

Stress in the Red-cockaded Woodpecker: Hormonal Mechanisms of Reproductive
Suppression in Helper Males and Impacts of Military Training Activities

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biological Sciences

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4 September 2007
Blacksburg, VA

Keywords: stress, corticosterone, cooperative breeding, human disturbance, *Picoides borealis*

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Abstract

The measurement of stress hormone levels in wild free-living animals is becoming an increasingly effective method for examining proximate mechanisms of animal behavior and the physiological impacts of human activities on wildlife. In these studies I measured plasma levels of the stress hormone corticosterone in the endangered red-cockaded woodpecker (*Picoides borealis*) to determine their role in the reproductive behavior of individuals in this species, and whether they are affected by human disturbance. In chapter one, I provide an introduction to the vertebrate stress response and I describe the natural history of the red-cockaded woodpecker. In chapter two, I compare corticosterone and reproductive hormone levels between breeding males and helper males to examine hormonal mechanisms of reproductive suppression in helper males. No hormonal differences existed between breeding and helper males. However, baseline corticosterone levels were lower in all males living in groups with two or more helper males, suggesting that male helpers reduce the workload of all other group members. In chapter three, I compare corticosterone levels between birds living in clusters subject to two different training restriction regimes on a military installation. Males living in clusters without training restrictions had lower baseline corticosterone than those living in clusters with training restrictions, suggesting that males habituate to chronic disturbance by downregulating baseline corticosterone levels.

Acknowledgements

I thank my advisor Ignacio Moore for helping me to formulate a research study that encompassed my passions and interests. I am grateful to Jeff Walters for providing me with the opportunity to join in on one of his ongoing research projects. I thank both Ignacio and Jeff, as well as Carola Haas, for their support, encouragement and constructive criticism in all aspects of my research. I thank Anne McNabb, whose enthusiasm in teaching my first introductory course in endocrinology was greatly inspiring. This work would not have been possible without the tremendous help of the Camp Lejeune field technicians Kristina Hudgins, Charlie Clarkson, and Cari Nycum. I am indebted to them for teaching me how to identify and capture birds, navigate through an often confusing field site, and free the work truck from unyielding mud puddles. I am especially grateful to Kristina for housing me during the last six months of my fieldwork. I thank John Townsend and Craig Ten Brink for their support and assistance in providing data. I thank Kerry Brust, Jen Maynard, Dan Kuefler, Koan Heindel, and Erika Gonzalez for field assistance. I thank the members of the Virginia Tech RIA lab, especially Jerry Husak, for teaching me lab protocol and answering a doubtlessly annoying number of technical questions. I am thankful to the members of the Virginia Tech Integrative, Behavioral and Organismal Biology group for their advice and emotional support, especially the Avian Ecology Lab, and Brian Olsen, Travis Belote, Pam Widder and Dylan Kessler for taking time out of their busy schedules to give me statistical advice. I could not have made it through the past three years, and especially the past several months, without the unfaltering love and support of Courtney Culp, Jen Griggs, Jen Perkins, Anna Chapman and Holly Goyert. I thank the Virginia Tech Graduate School, Sigma Xi, the Women in Science Program, the U. S. Department of Defense, Marine Corps Base Camp Lejeune, and the National Science Foundation for financial support.

Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
List of Tables.....	v
List of Figures.....	vi
Chapter 1: Introduction to stress physiology and natural history of the red-cockaded woodpecker.....	1
Introduction.....	1
The vertebrate stress response.....	2
Comparative endocrinology in studies of free-living animals.....	5
Study species.....	9
Study area.....	15
Research goals and objectives.....	16
Literature cited.....	17
Chapter 2: Hormonal mechanisms of reproductive suppression in helper males and effect of helper male presence on group members in the cooperatively breeding red-cockaded woodpecker.....	24
Abstract.....	24
Introduction.....	25
Methods.....	29
Results.....	34
Discussion.....	38
Literature cited.....	45
Chapter 3: Impacts of military training activities on stress hormone levels and body mass in the endangered red-cockaded woodpecker.....	55
Abstract.....	55
Introduction.....	56
Methods.....	60
Results.....	66
Discussion.....	72
Literature cited.....	80

List of Tables

Table 2.1. Sampling dates and number of days before/after clutch initiation or after hatch date for each breeding phase. Overlap in sampling dates across phases is due to asynchronous breeding across family groups.....	50
Table 2.2. Candidate models representing multiple hypotheses of effects on baseline corticosterone, stress-induced corticosterone, testosterone, estradiol and progesterone levels in males and females.....	51
Table 3.1. Candidate models representing multiple hypotheses of effects on baseline corticosterone levels, stress-induced corticosterone levels and body mass in males and females combined.....	86
Table 3.2. Candidate models representing multiple hypotheses of effects on baseline corticosterone levels, stress-induced corticosterone levels and body mass in males and females	87

List of Figures

Fig. 2.1. Baseline and maximal plasma corticosterone levels (mean $\pm$ SE) of breeding females, breeding males, and helper males during all phases of the breeding cycle. Sample sizes are included in the plots, listed in the following order: breeding females, breeding males, helper males. Note difference in scale between baseline and maximal corticosterone levels.....	52
Fig. 2.2. Baseline plasma corticosterone (mean + SE) of breeder males and helper males living in family groups with zero, one, or two to three helper males. Sample sizes are indicated above bars.	53
Fig. 2.3. Plasma testosterone, estradiol and progesterone levels (mean $\pm$ SE) of breeding females, breeding males, and helper males during all phases of the breeding cycle. Sample sizes are included in the plots, listed in the following order: breeding females, breeding males, helper males. Note difference in scale for each hormone.....	54
Fig. 3.1. Map of the Main Base of Marine Corps Base Camp Lejeune, showing major training areas and locations of sampled clusters. M = marked clusters; U = unmarked clusters. Numbers indicate the number of training activities scheduled in an area from September 2003-September 2004. See text for explanation of two areas with no number.....	88
Fig. 3.2. Plasma corticosterone levels of males and females living in marked and unmarked clusters during the pre- and post-breeding seasons. Sample size is indicated above error bars; error bars are standard error. Note difference in scale between males and females.....	89
Fig. 3.3. Plasma corticosterone levels of males and females in marked and unmarked clusters during the pre- and post-breeding seasons, plotted as a function of the number of training activities scheduled in the area in which an individual's cluster was located.....	90
Fig. 3.4. Baseline plasma corticosterone levels of males from groups of varying sizes during the pre- and post-breeding seasons. Sample size is indicated above error bars; error bars are standard error.....	91
Fig. 3.5. Body mass of males and females from groups of varying sizes. Mass is combined from both marked and unmarked clusters and pre- and post-breeding seasons. Sample size is indicated above error bars; error bars are standard error.....	92

Chapter 1: Introduction to stress physiology and natural history of the red-cockaded woodpecker

INTRODUCTION

Comparative field endocrinology is becoming an increasingly effective method in studies of animal behavior and conservation biology (Wingfield et al. 1997, Boonstra 2004). Hormones play a role in mediating the individual behaviors that shape a population's social structure, and affect the physiological functions and behaviors that allow an animal to adapt to changes in its environment and continue to reproduce and survive. Blood samples for measuring hormones are easy to obtain and collecting them has been shown to cause no long-term harm to individuals. Perhaps most importantly, hormone levels can provide information about a population's health or an individual's behavior much more quickly than the weeks or even years needed to obtain the same data by censusing or observation (Wingfield et al. 1997).

Stress hormone levels, in particular, can be used to address specific questions about behavior and conservation. For example, levels of stress hormones often vary among individuals of different social status due to differences in the energy required to carry out daily activities such as territory defense and foraging. These differing hormone concentrations can result in differences in reproductive behaviors (Goymann and Wingfield 2004). Stress hormone levels can therefore give insight into the effort required to maintain a certain social status and may explain intraspecific differences in breeding behavior.

Stress hormone levels are useful in studies of conservation because they are often elevated by environmental changes such as those caused by anthropogenic disturbances.

For example, stress hormone levels were increased in male Northern Spotted Owls (*Strix occidentalis caudalis*) that resided near a recent timber harvest or near a major logging road (Wasser et al. 1997). Elk (*Cervus elaphus*) in Yellowstone National Park and wolves (*Canis lupus*) in Yellowstone, Voyageurs, and Isle Royale National Parks were found to have higher stress hormone levels correlated with areas and times of snowmobile use (Creel et al. 2002). Chronically increased stress levels may have deleterious effects on the health and survival of individuals, and may disrupt or inhibit breeding, thereby reducing the population size. By measuring stress hormone levels, one may be able to identify populations or subpopulations that are under stress, and thus more likely to decline rapidly in the face of unpredictable or disruptive environmental changes. One can also predict a population's response to a specific environmental disturbance before it begins to decline or goes extinct (Wingfield et al. 1997). Predicting which species or populations are vulnerable to disturbance can help minimize humans' negative impacts on wildlife.

THE VERTEBRATE STRESS RESPONSE

Stress can be broadly defined as a challenge to homeostasis (Hadley 2000, McEwen and Wingfield 2003, Sapolsky 2004). It encompasses two basic components, a stressor and a stress response. A stressor is any environmental or social stimulus that poses a physiological or psychological threat to homeostasis. Such an event evokes a stress response, a characteristic set of physiological, hormonal, and behavioral reactions evolved to help an individual cope with and recover from the stressor (Nelson 2000). This response is highly conserved across vertebrate taxa (Romero 2004).

The vertebrate stress response has two major endocrine components divided into three stages. In the first stage, a stressful social or environmental event immediately evokes the release of the catecholamines epinephrine and norepinephrine from the adrenal medulla and sympathetic nervous system, respectively (Hadley 2000). These catecholamines mobilize stored glucose for energy utilization (Wingfield et al. 1997, Hadley 2000, Nelson 2000), and inhibit insulin secretion, preventing the removal of glucose from general circulation (Hadley 2000). Fat metabolism is stimulated, and the free fatty acids released into the blood are used by the liver for the formation of glucose (Hadley 2000). Free fatty acids are also used as sources of energy by the brain and cardiac muscle (Hadley 2000), increasing mental alertness and cardiac output (Wingfield et al. 1997, Hadley 2000). Blood flow to muscles and oxygen delivery to tissues increase. These physiological effects are very short-term, lasting only seconds to minutes, and prepare the animal for immediate and intense exertion in the face of a social or physical stressor (Wingfield et al. 1997, Hadley 2000, Nelson 2000).

The second stage of the stress response occurs within several minutes and may take minutes to hours to have effects. This second response takes place through the hypothalamic-pituitary-adrenal (HPA) axis. Nerve cells in the hypothalamus are activated to secrete corticotropin releasing hormone (CRH), which stimulates the anterior pituitary to secrete adrenocorticotrophic hormone (ACTH), which then stimulates the adrenal cortex to release glucocorticoids (cortisol in most mammals and corticosterone in most rodents, birds, amphibians, and reptiles) into the general circulation. Glucocorticoids regulate tissue responses to catecholamines released in the first stage of the stress response. Glucocorticoids are necessary for continued energy mobilization,

enhancing the actions of the previously released catecholamines by inhibiting glucose uptake by skeletal and adipose tissue and by increasing the synthesis of enzymes necessary for the continued mobilization of glucose and fatty acids (Hadley 2000). In addition to the mobilization of stored glucose, glucocorticoids trigger gluconeogenesis, usually at the expense of protein and fat (Wingfield 1994).

Other adaptive physiological and behavioral responses, lasting hours to days, facilitate survival in the face of the stressor. Metabolism and blood flow increase (Hadley 2000, Nelson 2000), and other physiological processes that are unnecessary for immediate survival, such as digestion, reproduction, immune function, growth, and response to injury (pain perception and injury reparation), are temporarily dampened (Wingfield et al. 1997, Nelson 2000, Sapolsky 2004). Parental behavior (Silverin 1986) and territorial defense (Wingfield and Silverin 1986, DeNardo and Sinervo 1994) decrease; territories may even be abandoned (Silverin 1986). Physical activity may increase to move to a more favorable habitat (Wingfield et al. 1997), or may decrease to save energy (Adams and Finn 1972). Food consumption is suppressed (Carr 2002), while foraging often increases (Wingfield et al. 1997), as animals may hoard food for the period after the stressful event passes (Maier and Watkins 1999). When the stress has subsided, glucocorticoid concentrations return to baseline levels, and normal activities may resume (Wingfield 1988).

While a moderate duration rise in GC levels is necessary for survival, if the stressor persists or occurs frequently and elevated glucocorticoid levels are prolonged an animal can enter into the third stage of the stress response, chronic stress, which can reverse earlier adaptive responses to a stressor and is often detrimental to the health of the

animal (Wingfield et al. 1997, Hadley 2000). Hyperglycemia, vascular derangements, and gastrointestinal ulceration may occur, and growth and metamorphosis may be inhibited (Wingfield et al. 1997). Temporary suppression of reproduction can transition into complete inhibition of reproduction. The lymphatic system may atrophy, resulting in failure of the body to provide antibodies during an infection (Hadley 2000); neuronal cell death may occur, and second messenger systems may be disrupted (Wingfield et al. 1997); muscle wasting due to catabolism and hyperglycemia can occur (Hadley 2000). Such actions can critically reduce overall body condition, which may decrease individual survival (Brown et al. 2005, Cabezas et al. 2007). Furthermore, the effects of stress can even extend beyond the individual, as chronic stress suffered by parents may affect offspring both *in utero* and shortly after birth and thus have effects across generations (Nelson 2000, Clinchy et al. 2004).

COMPARATIVE ENDOCRINOLOGY IN STUDIES OF FREE-LIVING ANIMALS

When studying stress in free-living animals, it is important to measure both baseline and maximal (stress-induced, i.e., after activation of the HPA axis) glucocorticoid levels (Romero 2004). Baseline levels often indicate the concentration of stress hormones that an animal maintains on a daily basis. Maximal levels often represent the magnitude of the stress response and reflect sensitivity to stressful events (Wingfield et al. 1997). These two measures have distinct physiological and behavioral correlates (Romero 2004). Chronic stress may affect both baseline and maximal levels, but it may

affect baseline glucocorticoid levels with no concomitant effect on maximal levels (Wingfield et al. 1997), or vice versa (Dunlap and Schall 1995).

At low (baseline) concentrations, glucocorticoids bind to Type I receptors (Romero 2004), and have permissive effects on thyroid hormones, resulting in sustained high metabolism (in homeotherms), and on liver gluconeogenesis and adipose tissue metabolism, helping to maintain high body temperature (in homeotherms) (Hadley 2000). Thus baseline glucocorticoid levels are most likely elevated to support increased physical activity, which may be necessary to survive seasonal environmental fluctuations (Romero 2002). However, a prolonged need for increased activity, such as maintenance of a social status that requires an individual to expend more energy on food and mate acquisition, care of offspring, or aggressive conspecific interactions may result in chronically elevated baseline levels of glucocorticoids (Romero 2002, McEwen and Wingfield 2003, Goymann and Wingfield 2004), which may cause the deleterious consequences described above. A comparison of baseline glucocorticoid levels across seasons or among individuals in a population may give insight into the unobserved challenges of daily living.

At high, stress-induced concentrations, glucocorticoids bind to Type II receptors. The binding of glucocorticoids to these receptors activates many of the stimulatory and inhibitory effects of glucocorticoids explained above (Romero 2004). A healthy individual should respond to an induced acute stressor, since the stress response has evolved to allow individuals to cope and recover from a stressor (Romero 2004, Cabezas et al. 2007). Therefore, lack of a response or a very small response to a stressor may indicate that an animal is in poor health, possibly because it has experienced a stressor

severe or persistent enough that the entire functioning of its HPA axis has been altered (Romero 2004). This may impair the future survival of the individual (Hopkins et al. 1997).

Once it is determined that an animal can exhibit a stress response, one can then examine whether the rise in glucocorticoids is moderate or above a physiological average. While a moderate rise is adaptive and necessary to respond to and recover from a stressful event, a sustained high maximal level may lead to the health defects described above for chronic stress. High maximal levels may reflect long-term adrenal enlargement (Boonstra et al. 1998) and are generally associated with poor physiological condition (Wingfield 1994, Dunlap and Wingfield 1995, Wingfield et al. 1997). Individuals that exhibit a very rapid and steep rise in plasma glucocorticoids in response to acute stressors may unnecessarily suppress physiological functions such as reproduction and immunity (Wingfield et al. 1997).

Just as important as the magnitude of an individual's stress response is the duration of the response, since glucocorticoids mediate their biological effects during the entire time they remain in circulation. An individual may exhibit a moderate peak glucocorticoid level, but may not respond to a stressor efficiently (perhaps due to deficits in negative feedback pathways). Glucocorticoids may then remain elevated for an extended period of time, and this individual may experience a greater overall concentration of glucocorticoids than an individual with a high peak concentration that quickly returns to baseline levels once a stressor is successfully dealt with (Romero 2004).

The context of the stressor should be taken into account when interpreting both baseline and maximal glucocorticoid concentrations in free-living animals. Generally, animals respond less to a potential stressor if it is a predictable challenge or if they have a high degree of control over the situation (Creel et al. 1996, Romero 2004). For example, an unpredictable weather event may provoke a higher stress response than a predictable seasonal change in climate and food availability (Romero 2002). Baseline stress levels may vary between the breeding and non-breeding season, and among stages of the breeding season, because differences in energy demands at this time (Romero 2002). The stress response may be primed during the breeding season to prepare an individual to respond to predictable seasonal challenges (Romero 2002), or it may be temporarily suppressed to allow an individual to attend to more important physiological functions such as reproduction (Moore and Jessop 2003).

Baseline and maximal glucocorticoid concentrations may be obtained in the field by a commonly used capture and restraint protocol (Wingfield 1994, Wingfield et al. 1997). An animal is captured and a baseline sample is taken within three minutes, before glucocorticoids begin to rise in blood (Hadley 2000). The animal is then restrained for approximately one hour, and serial blood samples are collected several times throughout this holding period, to determine the maximal glucocorticoid concentration that the animal is able to produce and to characterize the rate and steepness of the stress response (Wingfield et al. 1997). The animal's response (rise in glucocorticoid levels) to capture and human handling can be used to infer how that individual, and subsequently a population, would cope with other sources of stress (Wingfield 1994).

STUDY SPECIES

The red-cockaded woodpecker (*Picoides borealis*) is an ideal species in which to examine both the hormonal mechanisms of animal behavior and the impact of human activities on stress levels of wildlife. Red-cockaded woodpeckers engage in cooperative breeding, a unique social system in which individuals take on distinct behavioral roles within populations (Brown 1987). They are also an endangered species forced by habitat loss to reside largely in areas of frequent human activity (Conner et al. 2001).

Natural history

The red-cockaded woodpecker is named for the red “cockade” feathers located on each side of the adult male’s head. This small bird, characterized by its black and white barred wing pattern, black head cap, and large white cheek patch, is found in the open, mature pine forests of the southeastern United States. Red-cockaded woodpeckers reside predominantly in longleaf pine (*Pinus palustris*), as well as loblolly (*Pinus taeda*) and shortleaf pine (*Pinus echinata*), forests. In the trunks of these pines, the woodpeckers use their wedge-shaped, chisel-tipped bills to excavate cavities in which to nest and individually roost (Conner et al. 2001). Red-cockaded woodpeckers preferentially select old, live pines for cavity excavation (Walters et al. 1988, Walters 1990). Live pines produce large amounts of resin, used by birds to create a protective series of resin wells around the cavity entrance as a barrier to predators (Walters et al. 1988, Conner et al. 2001). Old trees contain more heartwood in which to build cavities than younger trees, and are more likely to contain red heart fungus (*Phellinus pini*) which decays the heartwood, making cavity excavation a less arduous task (Conner et al. 2001).

Nevertheless cavity excavation may take years (Walters et al. 1988, Conner et al. 2001, Harding and Walters 2002). Birds live in family groups in permanent territories consisting of a cluster of cavity trees (from herein referred to as clusters) and surrounding foraging habitat (Conner et al. 2001). Territory size varies greatly (Walters 1990), usually encompassing approximately 50 to 100 hectares (Walters et al. 2002).

Breeding system

Cooperative breeding is an uncommon reproductive system in which adults assist in the rearing of young that are not their own offspring. This social system has been described in approximately 3% of bird and mammal species and exists in several forms. Multiple group members of one or both sexes may reproduce, and help raise each other's young. More commonly, only one male and one female of a group reproduce, while other group members, often offspring of previous years, remain reproductively inactive and assist in parental duties (Emlen 1997).

Red-cockaded woodpeckers participate in the latter, more common form of cooperative breeding. Family groups consist of one breeding male and one breeding female, along with zero to five non-breeding helpers, who are mostly male offspring of at least one member of the breeding pair. Approximately 30% of family groups have helpers, and of these, most have one helper (Walters et al. 1988). Helpers assist the breeding pair in all aspects of territory defense, cavity construction and maintenance, and care of offspring, including feeding and incubation (Walters 1990, Conner et al. 2001); like the parents, helpers even develop a brood patch (Conner et al. 2001).

Despite a large contribution to parental duties, helpers do not attempt copulation or father offspring, even if the breeding bird of the opposite sex is unrelated to them (Haig et al. 1994). This is an unusual characteristic of red-cockaded woodpeckers. Helper males in other cooperatively breeding bird species often father offspring in both their own family groups and groups other than their own (Whittingham et al. 1997, Li and Brown 2000, Webster et al. 2004). In some species, helper males often attempt to mate but are usually unsuccessful due to un-receptivity of breeding females (Mays et al. 1991) or aggressive mate-guarding from breeding males (Reyer et al. 1986, Vleck et al. 1991). In other species, helper males are reproductively suppressed via low levels of the reproductive hormone testosterone (Reyer et al. 1986, Mays et al. 1991, Schoech et al. 1991, Wingfield et al. 1991). Breeding male red-cockaded woodpeckers, however, do not appear to mate-guard (Walters 1990), and breeding males and helper males maintain similar levels of testosterone throughout the breeding cycle (Khan et al. 2001). Thus the complete reproductive suppression of reproduction in red-cockaded helper males is uncommon, and its mechanisms are not yet understood.

In the spring, each family group builds a single nest, laying up to five (but usually between two and four) eggs (Walters 1990). Most female offspring disperse from their natal territory, traveling through one or more adjacent territories, moving a mean distance of 4.5 kilometers. Of those that survive dispersal (about one third), most become breeders in nearby territories. Some of those that disperse continue to travel from territory to territory even during the breeding season; these individuals are termed floaters. A very small number (about 1% overall and about 8% at the present study's field site) of female fledglings remain in their natal territory. About half of these become the breeder of their

group, while the others may act as helpers while awaiting a breeding opportunity (Walters et al. 1988). Each year about 10% of breeding females switch groups, which may occur to avoid inbreeding, explained below, or females may be driven from their groups by other females competing for a breeding position (Walters et al. 1988).

Survival of male fledglings is approximately 25% higher than in females. Male fledglings may disperse to become either floaters, solitary males (defending their own territory, unaccompanied by a mate), breeders in nearby territories, or to a lesser extent, helpers in other territories. A small number of male fledglings remain and inherit their natal territory as solitary males or breeders. The majority of surviving males, however, remain on their natal territory to act as helpers, and may remain as helpers for several years. When occupation of a breeding position does occur, it happens approximately as often by a helper inheriting the position in his natal territory, as by a helper moving to fill a vacancy in a nearby territory. A helper will only fill a breeding vacancy on a non-natal territory if the new family group contains no natal helpers. If multiple helpers are present, it is the oldest helper who fills an open breeding position. If the current breeding female is this helper's mother, she leaves the territory, often joining a new, nearby family group. Among males, once breeding status is obtained by either of several means, it is almost always held until the bird's death. Unlike in females, very few exceptions to this exist (Walters 1990, Conner et al. 2001).

Endangered species listing

The red-cockaded woodpecker was listed as endangered in 1968 and was granted legal protection in 1973 after the passing of the Endangered Species Act. As many as 1.6

million family groups may have once existed; now less than six thousand exist (USFWS 2003). Logging and fire suppression have been the two main causes of this species' decline. Habitat fragmentation and loss attributable to these actions have left today's existing populations mostly small and isolated from one another by a matrix of unsuitable habitat throughout its range (Walters 1990, Conner et al. 2001).

Logging of pine forests for lumber and clearing for agriculture began in the nineteenth century and continued into the early twentieth century. It has resulted in severe habitat loss, leaving only 3% of what was once a vast forest ecosystem. Remaining habitat is fragmented and degraded. Old pines and those infected with red-heart fungus, those most needed by red-cockaded woodpeckers, were actively removed. Short crop rotations were established, only allowing time for the establishment of young, dense forest (Conner et al. 2001).

Frequent fire favors the formation of pine woodlands and savanna communities, comprised of tall grasses and forbs with a pine overstory. Beginning in the mid-twentieth century, use of prescribed fire was greatly suppressed, as it was believed to negatively impact timber resources and development. When fire management was used, it took place in the nongrowing season, since winter fires are easier to control. These cool fires do not control the growth of hardwood species as spring fires do, and thus the altered fire regimes resulted in development of dense hardwood midstory in once open pine stands. For reasons not yet fully understood, red-cockaded woodpeckers will not remain in pine forests being encroached upon by surrounding hardwoods (Conner et al. 2001).

Currently, red-cockaded woodpecker habitat is protected and actively managed through actions such as prescribed burning and control of hardwood midstory (Conner et

al. 2001, Doresky et al. 2001, USFWS 2003). At the current study site, management plans updated in 1999 also include the placement of metal plate restrictors on red-cockaded woodpecker cavities to prevent larger cavity-nesting species from usurping them, the drilling of artificial cavities in existing clusters and the construction of recruitment clusters, previously unoccupied territories in which cavities are drilled to promote use by red-cockaded woodpeckers (Walters et al. 2005). In 2003 the USFWS designated the current study site as part of one of 12 primary core populations whose survival is considered necessary for persistence of red-cockaded woodpeckers (USFWS 2003). A population goal of 173 active clusters was assigned for this area. From 1996 to 2004 the population increased by 67%, reaching 70 potential breeding groups by 2004. Nonetheless, much more growth is needed to reach the population size set by the USFWS recovery goal. Therefore, populations are still being closely monitored, and foraging habitat and response of the birds to management plans are continually evaluated.

Living on human-occupied lands

The 15 military installations and other federal lands on which red-cockaded woodpeckers still exist are among the few areas that have been protected from recurring deforestation (USFWS 2003). While red-cockaded woodpecker habitat on military lands is protected and actively managed (described above) (Conner et al. 2001, Doresky et al. 2001, USFWS 2003), wildlife is still exposed to a high level of human activity. Training activities at the present study area (described below) include infantry operations, helicopter operations, and firing range operations, with the level of training intensity varying widely across the base. Activities are restricted around red-cockaded woodpecker

clusters. Use of vehicles, bivouacking, establishment of fixed positions, foxhole digging, girdling trees with wire, and burying cable are prohibited within 200 feet of a cavity cluster. Noise (such as artillery firing) and smoke are restricted within 600 feet of a cluster (Walters et al. 2005).

STUDY AREA

Marine Corps Base Camp Lejeune is located near Jacksonville, North Carolina, on the central coast of the state. The red-cockaded woodpecker population on base has been extensively monitored since 1986 (Walters et al. 2005). Camp Lejeune is composed of two parts, the Main Base, an area of 110,000 acres, including 26,000 acres of open water, and the Greater Sandy Run Area, encompassing 41,000 acres (Convery 2002, Walters et al. 2005). The Sandy Run Area does not contain a sufficient amount of suitable woodpecker habitat, and therefore it is only on the Main Base that red-cockaded woodpeckers reside. Woodpecker groups are divided into two subpopulations. Most groups live on the east side of the Main Base, in the Combat Town training area, the G-10 impact area, and the Northeast area. Much dispersal occurs among these areas. A smaller subpopulation resides on the west side of the Main Base, in the Verona Loop training area. There is relatively little dispersal between the subpopulations (Walters et al. 2005). A total of 72 red-cockaded woodpecker groups were present in 2005; 71 were present in 2006.

RESEARCH GOALS AND OBJECTIVES

The research presented here has two main objectives. The first is to examine the role of stress hormones in the cooperative breeding system of red-cockaded woodpeckers. Specifically, I wish to determine whether elevated stress hormone levels are responsible for the observed reproductive suppression of helper males. Additionally I wish to examine whether the number of helpers in a group affects the stress hormone levels of group members, including other helpers. This may provide more insight into the social interactions between group members: increased corticosterone in larger groups may indicate the presence of competition or conflict that has not been previously observed, while reduced baseline corticosterone in larger groups would provide a physiological correlate for the behavioral cooperation observed in this species. I also wish to describe the seasonal pattern of stress hormone levels throughout all stages of the breeding cycle, since few studies thus far have examined how stress hormone levels change across phases of the breeding season. These data will contribute to the limited data describing proximate causes of cooperative breeding behavior, and will provide further insight into this unique social system.

The second objective of this research is to examine whether military training activities are impacting the stress hormone levels of red-cockaded woodpeckers. I wish to determine the effectiveness of the training restrictions currently in place around woodpecker clusters, and will do so by determining whether birds not protected by training restrictions maintain higher concentrations of stress hormones than birds living in training-restricted clusters. However, since the current training restrictions are on a small scale (200-600 feet), I also plan to examine the impacts of military training on a

larger scale, by comparing stress hormone levels of birds residing on larger areas of varying training intensity on base to determine whether areas of lower training intensity contain birds with lower stress levels, and therefore may be more suitable for construction of recruitment clusters. These data will have implications for the management of this federally endangered species.

LITERATURE CITED

- Adams, L., and J. A. Finn. 1972. Behavioral indices of adrenal gland weight in the California ground squirrel. *Ecology* **53**:173-176.
- Boonstra, R. 2004. Coping with changing northern environments: the role of the stress axis in birds and mammals. *Integrative and Comparative Biology* **44**:95-108.
- Boonstra, R., D. Hik, G. R. Singleton, and A. Tinnikov. 1998. The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs* **68**:371-394.
- Brown, C. R., M. Bomberger, S. A. Raouf, L. C. Smith, and J. C. Wingfield. 2005. Effects of endogenous steroid hormone levels on annual survival in cliff swallows. *Ecology* **86**:1034-1046.
- Brown, J. L. 1987. *Helping and Communal Breeding in Birds*. Princeton University Press, Princeton, N. J.
- Cabezas, S., J. Blas, T. A. Marchant, and S. Moreno. 2007. Physiological stress levels predict survival probabilities in wild rabbits. *Hormones and Behavior* **51**:313-320.
- Carr, J. A. 2002. Stress, neuropeptides, and feeding behavior: a comparative perspective. *Integrative and Comparative Biology* **42**:582-590.

- Clinchy, M., L. Zanette, R. Boonstra, J. C. Wingfield, and J. N. M. Smith. 2004.
Balancing food and predator pressure induces chronic stress in songbirds.
Proceedings of the Royal Society of London, Series B **271**:2473.
- Conner, R. N., D. C. Rudolph, and J. R. Walters. 2001. The Red-cockaded Woodpecker:
Surviving in a Fire-maintained Ecosystem. University of Texas Press, Austin.
- Convery, K. M. 2002. Assessing Habitat Quality for the Endangered Red-cockaded
Woodpecker (*Picoides borealis*). M.S. Thesis. Virginia Polytechnic and State
University, Blacksburg, VA.
- Creel, S., N. M. Creel, and S. L. Monfort. 1996. Social stress and dominance. *Nature*
379:212.
- Creel, S., J. E. Fox, A. Hardy, J. Sands, B. Garrott, and R. O. Peterson. 2002.
Snowmobile activity and glucocorticoid stress responses in wolves and elk.
Conservation Biology **16**:809-814.
- DeNardo, D. F., and B. Sinervo. 1994. Effects of steroid hormone interaction on activity
and home range size of male lizards. *Hormones and Behavior* **28**:273-287.
- Doresky, J., K. Morgan, L. Ragsdale, H. Townsend, M. Barron, and M. West. 2001.
Effects of military activity on reproductive success of red-cockaded woodpeckers.
Journal of Field Ornithology **72**:305-311.
- Dunlap, K. D., and J. J. Schall. 1995. Hormonal alterations and reproductive inhibition in
male fence lizards (*Sceloporus occidentalis*) infected with the malarial parasite
Plasmodium mexicanum. *Physiological Zoology* **68**:608-621.
- Dunlap, K. D., and J. C. Wingfield. 1995. External and internal influences on indices of
physiological stress. 1. Seasonal and population variation in adrenocortical

- secretion of free-living lizards, *Sceloporous occidentalis*. Journal of Experimental Zoology **271**:36-46.
- Emlen, S. T. 1997. Predicting family dynamics in social vertebrates. Pages 228-253 in J. R. a. D. Krebs, N. B., editor. Behavior Ecology: An Evolutionary Approach. Blackwell Science, Malden, MA.
- Goymann, W., and J. C. Wingfield. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. Animal Behavior **67**:591-602.
- Hadley, M. E. 2000. Endocrinology, 5th edition. Prentice Hall, Upper Saddle River, NJ.
- Haig, S. M., J. R. Walters, and J. H. Plissner. 1994. Genetic evidence for monogamy in the cooperatively breeding red-cockaded woodpecker. Behavioral Ecology and Sociobiology **34**:295-303.
- Harding, S. R., and J. R. Walters. 2002. Processes regulating the population dynamics of red-cockaded woodpecker cavities. Journal of Wildlife Management **66**:1083-1095.
- Hopkins, W. A., M. T. Mendonca, and J. D. Congdon. 1997. Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. General and Comparative Endocrinology **108**:237-246.
- Khan, M. Z., F. M. A. McNabb, J. R. Walters, and P. J. Sharp. 2001. Patterns of testosterone and prolactin concentrations and reproductive behavior of helpers and breeders in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*). Hormones and Behavior **40**:1-13.

- Li, S., and J. L. Brown. 2000. High frequency of extrapair fertilization in a plural breeding bird, the Mexican jay, revealed by DNA microsatellites. *Animal Behaviour* **60**:867-877.
- Maier, S. F., and L. R. Watkins. 1999. Bidirectional communication between the brain and the immune system: implications for behavior. *Animal Behavior* **57**:741-751.
- Mays, N. A., C. M. Vleck, and J. W. Dawson. 1991. Plasma luteinizing hormone, steroid hormones, behavioral role, and nest stage in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). *Auk* **108**:619-637.
- McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. *Hormones and Behavior* **43**:2-15.
- Moore, I. T., and T. S. Jessop. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Hormones and Behavior* **43**:39-47.
- Nelson, R. J. 2000. *An Introduction to Behavioral Endocrinology*, 2nd edition. Sinauer Associates, Inc, Sunderland, MA.
- Reyer, H., J. P. Dittami, and M. R. Hall. 1986. Avian helpers at the nest: are they psychologically castrated? *Ethology* **71**:216-228.
- Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology* **128**:1-24.
- Romero, L. M. 2004. Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution* **19**:249-255.
- Sapolsky, R. M. 2004. *Why Zebras Don't Get Ulcers: A Guide to Stress, Stress-Related Diseases, and Coping*. W. H. Freeman, New York.

- Schoech, S. J., R. L. Mumme, and M. C. Moore. 1991. Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). *The Condor* **93**:354-364.
- Silverin, B. 1986. Corticosterone-binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period. *General and Comparative Endocrinology* **64**:67-74.
- USFWS. 2003. Recovery plan for the red-cockaded woodpecker (*Picoides borealis*): second revision. U.S. Fish and Wildlife Service, Atlanta, GA.
- Vleck, C. M., N. A. Mays, J. W. Dawson, and A. R. Goldsmith. 1991. Hormonal correlates of parental and helping behavior in cooperatively breeding harris' hawks (*Parabuteo unicinctus*). *Auk* **108**:638-648.
- Walters, J. R. 1990. Red-cockaded woodpeckers: a "primitive" cooperative breeder. *in* P. B. Stacey and W. D. Koenig, editors. *Cooperative Breeding in Birds*. Cambridge University Press, Great Britain.
- Walters, J. R., L. B. Crowder, and J. A. Priddy. 2002. Population viability analysis for red-cockaded woodpeckers using an individual-based model. *Ecological Applications* **12**:249-260.
- Walters, J. R., P. D. Doerr, and J. H. I. Carter. 1988. The cooperative breeding system of the red-cockaded woodpecker. *Ethology* **78**:275-305.
- Walters, J. R., B. Simmons, C. Nycum, and R. Meekins. 2005. The biology and management of the red-cockaded woodpecker on Marine Corps Base Camp Lejeune, North Carolina: Progress toward recovery under the new management plan.

- Wasser, S. K., K. Bevis, G. King, and E. Hanson. 1997. Noninvasive physiological measures of disturbance in the northern spotted owl. *Conservation Biology* **11**:1019-1022.
- Webster, M. S., K. A. Tarvin, E. M. Tuttle, and S. Pruett-Jones. 2004. Reproductive promiscuity in the splendid fairy-wren: effects of group size and auxiliary reproduction. *Behavioral Ecology* **15**:907-915.
- Whittingham, L. A., P. O. Dunn, and R. D. Magrath. 1997. Relatedness, polyandry and extra-group paternity in the cooperatively-breeding white-browed scrubwren (*Sericornis frontalis*). *Behavioral Ecology and Sociobiology* **40**.
- Wingfield, J. C. 1988. Changes in reproductive function of free-living birds in direct response to environmental perturbations. Pages 121-148 *in* M. H. Stetson, editor. *Processing of Environmental Information in Vertebrates*. Springer-Verlag, New York.
- Wingfield, J. C. 1994. Modulation of the adrenocortical response to stress in birds. Pages 520-528 *in* K. G. Davey, R. E. Peter, and S. S. Tobe, editors. *Perspectives in comparative endocrinology*. National Research Council Canada, Ottawa.
- Wingfield, J. C., R. E. Hegner, and D. M. Lewis. 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *Journal of Zoology, London* **225**:43-58.
- Wingfield, J. C., K. Hunt, C. Breuner, K. Dunlap, G. S. Fowler, L. Feed, and J. Lepson. 1997. Environmental stress, field endocrinology, and conservation biology. Pages

95-131 in J. R. Clemmons and R. Buchholz, editors. Behavioral Approaches to Conservation in the Wild. Cambridge University Press, Cambridge.

Wingfield, J. C., and B. Silverin. 1986. Effects of corticosterone on territorial behavior of free-living male song sparrows, *Melospiza melodia*. Hormones and Behavior **20**:405-417.

Chapter 2: Hormonal mechanisms of reproductive suppression in helper males and effect of helper male presence on group members in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*)

ABSTRACT

In the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*), male helpers are subordinate to male breeders, and do not mate with females. We investigated whether high levels of the stress hormone corticosterone suppress reproduction in helper males, whether the number of helper males present in a family group influences corticosterone levels of group members, and whether corticosterone levels changed seasonally. We compared baseline and maximal plasma levels of corticosterone between helper males and breeding males, and among helper males and breeders of both sexes living in groups of different sizes throughout the reproductive cycle. We also measured plasma levels of estrogen, progesterone and testosterone to examine other potential hormonal differences between helpers and breeders. Breeding status did not explain variation in any hormones, therefore our data do not support the hypothesis that helper males are reproductively suppressed via corticosterone or the other hormones investigated. However, the presence of two or more helper males in a group tended to reduce baseline corticosterone in breeding and helper males, but not breeding females, suggesting that helper males reduce the effort that males of both status classes put into parental care. Low corticosterone in helper males may also indicate that when several helpers are present they share aggression received from breeders and provide each other with social support. Seasonally, maximal corticosterone peaked during the nestling provisioning phase for

breeding and helper males, but not breeder females, suggesting that males show an increased response to stressors posing a threat to survival of offspring.

INTRODUCTION

Cooperative breeding has been described in over one hundred mammal and nearly three hundred bird species (Brown 1987). In this unique reproductive strategy, some adults, termed helpers, remain reproductively inactive and help raise the young of breeding pairs to whom they are often, but not always, related. Ultimate causes for delayed reproduction in helpers have been investigated (Brown 1987, Walters et al. 1988, Arnold and Owens 1998, Conner et al. 2001), but studies describing proximate mechanisms controlling these differences in reproductive behavior between breeders and helpers are limited.

Several hypotheses have been advanced to explain the mechanisms of suppression of reproductive behavior in male helpers. Behavioral interference may prevent even reproductively capable helper males from mating. Helpers may be physically blocked from mating by breeding males, via direct aggression or mate-guarding. In pied kingfishers (*Ceryle rudis*), unrelated helpers are aggressively driven from the nest area by male breeders until the female is no longer receptive (Reyer et al. 1986). Dominant Mexican jay (*Aphelocoma ultramarina*) males actively guard their mates (Vleck and Brown 1999).

In many species, helper males exhibit lower plasma levels of testosterone than breeder males (Reyer et al. 1986, Mays et al. 1991, Schoech et al. 1991, Wingfield et al. 1991, Schoech et al. 1996). Reduced testosterone levels may be the result of lack of

stimulation from breeding females (Wingfield and Ramenofsky 1985, Wingfield and Farner 1993, Schoech et al. 1996). Helpers may also exhibit high levels of progesterone (Schoech et al. 1991) or prolactin, two hormones associated with parental care (Hadley 2000) that may reduce testosterone levels or inhibit testosterone production (Nelson 2000). Low levels of testosterone may be accompanied by low body mass (Wingfield et al. 1991), small testes (Reyer et al. 1986, Wingfield et al. 1991, Schoech et al. 1996), or inability to produce sperm (Reyer et al. 1986), rendering helpers physiologically incapable of reproduction, independent of age.

Helper males may also be reproductively suppressed by the production of high levels of glucocorticoid stress hormones. Helpers are subordinate to breeders, and in socially living species, subordinates may have higher levels of the glucocorticoid hormone corticosterone (in birds) or cortisol (in most mammals) if they endure physical or psychological aggression from dominants or if they retain little control over resources such as food and mates (Goymann and Wingfield 2004). Since one of the main actions of glucocorticoids is to mobilize energy stores, subordinates may also produce high levels of these hormones if they must expend more energy than dominants to carry out daily activities such as foraging and territory defense (Goymann and Wingfield 2004). Stress hormones are well known for their suppressive effects on reproduction (Moore and Jessop 2003): they can inhibit the production of reproductive hormones or directly inhibit courtship and mating behaviors without affecting other hormones (Wingfield 1988, Moore and Jessop 2003).

The objective of this study was to investigate hormonal mechanisms that could explain reproductive suppression of subordinate helpers in the red-cockaded woodpecker

(*Picoides borealis*). The cooperatively breeding red-cockaded woodpecker lives in family groups that consist of a breeding pair and zero to five non-breeding helpers, who are mostly male offspring of at least one member of the breeding pair. Helpers assist the breeding pair in territory defense, cavity construction, and care of offspring, including feeding and incubation, but appear to never copulate (Lennartz et al. 1987, Lape 1990, Walters 1990) or father offspring, even if the breeding bird of the opposite sex is unrelated to them (Haig et al. 1994). Male breeders and helpers maintain similar levels of testosterone throughout the breeding cycle. Furthermore, breeders and helpers are of comparable body condition and exhibit similar levels of prolactin over the duration of the breeding cycle (Khan et al. 2001). While males are capable of successful reproduction even in their first year (Lape 1990, Walters 1990), birds may remain helpers until up to eight years of age (Conner et al. 2001), therefore sexual inactivity is not due to age or sexual immaturity (Khan et al. 2001). Breeding males do not appear to engage in mate-guarding to physically block helpers from mating (Walters 1990). Therefore, it is likely that there is likely hormonal mechanism other than testosterone or prolactin inhibiting helper males from courtship and mating.

The primary goal of this study was to determine if high levels of corticosterone are a physiological mechanism mediating the reproductive inhibition of male helpers. Helpers may experience chronic stress for various reasons. Helpers partake in a significant amount of helping duties and they must cope with occasional mild aggression, other than mate guarding, from breeders (Lape 1990). Although red-cockaded woodpeckers live in stable social groups, access to potential mates may be unpredictable for helpers. Helpers maintain spatially separated foraging niches from breeders,

suggestive of dominant birds maintaining greater control of food resources (Rudolph et al. 2007). As secondary objectives, we wished to determine whether the number of helper males present in a family group influences the stress levels of breeders, and whether the presence of additional helpers influences the stress levels of helpers. Since stress levels are often related to energetic demands (Goymann and Wingfield 2004), and since the breeders reduce their parental efforts in the presence of helpers (Khan and Walters 2002), stress levels of breeders may be lower in the presence of helpers. Alternatively, if the presence of helpers results in increased stress levels in male breeders, this could suggest that helpers present a challenge to the maintenance of breeding males' social position. If the presence of additional helpers reduces the stress levels of helpers, this will lend support to the hypothesis that additional subordinate animals provide each other with "social support" (Goymann and Wingfield 2004). Finally, since energetic demands and social interactions may vary through the breeding cycle (Silverin 1998, Romero 2002), we measured corticosterone levels throughout the reproductive cycle.

We compared plasma levels of baseline and stress-induced corticosterone between helper males and breeding males, and among helpers and breeders living in family groups of different sizes, during six phases of the breeding cycle. In addition, we compared testosterone levels between male helpers and male breeders to confirm, in a second population, Khan et al's (2001) previous finding that testosterone levels do not differ between male helpers and breeders. To investigate if there were other hormonal differences between male breeders and helpers, we also compared plasma levels of estrogen and progesterone between male helpers and breeders. Finally, we sampled

breeding females opportunistically, to see if their seasonal hormonal profiles differed from those of males.

METHODS

Study site

Our study was conducted on a population of red-cockaded woodpeckers on Marine Corps Base Camp Lejeune on the central coast of North Carolina, USA. Camp Lejeune is composed of two parts, the Main Base, an area of 110,000 acres, including 26,000 acres of open water, and the Greater Sandy Run Area, encompassing 41,000 acres (Convery 2002, Walters et al. 2005). It is only on the Main Base that red-cockaded woodpeckers reside. Woodpecker groups are divided into two subpopulations. Most groups live on the east side of the Main Base, in the Combat Town training area, the G-10 impact area, and the Northeast area. Much dispersal occurs among these areas. A smaller subpopulation resides on the west side of the Main Base, in the Verona Loop training area. There is relatively little dispersal between the two subpopulations (Walters et al. 2005). Camp Lejeune's red-cockaded woodpecker population has been extensively monitored since 1986 (Walters et al. 2005). Birds are removed from the nest as nestlings and banded with a numbered USFWS leg band and a unique color band combination. Each individual's group membership and social status is determined annually from behavioral observations, using criteria described in Walters et al. (1988).

Study design

We collected blood samples from male breeders, female breeders, and male helpers during the post-breeding phase in 2005 and during the pre-breeding, copulation, egg-laying/incubation, nestling provisioning, fledgling provisioning, and post-breeding phases in 2006 (see Table 1 for dates and sample sizes). Pre-breeding, copulation, egg-laying /incubation, and nestling provisioning were defined following Khan et al. (2001). Fledgling provisioning was defined as the period after young left the nest but were still fed by adults, approximately 4 to 11 weeks post-hatch (Walters 1990). Post-breeding was defined as any time after 11 weeks post-hatch. Birds were sampled approximately equally from the four main areas of base (Combat Town, Northeast, G-10, and Verona Loop) and from family groups ranging in size from two to four birds. Only one family group had three male helpers, and all birds from this group were sampled.

Blood collection

Birds were observed in the evenings to determine the tree cavities in which individuals were roosting. In the mornings, birds were captured by placing a net attached to an expandable pole over roosting cavities before birds arose at dawn. Birds were flushed into the net by kicking the trunk of the tree or banging the trunk with a wooden stake. While adults were laying and incubating eggs, birds were captured off the nest in the same manner, but were captured not only dawn but also throughout midmorning until approximately 1000 hours. Birds were never captured the morning immediately following an evening observation on their territory. Furthermore, birds that were

followed at night sometimes open-roosted in response to being followed, and would therefore be impossible to capture.

Blood was collected in heparinized microhematocrit tubes after puncture of the alar wing vein with a 26-gauge needle. Approximately 50 μ L of blood was collected within three minutes of capture to assess baseline plasma corticosterone levels. Another 200 μ L of blood was collected within ten minutes of capture to measure plasma levels of testosterone, estradiol, and progesterone. Birds were then held in an opaque cloth bag. Thirty minutes after the initial capture another 50 μ L of blood was collected to assess stress-induced plasma corticosterone levels. Blood samples were transferred to microcentrifuge tubes and kept on ice in a small cooler in the field (< 6 hours) until centrifuged to extract plasma. Plasma was kept frozen at -20°C until hormone analyses.

Hormone assays

Plasma collected at the 3- and 30-minute time points was analyzed for levels of corticosterone by direct radioimmunoassay. Plasma volumes of 9-35 μ L were equilibrated overnight with 2000 cpm radiolabeled corticosterone for individual recovery determination. Each sample was then extracted in dichloromethane and the organic phase was removed and dried in a warm water bath under nitrogen gas. The dried extracts were resuspended in buffer for the assay. The samples were divided into duplicate 200- μ L samples, and a 100- μ L aliquot was used to determine individual extraction efficiency. Serial dilutions for the standard curves were performed in triplicate. All samples including serial dilutions were incubated overnight with 100 μ L of antiserum and 100 μ L of radiolabeled steroid. Unbound steroid was separated using dextran-coated charcoal,

and the bound steroid was decanted into scintillation vials. The samples were resuspended in 5 mL of scintillation fluid and counted on a scintillation counter. A cubic spline curve was fitted to the standard curve points, and final steroid levels were calculated from this curve and adjusted based on sample volumes and individual recoveries. Plasma corticosterone levels were measured in four assay runs with intraassay and interassay variations of 18% and 20%, respectively. Limit of detectability was ~ 0.76 ng/mL.

Plasma collected at the 10-minute time point was analyzed for levels of testosterone, estradiol and progesterone. Plasma volumes of 36-164 μ L were used in these assays. The assay was similar to that used for corticosterone except that the hormones were separated from one another and from neutral lipids using column chromatography. The samples were passed through individual celite columns and increasing proportions of ethyl acetate in iso-octane were added to remove each steroid fraction. The purified extracts were dried and resuspended in buffer for the assay. Sample plasma testosterone, estradiol, and progesterone levels were measured in three assay runs. Intraassay variations were 8%, 12% and 25% for testosterone, estradiol and progesterone, respectively. Interassay variations were 14%, 22% and 29% for testosterone, estradiol and progesterone, respectively. Limits of detectability were ~ 0.07 ng/mL, ~ 0.08 ng/mL, and ~ 0.31 ng/mL for testosterone, estradiol and progesterone, respectively.

Statistical analyses

Hormone levels were transformed as necessary to meet assumptions of normality and homogeneity of variance. Females were analyzed separately from males, as they

were captured opportunistically and as our *a priori* interest was to compare hormone levels between male helpers and male breeders. The presence of more than two male helpers in a group is uncommon (Lennartz et al. 1987), and only five birds were captured from one family group with three male helpers. Therefore samples from this group were combined with samples from groups with two male helpers, and were collectively referred to as groups with two or more helpers. All statistical analyses were performed using SAS version 9.1.

We constructed an *a priori* set of 17 candidate models for males and 8 candidate models for females representing multiple hypotheses of effects on hormone levels (Table 2). The same set of models was used for each hormone. The candidate models were linear mixed models that included combinations of five independent variables for males and four independent variables for females: social status (males only), breeding phase, number of helpers in a group, year of data collection and age of individuals. Because of small sample sizes we restricted candidate models to two independent variables and all possible two-way interactions.

We used an information-theoretic approach to compare the fit of our candidate models to our data set. We used the corrected Akaike's Information Criterion (AIC_c), where the correction, symbolized by "c", allows for small sample sizes relative to the number of parameters (Burnham and Anderson 2002). AIC_c values were provided by PROC MIXED in SAS 9.1. The AIC_c values were used to calculate Akaike weights (w_i), which measure a model's relative probability of being the best model for the available data compared to the other candidate models. The model with the highest Akaike weight in each set of models was chosen as the best model; when more than one model had

similar high Akaike weights, all models were considered together (Burnham and Anderson 2002). The best model(s) for each hormone was then analyzed with analysis of variance (ANOVA) and followed by posthoc Tukey pairwise comparisons when appropriate.

RESULTS

Of the candidate models, the model incorporating breeding phase, number of helpers and their interaction (Table 2, models M5 and F1), was the model best supported by the available data for all hormones and for both males and females. This model was very strongly supported, with an Akaike weight of near or equal to 1.00 among the candidate models for every hormone. There was no support for an effect of status for any hormone in the male models, that is, breeder and helper males had similar hormone profiles.

Baseline corticosterone

Males. Model M5 (Akaike weight = 0.9997) accounted for 21% of the variation in baseline plasma corticosterone levels in breeder and helper males. ANOVA indicated that baseline corticosterone did not vary significantly due to breeding phase ($F_{5,73} = 0.88$, $p = 0.50$) (Fig. 1) or the interaction of breeding phase and number of helpers ($F_{10,73} = 0.51$, $p = 0.87$). However, there was a marginally significant trend for variation in baseline levels due to number of helpers ($F_{2,73} = 2.56$, $p = 0.086$), with lower baseline corticosterone when two or three helpers were present than when one helper was present (Tukey's $p = 0.071$) (Fig. 2).

Females. Model F1 (Akaike weight = 1.0000) accounted for 48% of the variation in baseline plasma corticosterone levels in females. However, neither breeding phase ($F_{5,38} = 1.25$, $p = 0.32$), number of helpers ($F_{2,38} = 0.23$, $p = 0.80$), or the interaction of these factors ($F_{7,38} = 1.32$, $p = 0.28$) significantly influenced baseline corticosterone in the ANOVA (Fig. 1).

Maximum corticosterone

Males. Model M5 (Akaike weight = 1.0000) accounted for 34% of the variation in maximal plasma corticosterone in males. Maximal corticosterone did not vary significantly due to number of helpers ($F_{2,76} = 1.51$, $p = 0.23$) or the interaction between breeding phase and number of helpers ($F_{9,76} = 1.05$, $p = 0.41$) in the ANOVA, but varied among breeding phases ($F_{5,76} = 3.20$, $p = 0.013$) (Fig. 1). Maximal corticosterone levels were significantly higher in the nestling provisioning phase than in the egg-laying/incubation, copulation and post-breeding phases (Tukey's $p < 0.05$) for both breeder males and helper males. There was a strong trend for higher maximal corticosterone levels in the nestling provisioning phase than in the pre-breeding phase (Tukey's $p = 0.052$).

Females. Model F1 (Akaike weight = 1.0000) accounted for 35% of the variation in maximal plasma corticosterone in females. However, neither breeding phase ($F_{5,38} = 1.22$, $p = 0.33$), number of helpers ($F_{2,38} = 0.85$, $p = 0.44$), or the interaction of these factors ($F_{7,38} = 0.92$, $p = 0.51$) significantly influenced maximal corticosterone in the ANOVA (Fig. 1).

Testosterone

Males. Model M5 (Akaike weight = 1.0000) accounted for 57% of the variation in plasma testosterone in males. In the ANOVA testosterone did not vary significantly due to number of helpers ($F_{2,79} = 0.33$, $p = 0.72$), but varied due to breeding phase ($F_{5,79} = 6.67$, $p < 0.0001$) (Fig. 3) and the interaction of phase and number of helpers ($F_{10,79} = 2.25$, $p = 0.026$). Plasma testosterone was significantly higher during the copulation phase than during the pre-breeding, egg-laying/incubation, fledgling provisioning, and post-breeding phase (Tukey's $p < 0.05$) for both breeder males and helper males. Plasma testosterone was significantly lower during the post-breeding phase than during the copulation, egg-laying/incubation and nestling provisioning phases (Tukey's $p < 0.05$). In the egg-laying/incubation phase, plasma testosterone was higher in groups with two or more helpers than in groups with one helper (Tukey's $p < 0.05$). Higher plasma testosterone in the copulation phase compared to other phases was only found in groups with one or two or more helpers during this phase (Tukey's $p < 0.05$). Lower plasma testosterone in the post-breeding phase compared to other phases was found only in groups with one or two or more helpers in this phase (Tukey's $p < 0.05$).

Females. Model F1 (Akaike weight = 1.0000) accounted for 69% of the variation in plasma testosterone in females. Breeding phase ($F_{5,39} = 3.15$, $p = 0.024$) (Fig. 3), number of helpers ($F_{2,39} = 5.54$, $p = 0.01$) and the interaction of phase and number of helpers ($F_{10,39} = 2.69$, $p = 0.032$) all significantly influenced plasma testosterone levels in the ANOVA. Plasma testosterone was higher in the copulation and fledgling provisioning phases than in the post-breeding phase (Tukey's $p < 0.05$). The effect of number of helpers disappeared with a Tukey's correction. The interaction of phase and number of

helpers was completely driven by the high mean testosterone level in groups with no helpers during the fledgling provisioning phase, which was significantly higher than that of females in groups of all sizes in several phases (Tukey's $p < 0.05$). The high mean was likely influenced by three individuals with especially high plasma testosterone levels.

Estradiol

Males. Model M5 (Akaike weight = 1.0000) accounted for 23% of the variation in plasma estradiol in males. However, neither breeding phase ($F_{5,78} = 1.20$, $p = 0.32$), number of helpers ($F_{2,78} = 1.01$, $p = 0.37$), or the interaction of these factors ($F_{10,78} = 1.19$, $p = 0.31$) significantly influenced plasma estradiol in the ANOVA (Fig. 2).

Females. Model F1 (Akaike weight = 0.9998) accounted for 50% of the variation in plasma estradiol in females. The number of helpers ($F_{2,39} = 0.16$, $p = 0.86$) and the interaction of breeding phase and number of helpers ($F_{10,39} = 0.77$, $p = 0.62$) did not influence plasma estradiol levels in the ANOVA. There was a strong trend for an effect due to breeding phase ($F_{5,39} = 2.49$, $p = 0.059$) (Fig. 2), with higher estradiol levels in the pre-breeding phase than in the egg-laying/incubation, fledgling provisioning and post-breeding phases (Tukey's $p < 0.05$).

Progesterone

Males. Model M5 (Akaike weight = 0.9969) accounted for 38% of the variation in plasma progesterone in males. Progesterone levels did not vary significantly due to number of helpers in the ANOVA ($F_{2,79} = 0.24$, $p = 0.79$), but did vary due to breeding phase ($F_{5,79} = 3.09$, $p = 0.015$) (Fig. 3). There was a marginally significant interaction of

breeding phase and number of helpers ($F_{10,79} = 1.85$, $p = 0.071$). Plasma progesterone was significantly higher during the nestling provisioning phase than during the fledgling provisioning phase (Tukey's $p < 0.05$) for both breeder males and helper males. Males living in groups with one helper had higher progesterone during the nestling provisioning phase than those in groups with one helper during the egg-laying/incubation phase and those in groups with no helpers during the fledgling provisioning stage (Tukey's $p < 0.05$).

Females. Model F1 (Akaike weight = 0.9980) accounted for 54% of the variation in plasma progesterone in females. The number of helpers ($F_{2,39} = 1.53$, $p = 0.24$) and the interaction of breeding phase and number of helpers ($F_{10,39} = 0.75$, $p = 0.63$) did not significantly influence progesterone levels in the ANOVA. There was a significant effect of breeding phase ($F_{5,39} = 3.12$, $p = 0.025$) (Fig. 3), with higher progesterone in the pre-breeding and copulation phases than in the post-breeding phase (Tukey's $p < 0.05$).

DISCUSSION

Reproductive suppression of helper males

The results of this study do not support our primary hypothesis that increased plasma corticosterone levels are associated with reproductive inactivity in helper male red-cockaded woodpeckers. To our knowledge, studies comparing corticosterone levels between breeders and helpers have been conducted in only three cooperatively-breeding bird species, Florida scrub jays (Schoech et al. 1991, Schoech et al. 1997), white-browed sparrow weavers (Wingfield et al. 1991), and Harris' hawks (Mays et al. 1991). No significant differences in either baseline or maximal (measured in scrub jays only)

corticosterone levels were found to exist between male helpers and breeders of these species. However, we predicted that red-cockaded woodpeckers would contradict these previous results because the reproductive suppression of these other species has been explained by other physiological and behavioral mechanisms that have not been observed in red-cockaded woodpeckers, such as low levels of testosterone (Mays et al. 1991, Schoech et al. 1991, Wingfield et al. 1991, Schoech et al. 1996) and low body mass (Wingfield et al. 1991) in helper males. The results of our current study confirmed Khan et al.'s (2001) finding that red-cockaded woodpecker helpers maintain levels of testosterone similar to those of breeder males. Our finding that helpers also maintain corticosterone levels similar to those of breeders, however, only further supports the emerging, consistent pattern that corticosterone levels do not differ between male breeders and helpers in cooperatively breeding birds. We also measured plasma estradiol and progesterone to examine alternative hypothesis for reproductive inhibition in male helpers, but found no support for an effect of these hormones on reproductive suppression of helper males.

Male red-cockaded helpers and breeders may exhibit no difference in their plasma corticosterone levels for several reasons. Red-cockaded woodpeckers live in stable social groups with established dominance relationships. Commonly, subordinates living in such permanent social groups do not maintain elevated stress hormone levels because the outcome of social interactions are predictable and severe agonistic encounters are uncommon (Creel 2001). Similar baseline corticosterone levels suggest that male helpers and breeders expend equal amounts of energy on caring for young, defending territories,

and excavating cavities; in fact, breeder males may expend slightly more energy on these activities (Khan et al. 2001).

At this point, as suggested by Khan et al. (2001), direct behavioral suppression by either breeder males or females (or both) appears to be the best hypothesis to explain why seemingly reproductively capable helper red-cockaded woodpeckers do not attempt to breed. Although red-cockaded breeder males do not appear to guard females and are not overtly aggressive toward helper males, mate guarding may take forms other than guarding females (Lape 1990). Lape (1990) noted several occasions on which breeder males followed or chased helper males. Also, although helper males have not been observed to attempt copulation with the breeder female (Lennartz et al. 1987, Walters et al. 1988), Lape (1990) noted several occasions in which a helper male approached a breeder female, and the breeder female became noisy and flew away; in one instance a female open-wing displayed. Such social interactions may not be of sufficient frequency or intensity to influence corticosterone levels in helper males, but may be direct enough to prevent mating attempts by helpers.

Effect of presence of helpers on baseline corticosterone

Our results revealed a trend for baseline corticosterone levels of breeding males to change as the number of male helpers in a family group increases. Baseline corticosterone levels are significantly lower in males living with two or three helper males than in those living with one helper male. Reduced baseline corticosterone levels generally correlate with reduced energy expenditure (Goymann and Wingfield 2004). The results of this study therefore provide a physiological correlate to the finding by

Khan and Walters (2002) that breeding males significantly reduced their incubation efforts and their nestling provisioning rates when assisted by helpers. The fact that the reduction in corticosterone did not occur until two or more helpers were present mirrors results by Conner et al. (2004) that a threshold number of helpers may be necessary before a significant increase in fledging success occurs. This threshold of at least two helper males before an increase in reproductive success is attained was also suggested for splendid fairy-wrens (Webster et al. 2004) and stripe-backed wrens (Rabenold 1984). Khan and Walters (2002) found that while breeder females reduced their incubation efforts, and tended to reduce their feeding rates, in the presence of helpers, the reduction in their workload was less than that of breeder males. This may explain why female corticosterone levels were not significantly influenced by the presence of helpers.

Data from all males were combined for analysis, but the effect of group size on helper males' corticosterone levels is actually a test of the effect of the presence or absence of *additional* helper males in a group. The presence of additional helpers tends to decrease the baseline corticosterone levels of all helper males. Since helpers reduce the workload of breeder males, presumably the presence of an additional helper also reduces the workload of other helpers. Also, when two or more helpers are present each helper may receive less aggression (described above) from the breeder male, and helpers may provide each other with social support (Goymann and Wingfield 2004).

The effect of helper presence may be confounded with habitat quality, since groups with more helpers tend to inhabit higher quality territories (Lennartz et al. 1987). However, previous studies that found a reduction in breeder workload (Khan and Walters 2002) and an increase in reproductive success (Lennartz et al. 1987) in the presence of

helpers showed that these effects still held when territory quality was accounted for. Thus, while higher quality habitat in the presence of two helpers may influence baseline corticosterone levels to some extent, the decreased workload of breeders appears to be a direct effect of helper presence, and therefore likely has a strong effect on baseline corticosterone levels.

Seasonal patterns of hormone secretion

The majority of avian species studied thus far seasonally modulate their baseline corticosterone levels, exhibiting a peak during the breeding season. This peak may occur because breeding is an energetically costly time of year since birds are often caring for young, and corticosterone may function to mobilize energy stores (Romero 2002). Our results are not consistent with the typical seasonal pattern of avian baseline corticosterone levels. Neither male nor female red-cockaded woodpeckers in our study displayed seasonal variation in baseline corticosterone. Although caring for young is energetically costly, perhaps other activities, such as cavity excavation and territory defense in which red-cockaded woodpeckers participate year-round are equally costly, such that the net energy expenditure remains relatively steady throughout the year. Furthermore, unlike most bird species, cooperative breeders share parental responsibilities among parents and helpers. This may result in red-cockaded woodpeckers expending relatively less energy individually during the breeding season than do other avian species.

Of the bird species reviewed by Romero (2002), approximately half exhibit their highest maximal corticosterone levels during the breeding season, and approximately half exhibit their lowest levels during this time. Male red-cockaded males exhibit an elevated

stress response during the nestling provisioning phase of the breeding season. This may reflect an increased need to respond quickly to a stressor that poses a potential threat to survival of offspring. Loss of red-cockaded nestlings is often due to conflicts with intruders, either other red-cockaded woodpeckers (Johnston 2006) or other cavity-nesting species, which intensify during the breeding season (Walters 1990). Males may mount a large stress response to guard their nest against intruders. Participating in defensive behavior conflicts with time spent caring for young, but in cooperative breeders, additional adult group members are available to tend to young while others chase intruders. Furthermore, unlike species in which the stress response is muted, which often have short breeding seasons and must invest much energy into a single nesting attempt (Silverin 1998), red-cockaded woodpeckers often re-nest if eggs or nestlings are lost (Walters 1990). Breeder females do not exhibit a seasonal change in their maximal corticosterone levels. Khan (2001) suggested that females are more sensitive to nest stimuli (e.g., vocalizations from nestlings) than are males. Thus they may be less likely than males to respond to stimuli outside the nest. Also, females must not only incubate, but also produce and lay eggs, and therefore have a physiologically higher energy investment in a clutch than do males. Females may be physically unable to mount an increased stress response during the breeding season.

The seasonal pattern of reproductive hormones in red-cockaded woodpeckers matched the typical endocrine profiles of most biparental, monogamously breeding avian species (Wingfield et al. 1990). The increase in male plasma testosterone and female plasma estradiol during the copulation and pre-breeding phases, respectively, correspond with the expression of sexual behaviors during this time of year (Wingfield et al. 1990,

Nelson 2000). Females' increase in plasma testosterone during the pre-breeding and copulation phases may be due to increased female-female competition for breeding positions during this time of year. [Unlike males, female "floaters", who sometimes affiliate with groups to whom they are unrelated, and female helpers may compete with breeding females for breeding status (Walters 1990).] The peak in female testosterone levels during the fledgling provisioning stage is curious; perhaps the three individuals with exceptionally high levels were experiencing some form of competition or aggression at that time. For example, during this time of year females often involve young in territorial boundary disputes with neighboring red-cockaded woodpeckers (Conner et al. 2001). Males exhibited elevated plasma progesterone during the nestling provisioning stage, as progesterone is involved in the promotion of parental behavior (Nelson 2000). That a concomitant peak in plasma progesterone was not seen at this time in females is surprising; however, only two females were captured during the nestling provisioning stage.

Conclusions

The results of this study add to the data describing proximate mechanisms of cooperative breeding, and lend further support to the hypothesis that corticosterone is not a mechanism mediating reproductive suppression of helpers in cooperatively breeding species. Red-cockaded woodpeckers remain unique among other cooperatively breeding bird species because the reproductive suppression of helpers also is unexplained by alternative hormonal differences that have been demonstrated in other species. Although we found no effect of social status on corticosterone levels of male red-cockaded

woodpeckers, this study provided physiological evidence for benefits of social living in this species. Reduced stress in both breeder and helper males in large groups, due to decreased workloads and possibly due to social support, may translate into fitness benefits for male members of these large groups. This study further supports behavioral and demographic evidence that red-cockaded woodpecker breeders benefit from the presence of helpers. To our knowledge this is the first study to examine the physiological effects of helpers on other helpers in addition to their effects on breeders, and the first to examine changes in corticosterone levels due to group size in a cooperatively breeding bird. Further research in these areas may provide interesting insight into the complex physiological effects of social interactions in cooperative breeders.

LITERATURE CITED

- Arnold, K. E., and I. P. F. Owens. 1998. Cooperative breeding in birds: a comparative test of the life history hypothesis. *Proceedings of the Royal Society of London* **265**:739-745.
- Brown, J. L. 1987. *Helping and Communal Breeding in Birds*. Princeton University Press, Princeton, N. J.
- Burnham, K. P., and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edition. Springer Science, New York.
- Conner, R. N., D. C. Rudolph, and J. R. Walters. 2001. *The Red-cockaded Woodpecker: Surviving in a Fire-maintained Ecosystem*. University of Texas Press, Austin.

- Convery, K. M. 2002. Assessing Habitat Quality for the Endangered Red-cockaded Woodpecker (*Picoides borealis*). M.S. Thesis. Virginia Polytechnic and State University, Blacksburg, VA.
- Creel, S. 2001. Social dominance and stress hormones. *Trends in Ecology and Evolution* **16**:491-497.
- Goymann, W., and J. C. Wingfield. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. *Animal Behavior* **67**:591-602.
- Hadley, M. E. 2000. *Endocrinology*, 5th edition. Prentice Hall, Upper Saddle River, NJ.
- Haig, S. M., J. R. Walters, and J. H. Plissner. 1994. Genetic evidence for monogamy in the cooperatively breeding red-cockaded woodpecker. *Behavioral Ecology and Sociobiology* **34**:295-303.
- Johnston, P. A. 2006. Reproductive success and intraspecific competition of the red-cockaded woodpecker. Master of Earth and Environmental Resource Management. University of South Carolina.
- Khan, M. Z., F. M. A. McNabb, J. R. Walters, and P. J. Sharp. 2001. Patterns of testosterone and prolactin concentrations and reproductive behavior of helpers and breeders in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*). *Hormones and Behavior* **40**:1-13.
- Khan, M. Z., and J. R. Walters. 2002. Effects of helpers on breeder survival in the red-cockaded woodpecker (*Picoides borealis*). *Behavioral Ecology and Sociobiology* **51**:336-344.
- Lape, J. J. 1990. *Mate Guarding in the Red-Cockaded Woodpecker*. M. S. North Carolina State University, Raleigh.

- Lennartz, M. R., R. G. Hooper, and R. F. Harlow. 1987. Sociality and cooperative breeding of red-cockaded woodpeckers, *Picoides borealis*. Behavioral Ecology and Sociobiology **20**:77-88.
- Mays, N. A., C. M. Vleck, and J. W. Dawson. 1991. Plasma luteinizing hormone, steroid hormones, behavioral role, and nest stage in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). Auk **108**:619-637.
- Moore, I. T., and T. S. Jessop. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior **43**:39-47.
- Nelson, R. J. 2000. An Introduction to Behavioral Endocrinology, 2nd edition. Sinauer Associates, Inc, Sunderland, MA.
- Rabenold, K. N. 1984. Cooperative enhancement of reproductive success in tropical wren societies. Ecology **65**:871-885.
- Reyer, H., J. P. Dittami, and M. R. Hall. 1986. Avian helpers at the nest: are they psychologically castrated? Ethology **71**:216-228.
- Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative Endocrinology **128**:1-24.
- Rudolph, D. C., R. N. Conner, R. R. Schaefer, and N. E. Koerth. 2007. Red-cockaded woodpecker foraging behavior. The Wilson Journal of Ornithology **119**:170-180.
- Schoech, S. J., R. L. Mumme, and M. C. Moore. 1991. Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). The Condor **93**:354-364.

- Schoech, S. J., R. L. Mumme, and J. C. Wingfield. 1996. Delayed breeding in the cooperatively breeding Florida scrub jay (*Aphelocoma coerulescens*): inhibition or the absence of stimulation? *Behavioral Ecology and Sociobiology* **39**:77-90.
- Schoech, S. J., R. L. Mumme, and J. C. Wingfield. 1997. Corticosterone, reproductive status, and body mass in a cooperative breeder, the Florida scrub-jay (*Aphelocoma coerulescens*). *Physiological Zoology* **70**:68-73.
- Silverin, B. 1998. Stress responses in birds. *Poultry and Avian Biology Reviews* **9**:153-168.
- Vleck, C. M., and J. L. Brown. 1999. Testosterone and social and reproductive behavior in *Aphelocoma* jays. *Animal Behavior* **58**:943-951.
- Walters, J. R. 1990. Red-cockaded woodpeckers: a "primitive" cooperative breeder. *in* P. B. Stacey and W. D. Koenig, editors. *Cooperative Breeding in Birds*. Cambridge University Press, Great Britain.
- Walters, J. R., P. D. Doerr, and J. H. I. Carter. 1988. The cooperative breeding system of the red-cockaded woodpecker. *Ethology* **78**:275-305.
- Walters, J. R., B. Simmons, C. Nycum, and R. Meekins. 2005. The biology and management of the red-cockaded woodpecker on Marine Corps Base Camp Lejeune, North Carolina: Progress toward recovery under the new management plan.
- Webster, M. S., K. A. Tarvin, E. M. Tuttle, and S. Pruett-Jones. 2004. Reproductive promiscuity in the splendid fairy-wren: effects of group size and auxiliary reproduction. *Behavioral Ecology* **15**:907-915.

- Wingfield, J. C. 1988. Changes in reproductive function of free-living birds in direct response to environmental perturbations. Pages 121-148 *in* M. H. Stetson, editor. Processing of Environmental Information in Vertebrates. Springer-Verlag, New York.
- Wingfield, J. C., and D. S. Farner. 1993. Endocrinology of reproduction in wild species. Pages 163-327 *in* D. S. Farner, J. R. King, and K. C. Parkes, editors. Avian Biology. Academic Press, New York.
- Wingfield, J. C., R. E. Hegner, A. M. J. Dufty, and G. F. Ball. 1990. The "Challenge Hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist* **136**:829-846.
- Wingfield, J. C., R. E. Hegner, and D. M. Lewis. 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *Journal of Zoology, London* **225**:43-58.
- Wingfield, J. C., and M. Ramenofsky. 1985. Testosterone and aggressive behavior during the reproductive cycle of male birds. Pages 92-104 *in* R. Gilles and J. Balthazart, editors. Neurobiology. Springer-Verlag, New York.

Table 2.1. Sampling dates and number of days before/after clutch initiation or after hatch date for each breeding phase. Overlap in sampling dates across phases is due to asynchronous breeding across family groups. Sample sizes are listed in the following order: breeding females, breeding males, helper males.

Phase		
Pre-breeding	Dates	21 February – 26 March
	Days before clutch initiation	35-60
	Sample size	6, 7, 2
Copulation	Dates	10 April -10 May
	Days before clutch initiation	3-22
	Sample size	9, 9, 2
Lay/incubation	Dates	6 May – 17 June
	Days after clutch initiation	3-14
	Sample size	9, 13, 8
Nestling provisioning	Dates	14 May – 21 July
	Days after hatching	1-27
	Sample size	2, 7, 4
Fledgling provisioning	Dates	8 June - 20 July
	Days after hatching	29-81
	Sample size	7, 8, 5
Post-breeding (2005)	Dates	26 July – 17 August
	Days after hatching	88-94
	Sample size	3, 3, 3
Post-breeding (2006)	Dates	26 July – 15 August
	Days after hatching	79-95
	Sample size	3, 1, 1

Table 2.2. Candidate models representing multiple hypotheses of effects on baseline corticosterone, stress-induced corticosterone, testosterone, estradiol and progesterone levels in males and females. Values in bold represent the best-fit model for each hormone.

Model number and structure ^a	Baseline corticosterone			Maximal corticosterone			Testosterone			Estradiol			Progesterone		
	AIC _c	Δ AIC _c	W _t	AIC _c	Δ AIC _c	W _t	AIC _c	Δ AIC _c	W _t	AIC _c	Δ AIC _c	W _t	AIC _c	Δ AIC _c	W _t
M1 s * p	220.4	16.6	0.0002484	542.2	35.5	1.9557E-08	231.2	27.5	1.0677E-06	420.5	34.6	3.0670E-08	129.5	12.2	0.002236
M2 s + p	231.8	28.0	8.3130E-07	583.1	76.4	2.570E-17	241.4	37.7	6.5095E-09	445.9	60.0	9.3572E-14	134.6	17.3	0.0001746
M3 s * h	230.1	26.3	1.9449E-06	608.8	102.1	6.7494E-23	267.1	63.4	1.7095E-14	455.5	69.6	7.7011E-16	146.3	29.0	5.0276E-07
M4 s + h	231.0	27.2	1.2403E-06	615.1	108.4	2.8923E-24	270.7	67.0	2.8258E-15	459.9	74.0	8.5331E-17	146.5	29.2	4.5492E-07
M5 p * h	203.8	0.0	0.9997	506.7	0.0	1.0000	203.7	0.0	1.0000	385.9	0.0	1.0000	117.3	0.0	0.9969
M6 p + h	228.3	24.5	4.7838E-06	576.5	69.8	6.9682E-16	240.7	37.0	9.2374E-09	443.7	57.8	2.8112E-13	136.9	19.6	5.5277E-05
M7 s	236.1	32.3	9.6833E-08	625.4	118.7	1.6774E-26	280.8	77.1	1.8111E-17	465.1	79.2	6.3378E-18	143.7	26.4	1.8448E-06
M8 p	233.0	29.2	4.5623E-07	588.2	81.5	2.0068E-18	240.9	37.2	8.3584E-09	449.2	63.3	1.7971E-14	133.4	16.1	0.0003181
M9 h	232.7	28.9	5.3006E-07	619.9	113.2	2.6238E-25	273.4	69.7	7.3255E-16	462.6	76.7	2.2121E-17	144.7	27.4	1.1189E-06
M10 s * a	233.3	29.5	3.9268E-07	616.7	110.0	1.2996E-24	285.7	82.0	1.5629E-18	462.5	76.6	2.3255E-17	149.8	32.5	8.7367E-08
M11 s + a	236.6	22.8	7.5414E-08	621.4	114.7	1.2394E-25	284.8	81.1	2.4511E-18	465.4	79.5	5.4550E-18	147.5	30.2	2.7592E-07
M12 h * a	229.1	25.3	3.2067E-06	609.7	103.0	4.3036E-23	278.8	75.1	4.9231E-17	456.2	70.3	5.4260E-16	154.1	36.8	1.0177E-08
M13 s * y	233.2	29.4	4.1281E-07	608.8	102.1	6.7494E-23	269.0	65.3	6.6113E-15	453.2	67.3	2.4322E-15	143.6	26.3	1.9394E-06
M14 s + y	235.1	31.3	1.5965E-07	616.0	109.3	1.8442E-24	271.5	67.8	1.8942E-15	458.6	72.7	1.6345E-16	144.3	27.0	1.3667E-06
M15 h * y	226.2	22.4	1.3674E-05	595.2	88.5	6.0600E-20	257.4	53.7	2.1837E-12	445.9	60.0	9.3576E-14	142.9	25.6	2.7521E-06
M16 h + y	231.2	27.4	1.1221E-06	610.2	103.5	3.3517E-23	262.8	59.1	1.4676E-13	456.4	70.5	4.9104E-16	145.4	28.1	7.8849E-07
M17 p + y	230.0	26.2	2.0447E-06	580.5	73.8	9.4305E-17	238.6	34.9	2.6397E-08	444.6	58.7	1.7925E-13	133.2	15.9	0.0003516
F1 p * h	78.3	0.0	1.0000	219.0	0.0	1.0000	84.2	0.0	1.0000	82.8	0.0	0.9998	64.7	0.0	0.9980
F2 p + h	101.8	23.5	7.8891E-06	280.7	61.7	3.9996E-14	115.8	31.6	1.3745E-07	109.7	26.9	1.4402E-06	79.0	14.3	0.0007833
F3 p	102.2	23.9	6.4590E-06	294.0	75.0	5.1756E-17	123.7	39.5	2.6466E-09	122.6	39.8	2.2774E-09	80.1	15.4	0.0004519
F4 h	113.7	35.4	2.0558E-08	316.9	97.9	5.5117E-22	138.0	53.8	2.0772E-12	123.8	41.0	1.2499E-09	94.8	30.1	2.9039E-07
F5 h * a	120.8	42.5	5.9051E-10	304.3	85.3	3.0015E-19	142.2	58.0	2.5437E-13	99.6	16.8	0.0002248	96.0	31.3	1.5937E-07
F6 h * y	107.2	28.9	5.3019E-07	291.9	72.9	1.4790E-16	127.7	43.5	3.5818E-10	112.7	29.9	3.2151E-07	82.8	18.1	0.0001172
F7 h + y	112.8	34.5	3.2241E-08	308.4	89.4	3.8640E-20	134.2	50.0	1.3888E-11	120.0	37.2	8.3565E-09	90.3	25.6	2.7552E-06
F8 p + y	100.0	21.7	1.9404E-05	286.5	67.5	2.2007E-15	121.4	37.2	8.3584E-09	117.5	34.7	2.9167E-08	79.3	14.6	0.0006742

^a Models M1-17 represent males and models F1-8 represent females. Model factors included: s = status, p = breeding phase, h =

number of male helpers in group, a = age of individuals, y = year of data collection. * indicates a factorial model and + indicates an additive model.

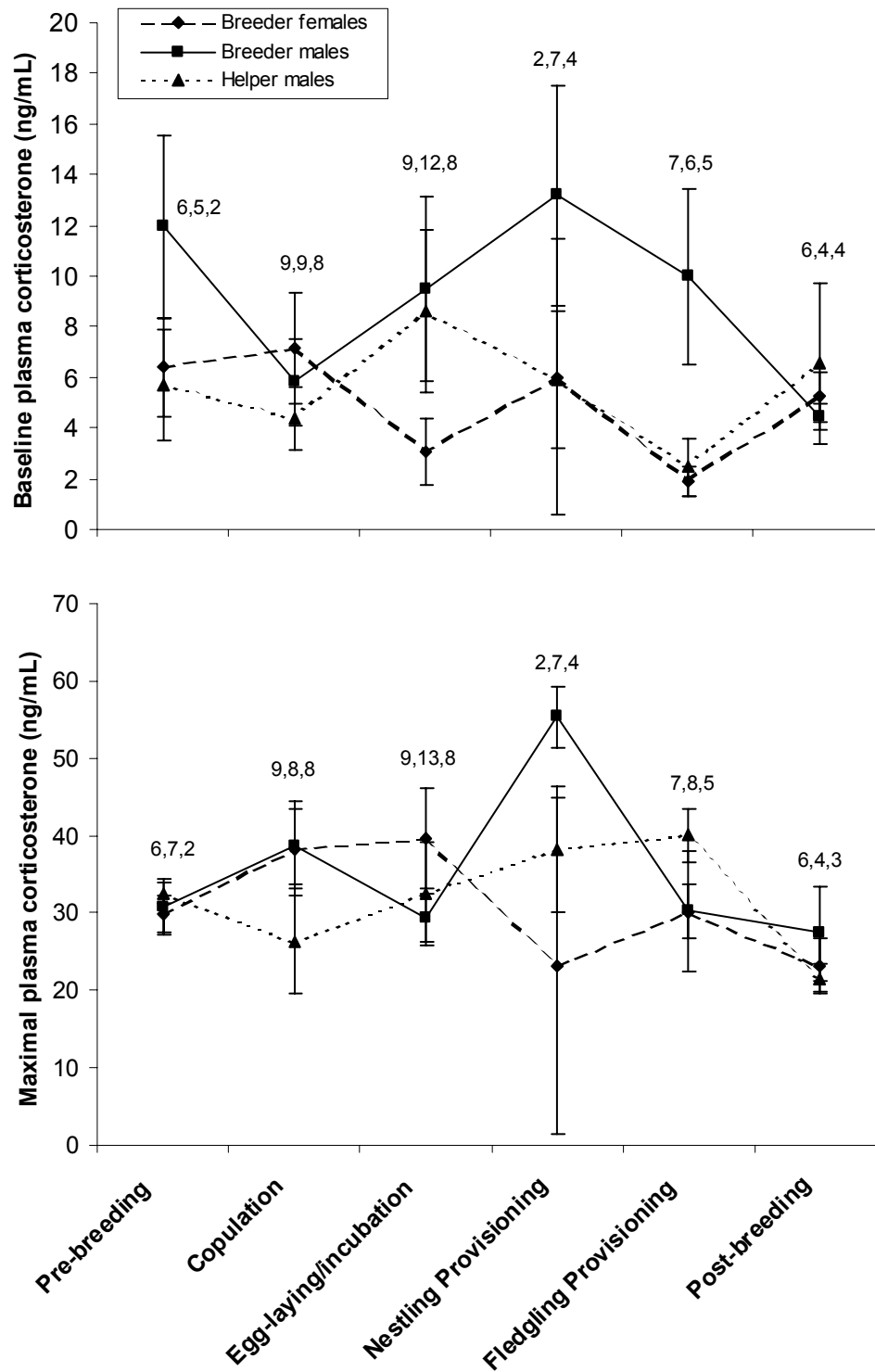


Fig. 2.1. Baseline and maximal plasma corticosterone levels (mean $\pm$ SE) of breeding females, breeding males, and helper males during all phases of the breeding cycle. Sample sizes are included in the plots, listed in the following order: breeding females, breeding males, helper males. Note difference in scale between baseline and maximal corticosterone levels.

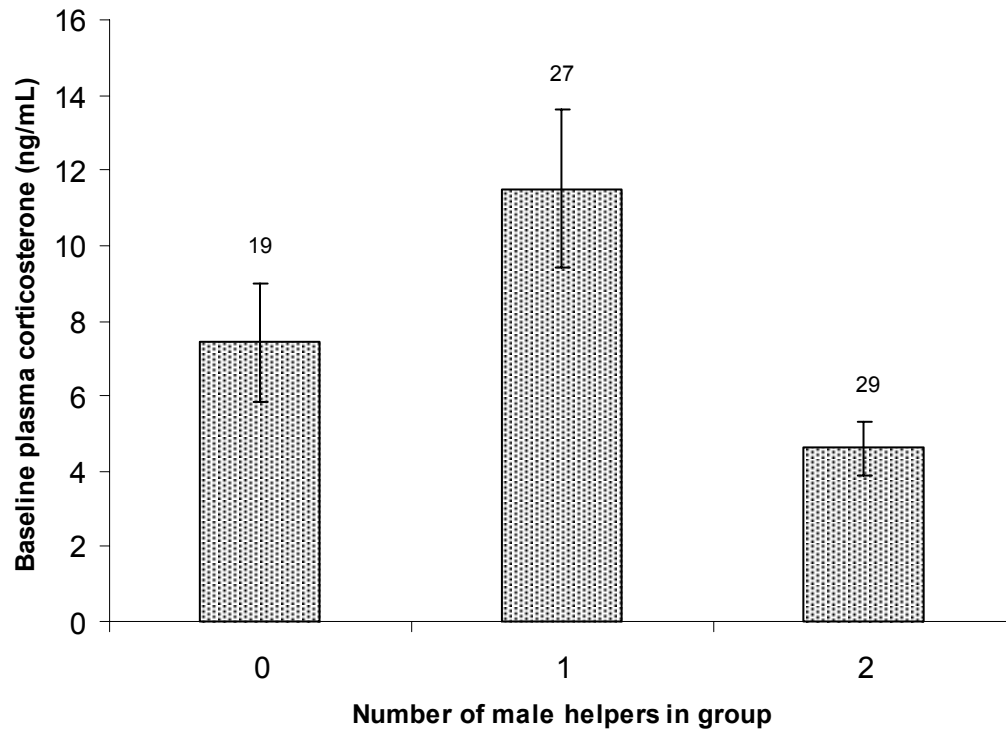


Fig. 2.2. Baseline plasma corticosterone (mean + SE) of breeder males and helper males living in family groups with zero, one, or two to three helper males. Sample sizes are indicated above bars.

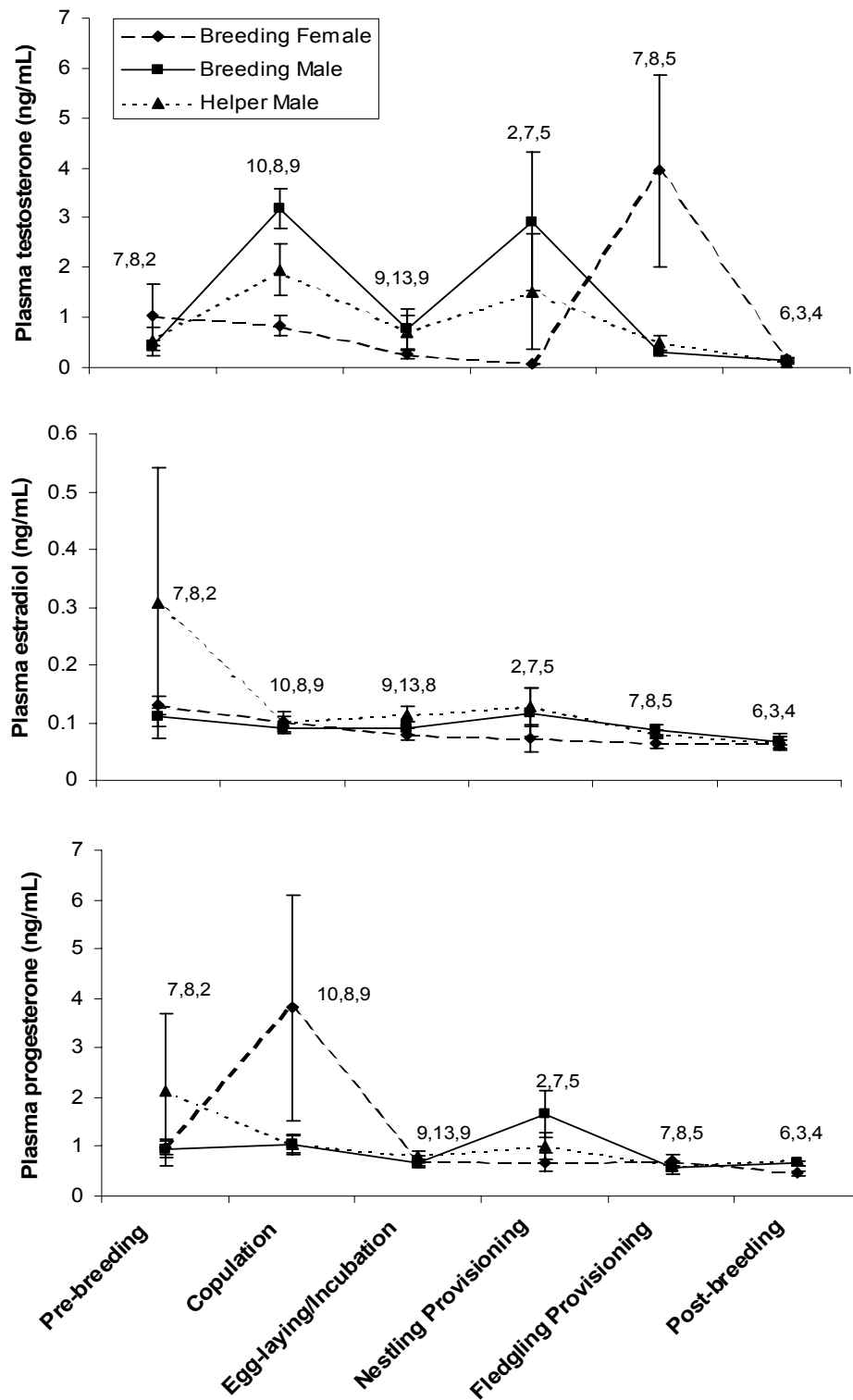


Fig. 2.3. Plasma testosterone, estradiol and progesterone levels (mean $\pm$ SE) of breeding females, breeding males, and helper males during all phases of the breeding cycle. Sample sizes are included in the plots, listed in the following order: breeding females, breeding males, helper males. Note difference in scale for each hormone.

Chapter 3: Impacts of military training activities on stress hormone levels and body mass in the endangered red-cockaded woodpecker

ABSTRACT

Many remaining populations of the endangered red-cockaded woodpecker (*Picoides borealis*) reside on military installations because these federal lands are protected from deforestation. Despite offering suitable habitat, military installations are sources of disturbances such as infantry activity, helicopter flights and firing range operations. We investigated physiological responses of red-cockaded woodpeckers to military training disturbance. We compared plasma levels of the stress hormone corticosterone and body mass between adult birds living in two cluster types, those protected by a buffer zone of training restrictions and those free of such restrictions. We also compared birds from both cluster types across larger regions in which different numbers of training activities are scheduled each year. Males, but not females, had lower baseline corticosterone in clusters with no buffer zone, but stress-induced corticosterone levels and body mass were unaffected by cluster type. The number of scheduled training activities in large regions did not influence any physiological measures in males or females. Our results suggest that either military training activities do not disturb red-cockaded woodpeckers, or that males habituate to chronic disturbance by downregulating baseline corticosterone levels, which may have negative long-term consequences on survival and reproductive fitness. We recommend that current training restrictions should remain in place until further studies continue to investigate the impacts of military training on red-cockaded woodpeckers.

INTRODUCTION

The current rate at which species are becoming endangered is alarming, driven largely by anthropogenic degradation of wildlife habitat (Vitousek et al. 1997). Censusing or observational studies take at least weeks and more typically years to obtain sufficient data to assess the impact of human activities on the health and survival of wildlife (Wingfield et al. 1997). To provide information more quickly, a growing number of studies have used levels of stress hormones, namely glucocorticoids, as a more immediate measure of disturbance (Wasser et al. 1997, Wingfield et al. 1997). In response to challenging or threatening stimuli, the release of glucocorticoids from the hypothalamic-pituitary-adrenal (HPA) axis is a healthy response that mobilizes energy stores and modifies behavior to cope with and recover from the stressor (McEwen and Wingfield 2003). However, chronically elevated glucocorticoid levels as a result of frequent or prolonged environmental disturbance can result in muscle wasting, inhibition of immune response and growth (McEwen and Wingfield 2003) and reduction of individual survival (Brown *et al.* 2005). The effects of stress can even extend beyond the individual, as chronic stress suffered by parents may affect offspring both *in utero* and shortly after birth (Nelson 2000, Clinchy et al. 2004), and may even inhibit reproduction altogether (Hadley 2000), thus having long-term effects across generations. These effects may begin to manifest before they can be detected at a behavioral or demographic level. Therefore measurement of glucocorticoid levels may allow us to predict a population's response to a specific environmental disturbance before the population begins to decline or goes extinct (Wingfield et al. 1997).

Various studies have documented increased glucocorticoid levels in wildlife exposed to human disturbances such as timber management activity (Wasser et al. 1997), road traffic (Millspaugh et al. 2001), snowmobile use (Creel et al. 2002), urbanization (Bonier et al. 2007) and tourism (Garcia Pereira et al. 2006). Other studies have found conflicting results, however. Some species exhibit lower glucocorticoid levels in the presence of poaching, road traffic (Wasser et al. 2004), tourism and public recreation (Romero and Wikelski 2002, Wasser et al. 2004, Walker et al. 2006), loud radio noise or novel objects (Cyr and Romero 2007). In some cases this may indicate that human activities have selected for individuals that are resistant to anthropogenic stress. For other species, individuals may have habituated to their human-modified environments via alteration of the functioning of the HPA axis (Walker et al. 2006). This may impair the animals' ability to respond efficiently to a future, novel stressor (Romero and Wikelski 2002). Furthermore, glucocorticoids are necessary to mobilize energy stores for survival and reproduction (Nelson 2000). If chronically stressed animals maintain reduced glucocorticoid concentrations, they may only be able to allocate energy toward their own survival, and consequently suffer reduced reproductive success (Cyr and Romero 2007).

The objective of the present study was to determine whether military activities influence the glucocorticoid levels of the endangered red-cockaded woodpecker (*Picoides borealis*). Red-cockaded woodpeckers require live, mature pines in which to excavate cavities for roosting and nesting. Logging of pine forests for lumber and agriculture over the past two centuries has resulted in severe habitat loss for this species (Conner et al. 2001). Many remaining populations now reside on military installations, since these federal lands have been protected from deforestation (Conner et al. 2001).

Despite offering large tracts of open forest, military installations are obvious sources of disturbances such as infantry activities, helicopter flights, and firing range operations (USFWS 2003). Thus these military installations provide a good opportunity to investigate the effects of human disturbance on a critically endangered species.

Several studies have investigated the impact of military training activities on red-cockaded woodpecker demography and behavior, but have produced inconclusive results. At Fort Benning Military Installation in Georgia, red-cockaded woodpecker family groups located near areas used for military training and in relatively undisturbed areas had similar numbers of eggs, nestlings and adults, nestling and adult masses, return rates of birds feeding young, and nest failure rates. However, the study also found that groups located in undisturbed areas experienced noise and vibration levels comparable to those experienced by groups in areas of frequent military training (Doresky et al. 2001). A study on Marine Corps Base Camp Lejeune, North Carolina, examined the effects of noise and movement on red-cockaded woodpeckers. Birds nesting near such disturbances showed temporarily reduced feeding rates, and some groups stopped tending to their nests, but overall no consistent differences were found between birds exposed or not exposed to disturbances (Walters 1992). Another study conducted on Camp Lejeune from 2000-2005 compared groups whose cavities trees (termed clusters because each group of birds owns a set of cavity trees, which are often clustered spatially (Walters 1990)) were protected by a buffer zone of training restrictions to those unprotected by such restrictions. Red-cockaded woodpecker groups in buffered clusters had longer incubation bout lengths and higher provisioning rates with increasing group size, but also suffered

higher partial brood loss; however, the effect on brood loss was not consistent across years (Perkins 2006).

Our goal was to investigate physiological responses of red-cockaded woodpeckers to military training disturbance. We compared baseline and acute stress-induced levels of corticosterone, the primary avian glucocorticoid stress hormone, of male and female adult red-cockaded woodpeckers living in clusters with and without training restrictions on Marine Corps Base Camp Lejeune. Baseline levels often indicate the concentration of glucocorticoids that an animal maintains on a daily basis (Wingfield et al. 1997); differences in these levels often reflect differences in energy expenditure (Goymann and Wingfield 2004). Stress-induced levels represent the magnitude of the stress response and can reflect sensitivity to stressful events (Wingfield et al. 1997); differences in these levels may indicate alteration of the functioning of the HPA axis (Romero 2004). We also compared body mass, as a measure of body condition, between birds in each cluster type. Since the current training restrictions are on a small scale, and noise and vibrations may carry long distances (Doresky et al. 2001), we also compared corticosterone levels and body mass among birds residing in larger areas of varying training intensity on base. Determining whether military training activities influence stress levels and/or body condition of red-cockaded woodpeckers will have implications for the management of this federally endangered species, such as deciding which areas of a base are most suitable for the construction of recruitment clusters (previously unoccupied habitats in which tree cavities have been artificially drilled to promote use by red-cockaded woodpeckers).

METHODS

Study site

Marine Corps Base Camp Lejeune is located on the central coast of North Carolina, U.S.A. Camp Lejeune comprises a portion of one of 12 designated primary core red-cockaded woodpecker populations whose survival is considered necessary for persistence of the species (USFWS 2003). Camp Lejeune is composed of two parts, the Main Base, an area of 110,000 acres, including 26,000 acres of open water, and the Greater Sandy Run Area, encompassing 41,000 acres (Convery 2002, Walters et al. 2005). The Main Base is composed largely of pure pine stands, as well as many mixed hardwood-pine stands. The Sandy Run Area does not contain a sufficient amount of suitable woodpecker habitat, and therefore it is only on the Main Base that red-cockaded woodpeckers reside. Woodpecker groups are divided into two subpopulations. Most groups live on the east side of the Main Base, in the Combat Town training area, the G-10 impact area, and the Northeast area. Much dispersal occurs among these areas (Walters et al. 2005). A smaller subpopulation resides on the west side of the Main Base, in the Verona Loop training area (Fig. 1). There is relatively little dispersal between the subpopulations (Walters et al. 2005). A total of 72 red-cockaded woodpecker groups were present in 2005; 71 were present in 2006.

On Camp Lejeune, red-cockaded woodpecker management includes restriction of training activities near woodpecker clusters. Activities such as vehicular traffic, establishment of fixed positions, foxhole digging, girdling trees with wire, and burying cable are prohibited within 200 feet of active cavity trees. A white band is painted around

trees 200 feet from the cavity trees to signify this buffer to military personnel. Smoke and noise, such as artillery firing, are restricted within 600 feet of active cavity trees.

Study design

We attempted to minimize seasonal variation in physiological measurements by sampling only during non-breeding periods. Pre-breeding samples were collected from 21 February - 26 March 2006, up to 35 days before clutch initiation. Post-breeding samples were collected from 26 July - 17 August 2005 and 26 July - 15 August 2006, beginning at 50 days post-hatch. Samples were collected from the clusters used in the 2000-2005 study on Camp Lejeune (Perkins 2006). As part of that study, forty-three clusters were divided into two groups, one group subject to the usual training restrictions (marked clusters) and one group relieved of the usual training restrictions (unmarked clusters). Both treatment groups included three types of clusters: recently constructed recruitment clusters, new clusters recently created by red-cockaded woodpeckers, and older existing naturally created clusters. Within each type, clusters were paired, one each from the marked and unmarked groups, by similar habitat (Perkins 2006). Recruitment clusters assigned to the unmarked group were not painted with the usual white bands signifying the training buffer. The white buffer bands of other clusters assigned to the unmarked group were covered with black paint. Both marked and unmarked clusters exist in moderate proximity to each other within larger areas (approximately three quarters to two square miles in size) in which varying numbers of annual training activities (from none up to nearly one thousand) are scheduled. Both cluster types were sampled within several of these larger areas. We used the number of training activities scheduled in each area from

September 2003 to September 2004 in our analyses. This was the most recent data on scheduled training activities that we were able to obtain, and the location and number of annual training activities remains relatively constant from year to year. Clusters in two areas were only compared for cluster type but not number of activities: data on the number of scheduled training activities was not available for one area in the Verona Loop; no activities are scheduled for the center of the G10 impact area, but while vehicular and foot traffic is absent, much noise and vibration is present. Therefore we did not feel it was accurate to categorize it as a low-activity area by assigning it with zero scheduled activities. Samples were collected approximately equally from each of the four main regions of base (Combat Town, G10, Northeast, and Verona) (Fig. 1).

Physiological measurements

Birds were observed in the evenings to determine the tree cavities in which individuals were roosting. In the mornings, birds were captured by placing a net attached to an expandable pole over roosting cavities before birds arose at dawn. Birds were flushed into the net by kicking the trunk of the tree or banging the trunk with a wooden stake. Five individuals in 2005 and one individual in 2006 were captured in the evening several minutes after entering their roosting cavities. Blood was collected in heparinized microhematocrit tubes after puncture of the wing vein with a 26-gauge needle. Approximately 50 μ L blood was collected within three minutes of capture to assess baseline corticosterone levels. Birds were then weighed with a spring scale (2006 only), after which they were held in an opaque cloth bag. Thirty minutes after the initial capture time another 50 μ L blood was collected to assess stress-induced corticosterone levels.

Birds captured in the evening were released immediately after obtaining a body mass measurement so that they could return to their roosting cavities before it became very dark; thus a stress-induced corticosterone sample was not obtained from these birds. Blood samples were transferred to microcentrifuge tubes and kept on ice in a small cooler in the field (< 6 hours) until centrifuged to extract plasma. Plasma was kept frozen at -20°C until hormone analyses.

Hormone assays

Plasma was analyzed for concentrations of corticosterone by direct radioimmunoassay. Plasma volumes of 9-35 µL were equilibrated overnight with 2000 cpm radiolabeled corticosterone for individual recovery determination. Each sample was then extracted in dichloromethane and the dichloromethane phase was removed and dried in a warm water bath under nitrogen gas. The dried extracts were resuspended in buffer for the assay. The samples were divided into duplicate 200-µL samples, and a 100-µL aliquot was used to determine individual recoveries. Serial dilutions for the standard curves were performed in triplicate. All samples including serial dilutions were incubated overnight with 100 µL of antiserum and 100 µL of radiolabeled steroid. Unbound steroid was separated using dextran-coated charcoal, and the bound steroid was decanted into scintillation vials. The samples were resuspended in 5 mL of scintillation fluid and counted on a scintillation counter. A cubic spline curve was fitted to the standard curve points, and final steroid concentrations were calculated from this curve and adjusted based on sample volumes and individual recoveries. Plasma corticosterone concentrations

were measured in four assay runs with intraassay and interassay variations of 18% and 20%, respectively. Limit of detectability was ~ 0.76 ng/mL .

Statistical analyses

As a preliminary analysis we tested for an effect of the time of capture (morning or evening) on baseline corticosterone levels. Four of the six evening samples were obtained from males in unmarked clusters during the post-breeding period of 2005. We performed an analysis of variance (ANOVA) on samples collected from males in unmarked clusters during this time to control for effects of cluster type (marked or unmarked), sex, and time period (pre-or post-breeding). There was no effect of time of capture on baseline corticosterone levels in these samples ($F_{1,9} = 0.07$, $p = 0.79$), thus we felt confident that the time of capture did not influence corticosterone levels and combined morning and evening samples for subsequent analyses.

We constructed an *a priori* set of 34 candidate models representing multiple hypotheses of effects on corticosterone levels and body mass in males and females (Table 1). The same set of models was used for baseline corticosterone, acute-stress induced corticosterone and body mass. The candidate models were linear mixed models that included combinations of five independent variables: the two treatment variables, cluster type and number of training activities, as well as the biological variables time period, sex, and group size (two, three or four birds), which are all known to influence corticosterone levels in red-cockaded woodpeckers (Chapter 2). Because of small sample sizes we restricted candidate models to three independent variables and all possible two-way interactions. Some models included biological variables only, to account for the

possibility that neither cluster type nor number of training activities influences corticosterone levels or body mass.

We used an information-theoretic approach to compare the fit of our candidate models to our data set. We used the corrected Akaike's Information Criterion (AIC_c) to correct for a small sample size relative to the number of parameters. AIC_c values were provided by PROC MIXED in SAS 9.1. The AIC_c values were used to calculate Akaike weights (w_i), which measure a model's relative probability of being the best model for the available data compared to the other candidate models. The model with the highest Akaike weight in each set of models was chosen as the best model; when more than one model had similar high Akaike weights, all models were considered together (Burnham and Anderson 2002). The best model(s) for each dependent variable was then analyzed with ANOVA and followed by posthoc Tukey pairwise comparisons when appropriate. All statistical analyses were performed using SAS version 9.1. Hormone concentrations were natural log-transformed as necessary to meet assumptions of normality and homogeneity of variance.

We also examined males and females separately for each dependent variable. Body mass differed significantly between males and females in the best fit model. Although neither baseline nor stress-induced corticosterone differed significantly due to sex, one or more of the best fit models for each hormone measurement included sex as a variable. Furthermore, several studies have shown that males and females have different hormonal responses to human disturbance (Wasser et al. 1997, Wasser et al. 2004, Bonier et al. 2007). We constructed another set of candidate models for each dependent variable for each sex, using time period, group size, and cluster type as independent variables

(Table 2, models M1-9 for males and F1-9 for females). We omitted number of training activities as an independent variable, since this variable only appeared in models with low Akaike weights in candidate models combining males and females. Separating our data set by sex obviously reduced sample size, and we therefore restricted these models to two independent variables. Candidate models were again compared using AIC_c and the best fit models were analyzed with ANOVA and followed by posthoc Tukey pairwise comparisons when appropriate.

RESULTS

Of the 43 clusters selected for comparison, 14 were either not occupied or were occupied by a solitary male, and therefore were not included in the study. We successfully captured birds from 23 of the remaining 29 clusters.

Baseline corticosterone

When males and females were combined, the model containing the variables cluster type, time period, group size, and their two-way interactions (model MF3) best described variation in baseline corticosterone levels (Akaike weight = 0.6170, $R^2 = 0.37$). The model containing the variables cluster type, time period, sex and their two-way interactions (model MF2) also described variation in baseline corticosterone moderately well (Akaike weight = 0.2270, $R^2 = 0.36$). In both models, the effect of cluster type was marginally significant when subjected to ANOVA ($F_{1,52} = 3.53$, $p = 0.067$ for MF3 and $F_{1,52} = 7.81$, $p = 0.085$ for MF2). Time period and the interaction of time period and cluster type significantly influenced baseline corticosterone in both models ($F_{1,52} = 7.37$,

$p = 0.0095$ and $F_{3,52} = 9.62$, $p = 0.0034$, respectively for MF3; $F_{1,52} = 6.73$, $p = 0.013$ and $F_{1,52} = 3.10$, $p = 0.0076$, respectively, for MF2). Baseline corticosterone levels were higher during the pre-breeding period and in marked clusters (Fig. 2). The change in baseline corticosterone between time periods was significant only for birds in unmarked clusters (Tukey's $p = 0.0009$ for MF3 and $p = 0.0015$ for MF2; $p = 0.96$ for MF3 and $p = 1.00$ for birds in marked clusters), and the difference in baseline corticosterone due to cluster type was significant only in the post-breeding period (Tukey's $p = 0.01$ for MF3 and $p = 0.02$ for MF2; $p = 0.79$ for MF3 and $p = 0.88$ for MF 2 for the pre-breeding period). Although group size was a variable in the best candidate model, it did not significantly affect baseline corticosterone in the ANOVA ($F_{2,52} = 0.93$, $p = 0.40$). The interactions of cluster type and group size ($F_{5,52} = 0.33$, $p = 0.72$) and time period and group size ($F_{5,52} = 0.73$, $p = 0.49$) also were not significant. Neither sex ($F_{1,52} = 0.08$, $p = 0.78$), the interaction of cluster type and sex ($F_{3,52} = 2.08$, $p = 0.16$), nor the interaction of time period and sex ($F_{3,52} = 0.50$, $p = 0.48$) significantly affected baseline corticosterone in the ANOVA. Models containing the number of training activities as an independent variable all had very low Akaike weights and therefore we conclude that this variable did not contribute to variation in baseline corticosterone levels (Fig. 3).

When males were analyzed separately, the three best-fit models approximately equally described variation in baseline corticosterone: the model containing time period, group size and their interaction (M5, Akaike weight = 0.3206, $R^2 = 0.40$); the model containing cluster type, time period, and their interaction (M1, Akaike weight = 0.3049, $R^2 = 0.42$); and the model containing cluster type, group size and their interaction (M3, Akaike weight = 0.3049, $R^2 = 0.39$). Cluster type significantly affected baseline

corticosterone in the ANOVA for both models in which it was a variable ($F_{1,31} = 6.14$, $p = 0.02$ for M1 and $F_{1,31} = 8.26$, $p = 0.008$ for M3), with levels being higher in marked clusters (Fig. 2). Time period also significantly affected baseline corticosterone in both models in which it was a variable ($F_{1,31} = 10.02$, $p = 0.0039$ for M5 and $F_{1,31} = 7.14$, $p = 0.012$ for M1), with levels being higher in the pre-breeding period (Fig. 2). The interaction of cluster type and time period was marginally significant ($F_{3,31} = 3.16$, $p = 0.086$). Differences in baseline corticosterone between time periods were significant only for males in unmarked clusters (Tukey's $p = 0.0053$; $p = 0.94$ for marked clusters), and differences in baseline corticosterone between cluster types were significant only in the post-breeding time period (Tukey's $p = 0.037$; $p = 0.95$ for the pre-breeding period). Group size did not significantly affect baseline corticosterone in either model in which it was a variable ($F_{2,31} = 0.34$, $p = 0.71$ for M5 and $F_{2,31} = 0.39$, $p = 0.68$ for M3), and the interaction of group size and time period was not significant either ($F_{5,31} = 2.55$, $p = 0.097$). However, the interaction of group size and cluster type was significant ($F_{5,31} = 3.40$, $p = 0.049$). This complicated interaction was driven especially by high baseline corticosterone levels in groups of four in unmarked clusters (Fig. 4).

When females were analyzed separately, the model containing time period, group size and their interaction (F5) best described the variation in baseline corticosterone (Akaike weight = 0.9504, $R^2 = 0.29$). However, in the resulting ANOVA neither of these variables (time period: $F_{1,20} = 1.73$, $p = 0.21$; group size: $F_{2,20} = 1.75$, $p = 0.21$) or their interaction ($F_{5,20} = 0.49$, $p = 0.62$) significantly affected baseline corticosterone in females. Models containing cluster type as an independent variable all had very low

Akaike weights and therefore we conclude that this variable did not contribute to variation in baseline corticosterone levels in females.

Stress-induced corticosterone

When males and females were combined, the three best-fit models approximately equally described variation in stress-induced corticosterone levels: the model containing cluster type and time period (MF18, Akaike weight = 0.2193, $R^2 = 0.14$); the model containing cluster type, time period and their interaction (MF17, Akaike weight = 0.1545, $R^2 = 0.15$); and the model containing time period, sex and their interaction (MF32, Akaike weight = 0.1470, $R^2 = 0.14$). In all models, only time period significantly affected stress-induced corticosterone in the ANOVA ($F_{1,48} = 7.17$, $p = 0.01$ for MF18; $F_{1,48} = 7.19$, $p = 0.01$ for MF17; $F_{1,48} = 6.56$, $p = 0.014$ MF32), with levels being higher in the pre-breeding period (Fig. 2). Effects of cluster type ($F_{1,48} = 0.07$, $p = 0.79$ for MF18 and $F_{1,48} = 0.01$, $p = 0.91$ for MF17), the interaction of cluster type and time period ($F_{3,48} = 0.44$, $p = 0.51$), sex ($F_{1,48} = 0.32$, $p = 0.57$), and the interaction of sex and time period ($F_{3,48} = 0.13$, $p = 0.72$) were not significant. Models containing group size and/or number of training activities had low Akaike weights, thus we conclude that these variables did not contribute to variation in stress-induced corticosterone levels (Fig. 3).

When males were analyzed separately, the four best-fit models approximately equally described variation in stress-induced corticosterone levels: the model containing time period (M8, Akaike weight = 0.3571, $R^2 = 0.19$); the model containing cluster type, group size and their interaction (M3, Akaike weight = 0.2060, $R^2 = 0.30$); the model containing cluster type, time period and their interaction (M1, Akaike weight = 0.1526,

$R^2 = 0.23$); and the model containing cluster type and time period (M2, Akaike weight = 0.1314, $R^2 = 0.19$). Cluster type did not significantly affect stress-induced corticosterone in the accompanying ANOVA for any models in which it was a variable ($F_{1,29} = 0.29$, $p = 0.60$ for M3; $F_{1,29} = 0.56$, $p = 0.46$ for M1; $F_{1,29} = 0.23$, $p = 0.63$ for M2). The interaction of cluster type and group size was significant in the overall model ($F_{1,29} = 4.68$, $p = 0.019$), although no individual comparisons remained significant at the 0.05 level after Tukey's post-hoc adjustment. The interaction of cluster type and time period was not significant ($F_{3,29} = 1.10$, $p = 0.30$). Time period significantly affected stress-induced corticosterone in all models in which it was a variable ($F_{1,29} = 6.46$, $p = 0.017$ for M8; $F_{1,29} = 5.82$, $p = 0.023$ for M1; $F_{1,29} = 6.23$, $p = 0.019$ for M2), with levels being higher in the pre-breeding period (Fig. 2). Group size did not significantly affect stress-induced corticosterone ($F_{1,29} = 0.23$, $p = 0.80$).

When females were analyzed separately, the two best-fit models approximately equally described variation in stress-induced corticosterone levels: the model containing cluster type, group size and their interaction (F3, Akaike weight = 0.5006, $R^2 = 0.19$); and the model containing time period, group size, and their interaction (F5, Akaike weight = 0.4762, $R^2 = 0.23$). However, none of these effects or interactions were significant in the ANOVAs (time period: $F_{1,18} = 0.11$, $p = 0.75$; cluster type: $F_{1,18} = 0.15$, $p = 0.70$; group size: $F_{2,18} = 0.61$, $p = 0.56$ for F3 and $F_{2,18} = 1.58$, $p = 0.24$ for F5; the interaction of cluster type and group size: $F_{5,18} = 0.01$, $p = 0.93$; the interaction of time period and group size: $F_{5,18} = 0.89$, $p = 0.36$) (Fig. 2).

Body mass

When males and females were combined the model containing time period, sex, group size and their two-way interactions best described variation in body mass (MF31, Akaike weight = 0.9927, $R^2 = 0.47$). The effect of sex ($F_{1,42} = 9.94$, $p = 0.0034$) and the interaction of sex and group size ($F_{5,42} = 6.74$, $p = 0.0034$) were significant in the ANOVA (Fig. 5). Males had a higher average body mass than females. Effects of group size ($F_{2,42} = 1.40$, $p = 0.26$) and time period ($F_{1,42} = 0.01$, $p = 0.94$), the interaction of time period and sex ($F_{3,42} = 2.54$, $p = 0.12$), and the interaction of time period and group size ($F_{5,42} = 0.01$, $p = 0.94$) were not significant. Models containing cluster type and/or number of activities as independent variables had very low Akaike weights, thus we conclude that these variables did not contribute to variation in body mass.

When males were analyzed separately, the two best-fit models approximately equally described variation in body mass: the model containing cluster type, group size and their interaction (M3, Akaike weight = 0.4815, $R^2 = 0.42$); and the model containing time period, group size and their interaction (M5, Akaike weight = 0.4145, $R^2 = 0.46$). Group size significantly affected body mass in the ANOVA for both models ($F_{2,24} = 5.27$, $p = 0.015$ for M3 and $F_{2,24} = 8.56$, $p = 0.0021$ for M5). Males in groups of four had significantly higher body mass than males in groups of two (Tukey's $p = 0.025$) or three (Tukey's $p = 0.03$) (Fig. 5). Effects of cluster type ($F_{1,24} = 0.02$, $p = 0.89$) and time period ($F_{1,24} = 0.88$, $p = 0.36$), the interaction of cluster type and group size ($F_{5,24} = 0.17$, $p = 0.84$) and the interaction of time period and group size ($F_{5,24} = 0.38$, $p = 0.55$) were not significant.

When females were analyzed separately, the model containing cluster type, group size and their interaction (F3) best described variation in body mass (Akaike weight =

0.6692, $R^2 = 0.43$). The model containing time period, group size and their interaction (F5) also described variation in body mass moderately well (Akaike weight = 0.2721, $R^2 = 0.34$). Group size was marginally significant in the ANOVA for model F3 ($F_{2,17} = 3.60$, $p = 0.057$), with females in groups of three having higher body mass than females in groups of two (Tukey's $p = 0.023$), but was not significant in the ANOVA FOR model F5 ($F_{2,17} = 2.72$, $p = 0.10$) (Fig. 5). Effects of cluster type ($F_{2,17} = 0.15$, $p = 0.71$) and time period ($F_{2,17} = 0.33$, $p = 0.58$), the interaction of cluster type and group size ($F_{5,17} = 2.83$, $p = 0.12$) and the interaction of time period and group size ($F_{5,17} = 0.19$, $p = 0.67$) were not significant.

DISCUSSION

Although no one model best described variation in most comparisons of candidate models, the major findings held across several best-fit models for each dependent variable. Thus we feel confident that our results accurately represent the impacts of military training activities on corticosterone levels and body mass in red-cockaded woodpeckers.

Effects of cluster type on corticosterone levels and body mass

It is difficult to discern whether male red-cockaded woodpeckers in marked clusters exhibit high baseline corticosterone levels, or whether those in unmarked clusters exhibit low corticosterone levels. However, since all clusters on Camp Lejeune have been subject to training restrictions for several decades, we consider marked clusters the

control against which to compare physiological measures. Therefore, we discuss the results in terms of reduced corticosterone in unmarked clusters.

If our above assumption is correct, then our results contradict those of several previous studies demonstrating that human disturbance elevates baseline glucocorticoid levels in wild free-living animals (Wasser et al. 1997, Millspaugh et al. 2001, Creel et al. 2002, Garcia Pereira et al. 2006, Bonier et al. 2007). However, if the presence of military activity were not perceived as stressful by red-cockaded woodpeckers, then one would predict that baseline corticosterone levels between birds in marked and unmarked clusters would be similar. Instead, birds living in unmarked clusters exhibit lower baseline corticosterone levels. This suggests that red-cockaded woodpeckers are experiencing long-term consequences of human disturbance (Romero 2004). That the presence of a marked buffer on a cluster, but not the number of activities scheduled in a larger area, explained variation in corticosterone levels suggests that it is the close presence of humans, and not noise and vibrations per se, that affect stress in red-cockaded woodpeckers. Alternatively, the noise and vibrations produced during training activities may carry far enough to be similar in all clusters, as found on Fort Benning Military Installation in Georgia (Doresky et al. 2001).

There are at least two possible explanations for the pattern of baseline corticosterone that we observed in red-cockaded woodpeckers: military activity may have selected for birds resistant to disturbance, or birds may have habituated to disturbance. It is unlikely that military activity within clusters has selected for birds resistant to human presence. Training restriction buffers have only been removed since 2000. From 2000 to 2005 a similar percentage of females in marked and unmarked clusters have moved off

their territories, and virtually no males have moved (Perkins 2006). In the present study only four individuals moved to different territories, one male and one female from each cluster type. This indicates that differences in corticosterone levels between marked and unmarked clusters probably exist because of physiological changes in individual corticosterone secretions, and not because individuals intrinsically sensitive to disturbance have been driven from unmarked clusters.

Baseline corticosterone levels may be lower in unmarked clusters because red-cockaded woodpecker living in these clusters have habituated to human disturbance. Adult European starlings (Cyr and Romero 2007) and Magellanic penguins (Fowler 1999) exhibit reduced baseline corticosterone in response to chronic stress. Birds may habituate to chronic stressors by downregulating baseline corticosterone (Cyr and Romero 2007), which may help prevent potentially adverse effects of sustained high corticosterone, such as protein catabolism or suppression of immunity or reproductive function (Wingfield et al. 1997, Hadley 2000). Habituation may occur most easily if stressors are intense and frequent (Walker et al. 2006), which likely describes the type of disturbances to which red-cockaded woodpeckers in unmarked cluster are exposed.

The effect of cluster type on baseline corticosterone was much stronger in the post-breeding period. This is likely because training activities on Camp Lejeune seem to be concentrated during the summer (personal observation). It is also possible that if baseline corticosterone levels are already very low at this time due to molt (see below), then differences due to military training disturbances may be magnified. The interaction of season and stressor was suggested by Romero et al. (2000), who found that adverse weather strongly affected baseline corticosterone in Lapland longspurs during molt, but

not during the breeding period. The difference in corticosterone levels due to cluster type is also more pronounced in small groups. Perhaps birds in small groups perceive military activity as a more intense stressor because there are fewer group members to assist in responding to human intruders.

It is not apparent at this time whether differences in baseline corticosterone levels will ultimately have positive or negative (or neutral) impacts on the fitness of individual red-cockaded woodpeckers or the dynamics of the population. While low baseline corticosterone is generally believed to be associated with better health (Wingfield et al. 1997), it may also have negative consequences. Very low baseline corticosterone levels could reduce survival of red-cockaded woodpeckers in the long-term by hindering their ability to deal with other environmental stressors. Cliff swallows with very low endogenous levels of baseline corticosterone had a reduced likelihood of annual survival compared to those with intermediate corticosterone levels, presumably because they were unable to reallocate energy toward dealing with ectoparasitism (Brown et al. 2005). Although military training activities do not appear to cause direct mortality in red-cockaded woodpeckers (Perkins 2006), effects on long-term survival may not yet be apparent since red-cockaded woodpeckers are long-lived. (Individuals may live up to 18 years.)

Low baseline corticosterone levels of adults may also indirectly impact offspring. Individuals with reduced glucocorticoid levels may allocate their energy to supporting their own survival, at the expense of caring for young (Cyr and Romero 2007). Red-cockaded woodpeckers within each sex have similar body mass in both cluster types, but the low corticosterone levels of males living in unmarked clusters correlate with the

reduced feeding rates and shorter incubation bouts found in unmarked clusters in Perkins' previous study on Camp Lejeune (Perkins 2006). This observation, coupled with a trend for lower overall productivity in unmarked clusters (Walters et al. 2005), may have negative consequences for the population if it continues. Even if reduced feeding and shorter incubation are not drastic enough to decrease survival of nestlings, they may result in less healthy offspring, the effects of which may not be seen immediately. However, the correlation of corticosterone levels in the present study and the parental activities in Perkins' study should be interpreted with caution, since the present study was conducted during non-breeding periods. Birds showing hormonal responses to disturbance during the non-breeding season may not have similar responses during the breeding season (Romero et al. 2000), and stress-induced corticosterone levels in red-cockaded woodpeckers are higher during the nestling provisioning phase of the breeding season than during non-breeding periods (Chapter 2).

Although we used marked clusters as a basis for comparison of physiological measures, we cannot preclude the possibility that baseline corticosterone levels in marked clusters are higher than average, which may be detrimental to the health of individuals (Wingfield et al. 1997). Marked clusters are likely still subject to some human disturbances, such as noise and vibrations due to training activities outside the clusters or traffic on nearby roads open to non-military use. Military personnel may also accidentally enter marked clusters occasionally, despite management regulations. If this is true, then marked clusters may more realistically be considered areas of moderate disturbance, rather than undisturbed areas. Magellanic penguins in moderately disturbed areas vocalize in response to human presence more often than those in either isolated areas or

areas of high human disturbance (Fowler 1999). Physiological responses to stress may at first change in areas of moderate disturbance (such as marked clusters), and habituation that occurs in areas of heavy disturbance (such as unmarked clusters) may mirror the physiological measures in truly isolated areas.

Red-cockaded woodpeckers did not show a change in stress-induced corticosterone levels between marked and unmarked clusters, in contrast to adults of several species which show reduced stress-induced corticosterone levels in response to human disturbance, reflecting alteration of HPA axis (Fowler 1999, Romero and Wikelski 2002, Walker et al. 2006). This may indicate that military training does not cause stress in this species. However, since birds in marked and unmarked clusters express different baseline, but not stress-induced, corticosterone levels, it is also possible that partial habituation has taken place. Complete habituation may require an extended period of time (Fowler 1999), and may have only begun to take place since training restriction buffers have been removed on Camp Lejeune. Again, it is also possible that marked clusters are more similar to areas of moderate disturbance, and that stress-induced corticosterone levels of birds in both marked and unmarked clusters are lower than those of birds in truly undisturbed habitats. The fact that the pattern of change in baseline and stress-induced corticosterone levels are not parallel is not incompatible with this hypothesis. Fowler (1999) found that both baseline and stress-induced corticosterone levels in Magellanic penguins differ across areas of no, moderate, and frequent human presence, but that baseline and stress-induced levels changed at different rates.

The fact that cluster type did not affect body mass in either males or females matches the results of various studies reporting no change in body condition due to

human disturbance, even when baseline or stress-induced corticosterone levels are affected (Fowler 1999, Romero et al. 2000, Walker et al. 2006, Cyr and Romero 2007). The lack of differences in corticosterone levels due to cluster type in females mirrors results in several other species exposed to human disturbance (Wasser et al. 1997, Wasser et al. 2004, Bonier et al. 2007).

Seasonal effects on corticosterone levels

The strong overall effect of time period on male corticosterone levels was not surprising. Birds captured in the post-breeding period likely had low corticosterone levels because they were molting or preparing to molt at this time. [Red-cockaded woodpeckers molt between early July and early October (Jackson 1994)]. Many avian species exhibit both reduced baseline corticosterone levels and a suppressed hormonal stress responses during molt, likely to increase the energy supply available for feather growth and avoid glucocorticoids' catabolic effects on protein (Silverin 1998, Romero 2002). At the same time, birds captured during the pre-breeding period may exhibit higher baseline corticosterone levels to support increased activity in preparation for breeding. Red-cockaded woodpeckers begin to exhibit courtship behaviors as early as late February or March, and soon after begin to prepare protective resin wells around the cavity that will be used for nesting (Conner et al. 2001). In addition, foraging conditions may be poor during these months (Skorupa and McFarlane 1976), and birds may have to increase the energy expended on foraging.

Recommendations for management and future study

It remains uncertain how the effects reported in this and Perkins' (2006) previous study will ultimately affect the demography of red-cockaded woodpeckers on Camp Lejeune. Along with continued population monitoring, we recommend that physiological measures be examined during the breeding time period and in truly undisturbed habitat, such as the nearby Croatan National Forest. Physiological measurements should also be taken from nestling and fledglings. Young birds may have different physiological responses to stress than adult birds (Mullner et al. 2004, Walker et al. 2005, Wada et al. 2007), and the effects of exposure to stress at a young age may have long-lasting consequences (Welberg and Seckl 2001, Bhatnagar et al. 2005, Spencer and Verhulst 2007).

We believe that any changes to current training restrictions on Camp Lejeune should be conservative. It is essential for Camp Lejeune to create many more recruitment clusters to meet the recovery goal established by the United States Fish and Wildlife Service. However, it is important to maintain the multiple-use of military installations, as the habitat that is maintained for training realism is the same habitat necessary for the persistence of many species. On Fort Benning, red-cockaded woodpecker groups with no previous exposure to military activity experienced nest failure when exposed to a large-scale training exercise, despite previous fledging success. This suggests that even if red-cockaded woodpeckers are able to habituate to military presence, they may be affected by novel disturbances (Doresky et al. 2001). Therefore, we recommend that current training restrictions should remain in place, but that a proportion of each year's new recruitment clusters could be created without training buffers until further studies more accurately

depict the impacts of military training on red-cockaded woodpeckers. Establishing recruitment clusters without training restrictions would most likely be more beneficial than postponing the establishment of new clusters until a location can be found where training restrictions will least interfere with military training. This strategy will likely carry some flaws—for example, our study found that smaller groups seem to be more affected by differences in training restrictions, and groups in new recruitment clusters tend to be smaller—but at present it may be the most harmonious solution for both red-cockaded woodpeckers and military personnel.

LITERATURE CITED

- Bhatnagar, S., T. M. Lee, and C. Vining. 2005. Prenatal stress differentially affects habituation of corticosterone responses to repeated stress in adult male and female rats. *Hormones and Behavior* **47**:430-438.
- Bonier, F., P. R. Martin, K. S. Sheldon, J. P. Jensen, S. L. Foltz, and J. C. Wingfield. 2007. Sex-specific consequences of life in the city. *Behavioral Ecology* **18**:121-129.
- Brown, C. R., M. Bomberger, S. A. Raouf, L. C. Smith, and J. C. Wingfield. 2005. Effects of endogenous steroid hormone levels on annual survival in cliff swallows. *Ecology* **86**:1034-1046.
- Burnham, K. P., and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edition. Springer Science, New York.

- Clinchy, M., L. Zanette, R. Boonstra, J. C. Wingfield, and J. N. M. Smith. 2004.
Balancing food and predator pressure induces chronic stress in songbirds.
Proceedings of the Royal Society of London, Series B **271**:2473.
- Conner, R. N., D. C. Rudolph, and J. R. Walters. 2001. The Red-cockaded Woodpecker:
Surviving in a Fire-maintained Ecosystem. University of Texas Press, Austin.
- Convery, K. M. 2002. Assessing Habitat Quality for the Endangered Red-cockaded
Woodpecker (*Picoides borealis*). M.S. Thesis. Virginia Polytechnic and State
University, Blacksburg, VA.
- Creel, S., J. E. Fox, A. Hardy, J. Sands, B. Garrott, and R. O. Peterson. 2002.
Snowmobile activity and glucocorticoid stress responses in wolves and elk.
Conservation Biology **16**:809-814.
- Cyr, N. E., and L. M. Romero. 2007. Chronic stress in free-living European starlings
reduces corticosterone concentrations and reproductive success. General and
Comparative Endocrinology **151**:82-89.
- Doresky, J., K. Morgan, L. Ragsdale, H. Townsend, M. Barron, and M. West. 2001.
Effects of military activity on reproductive success of red-cockaded woodpeckers.
Journal of Field Ornithology **72**:305-311.
- Fowler, G. S. 1999. Behavioral and hormonal responses of Magellanic penguins
(*Spheniscus magellanicus*) to tourism and nest site visitation. Biological
Conservation **90**:143-149.
- Garcia Pereira, R. J., J. M. Barbanti Duarte, and J. A. Negrao. 2006. Effects of
environmental conditions, human activity, reproduction, antler cycle and grouping

- on fecal glucocorticoids of free-ranging Pampas deer stags (*Ozotoceros bezoarticus bezoarticus*). *Hormones and Behavior* **49**:114-122.
- Goymann, W., and J. C. Wingfield. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. *Animal Behavior* **67**:591-602.
- Hadley, M. E. 2000. *Endocrinology*, 5th edition. Prentice Hall, Upper Saddle River, NJ.
- Jackson, J. A. 1994. Red-cockaded Woodpecker (*Picoides borealis*). *in*. The Academy of Natural Sciences
- The American Ornithologists' Union, Philadelphia, Washington D. C.
- McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. *Hormones and Behavior* **43**:2-15.
- Millspaugh, J. J., R. J. Woods, K. E. Hunt, K. J. Raedeke, G. C. Brundige, B. E. Washburn, and S. K. Wasser. 2001. Fecal glucocorticoid assays and the physiological stress response in elk. *Wildlife Society Bulletin* **29**:899-907.
- Mullner, A., K. E. Linsenmair, and M. Wikelski. 2004. Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (*Opisthocomus hoazin*). *Biological Conservation* **118**:549-558.
- Nelson, R. J. 2000. *An Introduction to Behavioral Endocrinology*, 2nd edition. Sinauer Associates, Inc, Sunderland, MA.
- Perkins, J. L. 2006. Effects of military training activity on red-cockaded woodpecker demography and behavior and new territory formation in the cooperatively breeding red-cockaded woodpecker. Virginia Polytechnic and State University, Blacksburg, VA.

- Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology* **128**:1-24.
- Romero, L. M. 2004. Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution* **19**:249-255.
- Romero, L. M., J. M. Reed, and J. C. Wingfield. 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. *General and Comparative Endocrinology* **118**:113-122.
- Romero, L. M., and M. Wikelski. 2002. Exposure to tourism reduces stress-induced corticosterone levels in Galapagos marine iguanas. *Biological Conservation* **108**:371-374.
- Silverin, B. 1998. Stress responses in birds. *Poultry and Avian Biology Reviews* **9**:153-168.
- Skorupa, J. P., and R. W. McFarlane. 1976. Seasonal variation in foraging territory of Red-cockaded Woodpeckers. *Wilson Bulletin* **88**:662-665.
- Spencer, K. A., and S. Verhulst. 2007. Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (*Taeniopygia guttata*). *Hormones and Behavior* **51**:273-280.
- USFWS. 2003. Recovery plan for the red-cockaded woodpecker (*Picoides borealis*): second revision. U.S. Fish and Wildlife Service, Atlanta, GA.
- Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human Domination of Earth's Ecosystems. *Science* **277**:494-499.
- Wada, H., T. P. Hahn, and C. W. Breuner. 2007. Development of stress reactivity in white-crowned sparrow nestlings: Total corticosterone response increases with

- age, while free corticosterone response remains low. *General and Comparative Endocrinology* **150**:405-413.
- Walker, B. G., P. D. Boersma, and J. C. Wingfield. 2005. Physiological and behavioral differences in Magellanic penguin chicks in undisturbed and tourist-visited locations of a colony. *Conservation Biology* **19**:1571-1577.
- Walker, B. G., P. D. Boersma, and J. C. Wingfield. 2006. Habituation of adult Magellanic Penguins to human visitation as expressed through behavior and corticosterone secretion. *Conservation Biology* **20**:146-154.
- Walters, J. R. 1990. Red-cockaded woodpeckers: a "primitive" cooperative breeder. *in* P. B. Stacey and W. D. Koenig, editors. *Cooperative Breeding in Birds*. Cambridge University Press, Great Britain.
- Walters, J. R. 1992. Evaluation of possible disturbance effects on Red-cockaded Woodpeckers in the mechanized infantry training area, Camp Lejeune (unpublished report, submitted to DOD, Marine Corps Base Camp Lejeune).
- Walters, J. R., B. Simmons, C. Nycum, and R. Meekins. 2005. The biology and management of the red-cockaded woodpecker on Marine Corps Base Camp Lejeune, North Carolina: Progress toward recovery under the new management plan.
- Wasser, S. K., K. Bevis, G. King, and E. Hanson. 1997. Noninvasive physiological measures of disturbance in the northern spotted owl. *Conservation Biology* **11**:1019-1022.
- Wasser, S. K., B. Davenport, and E. R. Ramage. 2004. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the

- Yellowhead Ecosystem, Alberta, Canada. *Canadian Journal of Zoology* **82**:475-492.
- Welberg, L. A. M., and J. R. Seckl. 2001. Prenatal stress, glucocorticoids and the programming of the brain. *Journal of Neuroendocrinology* **13**:113-128.
- Wingfield, J. C., K. Hunt, C. Breuner, K. Dunlap, G. S. Fowler, L. Feed, and J. Lepson. 1997. Environmental stress, field endocrinology, and conservation biology. Pages 95-131 *in* J. R. Clemmons and R. Buchholz, editors. *Behavioral Approaches to Conservation in the Wild*. Cambridge University Press, Cambridge.

Table 3.1. Candidate models representing multiple hypotheses of effects on baseline corticosterone levels, stress-induced corticosterone levels and body mass in males and females combined. Values in bold represent the best-fit model(s) for each dependent variable.

Model number and structure ^a	Baseline corticosterone			Stress-induced corticosterone			Body mass		
	AIC _c	ΔAIC _c	Wt	AIC _c	ΔAIC _c	Wt	AIC _c	ΔAIC _c	Wt
MF1 c*a*p	157.0	43.3	2.4424E-10	90.9	38.5	9.569E-10	226.5	61.3	4.8495E-14
MF2 c*p*s	119.9	2.0	0.2270	56.1	3.7	0.03448	182.5	17.3	1.7385E-04
MF3 c*p*g	117.9	0.0	0.6170	54.8	2.4	0.06605	178.9	13.7	1.0517E-03
MF4 a*p*s	162.4	44.5	1.3404E-10	89.0	36.6	2.4744E-09	219.9	54.7	1.3148E-12
MF5 a*p*g	170.9	53.0	1.9120E-12	101.0	48.6	6.1333E-12	225.2	60.0	9.2894E-14
MF6 c*a*s	170.7	52.8	2.1131E-12	96.2	43.8	6.7609E-11	214.4	49.2	2.0567E-11
MF7 c*a*g	175.6	57.7	1.8235E-13	103.0	50.6	2.2563E-12	218.1	52.9	3.2339E-12
MF8 a*s*g	177.3	59.4	7.7939E-14	103.1	50.7	2.1463E-12	208.9	43.7	3.2173E-10
MF9 c+a+p	141.8	26.0	1.3947E-06	66.3	13.9	2.1024E-04	214.1	48.9	2.3896E-11
MF10 c+p+s	131.3	13.5	7.2245E-04	54.6	2.2	0.07300	196.3	31.1	1.7520E-07
MF11 c+p+g	131.4	15.5	2.6578E-04	56.0	3.6	0.03625	194.1	28.9	5.2634E-07
MF12 a+p+s	143.9	26.1	1.3267E-06	66.0	13.6	2.4426E-04	208.5	43.3	3.9296E-10
MF13 a+p+g	144.0	31.6	8.4810E-08	67.5	15.1	1.1538E-04	206.3	41.1	1.1805E-09
MF14 c+a+s	150.5	33.1	4.0061E-08	73.4	21.0	6.0389E-06	209.7	44.5	2.1566E-10
MF15 c+a+g	150.3	32.6	5.1440E-08	74.6	22.2	3.3142E-06	206.2	41.0	1.2410E-09
MF16 a+s+g	152.7	39.1	1.9945E-09	74.5	22.1	3.4842E-06	203.2	38.0	5.5619E-09
MF17 c*p	121.7	3.8	0.09229	53.1	0.7	0.1545	199.4	34.2	3.7187E-08
MF18 c+p	130.3	12.4	1.2522E-03	52.4	0.0	0.2193	203.5	38.3	4.7872E-09
MF19 c*a	161.2	44.5	1.3404E-10	83.4	31.0	4.0690E-08	225.7	60.5	7.2346E-14
MF20 c+a	149.5	32.4	5.6850E-08	71.2	18.8	1.8142E-05	217.4	52.2	4.5892E-12
MF21 c*s	135.7	19.3	3.9753E-05	58.5	6.1	0.01039	192.5	27.3	1.1714E-06
MF22 c+s	138.1	23.9	3.9855E-06	58.9	6.5	8.5034E-03	198.9	33.7	4.7749E-08
MF23 c*g	134.9	17.8	8.4155E-05	55.8	3.4	0.04006	188.4	23.2	9.0993E-06
MF24 c+g	137.9	20.2	2.5347E-05	60.6	8.2	3.6345E-03	196.0	30.8	2.0356E-07
MF25 a*s	163.5	45.6	7.7334E-11	81.6	29.2	1.0008E-07	217.3	52.1	4.8245E-12
MF26 s+s	152.0	34.8	1.7123E-08	71.1	18.7	1.9072E-05	211.2	46.0	1.0187E-10
MF27 a*g	171.3	53.4	1.5654E-12	91.9	39.5	5.8041E-10	222.7	57.5	3.2423E-13
MF28 a+h	151.9	34.1	2.4298E-08	72.4	20.0	9.9565E-06	208.2	43.0	4.5655E-10
MF29 c	137.2	20.0	2.8013E-05	56.7	4.3	0.02555	206.7	41.5	9.6652E-10
MF30 a	1510	34.0	2.5544E-08	68.8	16.4	6.0233E-05	218.9	53.7	2.1678E-12
MF31 p*s*g	122.6	4.7	0.05884	54.4	2.0	0.08068	165.2	0.0	0.9927
MF32 p+s+g	130.9	13.0	9.2765E-04	53.2	0.8	0.1470	194.2	29.0	5.0067E-07
MF33 p*g	130.1	12.2	1.3839E-03	54.6	2.2	0.0730	192.4	27.2	1.2315E-06
MF34 s*g	133.4	17.0	1.2554E-04	56.6	4.2	0.02686	175.4	10.2	6.0523E-03

^a Model factors included: c = cluster type, a = number of scheduled training activities, p = time period, s = sex and g = group size. * indicates a factorial model and + indicates an additive model.

Table 3.2. Candidate models representing multiple hypotheses of effects on baseline corticosterone levels, stress-induced corticosterone levels and body mass in males and females analyzed separately.

Model number and structure ^a	Baseline corticosterone			Stress-induced corticosterone			Body mass		
	AIC _c	ΔAIC _c	Wt	AIC _c	ΔAIC _c	Wt	AIC _c	ΔAIC _c	Wt
M1 c*p	72.5	0.1	0.3049	29.5	1.7	0.1526	96.3	11.2	1.7806E-03
M2 c+p	76.2	3.8	0.04795	29.8	2.0	0.1314	102.4	17.3	8.4328E-05
M3 c*g	72.5	0.1	0.3049	28.9	1.1	0.2060	85.1	0.0	0.4815
M4 c+g	81.1	8.7	4.1375E-03	36.5	8.7	4.6092E-03	91.3	6.2	0.02169
M5 p*g	72.4	0.0	0.3206	31.5	3.7	0.05615	85.4	0.3	0.4145
M6 p+g	79.4	7.0	9.6803E-03	31.4	3.6	0.05903	89.0	3.9	0.06851
M7 c	84.2	11.8	8.7818E-04	33.4	5.6	0.02172	104.4	19.3	3.1023E-05
M8 p	80.2	7.8	6.4889E-03	27.8	0.0	0.3571	105.5	20.4	1.7898E-05
M9 g	85.6	13.2	4.3609E-04	34.7	6.9	0.01134	92.5	7.4	0.01191
F1 c*p	103.7	10.9	4.0833E-03	136.1	9.0	5.56124E-03	79.7	9.5	5.7893E-03
F2 c+p	110.6	17.8	1.2963E-04	143.9	16.8	1.1257E-04	83.7	13.5	7.8350E-04
F3 c*g	99.3	6.5	0.03685	127.1	0.0	0.5006	70.2	0.0	0.6692
F4 c+g	104.1	11.3	3.3431E-03	135.0	7.9	9.6390E-03	77.2	7.0	0.02021
F5 p*g	92.8	0.0	0.9504	127.2	0.1	0.4761	72.0	1.8	0.2721
F6 p+g	103.4	10.6	4.7441E-03	135.5	8.4	7.50689E-03	76.6	6.4	0.02728
F7 c	115.1	22.3	1.3663E-05	149.9	22.8	5.6045E-06	87.3	17.1	1.2951E-04
F8 p	113.8	21.0	2.6171E-05	151.6	24.5	2.3955E-06	87.9	17.7	9.5944E-05
F9 g	108.4	15.6	3.8942E-04	141.5	14.4	3.7375E-04	80.2	10.0	4.5087E-03

^a Models M1-9 represent males and models F1-9 represent females. Model factors included: c = cluster type, a = number of scheduled training activities, p = time period and g = group size. * indicates a factorial model and + indicates an additive model

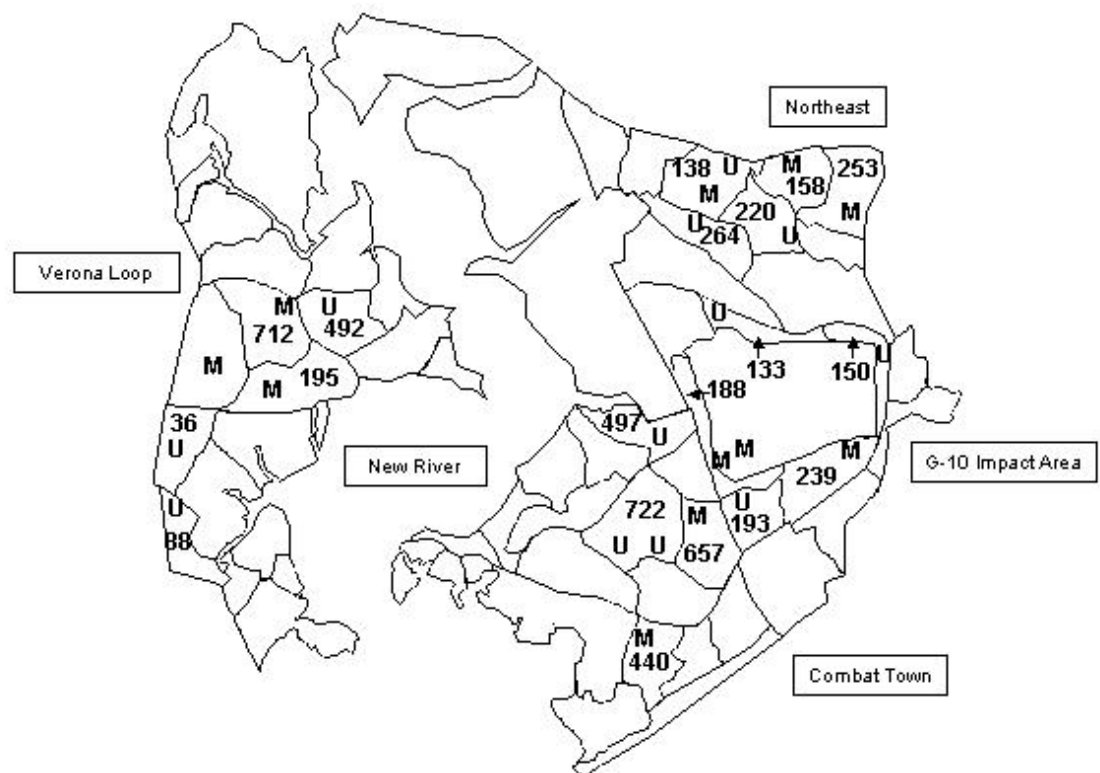


Fig. 3.1. Map of the Main Base of Marine Corps Base Camp Lejeune, showing major training areas and locations of sampled clusters. M = marked clusters; U = unmarked clusters. Numbers indicate the number of training activities scheduled in an area. See text for explanation of two areas with no number.

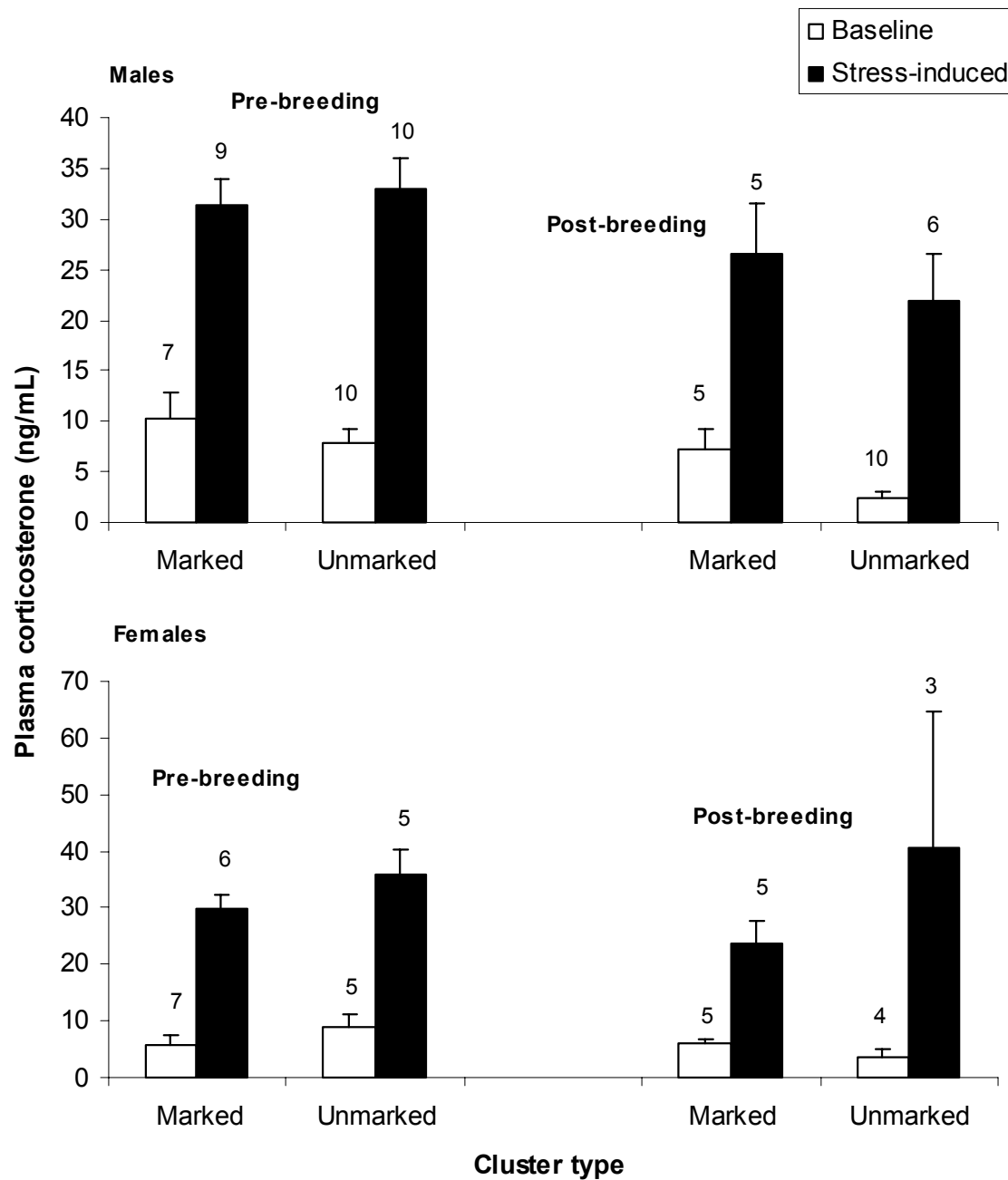


Fig. 3.2. Plasma corticosterone levels of males and females living in marked and unmarked clusters during the pre- and post-breeding seasons. Sample size is indicated above error bars; error bars are standard error. Note difference in scale between males and females.

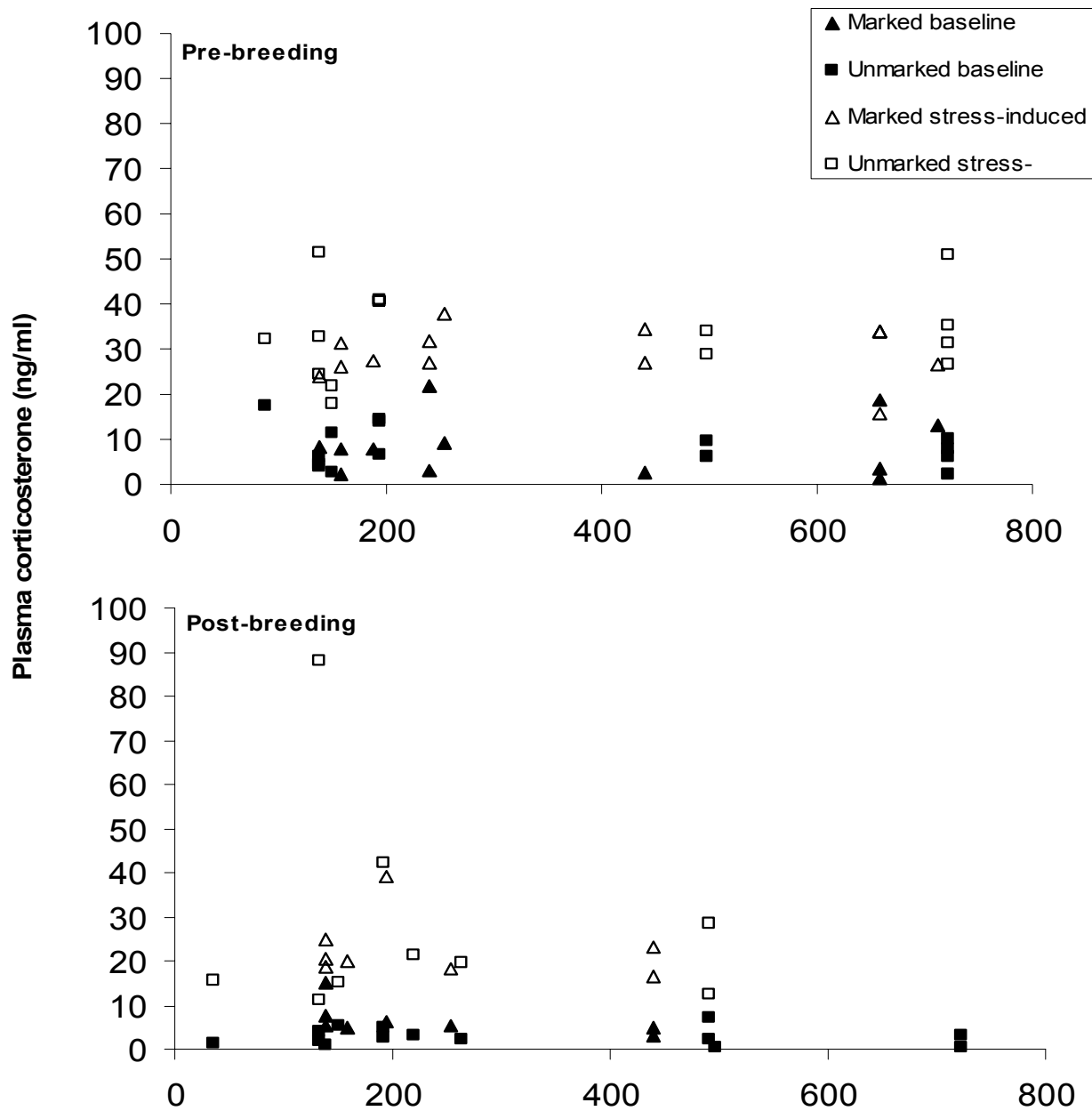


Fig. 3.3. Plasma corticosterone levels of males and females in marked and unmarked clusters during the pre- and post-breeding seasons, plotted as a function of the number of training activities scheduled in the area in which an individual's cluster was located.

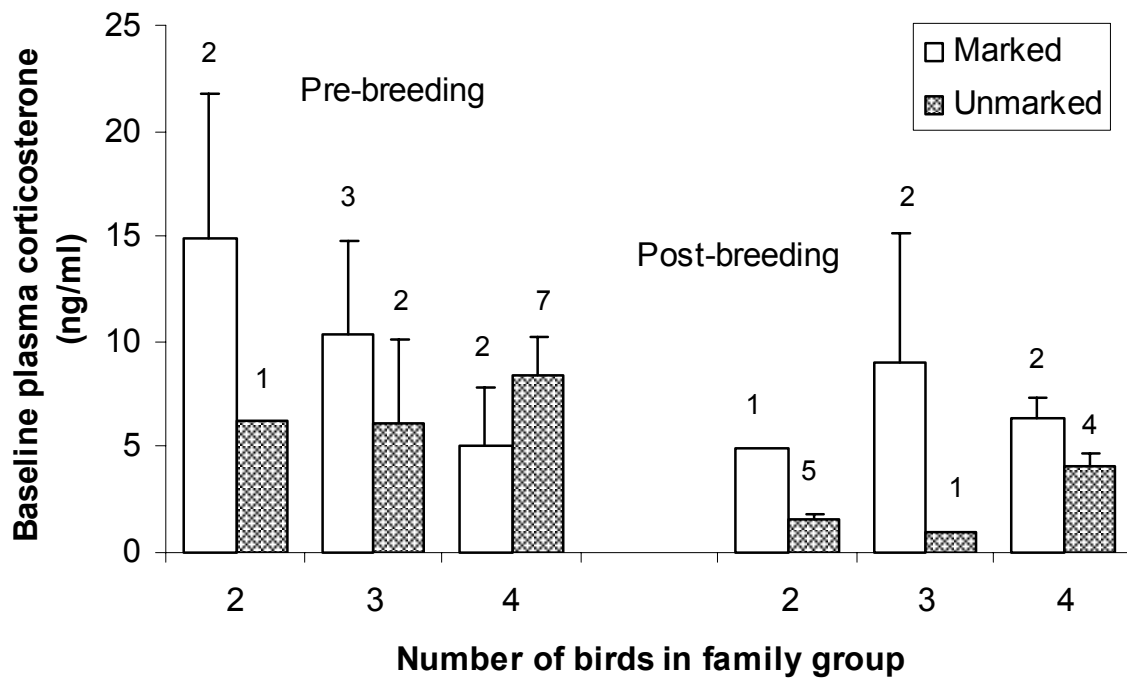


Fig. 3.4. Baseline plasma corticosterone levels of males from groups of varying sizes during the pre- and post-breeding seasons. Sample size is indicated above error bars; error bars are standard error.

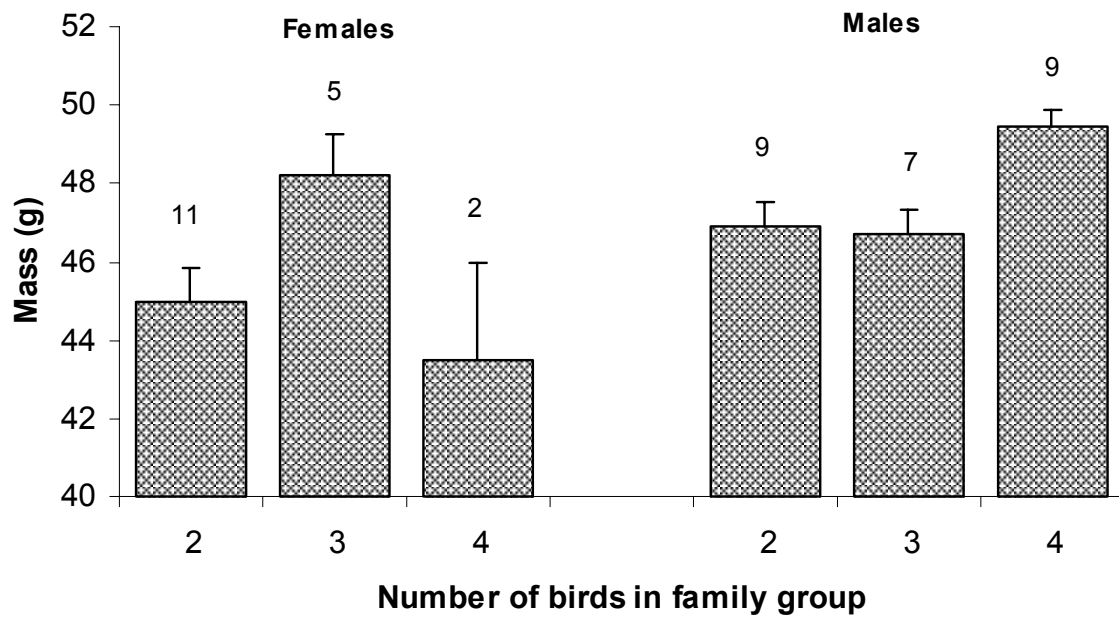


Fig. 3.5. Body mass of males and females from groups of varying sizes. Mass is combined from both marked and unmarked clusters and pre- and post-breeding seasons. Sample size is indicated above error bars; error bars are standard error.