and Ramenofsky, 2006;
155 Coverdill et al., 2011). At the scoto- to photophase transition, birds abruptly cease migratory

restlessness, begin to feed, drink and reduce locomotor activity. Plasma corticosterone levels track the onset and duration of migratory restlessness with a final peak at the scoto to photophase transition (Ramenofsky et al., 2008; Landys et al., 2004) (Fig. 1A). By contrast, GWCS held on winter or short-day photoperiods (6L:18D) show neither a quiescent phase nor nocturnal migratory restlessness (Ramenofsky et al., 2003). They do however show a peak of corticosterone at the conclusion of the scotophase (Landys et al., 2004). Others have also identified diel rhythms of corticosterone in GWCS held on both short and long-day photoperiods with the peak occurring at the close of the scotophase (Breuner et al., 1999; Landys et al., 2004) (Fig. 1 B, C).

Such results raise the question of whether the diel patterns of corticosterone concentrations in blood are a function of dawn and dusk (lights on and off) rather than being specific to migratory restlessness and its termination at dawn. Previous studies showed that birds exposed to an extended scotophase (constant dim light conditions) expressed continued migratory restlessness suggesting that environmental conditions (night time) may override or mask a daily cycle of locomotor activity (Coverdill et al., 2008). Thus we asked whether plasma corticosterone would track the locomotor patterns and remain elevated throughout the extended period of migratory restlessness (Ramenofsky et al., 2008). First we verified the patterns of locomotor activity and baseline levels of corticosterone in spring migrants held on the 18L:6D photoperiod (Fig 2A). Here again the pattern of elevated baseline corticosterone appeared throughout the scotophase and peaked just at the transition to the photophase. Locomotor activity followed the daily patterns with migratory restlessness apparent during the scotophase. In the final set of experiments, spring migrants initially held on the 18L:6D photoperiod and showing the normal pattern of migratory restlessness were then exposed to artificially extended

scotophases by 8, 26 or 48 hours (Ramenofsky et al., 2008). Extending the results of Coverdill et al. (2008), birds continued to express migratory restlessness throughout the prolonged scotophase while baseline levels of corticosterone remained elevated throughout (Ramenofsky et al., 2008, Fig. 2B). Therefore, it is likely that the sustained elevated plasma levels of corticosterone during the extended scotophase are related more to the continuous energetic demands of migratory flight that masks the endogenous rhythm (Coverdill et al., 2008). These data suggest that corticosterone may be acting at two levels. Firstly, sustained plasma levels during the scotophase represent a facultative response to the increased demands, energetic and otherwise, of migratory restlessness. Second, the diel function showing a peak at the end of the scotophase, acts at a level of greater amplitude, serving a preparatory function for daytime activity. Note also that the concentrations of corticosterone during migratory restlessness (or at night in general) are within the range of daily routines because stress levels, after capture, handling and restraint, are almost an order of magnitude higher (Fig. 2C). Therefore it appears that elevated corticosterone during extended migratory restlessness has some permissive/preparative role during migratory flight. This may also be advantageous for birds under severe environmental conditions requiring abrupt movements away from either a stopover or arrival destination.

These investigations also suggest that corticosterone may play an important permissive role in nomadic behavior versus territorial and reproductive behaviors as loose flocks arrive to locate and settle on hospitable sites for breeding. Moreover, this permissive action may be critical later when nesting during periods of nomadism after a severe weather event interrupts breeding. These complex interchanges of activity in the face of high environmental unpredictability (see below) will be an important focus for future investigations and may provide

insight into consequences of climate change and resultant increased frequency, duration and intensity of extreme weather events

The transition from migration to onset of breeding

As birds near their breeding grounds in the Arctic, migratory behavior becomes less directed and the first reproductive behaviors start to be expressed. This period is characterized by a sequence of events, called “arrival biology”, that includes arrival and local nomadic movements to find refugia; settlement in an area conducive to breeding when available; establishment of a territory; attraction of a mate followed by courtship, copulation and onset of nesting (Martin and Wiebe, 2004; Wingfield et al., 2004). All these events are dependent upon local conditions such as weather, predators and food. Although the reproductive system is “ready to go”, onset of nesting does not occur automatically and does not commence until environmental conditions are favorable, i.e. there appears to be a “brake” on onset of nesting, especially in females (Wingfield et al., 2016). This sequence of events is highly flexible and reversible because the arctic spring is notoriously unpredictable and songbirds need to be highly mobile looking for patchy resources to survive until environmental conditions improve and become conducive to settlement and breeding. The problems associated with arrival and a short breeding season can be summarized as follows (Wingfield and Hunt, 2002).

1. Birds arrive in spring when conditions are still severe compared with wintering grounds.
2. Food supply early in the season is unpredictable in space, amount and type.
3. The breeding season in the Arctic is brief and individuals must initiate nesting as soon as possible despite severe conditions.
4. Even when nesting has begun, they must ‘resist’ the acute stressors of high predation risk and inclement weather throughout the breeding season, but nonetheless should abandon breeding and

revert to local nomadic behaviour to find refugia and temporarily suspend territorial and reproductive behaviour when weather conditions become too severe to continue nesting.

Although migrant birds arrive at more or less the same time each year (Wingfield and Hunt, 2002; Wingfield et al., 2004), when they can actually settle, and where, depends upon snow depth and patterns of melt (Hahn et al., 1995). Although in the brief arctic spring we might expect rigid control mechanisms for gonadal growth and onset of breeding there are, nonetheless, many examples of flexibility. Extensive data collected at a field site at Toolik Lake on the North Slope of Alaska indicate that songbirds arrive with testes and ovaries in a near functional state regardless of local conditions. Females are capable of initiating final maturation (yolk deposition in ovarian follicles) and laying eggs within days of arrival, but the actual dates that first eggs are laid may vary across years depending upon snow melt. For example, in 1991, snow melted out by the first week in May but in 1992 deep snow cover and sub-freezing temperatures persisted until the last week of May and into June. Although timing of gonadal development is more or less the same in each year, the location at which they settle and nest is dependent upon local conditions such as snow cover. These data suggest that some arctic birds may be spatially opportunistic (nomadic) especially at the limits of breeding range at high latitudes (Hahn et al., 1995).

Hormonal bases of arrival biology in arctic-breeding birds.

Environmental cues such as changing day length (photoperiod) allow migrants to predict future events and time migration to arrive in mid-May. They also regulate reproductive function - preparations for the breeding season (Dawson et al., 2001). In contrast, responses to local unpredictable perturbations of the environment require rapid, facultative changes in behavior and physiology. The latter have been collectively termed the emergency life history stage (ELHS)

diverting the individual into a physiological and behavioral state that will promote survival (Wingfield et al., 2004; Romero and Wingfield, 2015). Although the ELHS is highly adaptive in most conditions, it may actually be detrimental in arriving arctic birds because breeding must begin as soon as conditions allow and not be delayed unless weather is very severe. Rapid transitions among behavioural states of loose flocks on the move, settlement and onset of reproductive behaviours, involves aspects of the ELHS, migration and breeding life history stages. Next we will focus on the role the hypothalamo-pituitary adrenal cortex (HPA) axis may play in orchestrating these complex transitions.

Vertebrates in general activate the HPA axis in response to environmental perturbations (Sapolsky et al., 2000). Adrenocorticotropin (ACTH) and endorphin are released from the precursor molecule pro-opiomelanocortin in the anterior pituitary under the control of corticotropin releasing factor (CRF) from the hypothalamus. ACTH acts primarily on the adrenocortical cells to promote synthesis and secretion of glucocorticoids (corticosterone in birds). Chronic elevation of corticosterone over weeks can have severe debilitating effects such as inhibition of the reproductive system, suppression of the immune system, promotion of severe protein loss from skeletal muscle, disruption of second cell messengers particularly the arachidonic acid cascade, neuronal cell malfunction and suppression of growth (Sapolsky et al., 2000). In birds and mammals glucocorticoids also have several behavioural and physiological effects that promote fitness at least in the short term. These include: suppression of reproductive behaviour without inhibiting the reproductive system, regulation of the immune system, increased gluconeogenesis, regulation of foraging behaviour, promotion of escape (irruptive) behaviour during the day, promotion of night restfulness and facilitation of recovery when the perturbation has passed (Sapolsky et al., 2000; Romero and Wingfield, 2015). These short-term

effects suppress "unnecessary" physiological and behavioural functions, activate alternate behavioural and physiological patterns that promote survival, and avoid the long-term, detrimental effects of stress-induced high levels of corticosterone.

Do migrants arriving in the Arctic have to resist the potential for stress (Holberton and Wingfield, 2003) despite extreme conditions? Our initial working hypothesis was “at arrival, adjustments of hormonal responses to stress allow individuals some degree of resistance until environmental conditions improve” (Wingfield et al., 2004). More specifically, we predicted that the stress-induced increase in corticosterone levels as measured by a standardized test of capture, handling and restraint, would be inhibited at arrival in the Arctic. This would provide resistance to the potential stress of severe weather with the benefit of enhanced reproductive success but at the potential cost of survival in the face of severe weather events. What we found was that the adrenocortical response to the standardized stress was greatly enhanced in males and to a lesser extent in females at arrival and up to the point of onset of nesting (Holberton et al., 2003; Wingfield and Ramenofsky, 2011). Given the established roles of corticosterone in both irruptive behavior and nocturnal restfulness (reviewed in Wingfield and Ramenofsky, 1999; Romero and Wingfield, 2015) it is likely that corticosterone may play a preparative role for nomadic behavior with elevated plasma levels on arrival and settlement, followed by a decline of the adrenocortical response to stress after settlement on a territory and onset of nesting (Holberton and Wingfield, 2003; Wingfield et al., 2004; Wingfield and Ramenofsky, 2011). Furthermore, birds nesting at the leading edge of their existing range or after range expansion, where environmental conditions are likely most severe in spring, show the most extreme example of this modulation of the HPA response to perturbations (Walker et al., 2015; Krause et al., 2016).

Most, if not all, stressors have the potential to elicit increases in corticosterone secretion although the time course and extent of the response may differ (Sapolsky et al., 2000; Romero and Wingfield, 2015). In arctic birds that had already begun nesting and were incubating or feeding nestlings, a reduction in the adrenocortical response to stress was greater in the sex showing most parental care (Romero and Wingfield, 2015; Wingfield et al., 2004), and in the most severe environments. In contrast, prolonged severe weather (chronic stress) resulted in elevated adrenocortical responses to the standardized stress test and birds abandoned their nests and reformed flocks roaming the snow-covered tundra (Astheimer et al., 1994). These studies suggest that in birds that are breeding, responsiveness to stress was initially suppressed, but can be reactivated. This indicates that flexibility in the intensity of the stress response is regulated but the mechanisms are as yet unknown. Note that a non-migratory sub-species of White-crowned Sparrow, *Z.l. nuttalli*, breeding in central coastal California, did not show this type of modulation of the response to stress during breeding (Wingfield et al., 2004). Similar enhancement of the response to capture stress at the beginning of the nesting season has now been demonstrated in other sub-arctic and arctic avian populations compared with their lower latitude congenors, e.g. Bush Warblers, *Cettia diphone*, in Hokkaido, Japan (Wingfield et al., 1995), Snow Buntings and Lapland Longspurs at the northern edge of their range at Thule, Greenland (Walker et al., 2015), white-crowned sparrows expanding northward in Alaska (Krause et al., 2016) and Red Knots, *Calidris canutus*, in northern Canada, (Reneerkens et al., 2002). In White-crowned Sparrows, the increase in stress response was accompanied by decreased sensitivity to glucocorticoid feedback (Astheimer et al., 1994) and enhanced response of the adrenal cortex to ACTH (Romero and Wingfield, 1999; Romero et al., 1997) allowing an overall increase in baseline as well as capture stress-induced plasma levels of corticosterone.

It is important to note that circulating glucocorticoids are bound to corticosteroid-binding globulin (CBG) and while bound to this protein, it is believed that they cannot enter cells and interact with receptors that mediate biological actions (Malisch and Breuner, 2010). If this is the case, then it is possible that CBG levels are modulated to buffer increases in responsiveness to stress. Given the pronounced modulation of the adrenocortical responses to stress during arrival and settlement we predicted that CBG levels would increase to buffer rapid and extensive changes in corticosterone secretion (Wingfield et al., 2004). The enhanced adrenocortical response to acute stress following arrival in the Arctic in male Gambel's white-crowned sparrows was accompanied by an increase in CBG levels consistent with the buffer hypothesis (Romero and Wingfield, 1999; Breuner et al., 2003). In contrast, a non arctic-breeding subspecies of White-crowned Sparrow, *Z.l. pugetensis*, did not increase responsiveness to stress in early spring and had a lower binding capacity of CBG resulting in higher free levels of corticosterone 30 minutes post capture than in Gambel's white-crowned sparrow (Breuner et al., 2003). These data are actually consistent with our original hypothesis that the adrenocortical response to stress was actually reduced even though total levels of corticosterone (bound and unbound to CBG) were high. In other words, the free corticosterone levels at baseline and after acute stress can be buffered by high levels of CBG ameliorating any immediate and potentially deleterious effects. Furthermore, there is evidence that the rise in CBG in early spring is regulated by rising testosterone levels in blood (Schoech et al., 1999) which would be appropriate as the onset of breeding draws near.

If the CBG buffer hypothesis is correct then under severe weather events in spring and rapid triggering of nomadic survival behavior, CBG levels in blood should be regulated down thus releasing bound corticosterone (Malisch and Breuner, 2010). There is evidence that CBG

binding capacity (a measure of how much CBG is present) can be regulated within hours if a stressor persists. In captive male Gambel's white-crowned sparrows food restriction (to mimic the effects of a storm that covers food resources), increased plasma corticosterone levels by 2 hours but had no immediate effect on CBG binding capacity. By 22 hours of food restriction, corticosterone levels were similar to controls but CBG levels had declined and free corticosterone titers were higher (Lynn et al., 2003).

Thus far the evidence suggests that corticosterone-behaviour systems associated with arrival biology in arctic birds are highly flexible, allowing high adrenocortical responses to stress at this time, but buffering the early effects with high CBG binding capacity. Although much more work needs to be done to assess how quickly the changes in CBG binding capacity occur and how they change through the rest of the arctic summer, this may be an intriguing system by which migrant birds arriving in the Arctic maintain high activity of the HPA axis but modulate the expression of the CBG gene and thus access of hormone to receptors. Other components of the HPA axis and target cells may also be regulated particularly corticosterone receptors.

In mammals and birds there are two intracellular genomic receptors for corticosterone. Once bound to corticosterone the receptor-hormone complex becomes a gene transcription factor regulating a broad spectrum of genes associated with the stress response and survival (e.g. Sapolsky et al., 2000; Breuner et al., 2003). These actions usually take hours for protein synthesis to be effective and therefore represent longer term responses. One receptor type is the mineralocorticoid receptor which has a high affinity for corticosterone and can bind (i.e. be activated) at baseline levels. A second receptor type is the glucocorticoid receptor which has a low affinity for corticosterone and binds at higher "stress" levels (Breuner et al., 2003). In vitro binding experiments show that the mineralocorticoid receptor-like receptor in white-crowned

sparrows has high affinity for corticosterone but has different specificity characteristics from the mammalian counterpart (Breuner et al., 2003). The glucocorticoid -like receptor has a lower binding affinity for corticosterone similar in specificity to that of mammals (Breuner et al., 2003) and is only activated at higher circulating concentrations of corticosterone. There were no significant differences in binding capacity of glucocorticoid receptors in either the liver or brain of Gambel's white-crowned sparrow (arctic-breeding) or Z.l. pugetensis (mid-latitude-breeding) males. Using mean levels of free corticosterone and glucocorticoid receptor capacity, it is possible to calculate the receptor occupancy (hormone bound) during stress. Although binding capacity of glucocorticoid receptors did not vary between the two taxa of White-crowned Sparrow, more glucocorticoid receptors were occupied by corticosterone at 30 minutes post capture in both liver and brain of Z.l. pugetensis (Breuner et al., 2003). These studies strengthen the argument that CBG buffering actually decreases sensitivity to stress in GWCS arriving in the Arctic.

There is also a non-genomic, membrane receptor mediating more rapid (within minutes) effects of corticosteroids (Breuner et al., 1998). If plasma levels of corticosterone are increased rapidly, but non-invasively in male Gambel's white-crowned sparrows fed meal worms injected with known doses of corticosterone, there followed an equally rapid increase in perch-hopping activity within 10 minutes compared with controls. This rapid action is not consistent with genomic receptor action that requires at least 30 minutes and usually several hours (Breuner et al., 1998; Breuner and Wingfield, 2000). Interestingly, the lower dose of corticosterone, that is consistent with intermediate plasma levels, was most effective in increasing locomotor activity. A higher dose that was consistent with high stress levels had no effect on activity (Breuner and Wingfield, 2000; Breuner et al., 1998). Furthermore, actions of this putative membrane-type

receptor appear to be greatest in spring (when arrival occurs) and less so in winter (Breuner and Wingfield, 2000). These studies suggest that rapid effects of corticosterone on activity may be related to final stages of spring migration in the Arctic when birds arrive on their breeding grounds, and are important in the regulation of locomotor activity associated with locating suitable breeding habitat, and familiarizing themselves with local food sources should conditions deteriorate.

Stress levels of corticosterone (as generated experimentally by subcutaneous implants of corticosterone) were predicted to elevate locomotor activity in male Gambel's white-crowned sparrows, but actually reduced activity during the day (Astheimer et al., 1992). However, if food was restricted then corticosterone-implanted white-crowned sparrows showed an increase in perch hopping activity as well as escape type behavior (Astheimer et al., 1992). This activity is different from that observed by Breuner et al. (1998) and suggests two distinct effects of corticosterone on activity (Romero and Wingfield, 2015). A question that arises from these studies is how one steroid hormone, corticosterone, can have effects on different types of activity and in different contexts (food present or not present). It is possible that corticosterone has permissive and preparative roles and the final behaviour exhibited depends on synergistic actions with other hormones or with central secretions that more directly affect behaviour. Additional experiments indicate that irruptive type behaviour (and its perch hopping equivalent) is also influenced by the brain peptide corticotropin-releasing factor (CRF) injected centrally into the third ventricle of male Gambel's white-crowned sparrows in the laboratory (Maney and Wingfield, 1998), as well as by central injections of CRF into the lateral ventricles of male Gambel's white-crowned sparrows in the field (Romero et al., 1998). Thus corticosterone action may play a permissive role for secretion of brain peptides, or other substances, that may provide

specificity of behaviour for particular environmental scenarios as well as some behavioural flexibility needed to respond to unpredictable weather events by switching back from territorial behaviour to flocking and wandering searching for refugia.

Another complex transition from arrival biology to breeding is the flexible interrelationships of flocking behaviour during severe weather and territorial behaviour and pair bonding as settlement occurs prior to nesting (Wingfield et al., 2004). It is well known that the gonads are near full maturation at arrival and plasma levels of testosterone in males can be high at this time (Wingfield et al., 2004). However, the rapid switch from social to territorial and pairing behaviour may be more related to actions of brain peptides such as mesotocin and vasotocin (Goodson et al., 2012). Although we have little information for arctic breeding songbirds, in other species from lower latitudes central actions of mesotocin may accompany pair bonding and vasotocin may be involved in aggression associated with territoriality (Goodson and Evans, 2004; Goodson et al., 2012 a,b). In free-living male Gambel's white-crowned sparrows central injections of vasotocin into the third ventricle of males had no effect on the response to a simulated territorial challenge compared with controls given saline (Romero et al., 1998). However, central injection of CRF did significantly reduce male responses to simulated territorial intrusions (Romero et al., 1998). The roles of these brain peptides may be pivotal for the transition from migration to breeding in arctic birds but more data are needed.

Transitions to breeding: potential role of a novel peptide.

As birds arrive in the Arctic, settle and transition to onset of nesting, then reproductive hormones become critical for final maturation. In female white-crowned sparrows this involves increases in luteinizing hormone and estradiol that culminate in final maturation of ovarian follicles leading to ovulation (Wingfield and Farner, 1993). In males luteinizing hormone and testosterone are

elevated as females become sexually receptive (Wingfield and Farner, 1993). Photoperiodic regulation of reproductive development in spring involves secretion of chicken gonadotropin-releasing hormone I (cGnRH-I), a major GnRH in passerines (Bentley et al., 2003). Although studies of the different forms of GnRH and its receptors have revealed a wealth of information as to their function, a directly inhibitory neuropeptide for gonadotropin release has, until recently, remained unknown. A neuropeptide that specifically inhibits avian pituitary gonadotropin release in vitro, gonadotropin-inhibitory hormone (GnIH) was discovered over 15 years ago (Tsutsui et al., 2000). The gonadotropin inhibitory system is an intriguing concept and provides us with an unprecedented opportunity to study the regulation of reproduction from an entirely novel standpoint. This is critical for arctic breeding songbirds because when onset of nesting occurs, sudden snow storms halt final maturation of the ovary, and birds abandon territories and form wide ranging flocks (Wingfield et al., 2004). How is this flexibility of switching from nomadism to settlement and breeding regulated while keeping the reproductive system in a near functional state? GnIH is a candidate for regulating the reproductive side of these transitions (as reviewed in Wingfield et al., 2016).

The GnIH gene has been cloned in the brain of GWCS (Osugi et al., 2004a), and it was demonstrated that only neurons of the paraventricular nucleus (PVN) in the hypothalamus express this gene. However, fibers emanating from these neurons project to multiple brain locations, including the median eminence and brainstem (Bentley et al., 2003). Such distribution suggests multiple functions within the central nervous system. In vivo experiments show that in white-crowned sparrows GnIH rapidly and consistently (within 2 minutes) attenuated the GnRH-induced rise in plasma luteinizing hormone (Osugi et al., 2004b). The mechanism(s) controlling GnIH production and release is unknown at present. Potentially, combinations of GnRH and

GnIH levels may regulate release of pituitary luteinizing hormone and follicle-stimulating hormone that in turn orchestrate gonadal growth and secretion of sex steroid hormones. Furthermore, temporary gene silencing of GnIH in brain of photostimulated Gambel's white-crowned sparrows results in increases in singing and reproductive behaviours accompanying onset of nesting (Ubuka et al., 2012). The role of GnIH associated with arrival in the Arctic, expression of reproductive behaviour, onset of breeding and its termination is particularly pertinent given the high degree of behavioural and physiological flexibility required to cope with such a severe environment (Wingfield, 2015; Wingfield et al., 2016). Growing evidence also suggests a positive interrelationship of GnIH and environmental stress (Calisi et al., 2008) that will likely shed new insight on arrival biology in the Arctic. Interrelationships of GnIH neurons and stress-induced increases in glucocorticoids deserve further investigation.

Conclusions

A concept of how migrant songbirds use the arctic landscape is emerging not only on arrival but also during breeding and when forced to abandon territories and wander because of severe weather events. We have the basis of a framework outlining how a highly flexible control system orchestrates complex transitions and reversals of behaviour and physiology in response to a highly unpredictable environment. Future investigations will further probe the mechanisms underlying these complex transitions in breeding behaviour and physiology. As global climate change progresses many species will be shifting their geographic range for breeding and with the increase in frequency, intensity and duration of weather events it is likely that transitions of life history stages will become more unpredictable and will need to be very flexible. The arrival biology of arctic birds may be a useful model providing insight into how these complex transitions may be regulated in a broader sense.

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Figures Legends

Figure 1. (A). Plasma levels of baseline corticosterone (ng/mL) in captive migratory White-crowned Sparrows on 18L:6D sampled at 6 time periods characterized by specific behaviors:

Mid-day, birds feeding and resting, Quiescence (Q) birds immobile, Early flight, 2h of scotophase birds expressing migratory restlessness, Late flight, last 4 h of scotophase with migratory restlessness, Flight End, onset of photophase, Morning, 2 h of photophase low level locomotor activity. Horizontal bars depicts photoperiod of 18L:6D, unfilled portion represents photophase and filled portion the scotophase. Means $\pm$ S.E., n = 6. * significant difference from Morning sample at $P < 0.05$, (from Ramenofsky et al., 2008, courtesy of Medimond S.r.l.).

(B). Basal CORT in short-day sparrows. Means $\pm$ S.E., n = 16. * $P < 0.0001$, 07:00 is different from all lights on values.

(C). Basal CORT in long-day sparrows. Means $\pm$ S.E., n = 16, except for 22:00, where n = 8, and 13:00, where n = 14. ** $P < 0.0001$, 01:00 is different from all lights on values, from Breuner et al., 1999, courtesy of Elsevier).

Figure 2. (A) Locomotor activity (average movement/min) during 6 thirty minute time periods and baseline levels of corticosterone (ng/ml) at the conclusion of the same thirty minute period in birds held on a 18L:6D Photoperiod: Mid-day, Q (quiescence), EF (2h of migratory restlessness), LF (4h migratory restlessness), FE (End of migratory restlessness at onset of photophase, Morning (2h of photophase). * locomotor activity levels significantly different from Mid-day, Quiescence and Morning samples at $p < 0.05$. Means $\pm$ S.E., n = 6 and # baseline levels of corticosterone significantly different from Morning sample at $P < 0.05$, (from Ramenofsky et al., 2008, courtesy of Medimond S.r.l.).

(B) Compilation of results from independent studies illustrating the locomotor activity (activity/min) and baseline corticosterone (ng/ml) measured at Mid-day and Quiescent phase

709 on18L and extending the scotophase by 8, 26 and 48 h (from Ramenofsky et al., 2008, courtesy
710 of Medimond S.r.l.).
711 (C) Cort in stress response (early migration) much greater than baseline in migratory restlessness
712 (redrawn from a compilation of several studies, Romero and Wingfield, 2015).

Regulation of complex behavioral transitions: migration to breeding.

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42 **Abstract**

43 *The evolution of migration combines the selective advantages of breeding with ephemeral*
44 *resources at high latitude or altitude with the benefits of overwintering at distant sites with*
45 *adequate resources to promote survival. Migration between sites involves changes in behaviour*
46 *especially transition from migration to nesting, or in autumn transition to wintering stage. We*
47 focus here on vernal transition. Because migration and onset of nesting are mutually exclusive
48 events, transition from a social, hyperphagic and highly mobile state to territoriality and pair-
49 bonding involves complex behavioral changes. In arctic regions, weather in spring is
50 unpredictable adding another dimension of complexity to the migration/breeding transition.
51 Nonetheless, the brief arctic summer requires that songbirds arrive on the breeding grounds
52 while conditions may still be unfavorable thus delaying nesting until conditions ameliorate. This
53 requires flexibility and coordination of migration behavior to be able to leave if weather
54 deteriorates, or become territorial, attract a mate and begin nesting within hours when conditions
55 permit. In white-crowned sparrows, neuroendocrine regulation of reproductive development
56 progresses throughout vernal migration so that males and females are mature when they arrive on
57 the nesting grounds, but final ovarian maturation leading to ovulation and reproductive behaviors
58 is suppressed. What provides the “brake” to onset of nesting? We focus on the adrenocortical
59 response to acute stress that is greatly increased at arrival, especially in males, probably to enable
60 flexibility in behavioural responses to severe weather. More recent evidence suggests that a
61 hypothalamic peptide, gonadotropin-inhibiting hormone (GnIH) may provide the brake for onset
62 of nesting without suppressing development of a functional reproductive system. Experimental
63 investigations show that gene silencing of GnIH in the brain increases singing and reproductive

behaviors accompanying onset of nesting. Thus we suggest that corticosteroids and GnIH play important regulatory roles specifically at the transition from vernal migration to onset of nesting.

Introduction.

Avian species that breed in the Arctic are almost all migrants spending the winter at lower latitudes (Piersma, 1994; Pielou, 1994; Dingle, 2014). On their return in spring, they are frequently faced with extreme conditions including complete snow cover and temperatures well below freezing. Even in warmer springs when snow cover is almost gone the potential for further snowstorms covering patchy food resources is very great. Despite these unpredictable conditions, arriving migrants need to locate a nesting area, establish territories and form pair bonds as early as possible and then initiate nesting activities when conditions are conducive for breeding to ensure greatest reproductive success. However, they must also adjust their physiology and behavior to withstand severe weather and sudden food shortages (O'Reilly and Wingfield, 1995; Wingfield and Hunt, 2002; Wingfield et al., 2004). Because blizzards and sub-freezing weather can occur unpredictably at any time during the spring, summer and early autumn, migrant birds are faced with “trade-offs” of needing to nest and ensure reproductive success or respond to severe local conditions favoring self-preservation to the detriment of reproductive success. Such trade-offs require rapid and complex transitions of behavior and physiology, but the mechanisms underlying such mobile flexibility, even nomadism, followed by territorial and reproductive behavior are only beginning to be understood.

On the North Slope of Alaska (66-71°N), snow may melt from the tundra as early as the first week in May, but subsequent storms (and temperatures down to -11°C or lower) may inundate early migrants. In other years, snow and sub-freezing temperatures may persist into late

87 May and early June (Hahn et al., 1995). Further north, as the coastal plain nears the Arctic Ocean,
88 temperature fluctuations are less extreme possibly due to the ameliorating effects of the ocean
89 (Stieglitz et al., 2003). The breeding season of passerines at the northern limit of their range is
90 three-four weeks longer than at Toolik Lake Field Station about 160 Km to the south. In other
91 Arctic *study areas* (e.g. field sites at Thule in NW Greenland, 76°N), at the northern edge of
92 breeding grounds for songbirds, weather is even more severe with some of the most truncated
93 breeding seasons known (Wingfield et al., 2004). Taken together, there is growing evidence that
94 *flexible* hormone-behavior adaptations have evolved in arctic-breeding birds to cope with
95 unpredictable weather, patchy trophic resources and fluctuating numbers of predators that all
96 vary not only with location in the Arctic, but also from year to year.

97 As migrants approach breeding sites, flight paths become more dispersed (Emlen et al.,
98 1976), and birds frequently become locally nomadic searching for refugia with food patches and
99 cover (Gwinner and Czeschlik, 1977; Wingfield et al., 2004; Wingfield and Hunt, 2002;
100 Cornelius et al., 2013). This is especially true if severe weather conditions prevail. Nonetheless,
101 migrants must endure and eventually settle when habitats conducive to breeding become
102 available (Wingfield et al., 2004; Romero and Wingfield, 2015). Nomadic movements tend to be
103 prolonged in years when weather is bad with deep snow pack, but songbirds can quickly
104 transition from arrival to settlement, territory formation and pair bonding behavior when nesting
105 habitat becomes accessible. Not infrequently later snow storms can blanket established territories
106 and birds revert to nomadism once more to locate refugia and food patches (Wingfield et al.,
107 2004; Breuner and Hahn, 2003). This may happen in response to severe weather events even
108 after nesting has begun. Thus, the ability to switch back to nomadic movements, form loose
109 foraging flocks and turn off territoriality and breeding is crucial for these high latitude / altitude

birds to survive capricious conditions and to maximize reproductive success (Astheimer et al., 1995; Hahn et al., 1995).

How are these complex transitions of behavior and physiology accomplished? Firstly, birds migrate seasonally and arrive at destinations in loose groups. Moving in small flocks, may be important to coordinate communication, orientation, arrival and subsequent events. To quote Low (2014) “Birds that live on grain maximize food-finding and safety by usually roaming in groups, a habit that suits colonization (arrive alone and you can expect to die alone)”. The arctic spring is short and unpredictable and coordination is important leading up to settlement, territoriality and pair-bonding. As such there appears to be facultative overlap of the final sub-stages of migration with settlement and onset of breeding. *Once settled and paired, commencement of nesting follows when resources are sufficient; however flexibility in timing at this juncture is reduced because incubation length and time to fledge young are more fixed* (Wingfield et al., 2004; Ramenofsky and Wingfield, 2006; Wingfield, 2008). Here we will highlight complex transitions in behavior and how they might be controlled from vernal migration to onset of nesting in arctic breeding songbirds.

Migration toward the breeding grounds.

Gambel’s White-crowned Sparrow, *Zonotrichia leucophrys gambelii*, (GWCS) is a long-distance nocturnal migrant. It travels great distances between its arctic breeding range and over-wintering sites extending from the lower 48 states into Mexico. In captivity, GWCS exhibit migratory behavior and physiology characteristic of free-living birds that include hyperphagia, fattening, and displays of nocturnal locomotor activity termed migratory restlessness also called *Zugunruhe*, a representation of migratory flight in captive birds (Ramenofsky and Németh, 2014; Agatsuma and Ramenofsky, 2006; Ramenofsky et al., 2003). The endocrine control of vernal migration has

133 been the subject of research for over 60 years, but the coordination of preparations for migration
134 and stopover biology resulting in refueling for the next flight remains unclear. Testosterone and
135 prolactin are involved in the fattening process and glucocorticoids and thyroid hormones are
136 probably important for most vernal events (see Wingfield et al., 1990; Ramenofsky and
137 Wingfield, 2006; Ramenofsky et al., 2012; Perez et al., in press). Here we will focus on the
138 hormonal bases of flexibility during the transition from migration to breeding rather than
139 migration or reproduction specifically.

140 Next we review the evidence for a role of corticosterone during migratory flight. There is
141 extensive evidence that baseline plasma levels of corticosterone are involved in metabolism (e.g.
142 glucose and fatty acid management), foraging and locomotor activity associated with migration
143 and facultative movements (Sapolsky et al., 2000; Landys et al., 2006; Ramenofsky, 2011;
144 Romero and Wingfield, 2015). Consistent with these actions, elevated levels of plasma
145 corticosterone have been documented *in a variety of long distance migrants when captured en*
146 *route during autumn, at departure from stopover sites in spring, or on arrival at the breeding*
147 *grounds* (Landys-Ciannelli et al., 2002; Falsone et al., 2009; Reneerkens et al., 2002) as well as
148 in captive birds expressing nocturnal migratory restlessness (Ramenofsky et al., 2008). In captive
149 GWCS held on a long-day photoperiod (18Light:6Dark) a clear expression of a diel sequence of
150 behaviors is evident (Agatsuma and Ramenofsky, 2006). Throughout the daylight hours
151 (photophase), birds feed, rest and show general locomotor activity. During the final 2 to 3 hours
152 of the photophase, they enter a quiescent stage and remain completely still until onset of the dark
153 or scotophase at which time they commence *migratory restlessness, including a bill-up position*
154 *in which the bill points skyward* (Landys et al., 2004; Agatsuma and Ramenofsky, 2006;
155 Coverdill et al., 2011). *At the scoto- to photophase transition, birds abruptly cease migratory*

156 *restlessness, begin to feed, drink and reduce locomotor activity.* Plasma corticosterone levels
157 track the onset and duration of migratory restlessness with a final peak at the scoto to photophase
158 transition (Ramenofsky et al., 2008; Landys et al., 2004) (Fig. 1A). By contrast, GWCS held on
159 winter or short-day photoperiods (6L:18D) show neither a quiescent phase nor nocturnal
160 migratory restlessness (Ramenofsky et al., 2003). They do however show a peak of
161 corticosterone at the conclusion of the scotophase (Landys et al., 2004). Others have also
162 identified diel rhythms of corticosterone in GWCS held on both short and long-day photoperiods
163 with the peak occurring at the close of the scotophase (Breuner et al., 1999; Landys et al., 2004)
164 (Fig. 1 B,C).

165 Such results raise the question of whether the diel patterns of corticosterone
166 concentrations in *blood are a function of dawn and dusk (lights on and off) rather than being*
167 *specific to migratory restlessness and its termination at dawn.* Previous studies showed that birds
168 exposed to an extended scotophase (constant dim light conditions) expressed continued
169 migratory restlessness suggesting that environmental conditions (night time) may override or
170 mask a daily cycle of locomotor activity (Coverdill et al., 2008). Thus we asked whether plasma
171 corticosterone would track the locomotor patterns and remain elevated throughout the extended
172 period of migratory restlessness (Ramenofsky et al., 2008). First we verified the patterns of
173 locomotor activity and baseline levels of corticosterone in spring migrants held on the 18L:6D
174 photoperiod (Fig 2A). Here again the pattern of elevated baseline corticosterone appeared
175 throughout the scotophase and peaked just at the transition to the photophase. Locomotor activity
176 followed the daily patterns with migratory restlessness apparent during the scotophase. In the
177 final set of experiments, spring migrants initially held on the 18L:6D photoperiod and showing
178 the normal *pattern of migratory restlessness were then exposed to artificially extended*

179 *scotophases by 8, 26 or 48 hours (Ramenofsky et al., 2008)*. Extending the results of Coverdill et
180 al. (2008), birds continued to express migratory restlessness throughout the prolonged
181 scotophase while baseline levels of corticosterone remained elevated throughout (Ramenofsky et
182 al, 2008, Fig. 2B). Therefore, it is likely that the sustained elevated plasma levels of
183 corticosterone during the extended scotophase are related more to the continuous energetic
184 demands of migratory flight that masks the endogenous rhythm (Coverdill et al., 2008). These
185 data suggest that corticosterone may be acting at two levels. Firstly, sustained plasma levels
186 during the scotophase represent a facultative response to the increased demands, energetic and
187 otherwise, of migratory restlessness. Second, the diel function showing a peak at the end of the
188 scotophase, acts at a level of greater amplitude, serving a preparatory function for daytime
189 activity. Note also that the concentrations of corticosterone during migratory restlessness (or at
190 night in general) are within the range of daily routines because stress levels, after capture,
191 handling and restraint, are almost an order of magnitude higher (Fig. 2C). Therefore it appears
192 that elevated corticosterone during extended migratory restlessness has some
193 permissive/preparative role during migratory flight. This may also be advantageous for birds
194 under severe environmental conditions requiring abrupt movements away from either a stopover
195 or arrival destination.

196 These investigations also suggest that corticosterone may play an important permissive
197 role in nomadic behavior versus territorial and reproductive behaviors as loose flocks arrive to
198 locate and settle on hospitable sites for breeding. Moreover, this permissive action may be
199 critical later when nesting during periods of nomadism after a severe weather event interrupts
200 breeding. These complex interchanges of activity in the face of high environmental
201 unpredictability (see below) will be an important focus for future investigations and may provide

insight into consequences of climate change and resultant increased frequency, duration and intensity of extreme weather events

The transition from migration to onset of breeding

As birds near their breeding grounds in the Arctic, migratory behavior becomes less directed and the first reproductive behaviors start to be expressed. This period is characterized by a sequence of events, called “arrival biology”, that includes arrival and local nomadic movements to find refugia; settlement in an area conducive to breeding when available; establishment of a territory; attraction of a mate followed by courtship, copulation and onset of nesting (Martin and Wiebe, 2004; Wingfield et al., 2004). All these events are dependent upon local conditions such as weather, predators and food. Although the reproductive system is “ready to go”, onset of nesting does not occur automatically and does not commence until environmental conditions are favorable, i.e. there appears to be a “brake” on onset of nesting, especially in females (Wingfield et al., 2016). This sequence of events is highly flexible and reversible because the arctic spring is notoriously unpredictable and songbirds need to be highly mobile looking for patchy resources to survive until environmental conditions improve and become conducive to settlement and breeding. The problems associated with arrival and a short breeding season can be summarized as follows (Wingfield and Hunt, 2002).

1. Birds arrive in spring when conditions are still severe compared with wintering grounds.
2. Food supply early in the season is unpredictable in space, amount and type.
3. The breeding season in the Arctic is brief and individuals must initiate nesting as soon as possible despite severe conditions.
4. Even when nesting has begun, they must ‘resist’ the acute stressors of high predation risk and inclement weather throughout the breeding season, but nonetheless should abandon breeding and

revert to local nomadic behaviour to find refugia and temporarily suspend territorial and reproductive behaviour when weather conditions become too severe to continue nesting.

Although migrant birds arrive at more or less the same time each year (Wingfield and Hunt, 2002; Wingfield et al., 2004), when they can actually settle, and where, depends upon snow depth and patterns of melt (Hahn et al., 1995). Although in the brief arctic spring we might expect rigid control mechanisms for gonadal growth and onset of breeding there are, nonetheless, many examples of flexibility. Extensive data collected at a field site at Toolik Lake on the North Slope of Alaska indicate that songbirds arrive with testes and ovaries in a near functional state regardless of local conditions. Females are capable of initiating final maturation (yolk deposition *in ovarian follicles*) and laying eggs within days of arrival, but the actual dates that first eggs are laid may vary across years depending upon snow melt. For example, in 1991, snow melted out by the first week in May but in 1992 deep snow cover and sub-freezing temperatures persisted until the last week of May and into June. Although timing of gonadal development is more or less the same in each year, the location at which they settle and nest is dependent upon local conditions such as snow cover. These data suggest that some arctic birds may be spatially opportunistic (nomadic) especially at the limits of breeding range at high latitudes (Hahn et al., 1995).

Hormonal bases of arrival biology in arctic-breeding birds.

Environmental cues such as changing day length (photoperiod) allow migrants to predict future events and time migration to arrive in mid-May. They also regulate reproductive function - preparations for the breeding season (Dawson et al., 2001). In contrast, responses to local unpredictable perturbations of the environment require rapid, facultative changes in behavior and physiology. The latter have been collectively termed the emergency life history stage (ELHS)

diverting the individual into a physiological and behavioral state that will promote survival (Wingfield et al., 2004; Romero and Wingfield, 2015). Although the ELHS is highly adaptive in most conditions, it may actually be detrimental in arriving arctic birds because breeding must begin as soon as conditions allow and not be delayed unless weather is very severe. Rapid transitions among behavioural states of loose flocks on the move, settlement and onset of reproductive behaviours, involves aspects of the ELHS, migration and breeding life history stages. Next we will focus on the role the hypothalamo-pituitary adrenal cortex (HPA) axis may play in orchestrating these complex transitions.

Vertebrates in general activate the HPA axis in response to environmental perturbations (Sapolsky et al., 2000). Adrenocorticotropin (ACTH) and endorphin are released from the precursor molecule pro-opiomelanocortin in the anterior pituitary under the control of corticotropin releasing factor (CRF) from the hypothalamus. ACTH acts primarily on the adrenocortical cells to promote synthesis and secretion of glucocorticoids (corticosterone in birds). Chronic elevation of corticosterone over weeks can have severe debilitating effects such as inhibition of the reproductive system, suppression of the immune system, promotion of severe protein loss from skeletal muscle, disruption of second cell messengers particularly the arachidonic acid cascade, neuronal cell malfunction and suppression of growth (Sapolsky et al., 2000). In birds and mammals glucocorticoids also have several behavioural and physiological effects that promote fitness at least in the short term. These include: suppression of reproductive behaviour without inhibiting the reproductive system, regulation of the immune system, increased gluconeogenesis, regulation of foraging behaviour, promotion of escape (irruptive) behaviour during the day, promotion of night restfulness and facilitation of recovery when the perturbation has passed (Sapolsky et al., 2000; Romero and Wingfield, 2015). These short-term

effects suppress "unnecessary" physiological and behavioural functions, activate alternate behavioural and physiological patterns that promote survival, and avoid the long-term, detrimental effects of stress-induced high levels of corticosterone.

Do migrants arriving in the Arctic have to resist the potential for stress (Holberton and Wingfield, 2003) despite extreme conditions? Our initial working hypothesis was “at arrival, adjustments of hormonal responses to stress allow individuals some degree of resistance until environmental conditions improve” (Wingfield et al., 2004). More specifically, we predicted that the stress-induced increase in corticosterone levels as measured by a standardized test of capture, handling and restraint, would be inhibited at arrival in the Arctic. This would provide resistance to the potential stress of severe weather with the benefit of enhanced reproductive success but at the potential cost of survival in the face of severe weather events. What we found was that the adrenocortical response to the standardized stress was greatly enhanced in males and to a lesser extent in females at arrival and up to the point of onset of nesting (Holberton et al., 2003; Wingfield and Ramenofsky, 2011). *Given the established roles of corticosterone in both irruptive behavior and nocturnal restfulness (reviewed in Wingfield and Ramenofsky, 1999; Romero and Wingfield, 2015)* it is likely that corticosterone may play a preparative role for nomadic behavior with elevated plasma levels on arrival and settlement, followed by a decline of the adrenocortical response to stress after settlement on a territory and onset of nesting (Holberton and Wingfield, 2003; Wingfield et al., 2004; Wingfield and Ramenofsky, 2011). Furthermore, birds nesting at the leading edge of their existing range or after range expansion, where environmental conditions are likely most severe in spring, show the most extreme example of this modulation of the HPA response to perturbations (Walker et al., 2015; Krause et al., 2016).

Most, if not all, stressors have the potential to elicit increases in corticosterone secretion although the time course and extent of the response may differ (Sapolsky et al., 2000; Romero and Wingfield, 2015). In arctic birds that had already begun nesting and were incubating or feeding nestlings, a reduction in the adrenocortical response to stress was greater in the sex showing most parental care (Romero and Wingfield, 2015; Wingfield et al., 2004), and in the most severe environments. In contrast, prolonged severe weather (chronic stress) resulted in elevated adrenocortical responses to the standardized stress test and birds abandoned their nests and reformed flocks roaming the snow-covered tundra (Astheimer et al., 1994). *These studies suggest that in birds that are breeding, responsiveness to stress was initially suppressed, but can be reactivated.* This indicates that flexibility in the intensity of the stress response is regulated but the mechanisms are as yet unknown. Note that a non-migratory sub-species of White-crowned Sparrow, *Z.l. nuttalli*, breeding in central coastal California, did not show this type of modulation of the response to stress during breeding (Wingfield et al., 2004). Similar enhancement of the response to capture stress at the beginning of the nesting season has now been demonstrated in other sub-arctic and arctic avian populations compared with their lower latitude congenors, e.g. Bush Warblers, *Cettia diphone*, in Hokkaido, Japan (Wingfield et al., 1995), Snow Buntings and Lapland Longspurs at the northern edge of their range at Thule, Greenland (Walker et al., 2015), white-crowned sparrows expanding northward in Alaska (Krause et al., 2016) and Red Knots, *Calidris canutus*, in northern Canada, (Reneerkens et al., 2002). In White-crowned Sparrows, the increase in stress response was accompanied by decreased sensitivity to glucocorticoid feedback (Astheimer et al., 1994) and enhanced response of the adrenal cortex to ACTH (Romero and Wingfield, 1999; Romero et al., 1997) allowing an overall increase in baseline as well as capture stress-induced plasma levels of corticosterone.

It is important to note that circulating glucocorticoids are bound to corticosteroid-binding globulin (CBG) and while bound to this protein, it is believed that they cannot enter cells and interact with receptors that mediate biological actions (Malisch and Breuner, 2010). If this is the case, then it is possible that CBG levels are modulated to buffer increases in responsiveness to stress. Given the pronounced modulation of the adrenocortical responses to stress during arrival and settlement we predicted that CBG levels would increase to buffer rapid and extensive changes in corticosterone secretion (Wingfield et al., 2004). The enhanced adrenocortical response to acute stress following arrival in the Arctic in male Gambel's white-crowned sparrows was accompanied by an increase in CBG levels consistent with the buffer hypothesis (Romero and Wingfield, 1999; Breuner et al., 2003). In contrast, a non arctic-breeding subspecies of White-crowned Sparrow, *Z.l. pugetensis*, did not increase responsiveness to stress in early spring and had a lower binding capacity of CBG resulting in higher free levels of corticosterone 30 minutes post capture than in Gambel's white-crowned sparrow (Breuner et al., 2003). These data are actually consistent with our original hypothesis that the adrenocortical response to stress was actually reduced even though total levels of corticosterone (bound and unbound to CBG) were high. In other words, the free corticosterone levels at baseline and after acute stress can be buffered by high levels of CBG ameliorating any immediate and potentially deleterious effects. Furthermore, there is evidence that the rise in CBG in early spring is regulated by rising testosterone levels in blood (Schoech et al., 1999) which would be appropriate as the onset of breeding draws near.

If the CBG buffer hypothesis is correct then under severe weather events in spring and rapid triggering of nomadic survival behavior, CBG levels in blood should be regulated down thus releasing bound corticosterone (Malisch and Breuner, 2010). There is evidence that CBG

binding capacity (a measure of how much CBG is present) can be regulated within hours if a stressor persists. In captive male Gambel's white-crowned sparrows food restriction (to mimic the effects of a storm that covers food resources), increased plasma corticosterone levels by 2 hours but had no immediate effect on CBG binding capacity. By 22 hours of food restriction, corticosterone levels were similar to controls but CBG levels had declined and free corticosterone titers were higher (Lynn et al., 2003).

Thus far the evidence suggests that corticosterone-behaviour systems associated with arrival biology in arctic birds are highly flexible, allowing high adrenocortical responses to stress at this time, but buffering the early effects with high CBG binding capacity. Although much more work needs to be done to assess how quickly the changes in CBG binding capacity occur and how they change through the rest of the arctic summer, this may be an intriguing system by which migrant birds arriving in the Arctic maintain high activity of the HPA axis but modulate the expression of the CBG gene and thus access of hormone to receptors. Other components of the HPA axis and target cells may also be regulated particularly corticosterone receptors.

In mammals and birds there are two intracellular genomic receptors for corticosterone. Once bound to corticosterone the receptor-hormone complex becomes a gene transcription factor regulating a broad spectrum of genes associated with the stress response and survival (e.g. Sapolsky et al., 2000; Breuner et al., 2003). These actions usually take hours for protein synthesis to be effective and therefore represent longer term responses. One receptor type is the mineralocorticoid receptor which has a high affinity for corticosterone and can bind (i.e. be activated) at baseline levels. A second receptor type is the glucocorticoid receptor which has a low affinity for corticosterone and binds at higher "stress" levels (Breuner et al., 2003). In vitro binding experiments show that the mineralocorticoid receptor-like receptor in white-crowned

sparrows has high affinity for corticosterone but has different specificity characteristics from the mammalian counterpart (Breuner et al., 2003). The glucocorticoid -like receptor has a lower binding affinity for corticosterone similar in specificity to that of mammals (Breuner et al., 2003) and is only activated at higher circulating concentrations of corticosterone. There were no significant differences in binding capacity of glucocorticoid receptors in either the liver or brain of Gambel's white-crowned sparrow (arctic-breeding) or *Z.l. pugetensis* (mid-latitude-breeding) males. Using mean levels of free corticosterone and glucocorticoid receptor capacity, it is possible to calculate the receptor occupancy (hormone bound) during stress. Although binding capacity of glucocorticoid receptors did not vary between the two taxa of White-crowned Sparrow, more glucocorticoid receptors were occupied by corticosterone at 30 minutes post capture in both liver and brain of *Z.l. pugetensis* (Breuner et al., 2003). These *studies* strengthen the argument that CBG buffering actually decreases sensitivity to stress in GWCS arriving in the Arctic.

There is also a non-genomic, membrane receptor mediating more rapid (within minutes) effects of corticosteroids (Breuner et al., 1998). If plasma levels of corticosterone are increased rapidly, but non-invasively in male Gambel's white-crowned sparrows fed meal worms injected with known doses of corticosterone, there followed an equally rapid increase in perch-hopping activity within 10 minutes compared with controls. This rapid action is not consistent with genomic receptor action that requires at least 30 minutes and usually several hours (Breuner et al., 1998; Breuner and Wingfield, 2000). *Interestingly, the lower dose of corticosterone, that is consistent with intermediate plasma levels, was most effective in increasing locomotor activity. A higher dose that was consistent with high stress levels had no effect on activity* (Breuner and Wingfield, 2000; Breuner et al., 1998). Furthermore, actions of this putative membrane-type

receptor appear to be greatest in spring (when arrival occurs) and less so in winter (Breuner and Wingfield, 2000). These studies suggest that rapid effects of corticosterone on activity may be related to final stages of spring migration in the Arctic when birds arrive on their breeding grounds, and are important in the regulation of locomotor activity associated with locating suitable breeding habitat, and familiarizing themselves with local food sources should conditions deteriorate.

Stress levels of corticosterone (as generated experimentally by subcutaneous implants of corticosterone) were predicted to elevate locomotor activity in male Gambel's white-crowned sparrows, but actually reduced activity during the day (Astheimer et al., 1992). However, if food was restricted then corticosterone-implanted white-crowned sparrows showed an increase in perch hopping activity as well as escape type behavior (Astheimer et al., 1992). This activity is different from that observed by Breuner et al. (1998) and suggests two distinct effects of corticosterone on activity (Romero and Wingfield, 2015). A question that arises from these studies is how one steroid hormone, corticosterone, can have effects on different types of activity and in different contexts (food present or not present). It is possible that corticosterone has permissive and preparative roles and the final behaviour exhibited depends on synergistic actions with other hormones or with central secretions that more directly affect behaviour. Additional experiments indicate that irruptive type behaviour (and its perch hopping equivalent) is also influenced by the brain peptide corticotropin-releasing factor (CRF) injected centrally into the third ventricle of male Gambel's white-crowned sparrows in the laboratory (Maney and Wingfield, 1998), as well as by central injections of CRF into the lateral ventricles of male Gambel's white-crowned sparrows in the field (Romero et al., 1998). Thus corticosterone action may play a permissive role for secretion of brain peptides, or other substances, that may provide

specificity of behaviour for particular environmental scenarios as well as some behavioural flexibility needed to respond to unpredictable weather events by switching back from territorial behaviour to flocking and wandering searching for refugia.

Another complex transition from arrival biology to breeding is the flexible interrelationships of flocking behaviour during severe weather and territorial behaviour and pair bonding as settlement occurs prior to nesting (Wingfield et al., 2004). It is well known that the gonads are near full maturation at arrival and plasma levels of testosterone in males can be high at this time (Wingfield et al., 2004). However, the rapid switch from social to territorial and pairing behaviour may be more related to actions of brain peptides such as mesotocin and vasotocin (Goodson et al., 2012). Although we have little information for arctic breeding songbirds, in other species from lower latitudes central actions of mesotocin may accompany pair bonding and vasotocin may be involved in aggression associated with territoriality (Goodson and Evans, 2004; Goodson et al., 2012 a,b). In free-living male Gambel's white-crowned sparrows central injections of vasotocin into the third ventricle of males had no effect on the response to a simulated territorial challenge compared with controls given saline (Romero et al., 1998). However, central injection of CRF did significantly reduce male responses to simulated territorial intrusions (Romero et al., 1998). The roles of these brain peptides may be pivotal for the transition from migration to breeding in arctic birds but more data are needed.

Transitions to breeding: potential role of a novel peptide.

As birds arrive in the Arctic, settle and transition to onset of nesting, then reproductive hormones become critical for final maturation. In female white-crowned sparrows this involves increases in luteinizing hormone and estradiol that culminate in final maturation of ovarian follicles leading to ovulation (Wingfield and Farner, 1993). In males luteinizing hormone and testosterone are

elevated as females become sexually receptive (Wingfield and Farner, 1993). Photoperiodic regulation of reproductive development in spring involves secretion of chicken gonadotropin-releasing hormone I (cGnRH-I), a major GnRH in passerines (Bentley et al., 2003). Although studies of the different forms of GnRH and its receptors have revealed a wealth of information as to their function, a directly inhibitory neuropeptide for gonadotropin release has, until recently, remained unknown. A neuropeptide that specifically inhibits avian pituitary gonadotropin release in vitro, gonadotropin-inhibitory hormone (GnIH) was discovered over 15 years ago (Tsutsui et al., 2000). The gonadotropin inhibitory system is an intriguing concept and provides us with an unprecedented opportunity to study the regulation of reproduction from an entirely novel standpoint. This is critical for arctic breeding songbirds because when onset of nesting occurs, sudden snow storms halt final maturation of the ovary, and birds abandon territories and form wide ranging flocks (Wingfield et al., 2004). How is this flexibility of switching from nomadism to settlement and breeding regulated while keeping the reproductive system in a near functional state? GnIH is a candidate for regulating the reproductive side of these transitions (as reviewed in Wingfield et al., 2016).

The GnIH gene has been cloned in the brain of GWCS (Osugi et al., 2004a), and it was demonstrated that only neurons of the paraventricular nucleus (PVN) in the hypothalamus express this gene. However, fibers emanating from these neurons project to multiple brain locations, including the median eminence and brainstem (Bentley et al., 2003). Such distribution suggests multiple functions within the central nervous system. *In vivo* experiments show that in white-crowned sparrows GnIH rapidly and consistently (within 2 minutes) attenuated the GnRH-induced rise in plasma luteinizing hormone (Osugi et al., 2004b). The mechanism(s) controlling GnIH production and release is unknown at present. Potentially, combinations of *GnRH* and

GnIH levels may regulate release of pituitary luteinizing hormone and follicle-stimulating hormone that in turn orchestrate gonadal growth and secretion of sex steroid hormones. Furthermore, temporary gene silencing of GnIH in brain of photostimulated Gambel's white-crowned sparrows results in increases in singing and reproductive behaviours accompanying onset of nesting (Ubuka et al., 2012). The role of GnIH associated with arrival in the Arctic, expression of reproductive behaviour, onset of breeding and its termination is particularly pertinent given the high degree of behavioural and physiological flexibility required to cope with such a severe environment (Wingfield, 2015; Wingfield et al., 2016). Growing evidence also suggests a positive interrelationship of GnIH and *environmental* stress (Calisi et al., 2008) that will likely shed new insight on arrival biology in the Arctic. Interrelationships of GnIH neurons and stress-induced increases in glucocorticoids deserve further investigation.

Conclusions

A concept of how migrant songbirds use the arctic landscape is emerging not only on arrival but also during breeding and when forced to abandon territories and wander because of severe weather events. We have the basis of a framework outlining how a highly flexible control system orchestrates complex transitions and reversals of behaviour and physiology in response to a highly unpredictable environment. Future investigations will further probe the mechanisms underlying these complex transitions in breeding behaviour and physiology. As global climate change progresses many species will be shifting their geographic range for breeding and with the increase in frequency, intensity and duration of weather events it is likely that transitions of life history stages will become more unpredictable and will need to be very flexible. The arrival biology of arctic birds may be a useful model providing insight into how these complex transitions may be regulated in a broader sense.

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Figures Legends

Figure 1. (A). Plasma levels of baseline corticosterone (ng/mL) in captive migratory White-crowned Sparrows on 18L:6D sampled at 6 time periods characterized by specific behaviors:

Mid-day, birds feeding and resting, Quiescence (Q) birds immobile, Early flight, 2h of scotophase birds expressing migratory restlessness, Late flight, last 4 h of scotophase with migratory restlessness, Flight End, onset of photophase, Morning, 2 h of photophase low level locomotor activity. Horizontal bars depicts photoperiod of 18L:6D, unfilled portion represents photophase and filled portion the scotophase. Means $\pm$ S.E., n = 6. * significant difference from Morning sample at $P < 0.05$, (from Ramenofsky et al., 2008, courtesy of Medimond S.r.l.).

(B). Basal CORT in short-day sparrows. Means $\pm$ S.E., n = 16. * $P < 0.0001$, 07:00 is different from all lights on values.

(C). Basal CORT in long-day sparrows. Means $\pm$ S.E., n = 16, except for 22:00, where n = 8, and 13:00, where n = 14. ** $P < 0.0001$, 01:00 is different from all lights on values, from Breuner et al., 1999, courtesy of Elsevier).

Figure 2. (A) Locomotor activity (average movement/min) during 6 thirty minute time periods and baseline levels of corticosterone (ng/ml) at the conclusion of the same thirty minute period in birds held on a 18L:6D Photoperiod: Mid-day, Q (quiescence), EF (2h of migratory restlessness), LF (4h migratory restlessness), FE (End of migratory restlessness at onset of photophase, Morning (2h of photophase). * locomotor activity levels significantly different from Mid-day, Quiescence and Morning samples at $p < 0.05$. Means $\pm$ S.E., n = 6 and # baseline levels of corticosterone significantly different from Morning sample at $P < 0.05$, (from Ramenofsky et al., 2008, courtesy of Medimond S.r.l.).

(B) Compilation of results from independent studies illustrating the locomotor activity (activity/min) and baseline corticosterone (ng/ml) measured at Mid-day and Quiescent phase

710 on18L and extending the scotophase by 8, 26 and 48 h (from Ramenofsky et al., 2008, courtesy
711 of Medimond S.r.l.).
712 (C) Cort in stress response (early migration) much greater than baseline in migratory restlessness
713 (redrawn from a compilation of several studies, Romero and Wingfield, 2015).
714

Fig. 1

Fig. 2