A BASELINE EVALUATION OF STRESS IN A FREE RANGING

POPULATION OF WILD PIGS

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

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Brian Lee Williams

Certificate of Approval:

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Stephen S. Ditchkoff	Jack W. Rogers, Jr.
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Wildlife Sciences	University Honors College

A BASELINE EVALUATION OF STRESS IN A FREE RANGING POPULATION OF WILD PIGS

Brian Lee Williams

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A BASELINE EVALUATION OF STRESS IN A FREE RANGING

POPULATION OF WILD PIGS

Brian Lee Williams

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Signature of Author

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Date

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VITA

Brian Lee Williams was born in Weaver, Alabama on December 8, 1983. He grew up in Weaver and graduated from Weaver High School in 2002. He then attended Auburn University and graduated summa cum laude with a Bachelor of Science degree in Wildlife Science in 2006. During his time at Auburn, he spent the summers of 2004 and 2005 working as a field technician under three Auburn graduate students who were examining a number of population characteristics among the wild pigs on Fort Benning, Georgia. His Honors Thesis project arose from this experience.

THESIS ABSTRACT

A BASELINE EVALUATION OF STRESS IN A FREE RANGING POPULATION OF WILD PIGS

Brian Lee Williams

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30 Typed Pages

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Wild pigs (*Sus scrofa*) can cause widespread ecological and economic damage in areas they have been introduced. However, despite the damage they cause, most efforts to control swine populations have proven ineffective. Hog populations quickly rebound from any attempts made at population reduction due to their high reproductive capacity, and it is for this reason that sustained intensive efforts to eliminate feral swine may be the only effective way to keep populations under control. However, to date no coherent swine eradication plans exist. This study examined stress levels in a free ranging population of wild pigs on Fort Benning, Georgia, using fecal glucocorticoid metabolite analysis in an effort to establish which portion(s) of the population was under the highest stress loads and at which time(s) of year these high stress levels occurred. Pigs on Fort Benning showed two distinct seasons of elevated farrowing activity (March/April and July/August). These two periods were labeled as the breeding season,

and pigs collected during these two periods were grouped separately from pigs collected throughout the rest of the year. Additionally, pigs were classified by age as either "sexually mature" or "sexually immature". For sexually mature females, fecal glucocorticoid levels were greater during the breeding seasons than during the rest of the year ($t_{25} = 1.71$, $P = 0.099$), and pregnant individuals had greater levels of fecal glucocorticoids than non-pregnant individuals ($t_{33} = -2.47$, $P = 0.019$). Sexually mature males had lower fecal glucocorticoid levels during the breeding season than during the rest of the year ($t_{38} = -2.30$, $P = 0.027$). This is likely due to a decrease in breeding activity during these seasons as the majority of receptive females were already pregnant. This is also evidenced by the fact that fecal glucocorticoid levels peaked among mature males during the two periods immediately preceding each breeding season. Fecal glucocorticoid concentrations did not differ among sexually immature individuals of either sex (males, $t_{23} = -0.26$, $P = 0.794$; females, $t_{37} = 1.14$, $P = 0.262$) between the breeding seasons and the rest of the year. Individuals collected from traps exhibited greater ($F_{1, 103} = 3.60$, $P = 0.060$) fecal glucocorticoid concentrations than individuals collected via hunter harvest. The timing of peak stress levels among both sexes of sexually mature animals and the lack of fluctuations in stress levels among sexually immature animals lead us to conclude that reproduction and the timing of peak reproductive activity are the major factors influencing stress among free-ranging wild pigs.

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I would especially like to thank Laura Hanson, Buck Jolley, and Bill Sparklin for giving me the opportunity to work on each of their projects and for the invaluable personal and professional experience I gained from each of them. I would also like to thank everyone who helped with the field work for this study including Buck Jolley and Chad Newbolt for helping with collection of my samples and Laura Hanson, Bill Sparklin, and Ben Malley for their assistance. I would also like to thank the personnel at the Land Management branch on Fort Benning for allowing me to use their facilities.

Style manual of journal used: Journal of Wildlife Management

Computer software used: Microsoft Word 2002 (text) and Statistical Analysis System (figures)

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Feral swine (*Sus scrofa*), or wild pigs, pose a number of ecological and economic threats in areas where they have become established in North America (US Department of Agriculture 1999, Engeman *et al*. 2001, Engeman *et al*. 2003). Introduced wild pigs pose both direct and indirect environmental threats to natural systems and native species through habitat destruction, predation, and competition (Oliver and Brisbin 1993, Choquenot *et al.* 1996, Taft 1999, Gabor and Hellgreen 2000). Even if pigs are successfully eradicated from an area, the negative environmental impacts associated with habitat destruction and predation on/competition with native species can linger for years (Hone and Stone 1989, Engeman *et al.* 2001, Engeman *et al*. 2003). Feral swine can have a substantial financial impact on agricultural operations due to the direct loss of crops, loss of livestock grazing areas, and/or damage control (Choquenot *et al*. 1996). Additionally, efforts to restore native habitats destroyed by feral hogs can easily cost tens of thousands of dollars per hectare (Engeman *et al*. 2003).

Despite the need to control wild pigs, techniques and tools that have been developed for this purpose have proven ineffective (Engeman *et al*. 2003), largely due to the fact that feral pigs are capable of reproducing faster than any other large mammal in North American (Wood and Barrett 1979, Hellgren 1999, Engeman *et al*. 2003). Swine management plans in which hogs are periodically harvested as game animals or undergo periods of intense removal, show little effect in reducing the impacts of hog populations

(Engeman *et al*. 2003). Hog populations quickly rebound from attempts at population reduction, and it is for this reason that sustained intensive efforts to eliminate feral swine may be the only effective way to keep populations under control (Engeman *et al*. 2003). However, to date no coherent swine eradication plans exist.

In the search for new eradication methods and techniques, it seems logical to focus efforts on any weak points that may exist within the natural life cycle of feral swine. One potential weak point may be those periods in which all or part of the population experiences high levels of stress. In mammals, high stress levels have been linked to many negative effects including suppression of the immune system (Munck *et al.* 1984, Peristein *et al.* 1993), pregnancy disruption (Wasser and Starling 1988, Dunbar 1989, deCatanzaro and MacNiven 1992), and growth inhibition (Breazile 1987, Young *et al.* 2004). To date no studies have examined natural variations in stress levels among free ranging feral swine.

Our goal was to examine timing and causes of stress within a population of free ranging feral swine. We used fecal glucocorticoid metabolite analysis, a non-invasive technique recently demonstrated to be applicable to wildlife (Goymann *et al*. 1999, Wasser *et al*. 2000, Luders *et al*. 2001, Millspaugh *et al*. 2002, Millspaugh *et al*. 2003, Washburn *et al*. 2003), to measure stress levels within a population of feral swine. Our specific objectives were to:

(1) determine if concentrations of fecal glucocorticoids vary seasonally in feral swine;

(2) and determine if concentrations of fecal glucocorticoids vary by sex and age in feral swine.

METHODS

Our study was conducted on the Fort Benning military reservation, located in west-central Georgia and eastern Alabama [32° 21' N, 84° 58' W]. Ft. Benning consisted mainly of the ridges, slopes, and bottomlands typical of the Fall Line Sandhill area of the East Gulf Coastal Plain in which it is located (Dilustro *et al.* 2002). Prior to military acquisition, which began in 1918, the land was mainly used for farming and grazing (Dilustro *et al.* 2002). The vegetation on the greater part of Ft. Benning consisted mainly of pure and mixed stands of longleaf (*Pinus palustris*), loblolly (*P. taeda*), and shortleaf pine (*P. echinata*; King *et al.* 1998). Stands of longleaf were planted and maintained in an effort to preserve and expand the mature longleaf pine ecosystem required by the endangered red-cockaded woodpecker (*Picoides borealis*; King *et al.* 1998, Dilustro *et al.* 2002). These stands were managed with prescribed burns every 2-4 years (King *et al.* 1998). Bottomlands consisted mainly of oak (*Quercus spp.*) and hickory (*Carya spp.*); other major plant species included sweetgum (*Liquidambar styraciflua*), flowering dogwood (*Cornus florida*), blackberry (*Rubus spp.*), gallberry (*Ilex spp.*), and wax myrtle (*Myrica cerifera*; King *et al.* 1998).

Wild pigs were collected from Fort Benning between June 2004 and January 2006 as part of a larger, ongoing study aimed at assessing the dynamics of the Fort Benning wild pig population. Hogs were collected using live-traps pre-baited with corn as part of a manipulative experiment to assess the impact of density reductions on population growth and recruitment rates. Additionally, hogs were collected via hunter harvest to examine diet, reproductive rates, and physiological parameters.

Upon collection of a hog, fecal samples were removed from the last 15 centimeters of the colon and frozen at -78° C until they could be processed. Approximately 10 grams of each frozen fecal sample was lyophilized for 24 hours. Freeze-dried samples were then returned to the freezer and stored at -78° C. Samples were then ground, sifted, and mixed. Glucocorticoids were extracted from the feces using a modified technique of Schwarzenberger *et al.* (1991). This involved placing 0.2 grams of dried feces in a test tube with 2.0 mL of 905 methanol and vortexing for 30 minutes. Samples were then centrifuged at 2,200 rpm for 20 minutes and the supernatant saved and stored at -84° C for later analysis. Glucocorticoids were measured using I^{125} corticosterone radioimmunoassay (RIA) kits (ICN #07-120103, ICN Biomedicals, Costa Mesa, CA). This assay has been shown to accurately measure fecal glucocorticoids in small birds (Washburn *et al.* 2002, Washburn *et al.* 2003), white-tailed deer (*Odocoileus virginianus*; Millspaugh *et al.* 2002), and elk (*Cervus elaphus*; Millspaugh *et al*. 2001). This technique followed the ICN protocol for the I^{125} corticosterone RIA, except that we halved the volume of all reagents.

Since pigs typically reach sexual maturity between 6-8 months of age, we classified an individual as "sexually mature" if it was >33 weeks of age (class 6) based on Matschke (1967). Individuals in class 6 or below (≤33 weeks) were grouped as "sexually immature", and all individuals in class 7 or above were considered sexually mature "adults". Sexually mature females were classified based on reproductive status as either pregnant or non-pregnant, and a *t*-test was conducted to determine if female reproductive status affected fecal glucocorticoid levels. Jolley (unpublished data) reported 2 peak periods of farrowing (March/April and July/August) in the Fort Benning pig population.

Based on this information, we considered these 2 periods as "breeding" seasons and grouped all individuals collected within each of these two periods separately from individuals collected throughout the rest of the year. We conducted *t*-tests for sexually mature and immature individuals within each sex based on date of collection (i.e., breeding season versus non-breeding season) to determine if breeding affected fecal glucocorticoid levels. We also conducted an analysis of variance (ANOVA) among individuals collected from traps and those collected via hunter harvest to assess if method of collection affected fecal glucocorticoid levels. To account for small samples sizes among some of our groupings, we set our alpha (α) value at 0.1 for all of our statistical analyses. All statistical analyses were performed using the Statistical Analysis System (SAS Institute Inc. 1990).

RESULTS

Fecal glucocorticoid concentrations differed (t_{33} = -2.47, P = 0.019) between pregnant (65.8 ng/mg ± 12.6 *SE*, n = 15) and non-pregnant (36.5 ng/mg ± 4.1, n = 20) sexually mature females. For sexually mature individuals, fecal glucocorticoid concentrations were greater (t_{25} = 1.71, P = 0.099) among females (66.0 ng/mg ± 18.9, n = 10) and less (t_{38} = -2.30, P = 0.027) among males (32.1 ng/mg ± 3.35, n = 9) during the 2 peaks of farrowing than during the rest of the year (Fig. 1). Fecal glucocorticoid concentrations did not differ among sexually immature (≤ class 6) individuals of either sex (males, t_{23} = -0.26, P = 0.794; females, t_{37} = 1.14, P = 0.262; Fig. 1) between the peaks of farrowing and the rest of the year. Individuals of both sexes collected by trapping had greater ($F_{1, 103}$ = 3.60, P = 0.060) fecal glucocorticoid concentrations than individuals harvested by hunters (Figure 2).

DISCUSSION

Our data suggest that fluctuations in fecal glucocorticoid levels that occurred within the Fort Benning population of wild pigs were likely driven by reproductive cycles. Fecal glucocorticoid levels among pregnant females were approximately 45% greater than those found in non-pregnant females, indicating that pregnancy is an important stressor among female wild pigs. Pregnancy in swine is known to be a stressful period during which females are often challenged physically to meet nutritional demands (Quesnel *et al*. 2005) and physiologically due to elevated levels of a suite of pregnancy-induced hormones (Morales *et al*. 2002). These stresses can have a number of detrimental effects, including decreased reproductive success (Quesnel *et al*. 2005), increased susceptibility to disease (Morales *et al*. 2002) and potential exposure to predators (Marchant-Forde and Marchant-Forde 2004).

When examined seasonally, overall fecal glucocorticoids levels among adult female pigs were elevated during 2 distinct periods. These peaks, which occurred during the two-month seasons of March/April and July/August corresponded with both of the intense farrowing seasons among Ft. Benning swine (Jolley, unpublished data). Originally, we had hypothesized that lactating females would exhibit the greatest levels of fecal glucorticoids due to the substantial nutritional constraints experienced by lactating females (Quesnel 2005, Quesnel *et al*. 2005). However, it is likely that most pregnant individuals were also lactating in this population. Although lactation data were not available for the individuals in this study, the fact that weaning normally does not occur until 2-4 months of age in pigs (Pond and Haupt 1968) combined with the short interlitter interval in the Fort Benning pig population (Jolley, unpublished data) suggests

that a majority of pregnant females were likely still lactating. The combined nutritional demands of gestation and lactation likely resulted in the bimodal pattern of fecal glucocorticoid secretion that we detected, which coincided with the two most intense farrowing seasons on Fort Benning.

Patterns of fecal glucocorticoid levels in adult male swine tended to be inversely associated with those of female swine. Adult males exhibited their lowest levels of fecal glucocorticoids during the two peaks (March/April and July/August) of farrowing but maintained greater levels of fecal glucocorticoids than females in the remaining sampling periods. We attribute this pattern to the stresses associated with breeding/sexual activity. As is the case in many polygynous species (Creel 2001), dominant male pigs secure the right to breed via physical confrontation/intimidation. It has been demonstrated that the frequent displaying and fighting associated with maintenance of the dominance hierarchy can elevate stress levels of dominant males in species in which breeding success is largely determined by dominance (Creel 2001, Mooring *et al*. 2006). In addition to physical stressors like display and fighting, adult male swine must also cope with the physiological stressors associated with sexual activity. One such stressor is elevated androgenic hormones (e.g., testosterone). Testosterone levels of male swine have been shown to fluctuate according to female sexual activity (Mauget and Boissin 1987), and since sexual activity of female swine can occur throughout the year on Fort Benning (Jolley, unpublished data), adult male swine must be physically able to cope with the effects of elevated levels of testosterone for extended periods. Numerous studies have shown that elevated testosterone levels that are sustained for extended periods can lead to suppression of the immune system (Buchanan *et al*. 2003, Berger *et al*. 2005), increased

aggression/fighting (Muller and Wrangham 2004, Ross *et al*. 2004, Oyegbile and Marler 2005) and decreased efforts to find food/anemia (Woodroffe and MacDonald 1995). Since Fort Benning swine successfully breed throughout the year, males could theoretically be subjected to consistently elevated levels of stress due to the physical and physiological factors associated with the maintenance or securing of dominance. Indeed, adult males did show stress levels greater than females in four of the six two-month seasons on Fort Benning. However, we believe that adult males exhibited decreased fecal glucocorticoid levels during the two peaks of farrowing (the two seasons when female fecal glucocorticoid levels were greatest) as a result of a decrease in breeding activity associated with fewer receptive females. Adult male fecal glucocorticoid levels were greatest during the seasons immediately preceding the peaks of farrowing, and this is likely due to a large percentage of female swine coming into estrous during these periods. Since that same large percentage of females is generally not receptive during farrowing, adult males likely use these periods of decreased breeding activity to recover from the negative effects associated with intense breeding activity. These periods of decreased breeding activity are likely necessary for physical recuperation and to ensure that they are physically able to compete for breeding opportunities in the future.

Both sexes of sexually immature individuals exhibited lower fecal glucocorticoid levels than sexually mature individuals, but the decrease was more pronounced in males. Since males typically reach sexual maturity around 6-8 months of age (Pond and Houpt 1968), most of the males included in our analysis of young individuals were most likely sexually immature. The lower stress levels among immature males tends to support the idea that breeding/sexual activity is one of the most important stressors among adult,

sexually mature and active males. It is possible that some class 6 (20-33 weeks old) individuals were sexually mature upon collection. However, these individuals were not yet at full adult body size and were likely dominated by larger males. Along these lines, Mooring *et al*. (2006) found substantially lower levels of fecal glucocorticoids in subordinate bison bulls than in dominant individuals. Pigs exhibit a polygynous mating system similar to that of bison and many other large mammals, and it seems probable that if any of the males we included in our analysis of young individuals were indeed sexually mature, they were still likely to exhibit fecal glucocorticoid levels lower than adults due to the suppression of breeding activity by larger, more dominant males.

The greater levels of fecal glucocorticoids observed in animals collected from traps likely resulted from the periods of time spent in the trap before collection. Fecal glucocorticoids provide a measure of stress level over a period of 24-48 hours (Harper and Austad 2000, Millspaugh *et al*. 2001). Our trapping schedule resulted in traps being checked the day after they were set. It is possible that pigs could have entered a trap immediately after it was set and been confined in the trap for a maximum of 24 hours. However, because our greatest trapping success occurred at night, it is likely that most pigs were confined to the trap for periods ranging from 4-14 hours. Animals left in traps for this amount of time are likely to show elevated levels of fecal glucocorticoids associated with the stress of being confined. Since the majority of our samples were collected from trapped individuals, it is possible that our mean values are greater than what we would have detected if only harvested individuals had been used in the analyses: limited sample sizes of animals collected from hunters precluded analyses and interpretations as described earlier. However, we feel the patterns revealed in our

analyses are likely representative of the actual fluctuations in fecal glucocorticoid levels throughout the year. Since they are not exposed to the stress of being confined, animals collected via hunter harvest likely provide the most precise measure of fecal glucocorticoid levels among wild pigs, but swine collected from traps may assist in revealing any seasonal/sexual patterns in fecal glucocorticoid fluctuations that may exist. In conclusion, we found that female wild pigs experienced peak stress levels during pregnancy/farrowing. Males experienced peak stress levels during the periods immediately preceding female peaks, and this was likely due to an increase in reproductive activity associated with an increase in receptive females during these periods. Male stress levels were lowest during the periods that female levels were highest, and this dramatic decrease in stress levels among males likely corresponds with a decrease in male reproductive activity while many females are pregnant/farrowing. Considering this evidence, and considering that neither sex of sexually immature animals showed any significant fluctuations in stress levels throughout the year, our results lead us to conclude that reproduction and the timing of peak reproductive activity are the major factors influencing stress levels among wild pigs.

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Figure 1. Fecal glucocorticoid levels of sexually mature (a) and sexually immature (b) wild pigs collected from Fort Benning, Georgia, USA, between June 2004 and January 2006.

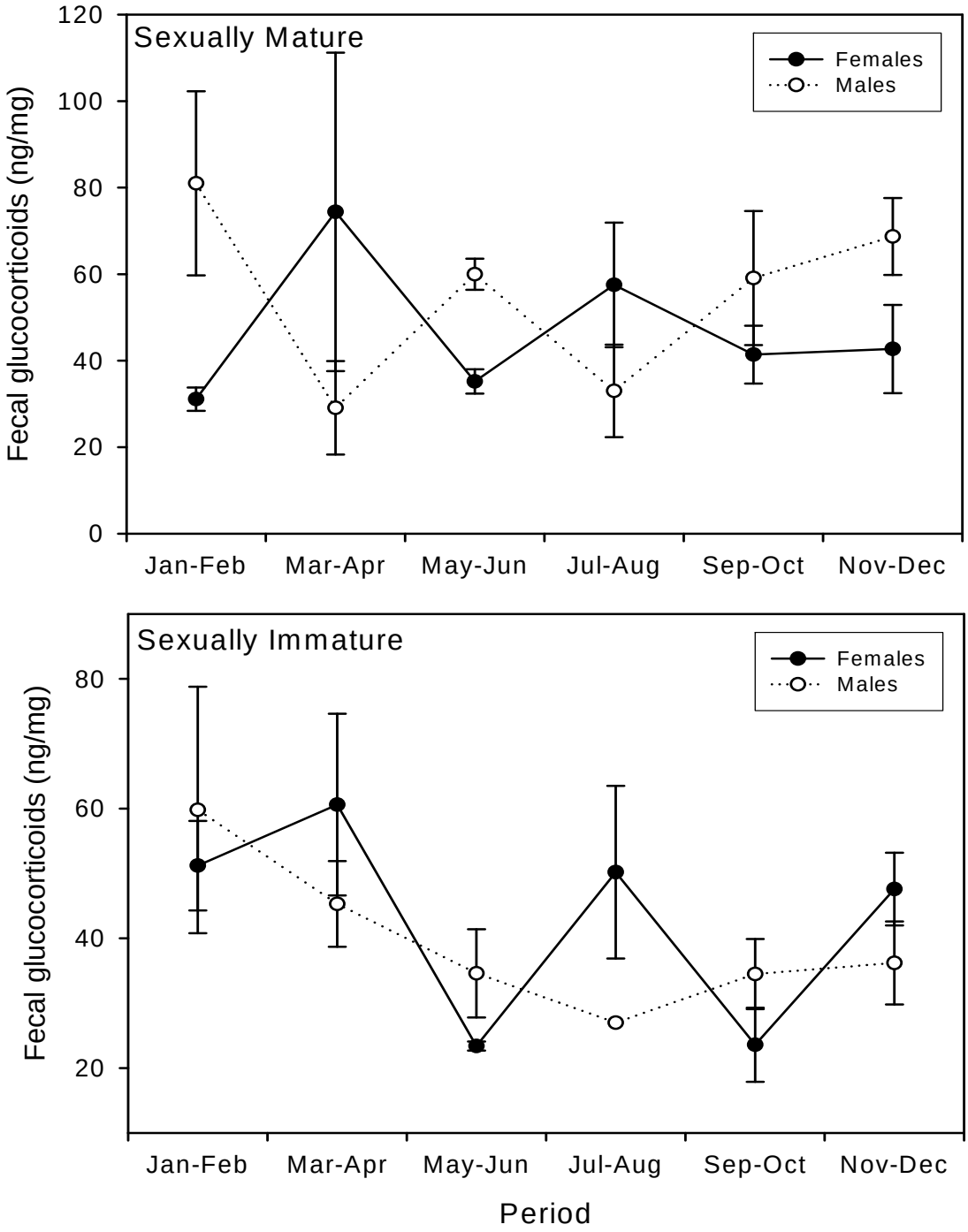
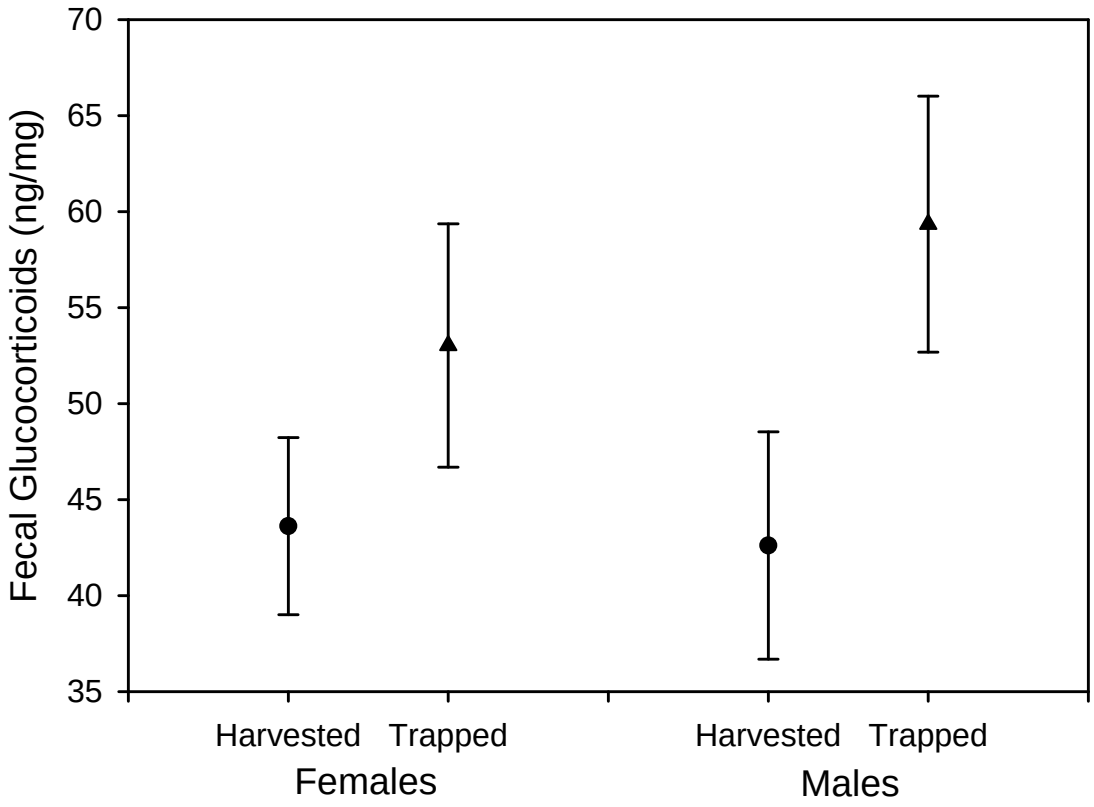

Sexually Mature
Females
Males
Fecal glucocorticoids (ng/mg)
120
100
80
60
40
20
0
Jan-Feb
Mar-Apr
May-Jun
Jul-Aug
Sep-Oct
Nov-Dec
Sexually Immature
Females
Males
Fecal glucocorticoids (ng/mg)
80
60
40
20
Jan-Feb
Mar-Apr
May-Jun
Jul-Aug
Sep-Oct
Nov-Dec
Period

Figure 2. Fecal glucocorticoid levels of wild pigs collected from traps compared to those of pigs collected via hunter harvest on Fort Benning, Georgia, USA between June 2004 and January 2006.


70
65
60
55
50
45
40
35
Fecal Glucocorticoids (ng/mg)
Harvested
Trapped
Females
Harvested
Trapped
Males