

Baseline Corticosterone Peaks in Shorebirds with Maximal Energy Stores for Migration: A General Preparatory Mechanism for Rapid Behavioral and Metabolic Transitions?

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Accepted July 24, 2000

In captive red knots (*Calidris canutus*, Scolopacidae) showing a regulated body mass increase of 50% related to their migration from temperate staging sites to tundra breeding grounds, plasma corticosterone concentrations increased from less than 10 ng · ml⁻¹ to levels as high as 30 ng · ml⁻¹ when the energy storage for migration was complete. These birds did not fly, but concentrations dropped to very low levels (<5 ng · ml⁻¹) as soon as the birds started their voluntary fasts to the low body masses preceding the early wing and body molts normally occurring after an unsuccessful breeding season. As the elevated levels of corticosterone are associated with stable body mass rather than with the preceding increase or subsequent decrease, it is suggested that a major role of corticosterone during the final stages just before departure may be to prepare birds for long-distance flights. Birds heading into the Arctic to breed face potentially arduous flights into unpredictable environmental and social conditions. Activation of the hypothalamic–pituitary–adrenal axis, as measured by elevated levels of corticosterone, may induce the suite of behavioral and metabolic changes necessary to negotiate these challenges successfully.

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Key Words: corticosterone; environmental unpredictability; migration; shorebirds; stress.

The preparation for, and the execution of, long-distance flights in migrating birds involves a large number of physiological changes (Ramenofsky, 1990; Wingfield *et al.*, 1990; Jenni and Jenni-Eiermann, 1998; Piersma, 1998). The glucocorticosteroid hormone, corticosterone, is thought to be important in the physiological orchestration of avian migration because of the identified elevated level of activity of the avian adrenal gland either prior to (John, 1965; Naik and George, 1963) or during (Peczely, 1976) migration. Shorebird species (Scolopacidae) display temporary elevations of this glucocorticoid, at least during northward migration and immediately after arrival from flights of several 1000 km (O'Reilly and Wingfield, 1995; Ramenofsky *et al.*, 1995; Tsipoura *et al.*, 1999). Long-term captive red knots (*Calidris canutus*) that maintain natural mass and molt cycles, even though they are unable to fly beyond the boundaries of the aviaries, also show elevated levels of plasma corticosterone during both northward and southward migration, periods which are indicated by peaks in body mass (Piersma and Ramenofsky, 1998). Furthermore, elevated levels of baseline corticosterone have been found in several passerines during the migration period (Romero *et al.*, 1997; Holberton, 1999; Ramenofsky *et al.*, 1999), but not consistently across species or seasons (Schwabl *et al.*, 1991; Gwinner *et al.*, 1992).

Even though there are several indications, based on correlations between adrenal gland activity and migratory period, that corticosterone may play a vital role in migration, the function of the glucocorticoids or even the timing of these effects remains unclear. Although often discussed in singular terms, migration is really made up of a sequence of distinct life cycle "substages" (Ens *et al.*, 1994; Jacobs and Wingfield, 2000). In the course of any substantial migration event, migratory birds (1) physiologically prepare for the flight (feed, gain mass, and perhaps molt), (2) become aphagic and make decisions about exact timing of departure, (3) depart and fly, and (4) arrive at their destination. Such stages can even be recognized in migrants held in the laboratory (e.g., Gwinner, 1990; Klaassen and Biebach, 1994). It is with respect to corticosterone levels during the various substages that the literature falls short. During which substage(s) is corticosterone elevated and which role might it then play?

Dallman *et al.* (1993) and Leibowitz *et al.* (1984) have shown that administration of corticosterone to adrenalectomized rats has a permissive effect on feeding, but this is at basal levels of corticosterone. The literature on birds is controversial. In some cases administration of corticosterone seems to increase foraging, yet in others such an effect is unclear (Nagra *et al.*, 1963; Wingfield and Silverin, 1986; Gross *et al.*, 1980; Kafri *et al.*, 1988; Gray *et al.*, 1990). A clear dichotomy was found by Astheimer *et al.* (1992) who studied Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Implanted corticosterone had no effect on feeding intensity in well-fed birds; only after a 24-h fast did corticosterone implantation enhance feeding activity. However, the ecological context of this experiment is very different from that experienced by a migratory bird fattening for migration, and the relevance of the findings of Astheimer *et al.* (1992) remains unclear for long-distance migrants.

Long-distance flying birds catabolize stored fat and the proteinaceous tissue of most of their organs (Battley *et al.*, 2000). Corticosterone may play a role in mobilizing these fuels by directing catabolic activities associated with gluconeogenesis or the retrieval of glucose from protein and amino acids (Holmes and Philips, 1976; McMahan *et al.*, 1988). Few studies have addressed this issue. For actively exercising homing

pigeons (*Columba livia*), Haase *et al.* (1986) have shown that corticosterone increases with duration of flight.

This study on captive red knots (*C. canutus*; Scolopacidae) aims to fill a gap in that it describes the relationship between endogenous levels of corticosterone and three distinct substages that are part of a complete long-distance migration event: (1) feeding + fattening (mass gain), (2) ready for takeoff + waiting for good departure conditions (level mass), and (3) "flight" (mass loss). Even in small outdoor aviaries red knots in the north temperate zone maintain cycles of molts, plumages, and body mass (Piersma *et al.*, 1995). In these cycles the preparations for the northward migration to the tundra breeding grounds can be recognized (Weber and Piersma, 1996; Piersma and Ramenofsky, 1998). In late May–early June the birds show sharply elevated body mass levels. This is associated with an apparent urge to migrate northward, as during the period of stable peak mass birds show strongly increased frequencies of restlessness (including flights) and even song and sexual harassments of flock mates (both of which are also observed in the field; Piersma *et al.*, 1991). These behaviors stop at the onset of the voluntary mass losses that involve decreases in the sizes of organ tissue and the amount of fat (Weber and Piersma, 1996). From April to July 1996 plasma samples were collected at weekly intervals to examine how baseline levels of corticosterone change during various substages of the restrained migration shown by captive red knots.

METHODS

Birds and aviaries. All experimental birds were captured during darkness with mistnets on Normerveen, Wieringen, a high-tide roost in the western Dutch Wadden Sea. One bird was caught there on 26 October 1994, 12 were caught on 6 November 1994, and another bird was caught on 27 October 1995. Given the time and place of capture, all birds belonged to the *islandica* subspecies that breeds on high arctic tundra in northern Greenland and northeast Canada and spends the nonbreeding season (August through April) in the large estuaries of western Europe (Davidson and Wilson, 1992). Thirteen of the 14 experimental red knots had been held in captivity for more

than 1 year before this study, such that acclimatization to captive conditions was assured (cf. Piersma and Ramenofsky, 1998).

Birds were individually marked with numbered aluminum bands around the right tibia, and seven each were randomly assigned to two separate aviaries within 1 month of capture. Based on dissection of a single bird that died after the experiment and post-study molecular assays using small blood samples of the remaining 13 (Baker *et al.*, 1999), 5 of the 14 birds were female. One aviary contained four females and three males, and another aviary contained one female and six males. The outdoor aviaries measured 2 by 4 m, with a height of 2 m. Rubber-coated concrete floors that occupied $\frac{3}{4}$ of the surface of the aviaries were kept wet with seawater sprinklers along the edges. The floors were cleaned and disinfected weekly. The remaining $\frac{1}{4}$ of their surfaces contained shallow basins with Wadden Sea sand and running seawater (see Piersma and Ramenofsky, 1998, Fig. 3b for a photograph). Each aviary contained a small freshwater basin for drinking and bathing. The red knots were fed protein-rich trout food pellets *ad lib*. This is an apparently attractive alternative to their normal shellfish diet (Piersma *et al.*, 1993).

Once per week the birds were taken out of the aviaries for a few hours and screened for occurrence of injuries, molt, and body mass. Body mass was measured on an electronic balance to the nearest gram, and the extent of breeding plumage was scored on a 1–7 scale (a 1 indicating a full gray winter plumage, a 2 indicating a trace of breeding plumage, a 3 indicating a quarter breeding plumage, up to a 7 indicating a full rusty-red breeding plumage). During the absence of the birds the aviaries were cleaned and disinfected and the food trays refilled.

Blood sampling. From 15 May to 10 July 1996 blood samples were taken once per week on the day of bird screening and aviary cleaning. Birds were not disturbed before they were captured in the aviary, which occurred between 9.00 h and 11.00 h to circumvent problems that daily rhythms in corticosterone levels might give (cf. Marra *et al.*, 1995; Ramenofsky *et al.*, 1999). For every individual we carefully timed the interval between the moment that we entered the aviary and the completion of bleeding. Birds were bled from the wing vein into heparinized capillaries, collecting 200–300 μ l of blood on each occasion. Samples

were centrifuged at 6900g for 15 min within 1–2 h after bleeding. Plasma was aspirated from each sample and stored (with 10 μ l of a 3% solution of β -propiolactone to satisfy United States import regulations) at -80° until transported frozen to Seattle.

Radioimmuno assay (RIA). Concentration of corticosterone was determined by specific RIA according to the procedures described by Wingfield and Farner (1975), but with the modifications of Ball and Wingfield (1987) and Wingfield *et al.* (1992). Plasma was thawed and 20 μ l of each sample of plasma was pipetted into glass centrifuge tubes to which 280 μ l distilled water was added. A total of 2000 cpm [3 H]corticosterone (NEN; NET-399) was added to each sample and allowed to equilibrate overnight at 4° . The lipid fraction in each sample was extracted with 4 ml of distilled dichloromethane which was then dried under nitrogen gas at 37° . The dried extracts were resuspended in 550 μ l phosphate-buffered saline with 0.1% gelatin. Duplicate subsamples of 200 μ l were measured by RIA. The remaining 100 μ l was pipetted into a glass vial to which we added 4.5 ml scintillation fluid. CPM from each vial were corrected for dilution and provided an estimate of percentage recovery of steroid following extraction for each sample. Concentration of corticosterone was determined from a standard curve that ranged from 7.8 to 2000 pg/ml and each sample was adjusted for percentage of recovery. All samples could be analyzed in a single assay. The percentages of recovery ranged from 70 to 100%.

Statistics. Having verified that the data obeyed the normality criterion, individual and time effects on corticosterone level were analyzed using standard analysis of variance (ANOVA).

RESULTS

During 1996, experimental red knots showed the expected slow increase in body mass from late April to late May (Fig. 1A). The total increase equals about 50% of the initial body mass. A period of stable high mass was documented from late May to early June followed by a rapid drop in mass. There were no detectable sex- or aviary-related differences in the body mass curves, although all individuals (except the one that was captured in late 1995) reached slightly higher peak masses

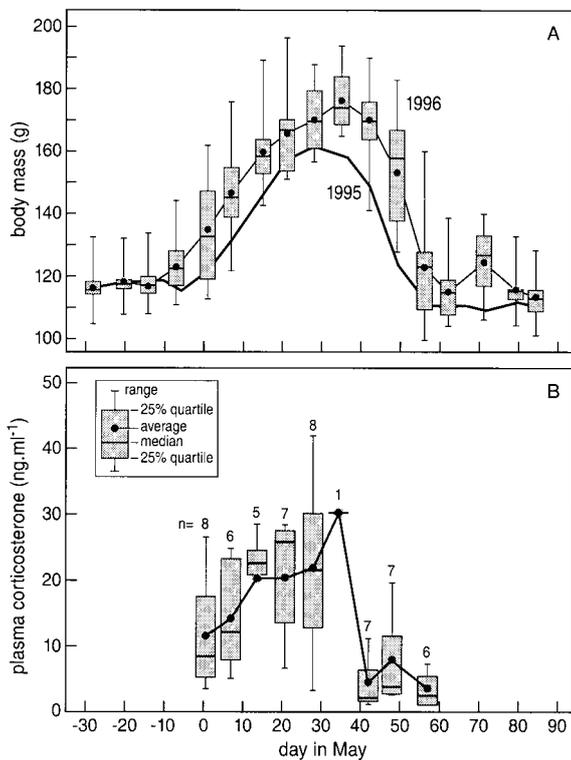


FIG. 1. (A) Seasonal change in average body mass of the 14 experimental red knots in 1996. For comparison, the average body mass trajectory for 13 of the 14 birds in 1995 is given by the thick line. (B) Plasma corticosterone concentrations from all 14 red knots as measured in plasma samples collected within 4 min after cage entry; n values are indicated above the range bars.

in 1996 than in 1995. This slow increase in the height of the spring mass peak over the first few years in captivity appears typical for captive red knots belonging to the *islandica* subspecies (Piersma *et al.*, 1995; Piersma and Ramenofsky, 1998).

Following entry into the aviary, plasma levels of corticosterone rose after 4 min of capture and handling (Fig. 2). The timing and the rate of the increase is typical of the stress response to capture and handling described in many other species (Wingfield, 1994). In the statistical analyses that follow, only plasma samples that were collected within the first 4 min after entry of the aviaries are used and defined as baseline levels. As there were no detectable differences in plasma corticosterone concentration among the 14 individuals in the entire sample (ANOVA, $F_{13,91} = 0.983$, $P = 0.475$), it was justified to analyze for other effects without taking individual variation into ac-

count. A further analysis of variance showed that the variation in corticosterone concentrations between dates was larger than the variation within dates ($F_{8,46} = 3.430$, $P = 0.004$). Plasma corticosterone peaked at about $30 \text{ ng} \cdot \text{ml}^{-1}$ in late May and early June, i.e., during the period of stable peak body masses (Fig. 1B). The peak level is roughly three times the level during the beginning of the mass increase (ca. $10 \text{ ng} \cdot \text{ml}^{-1}$).

A plot of individual baseline corticosterone on the rate of body mass change during the preceding week (Fig. 3) illustrates that, during periods of mass loss, corticosterone levels were even lower than $5 \text{ ng} \cdot \text{ml}^{-1}$. Furthermore, corticosterone was highest when individual body mass values were high and stable (Fig. 3A; ANOVA, $F_{1,36} = 6.380$, $P = 0.016$). A multivariate analysis including sex (Fig. 3B) and the interaction between phase and sex showed no effects of either of these two factors ($F_{1,34}$ ratios of 1.500 and 0.712 and P values of 0.229 and 0.405, respectively).

The captive birds were rather asynchronous in the timing of their body mass decreases. Thus, the patterns of body mass and corticosterone levels are illustrated in the four individuals for which there are reasonably complete series of baseline samples (Fig. 4). In all four cases plasma corticosterone peaked during the period of stable peak mass, rising to the high values

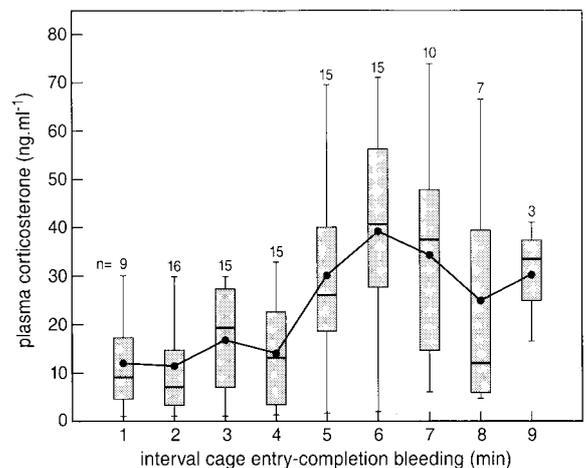


FIG. 2. Increase in plasma corticosterone levels of red knots as a function of the time between entry of the aviaries and completion of bleeding to the nearest minute. Note that lengths of intervals were random with respect to time of year and individual. The data are presented as box plots as explained in Fig. 1B; n values are indicated above the range bars.

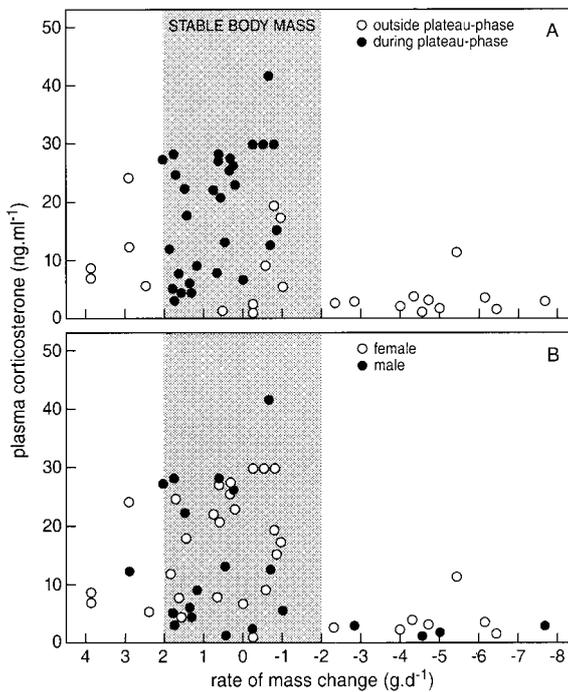


FIG. 3. Plasma corticosterone levels as a function of the average daily mass change over the 7 days preceding the day of bleeding in captive red knots, plotted separately for individuals during and outside the plateau phase in body mass (A) and separately for sex (B). The plateau phase is defined as the period after 25 May when individual body mass values are larger than 140 g.

only after peak mass had been reached and dropping to particularly low values as soon as body mass dropped. Figure 4 suggests that there are no relationships between either body mass or corticosterone concentrations and timing of the molt into a breeding plumage or completeness of this plumage change.

DISCUSSION

High corticosterone concentrations occur only at a time when captive red knots have maximum fuel stores. The findings thus focus on the substage of the long-distance migration event during which the birds are fully “fueled up.” The birds may then be ready for takeoff, although perhaps still undergoing internal reorganization not reflected by changes in body mass (Piersma, 1998). The importance of distinguishing between the various substages of a migratory event has

been overlooked in the literature thus far (Romero *et al.*, 1997; Piersma and Ramenofsky, 1998; Holberton, 1999).

The disassociation between elevations of corticosterone level and body mass changes suggests that corticosterone has little to do with energy management. To develop the hypothesis about the particular role of corticosterone in the context of long-distance migration, it is apt to first consider what long-distance migrant shorebirds do when they have accumulated sufficient nutritional stores to fly directly to the breeding grounds. In the captive red knots this phase commences once they have reached stable peak body mass levels but are unable to fly off due to their confinement. It is unlikely that the elevated levels before departure directly reflect adjustments to the breeding period per se (cf. Romero *et al.*, 1997). In this case levels would have been predicted to remain high even during mass loss. In fact, baseline corticosterone levels are rather low during the breeding season in high-arctic breeding red knots (J. Reneerkens and R. I. G. Morrison, unpublished data).

In nature, the success of such long migratory flights may depend to a large extent on the environmental conditions during takeoff and *en route* and on the number and quality of the migratory flock mates (Piersma *et al.*, 1990). Also, being ready to depart means that birds must make a transition from having regular routines in relatively benign environments (Piersma *et al.*, 1994) to a flight lasting several days to the tundra breeding grounds where environmental conditions are highly unpredictable (Green *et al.*, 1977; Myers and Pitelka, 1979; Piersma and Morrison, 1994). Additionally, the birds may face severe competition for suitable territories and mates immediately after arrival (Whitfield and Brade, 1991).

Shorebirds that are ready to depart for the breeding grounds must be able to integrate many internal and external cues from the physical and social environments to determine when to fly. The required level of “adaptive anxiety” in making the decision to take off may thus be much higher than under normal conditions and may represent an upregulation of processes that have been formerly associated with stress (Wingfield *et al.*, 1997). Here we propose that the hypothalamic-pituitary-adrenal axis is functionally activated to enable the birds to appropriately face the challenges imposed by the unpredictable environmen-

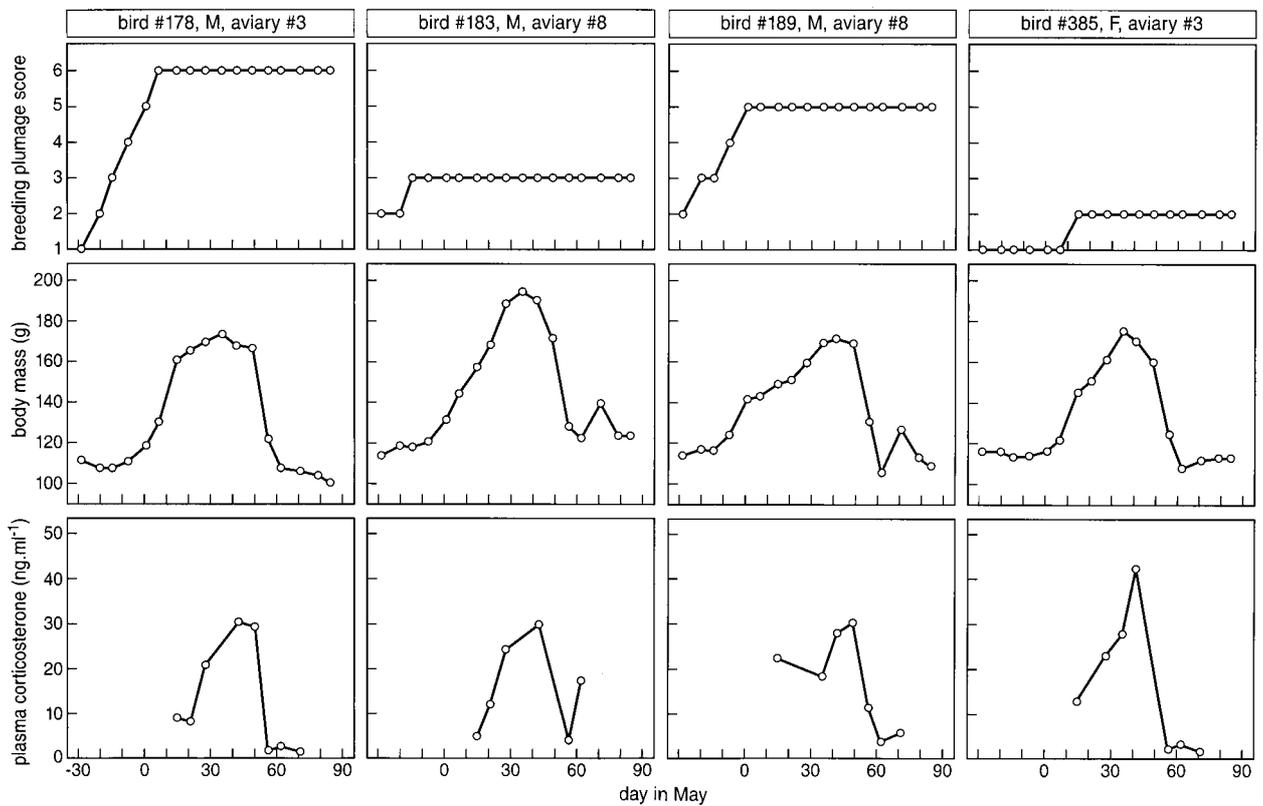


FIG. 4. Comparison of the seasonal changes in plumage score, body mass, and plasma corticosterone levels in 4 of the 14 captive red knots in April–July 1996. Plumage scores indicate extent of breeding plumage, with score 1 representing full winter plumage and score 6 representing almost full breeding plumage (trace of winter plumage). These 4 birds were selected on the basis of the availability of (almost) weekly plasma samples collected within 4 min after cage entry.

tal and social conditions resulting from long-distance flights toward the breeding grounds (cf. Astheimer *et al.*, 1995).

This hypothesis can address what Ramenofsky *et al.* (1995) called the “confusing” observation of high corticosterone levels in bar-tailed godwits (*Limosa lapponica*) that were ready to depart for a 5000-km-long flight from the Dutch Wadden Sea to the Siberian breeding grounds (observations that have since been substantiated; M. Landys, M. Ramenofsky, T. Piersma, and J. Jukema, unpublished data). Comparison of maximal levels of corticosterone at two sites during northward migration of western sandpipers (*Calidris mauri*) identified a significant elevation at the more northerly location (O’Reilly, 1995; O’Reilly and Wingfield, 1995). At this site, birds had greater distances to cover before reaching the tundra breeding grounds, and body mass and fat score were greater than at the more southerly location. This suggest that these birds

were preparing for the longer flights and potentially for the more uncertain environmental circumstances upon arrival. Although the data on godwits and sandpipers were collected in the field from birds actively engaged in migratory flights, the finding of elevated body mass and corticosterone are intriguingly reminiscent of the captive red knots. That the captive birds immediately responded with low corticosterone levels as soon as their body masses started to drop may be interpreted as reflecting the fact that by then they had given up the long northward flight. Indeed, the birds are forced to remain in the confines of the aviaries and will start the molt of contour and flight feathers within a couple of weeks, as is the habit of individuals that “opt out,” skip a breeding season, and remain on temperate staging or wintering areas (T. Piersma, unpublished data).

Our results are also in line with recent correlational data on corticosterone in relation to the dispersal of

birds. Based on the finding that free-living juvenile screech owls (*Asio kennicottii*) disperse at the time that captive juveniles show high levels of corticosterone in combination with high locomotor activity, Belthoff and Dufty (1998) proposed that when a growing individual has stored enough fat, elevated plasma corticosterone concentrations induce the locomotor activity required for dispersion. Indeed, Bruener *et al.* (1998) demonstrated an immediate influence upon locomotor activity following the noninvasive administration of moderate doses of corticosterone in fully grown Gambel's white-crowned sparrows. Similarly, in American kestrels (*Falco sparverius*), juveniles showed significantly increased circulating corticosterone when they were ready to leave the nest (Heath, 1997). On the basis of behavioral observations in a field experiment with willow tits (*Parus montanus*), Silverin (1997) proposed that corticosterone redirects juveniles from (the presumably preferred) establishment in a flock to dispersal into unpredictable situations where survival may be lower.

In the latter cases, as well as with respect to the fattened captive knots, the trigger for the elevation of corticosterone levels is unknown. The effects, however, may be behavioral (as described previously for correlational data) and perhaps metabolic (preparing for mobilization of energy stores and/or influencing organ remodeling prior to migration flight). We thus propose that in birds corticosterone fulfills particular and rather specific roles in inducing the suite of psychological, behavioral, and metabolic changes that are necessary for successful movements into the unknown.

ACKNOWLEDGMENTS

We thank Ciska Raaijmakers, Anita Koolhaas, Anne Dekinga, Pieter Honkoop, Piet Duiven, and Bernard Spaans for much practical help during this study, Theo Mulder for hospitality during the nights at Normerven, Annette D. Greenslade and Allan J. Baker for molecularly sexing the experimental birds, and Meta Landys, John C. Wingfield, and Jaap M. Koolhaas for stimulating feedback and comments on drafts. This research was supported by a PIONIER grant to T.P. from the Netherlands Organization for Scientific Research (NWO) and was carried out under the auspices of the DEC (Animal Experiment Committee) of the Dutch Royal Academy of Sciences (KNAW). J.R. received a travel grant from the Schuurman

Schimmel-Van Outeren Stichting. M.R. was supported by a University of Washington Graduate School Research Fellowship. This is NIOZ publication 3353.

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GENERAL AND COMPARATIVE ENDOCRINOLOGY

Published monthly by Academic Press, 6277 Sea Harbor Drive, Orlando, FL 32887-4900. Number of issues published annually: 12. Editors: Dr. F. L. Moore, Department of Zoology, Oregon State University, Corvallis, OR 97331-2914; and Dr. I.W. Henderson, Institute of Endocrinology, Department of Animal and Plant Sciences, Western Bank, University of Sheffield, Sheffield S10 2TN, United Kingdom. Owned by Academic Press, 525 B Street, Suite 1900, San Diego, CA 92101-4495. Known bondholders, mortgagees, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages, and other securities: None.

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