

MODULATION OF THE ADRENOCORTICAL RESPONSES TO ACUTE STRESS IN NORTHERN AND SOUTHERN POPULATIONS OF *ZONOTRICHIA*

John C. Wingfield^{1,5}, Ignacio T. Moore², Rodrigo A. Vasquez³, Pablo Sabat³, Shalin Busch¹, Aaron Clark¹, Elizabeth Addis¹, Federico Prado¹, & Haruka Wada⁴

¹Department of Biology, University of Washington, Seattle, Wa 98195, U.S.A.

²Department of Biological Sciences, Virginia Tech, 2125 Derring Hall, Blacksburg, VA 24061, USA.

³Departamento de Ciencias Ecologicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

⁴Department of Fisheries and Wildlife, Virginia Tech, 100 Cheatham Hall, Blacksburg, VA 24061-0321, USA.

Resumen. – Modulación de la reacción adrenocortical al estrés agudo en poblaciones norteañas y sureñas de *Zonotrichia*. – La forma como los animales responden a las perturbaciones del medio ambiente es relevante no solo a los efectos del cambio del clima global sino también a los disturbios producidos por los humanos. Los mecanismos fisiológicos causantes de estas reacciones facultativas a perturbaciones impredecibles del medio ambiente nos ayudan a entender el porqué algunas poblaciones son más capaces de adaptarse que otras. Esto es importante, no solo para la biología básica, sino también para la conservación. Poblaciones norteañas del Gorrión corona blanca (*Zonotrichia leucophrys*), muestran varios grados de modulación de la reacción adrenocortical al estrés agudo a comienzos de la temporada reproductiva. Estas variaciones están relacionadas a las temporadas reproductivas restringidas en zonas de alta latitud y altitud (regulación elevada de la reacción al estrés) y posiblemente al grado del cuidado paterno (regulación disminuida de la reacción al estrés). Investigaciones en varias especies del hemisferio norte indican que estos tipos de modulación son comunes entre los vertebrados. Sin embargo, se sabe muy poco de la modulación de la reacción adrenocortical al estrés en el hemisferio sur y las aves del Neotrópico representan un modelo ideal para examinar si los mismos patrones de reacción hormonal al estrés en el hemisferio norte también se encuentran en el hemisferio sur. Poblaciones tropicales y de altitud del Gorrión maizero (*Z. capensis costaricensis*), un congénere sureño del Gorrión corona blanca, tienen temporadas reproductivas extensas, pero no muestran regulación elevada temprana de la reacción adrenocortical al estrés. Este patrón es más parecido al de poblaciones del Gorrión corona blanca en zonas de mediana latitud y baja altitud. Lo que está aún bajo investigación es si las poblaciones australes del Gorrión maizero en zonas de alta latitud y altitud modulan estos procesos, bajo supuestas restricciones de estacionalidad similares a aquellas que se encuentran en zonas desde mediana a alta latitud en el norte.

Abstract. – How animals respond to perturbations of the environment is relevant to the effects of global climate change and human disturbance. The physiological mechanisms underlying facultative responses to unpredictable perturbations of the environment will allow us to understand why some populations are able

⁵Corresponding author's e-mail: jwingfie@u.washington.edu

to cope more than others. This is important for basic biology as well as for conservation. Northern populations of White-crowned Sparrow (*Zonotrichia leucophrys*), show varying degrees of modulation of the adrenocortical response to acute stress early in the breeding season. These variations are related to a short breeding season at high latitudes and altitudes (up-regulation of the stress response), and possibly degree of parental care (down-regulation of the stress response). Investigations of many taxa from the northern hemisphere indicate these types of modulation are widespread among vertebrates. However, modulation of the adrenocortical response to stress is much less well-known in the southern hemisphere and Neotropical birds present an ideal model system to test whether patterns of hormonal responses to stress in the northern hemisphere are consistent worldwide. Equatorial, high altitude, populations of the Rufous-collared Sparrow (*Z. capensis costaricensis*), a southern congener of the White-crowned Sparrow, have long breeding seasons, but show no early breeding up-regulation of the adrenocortical responses to stress. This pattern is more similar to mid-latitude, low altitude, populations of White-crowned Sparrows. Whether austral high latitude and altitude populations of the Rufous-collared Sparrows modulate these processes, under presumably similar constraints of mid- to high latitude seasonality in the north, is currently under investigation. *Accepted 20 October 2007.*

Key words: Stress, corticosteroids, coping, global climate change, human disturbance, *Zonotrichia*.

INTRODUCTION

One of the most important tasks for an animal is to adjust morphology, physiology and behavior in relation to a changing environment – both physical and social. Such changes include the predictable, such as day and night cycles, seasons etc., and the daily and seasonal routines that accompany them (Wingfield *et al.* 2001, Wingfield 1991). Additionally, it is crucial that individuals also be able to cope with unpredictable events in the environment such as natural perturbations due to storms, predators, social instability and, in recent times, human disturbance, pollution and global climate change (Wingfield *et al.* 2001; McEwen & Wingfield 2003). How organisms perceive environmental cues that portend predictable and unpredictable events has received scant attention with the possible exceptions of effects of photoperiod (day length changes) and some social interactions (Wingfield *et al.* 1991). However, determining the mechanisms underlying these responses to changing environments, including unpredictable perturbations, is critical if we are to understand the impacts of global climate change and are fundamental for focusing con-

servation efforts (see Walker *et al.* 2005).

Investigations of wild avian species both in the field and laboratory have begun to reveal potential mechanisms by which organisms, particularly birds, cope with a changing environment. A framework is emerging of how diverse environmental factors may interact to regulate the life cycles of vertebrates from polar regions to the tropics (Fig. 1). Predictive information from the environment allows organisms to initiate morphological, physiological and behavioral changes for a specific life history stage (such as breeding, migration, molt and winter) in anticipation of the event (Fig. 1). For example, an animal must develop the reproductive system and activate relevant breeding behavior before the breeding season actually begins so the onset of nesting can occur immediately when environmental conditions are favorable. This predictive information has two major types: 1) initial predictive such as changing day length with seasons that begins development of a life history stage, maintains it and then terminates that stage so the next can develop (Fig. 1, e.g., Wingfield & Silverin 2002, Wingfield 2007), and 2) local predictive information such as rainfall, temperature, food availability, nest

Classification of environmental factors regulating life cycles

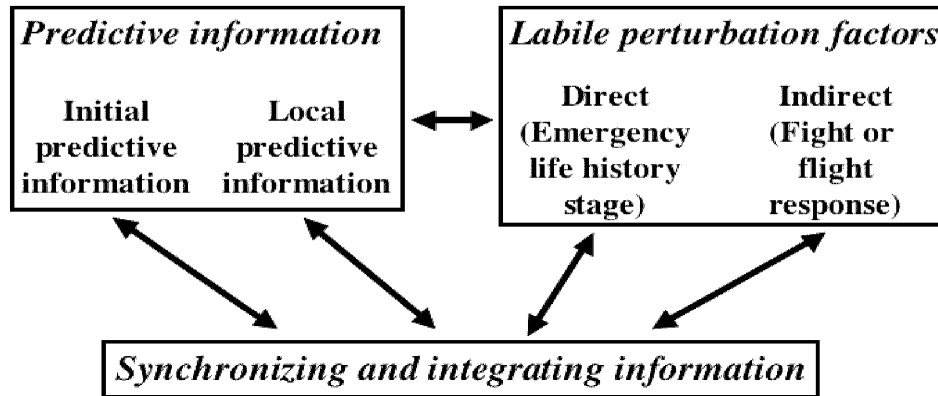


FIG. 1. Schematic diagram of how diverse environmental factors may interact to regulate life cycles. Predictive information from the environment allows organisms to initiate development of a life history stage (e.g., breeding, migration). Initial predictive information such as changing day length begins development of a life history stage, maintains it and then terminates that stage so the next can develop. Local predictive information (rainfall, temperature, food availability) speeds up or slows down development and termination of life history stages and regulates onset of the life history stage such as actual nesting. Labile perturbation factors are unpredictable events that organisms must cope with on a facultative basis and coping mechanisms occur during and after the event. Direct perturbations such as storms force an individual to abandon its life history stage. Indirect perturbations such as a predator attack trigger a flight or flight response. Social interactions synchronize individuals of a group in specific life history stages (e.g., synchronize a breeding pair or group) and integrate behavioral changes within a life history stage such as the transition from sexual to parental behavior when breeding. See Wingfield *et al.* (1999) and text for details.

sites etc. (Fig. 1) that can speed up or slow down development and termination of life history stages (e.g., warm springs speed up reproductive development), and regulate actual onset of the life history stage such as onset of nesting. These cues work in conjunction with photoperiod and endogenous rhythms to allow an animal to properly time seasonal processes (Gwinner & Dittami 1990, Wingfield *et al.* 1999). Labile perturbation factors are unpredictable events that organisms must cope with on a facultative basis (Fig. 1). That is, they cannot anticipate the event, at least not in the long term, and coping mechanisms occur during and after the event. There

are two types of perturbation factors. Direct, such as storms that lower food resources and force an individual to abandon its life history stage, and indirect, such as a flight-or-flight response to a predator or dominant conspecific. Predictive information and perturbation factors interact in complex ways that still remain largely unknown (Fig. 1). Finally, social interactions synchronize individuals of a group in specific life history stages (e.g., synchronize a breeding pair or group) and integrate behavioral changes within a life history stage such as the transition from sexual to parental behavior when breeding (Fig. 1). These synchronizing and

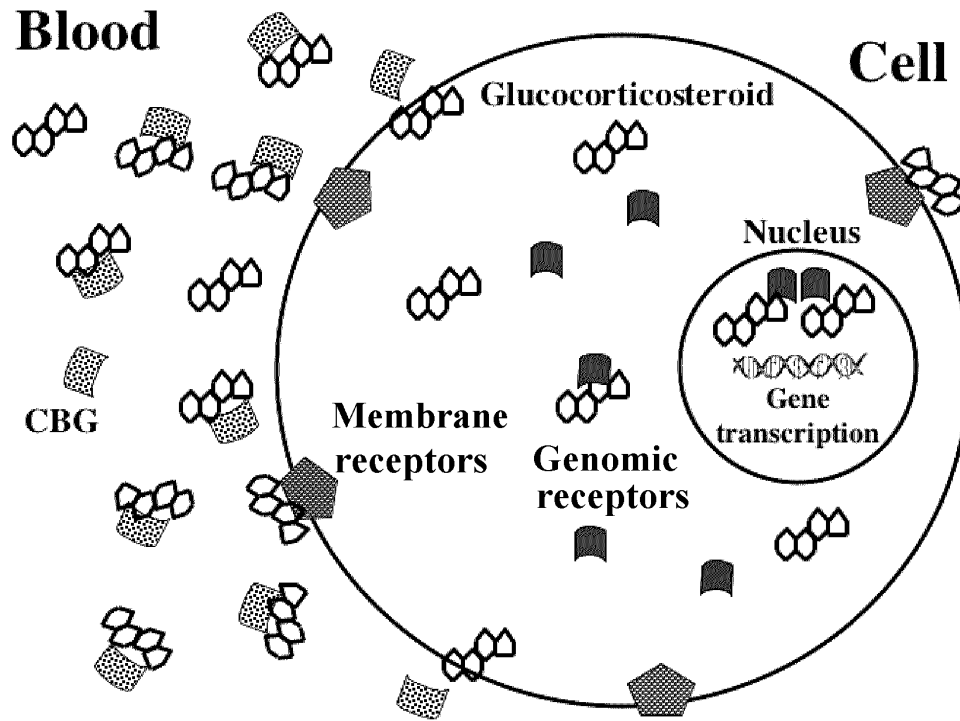


FIG. 2. Glucocorticoids such as corticosterone are secreted by the adrenal cortex and circulate in the blood mostly bound to a carrier protein, corticosteroid-binding globulin (CBG). When bound in this way it is generally thought they cannot enter target cells. Free (i.e., unbound) corticosterone can easily pass through cell membranes and interact with a membrane receptor (that mediates very rapid, within seconds to minutes) effects, or with an intracellular genomic receptor. There are two types of genomic receptors, the mineralocorticoid type (MR) and the glucocorticoid type (GR) both of which regulate gene transcription. This process and the later translation of mRNA into protein requires at least 30 mins and usually several hours – much slower than membrane receptor effects. See text for more explanation.

integrating factors interact extensively with both predictive and perturbation factors. See Wingfield *et al.* (1999) for details.

Here the focus will be on mechanisms by which vertebrates, particularly birds, cope with perturbation factors. A well known neural and endocrine system, the hypothalamo-pituitary-adrenal cortex axis (HPA) has been very well studied as a major mechanistic system regulating coping physiology and behavior (Sapolsky *et al.* 2000, Romero 2002, McEwen & Wingfield 2003). This HPA axis

and its responsiveness to perturbation factors in the environment can also be modulated on a seasonal and individual basis (Wingfield & Romero 1999, Wingfield & Sapolsky 2003). However, most investigations have been conducted in the northern hemisphere and much less in the tropics and southern hemisphere (see also Moore & Jessop 2003). Neotropical birds offer many intriguing model systems where closely related taxa have ranges extending into the northern and southern hemispheres, thus offering ideal

opportunities to determine whether the hormonal mechanisms are similar (evolutionary constraints hypothesis *sensu* Reed *et al.* 2006, Hau 2007), or whether multiple unique mechanisms have evolved in response to similar perturbations (evolutionary potential hypothesis, Hau 2007).

GLUCOCORTICOIDS AND COPING WITH STRESS

Glucocorticoids such as corticosterone are secreted by the adrenal cortex in response to a cascade of peptide secretions originating in the hypothalamus of the brain, including the anterior pituitary gland, and finally reaching the adrenal cortical cells through the peripheral blood stream (Norris 1997). They circulate in the blood mostly bound to a carrier protein called corticosteroid-binding globulin (CBG) (Breuner & Orchinik 2002). When bound in this way it is generally thought they cannot enter target cells (Fig. 2). Free (i.e., unbound) corticosterone can easily pass through cell membranes and interact with a membrane receptor (that mediates very rapid effects, within seconds to minutes), or with an intracellular genomic receptor (that mediates slower effects usually taking several hours) (Fig. 2, e.g., Breuner *et al.* 2003). There are two types of genomic receptors, the mineralocorticoid type (MR) and the glucocorticoid type (GR). Both of these genomic receptor types form homodimers once they bind corticosterone, resulting in conformational changes that allow them to enter the nucleus and interact with DNA to thus regulate gene transcription (Fig. 2). This process and the later translation of mRNA into protein require at least 30 min and usually several hours – much slower than membrane receptor effects.

The actions of glucocorticoids can be classified in three major types (Norris 1997, Sapolsky *et al.* 2000). They are important during development, especially just prior to birth.

Post-natal baseline levels that change with time of day and season are involved in regulation of glucose (particularly glucose transporters in cells), osmoregulatory effects through ion channel activity, and interactions with the immune system. However, glucocorticoid secretion increases even further in response to a very broad spectrum of environmental perturbations often termed stress. Very high circulating concentrations of glucocorticoids have a different suite of effects from chronic stress with high glucocorticoid secretion for many days or weeks. The latter effects are deleterious and may result in death and include neuronal damage, inhibition of the immune system, protein degradation (muscle wasting), suppression of growth and complete loss of reproductive function (e.g., Sapolsky *et al.* 2000, McEwen 2006). Although chronic stress is very important in medicine and agriculture, animals in natural conditions usually die before the symptoms of chronic stress appear. Less well-studied is a suite of more rapid effects (minutes to days) of high circulating glucocorticoids in response to an environmental perturbation that actually trigger physiological and behavioral traits (called the emergency life history stage, Wingfield 2003) that promote coping and survival in the face of stress (Sapolsky *et al.* 2000, Romero 2002). These effects include suppression of non-vital processes such as reproduction (but not to the extent of regression of the reproductive system), mobilization and production of glucose from stores such as fat and protein, promotion of survival strategies such as increased activity associated with leaving the area of the perturbation, finding a shelter etc., decreased extended metabolic rate at night (increased restfulness), and initial promotion of immune system function in anticipation of injury and/or infection (Wingfield & Ramenofsky 1999, Sapolsky *et al.* 2000, Dhabhar 2002, Wingfield 2003). The combination of these more rapid physiological and behavioral effects pro-

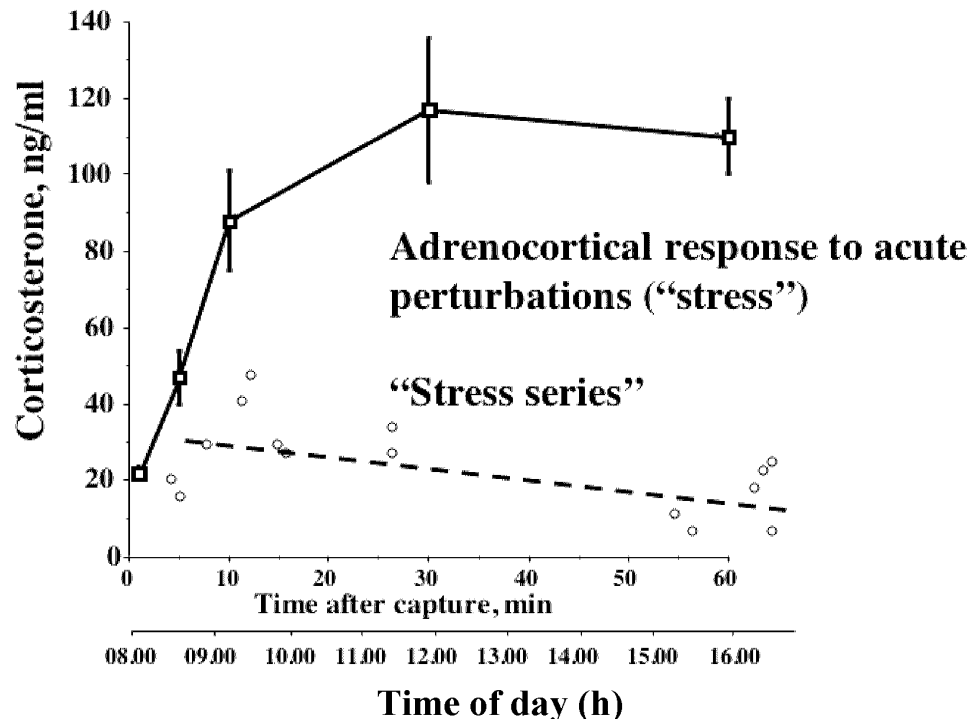


FIG. 3. A typical response to acute stress in a vertebrate such as Neotropical songbird. We use capture and handling during normal banding activities as a uniform stressor that we can apply to all individuals. Small blood samples (30–40 μ l) are collected from a wing vein as soon as possible after capture (usually within 1–2 min with a 3 min maximum) and then at intervals at 5, 10 and 60 min after capture. During the process of capture and handling the bird responds as it would to a predator – i.e., an acute stress. Measurement of plasma levels of corticosterone in those small samples show a rapid increase reaching a maximum within 10–60 min (solid line). The plasma levels of corticosterone at capture are representative of pre-capture, baseline levels, and when plotted as a function of time of day of capture (broken line), show no major changes compared with stress levels. This is termed a “stress series” and has proved to be a useful tool to compare the stress responsiveness of vertebrates at population levels (seasons, habitat differences, gender etc.) and at individual levels (age, parasite load, body condition etc.). See Wingfield (1994) and Wingfield & Romero (2001) for more details of methods.

motes survival and avoids the deleterious effects of chronic stress.

Given the important role glucocorticoids have during responses to stress, we would expect plasma concentrations to rise following a perturbation. This increase is thought to increase chances of survival. A typical response to acute stress in a vertebrate such as Neotropical songbird is shown in Figure 3.

We use capture and handling during normal banding and measurement activities as a uniform stressor that we can apply to all individuals regardless of gender, population, season etc. (Wingfield 1994). Small blood samples (30–40 μ l) are collected from a wing vein as soon as possible after capture (usually within 1–2 min with a 3 min maximum), and then at intervals at 5, 10 and 60 min after capture.

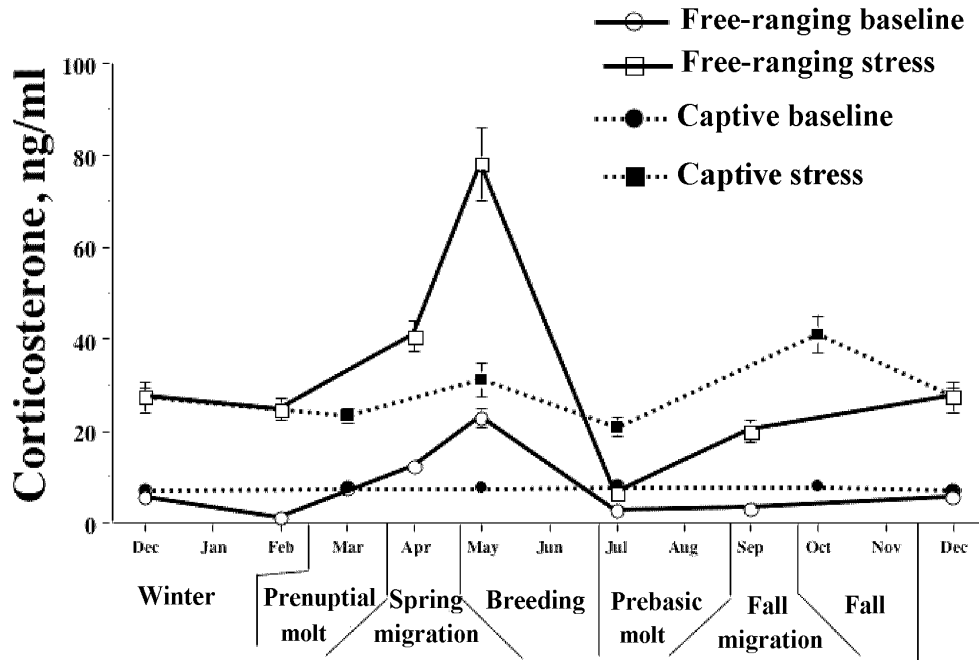


FIG. 4. Seasonal changes in circulating baseline and stress levels of corticosterone (after 30 min of capture, handling and restraint) in free-ranging and captive male White-crowned Sparrows (*Zonotrichia leucophrys gambelii*). Note that in free-ranging males, corticosterone titers increase in spring and both baseline and stress levels peak at the time of arrival on the breeding grounds on the North Slope of Alaska. Seasonal lows occur during the annual molt in July with intermediate levels the rest of the year. Note that in captive birds, this dramatic seasonal modulation of the adrenocortical response to stress is almost completely absent. These data suggest that environmental factors associated with the annual cycle in the field are important regulators of responsiveness of the hypothalamo-pituitary-adrenal axis in these birds. From Romero & Wingfield (1999), courtesy of Elsevier Press.

Throughout this time other data such as body weight, molt status etc. can be collected or the bird can be held quietly in a cloth bag. During the process of capture and handling, the bird presumably responds as it would to a predator – i.e., an acute stress. At each time point, the volume of blood is small (30–40 μ l) so that for bird weighing more than 15 g, the total amount of blood collected is less than 1% of body weight – that is in compliance with most Institutional Animal Care and Use Committee requirements. If the bird weighs less than 15 g, we collect samples at only 4 time points (or less) and reduce the total volume of each sam-

ple to 20–30 μ l. In the vast majority of species studied, this sampling regimen does not debilitate the bird in any way, and thus is a potentially very powerful tool to monitor and assess responses to stress in free-living populations (Wingfield 1994, Wingfield & Ramenofsky 1999).

Measurement of plasma levels of corticosterone in those small samples shows a rapid increase reaching a maximum within 10–60 min (solid line, Fig. 3). The plasma levels of corticosterone at capture are representative of pre-capture, baseline levels and, when plotted as a function of time of day of capture

(broken line, Fig. 3), show modest changes compared with stress levels. This is termed a 'stress series' and has proved to be a useful tool to compare the stress responsiveness of the HPA axis of vertebrates at population levels (seasons, habitat differences, gender etc.) and at individual levels (age, parasite load, body condition etc.). See Wingfield (1994) and Wingfield & Romero (2001) for more details of methods.

MODULATION OF ADRENOCORTICAL RESPONSE TO STRESS DURING THE ANNUAL CYCLE

Using the "stress series" as a tool in free-living populations of birds, it soon became apparent that the responsiveness of the HPA axis to the same perturbation can be modulated on a seasonal basis as well as among individuals (Wingfield 1994, Wingfield & Romero, 1999). Such modulation is also observed in many other vertebrates (Sapolsky *et al.* 2000, Moore & Jessop 2003, Wingfield & Sapolsky 2003). Seasonal changes in circulating baseline and stress levels of corticosterone (measured after 30 min of capture, handling and restraint) in free-ranging and captive male White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) are presented in Fig. 4. Note that in free-ranging males, corticosterone titers increase in spring, and both baseline and stress levels peak at the time of arrival on the breeding grounds on the North Slope of Alaska. Seasonal lows occur during the annual molt in July with intermediate levels the rest of the year (Fig. 4). Note that in captive birds, this dramatic seasonal modulation of the adrenocortical response to stress is almost completely absent (Fig. 4). These data suggest that environmental factors associated with the annual cycle in the field are important regulators of responsiveness of the HPA axis in these birds.

An obvious question that arises from these data is why modulate the adrenocortical response to stress? Hypotheses have been presented by Wingfield *et al.* (1995) and Wingfield & Sapolsky (2003). In northern White-crowned Sparrows (*Z.l. gambelii*), it is thought that up-regulation of the HPA axis and heightened adrenocortical responses to the standardized stress series occur just prior to onset of breeding accompanied by a decrease in sensitivity to negative feedback from corticosterone (Astheimer *et al.* 1994), and an increase in CBG concentrations that protect the individual from high stress-induced levels of corticosterone (Breuner *et al.* 2003). This may allow great behavioral and physiological flexibility in a songbird that arrives on its breeding grounds in the Arctic in spring when weather conditions may be inhospitable and unpredictable. Such conditions would normally trigger a stress response and breeding would be delayed. However, mechanisms may exist whereby if the perturbation becomes more chronic (i.e., a prolonged storm and reduced food supply for more than a day) then CBG levels decline resulting an increase in free-corticosterone that can then enter target cells and trigger coping behavior and physiology (Lynn *et al.* 2003). Curiously, White-crowned Sparrows in mid-latitudes (*Z. l. pugetensis*), that have much longer breeding seasons with less severe weather in spring, do not modulate the adrenocortical response to stress through the breeding season (Breuner *et al.* 2003). Once spring and summer have begun in these Arctic breeding grounds, both males and females show a dramatic down-regulation of the HPA axis and the adrenocortical response to stress (Romero & Wingfield 1998, Holberton & Wingfield 2004), at least partially through decreased sensitivity of the adrenal cortex to pituitary adrenocorticotrophic hormone (ACTH) (Romero & Wingfield 1999). Such decreased

sensitivity of the HPA axis to stress when breeding has been demonstrated in several vertebrates and is thought to increase fitness by promoting successful reproduction in the face of potentially disruptive acute perturbations (Wingfield *et al.* 1995, Sapolsky *et al.* 2000, Wingfield & Sapolsky 2003).

It is intriguing to suggest that the tropical and southern hemisphere congeners of the White-crowned Sparrow, the Rufous-collared Sparrow (*Z. capensis*), should also modulate its adrenocortical responses to stress, especially in the more severe environments of the extreme south. Equatorial, high altitude populations of *Z. c. costaricensis* in Ecuador have breeding seasons long enough to raise several broods of young (Moore *et al.* 2004). As in northern latitude *Zonotrichia*, equatorial congeners show seasonal modulation in total corticosterone and binding globulin capacity (Wada *et al.* 2006). However, the modulation is diminished in the equatorial birds with only higher corticosterone levels during breeding than during molt. These seasonal differences disappear when free-corticosterone levels are calculated. In addition, there are no gender based differences. Thus, as predicted equatorial *Zonotrichia* express a hormonal stress response that is more similar to mid-latitude than high-latitude congeners. This indicates that environmental factors besides photoperiod such as seasonal changes in rainfall may influence stress response. It is predicted that if the adrenocortical response to stress and the mechanisms underlying the physiological and behavioral coping traits that are expressed are highly conserved, then southern Rufous-collared Sparrows in severe environments should also modulate their adrenocortical response to stress. However, it is also possible that these austral populations may have evolved a very different suite of mechanisms underlying coping strategies. These ideas are currently being tested.

CONCLUSIONS.

The adrenocortical response to acute stressors triggers coping mechanisms by promoting survival physiology and behavior in the face of environmental perturbations. These responses can be modulated in relation to reproductive function especially in species that breed in severe habitats such as high latitude. Congeners in more benign habitats at mid-latitudes do not modulate the response to stress in this way. Several investigations have explored the endocrine and cell mechanisms underlying these events in northern taxa. However, details of the adrenocortical response to stress, its modulation and mechanisms down to the cell level remain essentially unknown in southern South American species. Many opportunities exist to tease apart these mechanisms and answer an intriguing question – have austral populations of birds solved problems of behavioral and physiological flexibility in severe environments by the same mechanisms, or are they different? Answers to such questions will also make important contributions of conservation biology of Neotropical birds by providing new tools with which to monitor wild populations in the face of global change.

ACKNOWLEDGMENTS

Much of the research cited in this review was supported by grant numbers OPP- 9911333 and IBN-0317141 from the National Science Foundation to J.C. Wingfield. RAV acknowledges support from IEB - grant P05-002-ICM.

REFERENCES

- Astheimer, L. B., W. A. Buttemer, & J. C. Wingfield. 1994. Gender and seasonal differences in the adrenocortical response to ACTH challenge in an Arctic passerine, *Zonotrichia leucophrys gambelii*.

- Gen. Comp. Endocrinol. 94: 33–43.
- Breuner, C.W., & M. Orchinik. 2002. Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J. Endocrinol.* 175: 99–112.
- Breuner, C.W., M. Orchinik, T. P. Hahn, S. Meddle, I. T. Moore, N. Owen-Ashley, T. S. Sperry, & J. C. Wingfield. 2003. Differential mechanisms for regulation of the stress response across latitudinal gradients. *Am. J. Physiol.* 285: R594–R600.
- Dhabhar, F.S. 2002. Stress-induced augmentation of immune function. The role of stress hormones, leukocyte trafficking, and cytokines. *Brain Behav. Immun.* 16: 785–798.
- Gwinner, E., & P. Dittami. 1990. Endogenous reproductive rhythms in a tropical bird. *Science* 249: 906–908.
- Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* 29: 133–144.
- Holberton, R., & J. C. Wingfield. 2003. Modulating the corticosterone stress response: a mechanism for balancing risk and reproductive success in Arctic breeding sparrows? *Auk* 120: 1140–1150.
- Lynn, S. E., C. W. Breuner, & J. C. Wingfield. 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone-binding globulin in a migratory songbird. *Horm. Behav.* 43: 150–157.
- McEwen, B. S., J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43: 2–15.
- Meddle, S. L., M. Romero, L. B. Astheimer, W. A. Buttemer, & J. C. Wingfield, J.C. 2002. Steroid hormone interrelationships with territorial aggression in an Arctic-breeding songbird, Gambel's White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. *Horm. Behav.* 42: 212–221.
- Moore, I. T., & T. S. Jessop. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Horm. Behav.* 43: 39–47.
- Moore, I. T., H. Wada, N. Perfito, D. S. Busch, T. P. Hahn, & J. C. Wingfield. 2004. Territoriality and testosterone in an equatorial population of Rufous-collared Sparrows, *Zonotrichia capensis*. *Anim. Behav.* 67: 411–420.
- Norris, D. O. 1997. *Vertebrate Endocrinology*. 3rd ed. Academic Press, New York, New York.
- Reed, W. L., M. E. Clark, P. G. Parker, S. A. Raouf, N. Arguedas, D. S. Monk, E. Snajdr, V. Nolan, Jr., & E. D. Ketterson. 2006. Physiological effects on demography: a long term experimental study of testosterone effects on fitness. *Am. Nat.* 167: 667–683.
- Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128: 1–24.
- Romero, L. M., & J. C. Wingfield. 1998. Seasonal changes in adrenal sensitivity alter corticosterone levels in Gambel's White-crowned Sparrows (*Zonotrichia leucophrys gambelii*). *Comp. Biochem. Physiol.* 119 C: 31–36.
- Romero, L. M., & J. C. Wingfield. 1999. Alterations in hypothalamic-pituitary-adrenal function associated with captivity in Gambel's White-crowned Sparrows (*Zonotrichia leucophrys gambelii*). *Comp. Biochem. Physiol. Part B* 122: 13–20.
- Sapolsky, R. M., L. M. Romero, & A. U. Munck. 2000. How do glucocorticosteroids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Rev.* 21: 55–89.
- Wada, H., I. T. Moore, C. W. Breuner, & J. C. Wingfield. 2006. Stress responses in tropical sparrows: comparing tropical and temperate *Zonotrichia*. *Physiol. Biochem. Zool.* 79: 784–792.
- Walker, B. G., P. D. Boersma, & J. C. Wingfield. 2005. Field endocrinology and conservation biology. *Integ. Comp. Biol.* 45: 12–18.
- Wingfield, J. C. 1994. Modulation of the adrenocortical response to stress in birds. Pp. 520–528 in Davey, K.G., R. E. Peter, & S. S. Tobe (eds.). *Comparative Endocrinology*. National Research Council Canada, Ottawa, Ontario.
- Wingfield, J. C. 2003. Control of behavioural strategies for capricious environments. *Anim. Behav.* 66: 807–816.
- Wingfield, J. C., & R. Ramenofsky. 1999. Hormones and the behavioral ecology of stress. Pp. 1–51 in Balm, P. H. M. (ed.). *Stress physiology in animals*. CRC Press, Boca Raton, Florida.
- Wingfield, J. C., & L. M. Romero. 2001. Adrenocortical responses to stress and their modula-

- tion in free-living vertebrates. Pp. 211–236 *in* McEwen, B. S. (ed.). Handbook of physiology. Section 7: The endocrine system. Volume 4: Coping with the environment: Neural and endocrine mechanisms. Oxford Univ. Press, Oxford, UK.
- Wingfield, J. C., & R. M. Sapolsky. 2003. Reproduction and resistance to stress: when and how. *J. Neuroendocrinol.* 15: 711–724.
- Wingfield, J. C., K. M. O'Reilly, & L. B. Astheimer. 1995. Ecological bases of the modulation of adrenocortical responses to stress in Arctic birds. *AM. Zool.* 35: 285–294.
- Wingfield, J. C., J. D. Jacobs, A. D. Tramontin, N. Perfito, S. Meddle, D. L. Maney, & K. Soma. 1999. Toward and ecological basis of hormone-behavior interactions in reproduction of birds. Pp. 85–128 *in* Wallen, K., & J. Schneider (eds.). *Reproduction in context*. M. I. T. Press, Cambridge, Massachusetts.