Serum Corticosterone Concentrations Related to Seasonal Variation in Reproductive Development in Natural Populations of Peromyscus leucopus noveboracensis

Serena Khosla
College of William & Mary - Arts & Sciences

Follow this and additional works at: https://scholarworks.wm.edu/etd

Part of the Developmental Biology Commons

Recommended Citation
https://dx.doi.org/doi:10.21220/s2-7fck-3t81

This Thesis is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Dissertations, Theses, and Masters Projects by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
SERUM CORTICOSTERONE CONCENTRATIONS RELATED TO
SEASONAL VARIATION IN REPRODUCTIVE DEVELOPMENT IN
NATURAL POPULATIONS OF

PEROMYSCUS LEUCOPUS NOVEBORACENSIS

A Thesis
Presented to
The Faculty of the Department of Biology of
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Sareena Khosla

1995
APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Arts

Author

Approved, April 1995

Eric L. Bradley

C. Richard Terman

Margaret S. Saha
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>RESULTS</td>
<td>17</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>56</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>75</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>76</td>
</tr>
<tr>
<td>VITA</td>
<td>83</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I would especially like to express my deepest appreciation to Dr. Eric Bradley, my co-advisor and mentor, for his continuous guidance, encouragement and support throughout my time here as a graduate student and for his unfailing confidence in my abilities. I would also like to sincerely thank Dr. C. Richard Terman, also my co-advisor, for his indispensable assistance throughout this study and in the preparation of this manuscript. Thanks are also extended to Dr. Margaret Saha for her support and reading of the manuscript. In addition, I would like to thank Jewel Thomas for her technical assistance without which I would not have been able to defend this thesis. I am also grateful to Brad Cherry for his help with the histology and image analysis during part of this study and for his sustained support and friendship. Additionally, I would like to thank my family for their endless love, caring and encouragement. Finally, I would like to extend my gratitude to my friends, both here at William and Mary and elsewhere, for their friendship and support given to me throughout this study.
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Body weight, paired testes, seminal vesicle and adrenal weights and serum corticosterone concentration of spring, summer and autumn males (values are mean ± S.E.M.)</td>
<td>23</td>
</tr>
<tr>
<td>2. Leydig cell area, seminiferous tubule area and number of spermatids with acrosome formation in spring, summer and autumn males (values are mean ± S.E.M.)</td>
<td>24</td>
</tr>
<tr>
<td>3. Body weight, paired ovary, uterus and adrenal weights and serum corticosterone concentrations of spring, summer and autumn females (values are mean ± S.E.M.)</td>
<td>25</td>
</tr>
<tr>
<td>4. Correlation coefficients and P values for spring, summer and autumn males</td>
<td>26</td>
</tr>
<tr>
<td>5. Correlation coefficients and P values for spring, summer and autumn males</td>
<td>27</td>
</tr>
<tr>
<td>6. Correlation coefficients and P values for spring, summer and autumn females</td>
<td>28</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Standard curve and <em>P. leucopus</em> serum serial dilution curve for corticosterone radioimmunoassay</td>
<td>30</td>
</tr>
<tr>
<td>2.</td>
<td>Mean body weights of males</td>
<td>32</td>
</tr>
<tr>
<td>3.</td>
<td>Mean adrenal weights of males</td>
<td>34</td>
</tr>
<tr>
<td>4.</td>
<td>Mean seminal vesicle weights</td>
<td>36</td>
</tr>
<tr>
<td>5.</td>
<td>Mean testis weights</td>
<td>38</td>
</tr>
<tr>
<td>6.</td>
<td>Mean serum corticosterone concentration in males</td>
<td>40</td>
</tr>
<tr>
<td>7.</td>
<td>Mean Leydig cell area</td>
<td>42</td>
</tr>
<tr>
<td>8.</td>
<td>Mean seminiferous tubule cellular area</td>
<td>44</td>
</tr>
<tr>
<td>9.</td>
<td>Mean number of spermatids with acrosomes</td>
<td>46</td>
</tr>
<tr>
<td>10.</td>
<td>Mean body weights of females</td>
<td>48</td>
</tr>
<tr>
<td>11.</td>
<td>Mean ovary weights</td>
<td>50</td>
</tr>
<tr>
<td>12.</td>
<td>Mean uterus weights</td>
<td>52</td>
</tr>
<tr>
<td>13.</td>
<td>Mean adrenal weights of females</td>
<td>54</td>
</tr>
<tr>
<td>14.</td>
<td>Mean serum corticosterone concentration in females</td>
<td>56</td>
</tr>
</tbody>
</table>

vi
ABSTRACT

This study examined the role of adrenal function in the reproductive development of natural populations of the white-footed mouse, *Peromyscus leucopus* noveboracensis. The animals utilized in this study were collected during spring, summer and autumn. Animals from the spring and fall were reproductively developed while those from the summer were reproductively suppressed. Tissue collection involved (1) gravimetric analysis of body weights, paired adrenal weights and reproductive organ weights, (2) determination of serum corticosterone concentration via radioimmunoassay, and (3) histological evaluation of testes from a select group of males. Data were collected and assessed. The mean body weight of reproductively suppressed males was significantly lower than that of reproductively developed males. No significant difference in mean body weight was found between suppressed and developed females nor between developed females from the spring and autumn. There was no statistically significant difference between the adrenal weights of males from all three seasons. Similarly, no significant difference was found between the females. The mean paired testes and seminal vesicle weights of reproductively suppressed males were significantly lower than those of spring and autumn males. The reproductive organ weights for developed spring males were also significantly lower than the corresponding weights for autumn males. Reproductively suppressed females similarly showed significantly reduced mean paired ovary and uteri weights than those of developed females. The mean paired uterus weight of developed spring females was also significantly greater than that of developed females from autumn.

Analysis of serum corticosterone concentration revealed a significantly lower mean serum corticosterone concentration value for autumn males than for spring and summer males. There was no statistically significant difference between the mean serum corticosterone concentration of females from the three seasons.

Histological analysis of testes showed a significantly lower mean seminiferous tubule area comprised of cells, mean Leydig cell area and mean number of spermatids with acrosome formation in suppressed males than in developed males.

Contrary to a previous study of laboratory populations of *P. leucopus*, this study did not indicate an association between adrenal function and the reproductive suppression observed in natural populations of *P. leucopus*. It did, however, support the trend of consistently elevated levels of serum corticosterone reported for reproductively suppressed *P. leucopus*. 
SERUM CORTICOSTERONE CONCENTRATIONS RELATED TO SEASONAL VARIATION IN REPRODUCTIVE DEVELOPMENT IN NATURAL POPULATIONS OF PEROMYSCUS LEUCOPUS NOVEBORACENSIS
INTRODUCTION

Small mammal populations of certain species show a variety of changes in density over time, some showing cycles that are more or less regular while others fluctuate annually or erratically (Krebs 1985). While numerous hypotheses have been postulated to explain these population fluctuations (e.g. food, predation, spacing behavior, phenotypic behavior, genotypic behavior, and multiple factors), currently, there is no universally accepted hypothesis regarding their cause (Taitt and Krebs, 1985).

The prairie deermouse, *Peromyscus maniculatus bairdii*, seldom undergoes natural population outbreaks (Blair, 1940), and shows a much lower population density fluctuation over an extended period of time than many species of small mammals (Terman, 1968). The white-footed mouse, *Peromyscus leucopus noveboracensis*, experiences a low to average range of population density fluctuation. However, unlike the prairie deermouse, the white-footed mouse undergoes this population fluctuation over a shorter time span (Terman, 1966). Laboratory studies of populations of both of these species provided with surplus food, water, and nesting sites have demonstrated a characteristic intrinsic ability to regulate population growth (Terman, 1965, 1973, 1979, 1987, 1993; Wolfe, 1981; Ransone and Bradley, 1991).
This growth curtailment occurs through various mechanisms including cessation of reproduction by adult parous females, high mortality of young before reaching reproductive age, and inhibition of reproductive development and function in large numbers of young regardless of density levels (Terman, 1965, 1973, 1979, 1987; Wolfe, 1981). The physiological mechanism of this reproductive inhibition has been the subject of much interest.

Disruption of reproductive function in many species is often a result of exposure to stress but the particular mechanisms that mediate these effects are not fully understood (Christian, 1971; Rivier et al., 1986). Increasing population densities increase the probability that individuals will interact. These interactions may distress the animals and therefore engender a stress response that may, ultimately, serve to control and limit population growth. Christian's stress hypothesis, one of the most well-known theories of population regulation, was proposed to explain population fluctuations by an intrinsic mechanism (Krebs and Myers, 1974; Christian, 1980; Taitt and Krebs, 1985). According to Christian (1964, 1980; Christian et al., 1965), an increased rate of interaction results in increased social stress which induces the General Adaptation Syndrome described by Selye (1946). This syndrome results in an eventual exhaustion of the adrenal-pituitary system and leads to increased mortality due to lowered resistance to disease and general susceptibility, and therefore, decreased
reproduction, which may be due to the inhibition of the pituitary-gonadal function (Christian 1964, 1980; Christian et al., 1965; Krebs and Myers, 1974; Tamarin, 1983; Taitt and Krebs, 1985).

Initially, Christian (1964; Christian et al., 1965) proposed that increased mortality was caused by the exhaustion of the pituitary-adrenal axis due to prolonged stimulation (chronic stress). However, he has more recently emphasized the importance of increased susceptibility to infection and parasitism, and diseases which are consequences of increased pituitary-adrenocortical stimulation (Christian 1971, 1978, 1980). This stimulation leads to an increased production of glucocorticoids, such as corticosterone. Glucocorticoids play two major physiological roles. First, they are essential for various activities associated with the diurnal cycle such as increased locomotion, exploratory behavior, appetite, and foraging behavior (McEwan, Brinton and Sapolsky, 1988; Boonstra and Boag, 1992). Second, they are critical because they allow animals to adapt to acute stressors by stimulation of hepatic gluconeogenesis, inhibition of glucose absorption by peripheral tissues and suppression of inflammatory responses and immune reactions by (1) inhibiting the production and/or activity of most of the mediators active on vascular and other systems (i.e. kinins, lymphokines, serotonin, histamine, catecholamines, immunologic mediators, prostaglandins, vasopressin etc.), (2) the movement of leukocytes to inflamed areas, and (3) the function of immunocompetent cells at the site of inflammation (Munck, Guyre and Holbrook,
Christian suggests that high levels of corticosterone precipitate increased mortality by suppressing the immune and inflammatory responses. This increases the vulnerability of animals to disease and impairs their reproductive capacity thereby inducing population declines (Christian, 1980). He also suggested that the magnitude of corticosterone response should vary with density since populations of higher density may be exposed to a greater amount of social strife (Christian, 1975). A number of studies have shown that either an increased adrenal mass or increased serum glucocorticoid concentration has been positively correlated with population density in house mice, *Mus musculus* (Christian 1955a, 1955b, 1956), lemmings, *Lemmus trimucronatus* (Andrews and Belknap, 1979), and voles, *Microtus breweri* and *Microtus pennsylvanicus* (To and Tamarin, 1977).

Previous studies have examined the relationship between the gravimetric, histological and endocrine characteristics exhibited by laboratory populations of reproductively suppressed animals (Terman, 1979, 1987; Bradley and Terman, 1981a; Ransone and Bradley, 1992). Studies concerning the growth of experimental laboratory populations of *P. maniculatus bairdii* have demonstrated an adrenocortical hyperfunction in suppressed population animals when compared with reproductively developed adults. While serum corticosterone concentrations were highly elevated in both sexes, no concurrent hypertrophy of the adrenal gland was observed (Sung *et al.*, 1977; Bradley and Terman, 1981a). Similarly, a recent study on laboratory raised
populations of *P. leucopus* demonstrated a significantly higher mean corticosterone concentration in reproductively suppressed males compared with the corresponding value for reproductively developed males with no concomitant increase in adrenal weight (Ransone and Bradley, 1992).

Stress-induced pituitary-adrenal hyperfunction has been suggested to be due to the direct suppressive effects of ACTH on the pituitary (inhibition of gonadotropin secretion) or due to ACTH-stimulated sex steroids (Christian, 1963; Christian and Davis, 1964). Elevated levels of ACTH have been noted in stressed animals (Cook *et al.*, 1973). In *P. maniculatus*, ACTH has been shown to cause adrenal hypertrophy when administered exogenously (Coppes and Bradley, 1984). Indeed, ACTH administration has also been shown to cause reproductive inhibition in males of various species (Christian *et al.*, 1965; Pasley and Christian, 1972; Schaison *et al.*, 1978; Collu *et al.*, 1979). However, a study by Coppes and Bradley (1984) showed no differences in serum levels of ACTH between control and reproductively suppressed laboratory population *P. maniculatus*.

Taken together, the lack of adrenal hypertrophy in suppressed *P. maniculatus* as well as the lack of ACTH elevation in the suppressed animals seem to suggest that elevated serum levels of ACTH are not the primary cause of the characteristic reproductive inhibition seen in this species.
Christian's Stress hypothesis has not won universal acceptance due to the above mentioned inconsistencies and to the difficulty of extrapolating to natural populations the results obtained from studies on laboratory populations. One of the most common criticisms is that Christian's hypothesis seems to work mainly at high densities in confined populations of some species and that these densities differ from those found in natural populations (Christian, 1964; Tamarin, 1983; Lee and Cockburn, 1985). As mentioned above, laboratory populations of Peromyscus maniculatus and Peromyscus leucopus have shown an exquisite sensitivity to population increase resulting in suppression of reproduction and regulation of growth at widely different densities in the presence of surplus food and water (Terman, 1965, 1973, 1979, 1987, 1993; Sung et al., 1977; Bradley and Terman, 1981a; Wolfe, 1981; Ransone and Bradley, 1992).

Studies of natural populations of both of these species have been undertaken to ascertain the relevance of these laboratory findings to natural populations (Andrews et al., 1975; Terman, 1993).

A seven year study of a natural population of Peromyscus leucopus noveboracensis supports the existence of a yearly bimodal curve of reproduction with significantly lower percentages of males and females in reproductive condition during May, June, and July than during February-April and August-October. This characteristic reproductive curtailment occurred at distinctly different densities. A lack of a significant relationship between number of adults/hectare and the percent of
animals in reproductive condition strongly suggests that the factors responsible for this inhibition of reproduction are not directly related to absolute density.

The purpose of the current study was to determine whether elevated serum corticosterone is associated with the reproductively suppressed condition in natural populations of *P. leucopus*. This was done by examining the relationships between selected gravimetric and histological characteristics and the serum corticosterone concentration in reproductively suppressed and reproductively developed animals sampled from natural populations.
MATERIALS AND METHODS

Experimental Animals

The animals in this study were collected from natural populations of white-footed mice (*Peromyscus leucopus noveboracensis*) by live-trapping in wooded areas near the Laboratory of Endocrinology and Population Ecology of the College of William and Mary in Williamsburg, Virginia.

Trapping was conducted in 1992, 1993 and 1994 during spring (March-April) summer (June-July) and autumn (August). The animals utilized in 1992, part of 1993 and 1994 were collected as part of a long term study of *P. leucopus* by C.R. Terman within an 11 hectare area as described by Terman (1993). Animals collected during part of 1993 were obtained by repeatedly setting 56 live-traps at irregular intervals in several different wooded areas. The traps were baited with a mixture of vegetable shortening and peanut butter. With few exceptions, trap inspections and collection began within 3 hours of dawn. Animals were removed from the traps, their sex was immediately determined, after which they were taken directly to quarantine in the laboratory. Following each trap inspection, the trap was reset with food. For each of these collection periods, five adult animals of each sex were utilized for this study.
The animals were individually marked by toe clipping for future identification. After the animal's sex was noted, it was weighed to the nearest gram. Age class was estimated by pelage color (adults: brown; juveniles: grey; young adults: molting from grey to brown). Males were classified according to whether the testes were scrotal or nonscrotal. Females were checked for vaginal perforation, evidence of pregnancy (including uterine scars after sacrifice) and lactation.

Following documentation of these characteristics, the animals were subjected to a 30-40 hour quarantine period during which they were kept singly in cages in which food and tap water were supplied *ad libitum*. Following this period, blood was collected from each animal.

**Collection of Tissues**

Each animal was identified and then rapidly anesthetized using diethyl ether. A ventral abdominal incision was made and blood was collected from the left renal artery using a sterile 1 ml plastic tuberculin syringe without needle (Scientific Products). All blood was collected within 2 minutes of initial contact with the animal. After collection, the blood was placed into a 1.5 ml polypropylene microcentrifuge tube (Scientific Products) and allowed to clot for at least two minutes before centrifugation at
9000 x g for two minutes to separate the serum from the cells. The serum was pipetted off, frozen, and then stored below -35 C until assayed.

The body was weighed to the nearest 0.1 gram and placed in a 10% buffered formalin solution. Later, gross dissections were performed in which the heart, spleen, right lobe of liver, right kidney, adrenals and reproductive structures of each animal were removed and placed directly in a 10% buffered formaldehyde solution. All organs were allowed to fix for at least 72 hours before being finely dissected and weighed. Paired adrenal glands, testis, seminal vesicles, paired ovaries and uterus were weighed to the nearest 0.01 mg using an electronic balance. Two weights were taken for each organ and the average recorded. The condition of the organs was noted. Scarred uteri and those of pregnant females were not weighed.

Radioimmunoassay for Corticosterone

Corticosterone antiserum (B3-163, lot 163-077, Endocrine Sciences, Tarzana, CA) and 1,2,6,7, tritiated corticosterone (NET-399, lot 2943-076), New England Nuclear) were used for the assay. Thin-layer chromatography was used to purify the radioactive material and to validate the authenticity of the corticosterone standard.

An antibody titer was performed to confirm that the antibody dilution recommended by the manufacturer was appropriate. After reconstituting the
lyophilized sample according to the manufacturer's specifications, the antibody was
diluted in duplicate to 1:40, 1:80, 1:120, 1:160, and 1:200. Nine thousand
disintegrations per minute of tritiated corticosterone was added to each tube. Thirty
percent non-binding of the total number of disintegrations occurred at a 1:80 dilution.
Since this level of binding was similar to the 1:85 dilution specified by the
manufacturer, 1:85 dilution was used throughout the assay.

A dilute antiserum solution was prepared by diluting 100 ul of the reconstituted
antisera with 8.0 ml of borate buffer (0.05M, pH 8.0), 200 ul of 10% bovine serum
albumin (A-7888, Sigma; in borate buffer), 200 ul of 2.5% bovine gamma globulin
(G-5009, Sigma; in normal saline). Four hundred thousand dpm of tritiated
corticosterone was added to provide approximately 9,000 counts per assay tube.

All values reported here were determined in a single assay. Standards for the
assay were run in duplicate with 0.0625, 0.125, 0.250, 0.500, 1.000 and 2.000ng/tube of
authentic corticosterone (Chem Service, West Chester, PA) diluted with redistilled
methanol. All standards were prepared in 12 X 75 mm polystyrene conical bottom
culture tubes (Scientific Products). The methanol was evaporated in a speed vac
(Savant Instruments, Inc.) at the medium drying rate for about 30 minutes. A constant
volume of 70 ul of borate buffer (0.05 M, pH 8.0) was added to each standard following
removal of the methanol.
A standard pool of male *P. leucopus* sera (SSER) was assayed in duplicate at 1.00, 0.50, 0.25 and 0.125 times the natural concentration. The serum samples collected from the field, together with the standard sera pool were prepared by diluting 20 ul of serum with 180 ul of borate buffer (0.05M, pH 8.0) in a 12 X 75 mm polysterene conical bottom culture tube. Following brief vortex mixing, 20 ul aliquots of diluted serum were transferred from each tube to duplicate 12 X 75 mm tubes and then extracted according to the method of Sheldon and Coppinger (1977). The enzyme, Subtilisin, Carlsburg (P-5380, Sigma Chem.) was diluted to 8 units of activity per sample in 0.05 M borate buffer. Fifty ul of the diluted enzyme was added to each sample tube and the samples were then incubated for one hour in a 37 C water bath and then incubated for 3.5 minutes in an 89 C water bath to inactivate the enzyme. The tubes were immediately cooled with running tap water.

Two hundred ul of the dilute antiserum mixture was aliquoted to each corticosterone standard and serum sample tube. All tubes were covered with parafilm, vortex mixed and incubated at 4 C for 16 hours. The repetitive pipetting in the assay was accomplished using an automatic pipette (Micromedic Systems model 25004).

Following incubation, 250 ul of saturated ammonium sulfate was added to each tube to precipitate the antigen-antibody complex. The same volume of ammonium sulfate was also added to three counting vials containing direct aliquots of the dilute antiserum in order to measure the total radioactivity added to each tube. The assay
tubes were covered with parafilm and centrifuged in an HS-4 rotor at 9,000 x g at 4 C for 20 minutes in a Sorvall RC-5B (Dupont, Inc., Newtown CT).

Four hundred ul of the supernatant from each tube was transferred to a plastic scintillation vial and 10 ml of Ready-Solve EP scintillation cocktail (Beckman) was added to each vial as well as the total count vials prepared earlier. After vortex mixing, each vial was placed in a liquid scintillation counter (Beckman, LS-3133T) and counted for tritium to a 1% error.

**Histological Analysis of Gonads:**

The testes were initially dehydrated in solutions of increasing ethanol concentration. This was conducted both manually and using a RMC 3189 Ultraprocessor. Following dehydration, the samples were impregnated with Historesin (Reichert-Jung, Heidelberg). The testes were sectioned at 6 um using a Sorvall JB-4 Microtome and stained for one minute using 0.1% toluidine blue. The tissues were then evaluated at magnifications of 20X and 40X using Bioquant Software (R&M Biometrics Inc., 1988)

For each animal, cross-sections of 20 seminiferous tubules, taken from cross-sections close to the middle of the testis, were microscopically inspected. A tubule was only analyzed if its minor/major axis ratio was > 0.70. This ratio was measured using
an optical micrometer. Data analysis of testis histology was based on three parameters: the mean number of spermatids with acrosome formation, the mean seminiferous tubule area comprised of cells, and the mean Leydig cell area. For each animal, the means of the twenty values for each parameter were calculated.

STATISTICS

The counts per minute (cpm) of each standard and serum sample were automatically converted to disintegrations per minute (dpm) using the external standards channels ratio (ESCR) value calculated by the counter. All sample and standard dpm were converted to percent of total average dpm in dilute antiserum aliquots and then logit transformed (Ransone and Bradley, 1992). Standard corticosterone concentration values were transformed to the natural log of the concentration. A multiple regression was run to compare the slope of the curves of both authentic corticosterone and serially diluted serum standards. The slopes of these two curves did not vary significantly indicating that the serially diluted plasma did not behave differently from serially diluted authentic hormone. Unknown sample concentrations were then calculated using the regression equation \( y = -.73556x + .02118 \) derived from the authentic corticosterone standard curve values. All calculated values were converted to ng/ml of sera.
In cases where a one-way ANOVA indicated heterogeneous variance the Kruskall Wallis test was used for comparisons. Correlation data were analyzed using Spearman's nonparametric ranked correlation test. Data are reported as the mean value ± the standard error of the mean. In these cases, $P < 0.05$ was considered statistically significant. When no heterogeneity among variances was found, animals from different seasons were compared using Tukey's test and parametric correlations were performed, where $P < 0.01$ was statistically significant.
RESULTS

The individual organ and body weights and corticosterone values for the three different time periods (spring, summer and autumn) in 1992 and were compared with the values from the corresponding time periods in 1993-1994. For a given time period, no significant differences existed between the values for the two years. Therefore, the data from similar time periods from both years were combined and all subsequent analysis is based on these combined data (see Appendix A: for all individual values).

Comparison between males from different time periods:

Gravimetric Analysis:

The mean body weight of summer males was significantly lighter than that of spring and autumn males (Table 1). There were no statistically significant differences between both the mean absolute and the mean relative adrenal weights from the three time periods (Table 1).

The mean paired testes weights and seminal vesicle weights of summer males were significantly ($P < 0.05$) lighter than those of spring and autumn males. Both of
these weights were also significantly different between spring and autumn males, with the spring weights being lighter (Table 1).

A significant ($P < 0.001; r = 0.6925$) correlation existed between body weight and seminal vesicle weight and between body weight and testes weights ($P < 0.0001; r = 0.7982$). Paired testes weight and paired seminal vesicle weight were also significantly ($P < 0.001; r = 0.8614$) correlated (Table 4).

**Analysis of Serum Corticosterone Concentration:**

The mean serum corticosterone concentration value for autumn males was significantly ($P < 0.05$) lower than for spring and summer males (Table 1).

A significant ($P < 0.01; r = -0.4581$) negative correlation existed between paired seminal vesicle weight and corticosterone concentration. Similarly, paired testes weight and corticosterone concentration were also significantly ($P < 0.01; r = -0.5115$) negatively correlated (Table 4).

Serum corticosterone concentration for all males was also significantly ($P < 0.05; r = -0.3635$) negatively correlated with adrenal weight.
Histological Analysis of Testes

Since no gravimetric differences were found between animals collected from the same time period of different years, histological analysis was only performed on testes from animals collected in 1993-4.

The mean number of spermatids with acrosome formation for males from the summer (July 1993) was significantly lower than that for autumn (August 1993) and spring (March 1994). Reproductively suppressed males from the summer had a significantly lower tubular area comprised of cells than those animals from spring or autumn. Animals from the summer had a significantly lower mean Leydig cell area than animals from spring or autumn (Table 2).

For all males whose testes were examined histologically, a significant correlation was found between seminal vesicle weight and mean number of spermatids with acrosome formation ($P = 0.003; r = 0.7427$), mean tubular area ($P = 0.001; r = 0.7635$) and mean Leydig cell area ($P = 0.010; r = 0.6156$; Table 5). A significant correlation was also found between testis weight and mean number of spermatids with acrosome formation ($P < 0.001; r = 0.9580$), mean tubular area ($P < 0.001; r = 0.9600$), and mean Leydig cell area ($P < 0.001; r = 0.8230$, Table 5).
Comparisons between females from different time periods:

**Gravimetric Analysis:**

The female body weights were not significantly different between the three time periods (Table 3). There were no statistically significant differences between the adrenal weights of females from the three time periods (Table 3). Body weights for all females were significantly (P < 0.05) correlated with adrenal weights.

The mean paired ovary weights (1.86mg) and uterus weights (7.23mg) of summer females were significantly lighter than those of spring (ovary: 6.77mg; uterus: 60.80mg) females and autumn (ovary: 8.44mg; uterus: 36.39mg) females (Table 3). The mean paired uterus weight of spring females was also significantly greater from that of autumn females. (Table 3).

A significant (P < 0.05; r = 0.4265) correlation existed between body weight and ovary weight and between body weight and uterus weight (P < 0.05; r = 0.4956) for all females (Table 6). Similarly, a significant (P < 0.01; r = 0.7538) correlation existed between uterus weight and ovary weight (Table 6).
**Analysis of Serum Corticosterone Concentration:**

There were no statistically significant differences between the mean corticosterone concentrations of all three time periods (Table 3).
Autumn

| Time Period | Adrenal weight (mg) | Seminal vesicle weight (mg) | Testes weight (mg) | Body weight | (g) 
|-------------|---------------------|---------------------------|-------------------|------------|-------
| Autumn      | 13.6 ±1.09          | 22.9 ±2.99                | 22.0 ±1.22        | 497.4 ±23.43 | 18.2 |
| Summer      | 11.9 ±6.6           | 11.2 ±2.4                 | 33.6 ±2.8         | 20.6 ±10.7  | 11.7  |
| Spring      | 11.9 ±6.6           | 11.2 ±2.4                 | 33.6 ±2.8         | 20.6 ±10.7  | 11.7  |

Body or organ weights followed by the same letter are not significantly different (at p > 0.05 Turkey)

Table I. Mean body weight, paired testes weight, seminal vesicle weight,
Values followed by the same letter are not significantly different at $P \geq 0.05$ (Tukey).

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Leydig Cell Area (mm$^2$)</th>
<th>Seminiferous Tubule Area (mm$^2$)</th>
<th>Number of Spermatids</th>
<th>Seminiferous Tubule Area with Acrosomes (mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>143.8 $\pm$ 13.98</td>
<td>70 + 24.26</td>
<td>9.5 $\pm$ 6.2</td>
<td>320.1 $\pm$ 51.70</td>
</tr>
<tr>
<td>Summer</td>
<td>102.3 $\pm$ 24.26</td>
<td>60.9 $\pm$ 0.09</td>
<td>8.5 $\pm$ 5.5</td>
<td>93.8 $\pm$ 22.33</td>
</tr>
<tr>
<td>Spring</td>
<td>370.0 $\pm$ 13.98</td>
<td>1.1 $\pm$ 0.17</td>
<td>1.1 $\pm$ 0.1</td>
<td>370.0 $\pm$ 13.98</td>
</tr>
</tbody>
</table>

Table 2. Means $\pm$ SE for Leydig Cell Area, Seminiferous Tubule Area and Number of Spermatids.
Numbers in brackets refer to the number of animals that were not pregnant.
Numbers in parentheses refer to the number of animals evaluated.

Table 3. Mean body weight, paired ovaries weight, uterus weight, paired.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Autumn</th>
<th>Summer</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>105.32 ± 1.13</td>
<td>105.66 ± 1.07</td>
<td>101.00 ± 1.00</td>
</tr>
<tr>
<td>Adrenal weight (mg)</td>
<td>14.3 ± 7.6</td>
<td>11.0 ± 7.2</td>
<td>13.9 ± 6.8</td>
</tr>
<tr>
<td>Uterus weight (mg)</td>
<td>3.72 ± 0.4</td>
<td>2.42 ± 0.7</td>
<td>5.61 ± 0.6</td>
</tr>
<tr>
<td>Serum corticosterone concentration (ng/ml)</td>
<td>1.13 ± 0.0</td>
<td>1.23 ± 0.2</td>
<td>1.91 ± 0.1</td>
</tr>
</tbody>
</table>

* 0.05 (Tukey)

Body or organ weights followed by the same letter are not significantly different (at p > 0.05).

*Note: Autumn and summer females.

Adrenal weight and serum corticosterone concentration of spring.
<table>
<thead>
<tr>
<th>p</th>
<th>0.0363</th>
<th>Adrenal weight</th>
<th>Serum weight</th>
<th>Testes weight</th>
<th>Seminal Vessel weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>0.0381</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.0065</td>
<td>0.01</td>
<td>0.009</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Correlation coefficients (r) and Probabilities (p) for spring, summer, and autumn males.
<table>
<thead>
<tr>
<th>Weight</th>
<th>Semi-Para</th>
<th>Tests Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10 ≤ p ≤ 0.05</td>
<td>0 ≤ p ≤ 0.01</td>
<td>p = 0.050</td>
</tr>
<tr>
<td>0.05 ≤ p ≤ 0.10</td>
<td>0.01 ≤ p ≤ 0.005</td>
<td>p = 0.010</td>
</tr>
</tbody>
</table>

**Table 5.** Correlation coefficients (r) and Probabilities (p) for spring, summer, and autumn.
<table>
<thead>
<tr>
<th></th>
<th>Adrenal weight</th>
<th>Uterus weight</th>
<th>Ovary weight</th>
<th>Serum corticosterone concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Body weight</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( r = 0.4265 ) [ p \leq 0.05 ]</td>
<td></td>
</tr>
<tr>
<td>Spearman's Ranked Correlation Coefficients and P values</td>
<td>{ 0.7538 } [ p = 0.001 ]</td>
<td>{ 0.2663 } [ p = 0.179 ]</td>
<td>{ 0.0418 } [ p = 0.437 ]</td>
<td>{ 0.0444 } [ p = 0.18 ]</td>
</tr>
</tbody>
</table>
Figure 1. Standard curve and *P. leucopus* serum serial dilution curve for corticosterone radioimmunoassay
Figure 2. Mean body weights of males
Mean Body Weights of Males

Season

Body Weight (g)

Spring
Summer
Autumn
Figure 3. Mean adrenal weights of males
Mean Adrenal Weights of Males

Adrenal Weight (mg)

Season

Spring

Summer

Autumn
Figure 4. Mean seminal vesicle weights
Mean Seminal Vesicle Weights

Season

Seminal Vesicle Weight (mg)

Spring
Summer
Autumn
Figure 5. Mean testis weights
Mean Testis Weights

![Graph showing mean testis weights across seasons.](image-url)
Figure 6. Mean serum corticosterone concentration in males
Mean Serum Corticosterone Concentration in Males

Season

Spring
Summer
Autumn

Serum Corticosterone Concentration (ng/ml)
Figure 7. Mean Leydig cell area
Mean Leydig Cell Area

![Bar chart showing the mean Leydig cell area for different seasons. The chart indicates that the area is highest in Autumn, followed by Spring, and lowest in Summer.](chart.png)
Figure 8. Mean seminiferous tubule cellular area
Mean Seminiferous Tubule Cellular Area

Season

Seminiferous Tubule Cellular Area (mm)
Figure 9. Mean number of spermatids with acrosomes
Mean Number of Spermatids with Acrosomes

Number of Spermatids with Acrosomes

Season

Spring
Summer
Autumn
Figure 10. Mean body weights of females
Mean Body Weights of Females

- Spring
- Summer
- Autumn

Body Weight (g)
Figure 11. Mean ovary weights
Mean Ovary Weights

<table>
<thead>
<tr>
<th>Season</th>
<th>Ovary Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>7</td>
</tr>
<tr>
<td>Summer</td>
<td>2</td>
</tr>
<tr>
<td>Autumn</td>
<td>9</td>
</tr>
</tbody>
</table>
Figure 12. Mean uterus weights
Mean Uterus Weights

<table>
<thead>
<tr>
<th>Season</th>
<th>Uterus Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>~60</td>
</tr>
<tr>
<td>Summer</td>
<td>~10</td>
</tr>
<tr>
<td>Autumn</td>
<td>~40</td>
</tr>
</tbody>
</table>
Figure 13. Mean adrenal weights of females
Mean Adrenal Weights of Females

Adrenal Weight (mg)

Season

Spring  Summer  Autumn

Adrenal Weight (mg)
Figure 14. Mean serum corticosterone concentration in females
Mean Serum Corticosterone Concentration in Females

<table>
<thead>
<tr>
<th>Season</th>
<th>Serum Corticosterone Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>900</td>
</tr>
<tr>
<td>Summer</td>
<td>800</td>
</tr>
<tr>
<td>Autumn</td>
<td>700</td>
</tr>
</tbody>
</table>
DISCUSSION

Studies of reproductive suppression in natural populations of *Peromyscus leucopus noveboracensis* near Williamsburg, Virginia, indicate a yearly bimodal curve of reproductive function in which significant reproductive suppression occurs during the months of May, June and July compared with February-April and August-October (Terman, 1993). Other studies have demonstrated a mid-summer breeding hiatus in this species but some variation exists in the specific months during which suppression occurs (Burt, 1940; Jackson, 1952; Brown, 1964; Rintamaa et al., 1976; Batzli, 1977; Cornish and Bradshaw, 1978; Drickamer, 1978; Wolff, 1985a, 1986; Wolff and Durr, 1986; Cranford and Wolff, 1986; Krohne et al., 1988; Millar, 1989).

In the present study, animals collected from the field near Williamsburg during June and July were reproductively suppressed compared to those collected in March-April and August. Reproductive suppression was assessed by: (1) external characteristics (abdominal testes and imperforated vaginae with no evidence of lactation); and, (2) markedly reduced weights of the reproductive organs (testes and seminal vesicles; ovaries and uteri). Animals regarded as "developed" had scrotal testes or were pregnant, lactating or had open vaginae. Developed animals also had significantly larger reproductive organs than reproductively suppressed animals (Table 1).
Histological evaluation of the testes of males collected during 1993 and 1994 reflected the gravimetric differences between the summer males (reproductively suppressed) and those from spring and autumn (reproductively developed), and further indicated the drastic nature of this reproductive inhibition (Table 2).

**Organ Weight Comparisons Between Animals from Different Seasons:**

*Body and adrenal weight comparisons for males and females*

The mean body weight of summer males was significantly (P < 0.05) less than the mean weight for spring and autumn males (Table 1). No significant difference was found between the mean body weights of females from the different time periods (Table 2). For both males and females, body weight was significantly, positively correlated with reproductive organ weights (Tables 4, 5 & 6). However, only female body weights were significantly correlated with adrenal weights (Table 6).

The data reported here for the mean body weights of male and female *P. leucopus* taken from natural populations compare favorably with the respective values for male and female *P. leucopus* indicated by other studies of natural and laboratory populations. Ransone and Bradley (1992) found similar results for suppressed *P. leucopus* of laboratory populations. However, unlike the current study, they showed a highly significant (P < 0.001) difference between the mean body weight
of suppressed males versus reproductively proven males. Terman's (1993) study showed a significant \( P < 0.05 \) difference in mean body weight between suppressed males from May, June and July (1983-89) and developed males from August. Significant \( P < 0.05 \) differences were also found between developed animals from April and developed males from August and September, with the April animals having lower mean body weights. With regards to females, the results of the current study support those reported by Terman (1993) and Ransone and Bradley (1992) for female *P. leucopus* and suggest that, for females of this species, the reproductive curtailment may not be directly correlated with a decrease in body weight (Ransone and Bradley, 1992). For both males and females, body weight was significantly, positively (Tables 4 & 5) correlated with reproductive organ weights.

The mean absolute adrenal weight was not significantly different between males (Table 1) or females (Table 2) from spring, summer and autumn. These results correspond with those reported for reproductively proven and suppressed animals from laboratory populations of *P. leucopus* (Ransone and Bradley, 1992). However, suppressed animals from the field generally had larger absolute adrenal weights than those reared in the laboratory. The mean relative adrenal weight was not significantly different between males or females from all three seasons.

It is possible that adrenal zone area differences may exist between animals that have similar absolute adrenal weights. However, a previous study indicated
significantly (P < 0.05) larger total area, absolute cortex, zona glomerulosa and zona fasciculata plus reticularis in reproductively proven *P. leucopus* males and females and *P. maniculatus* males when compared with reproductively suppressed males and females (Rouleau, 1990). These results suggest that in reproductively suppressed males for both species and suppressed female *P. leucopus* there is no hypertrophy of the zona fasciculata of the adrenal gland. It is important to note that for both of these species, significantly (P < 0.05, *P. leucopus*; P < 0.001, *P. maniculatus*) higher levels of corticosterone have been reported for reproductively suppressed males selected from laboratory populations versus proven control males (Ransone and Bradley, 1992; Bradley and Terman, 1981a). Also, significantly (P < 0.001) higher levels of corticosterone have been reported for suppressed *P. maniculatus* laboratory females (Bradley and Terman, 1981a).

These findings and the results of the current study do not support the hypothesis that a stress-induced adrenal hypertrophy, per se, is fundamental to the reproductive suppression characteristic of both laboratory and field animals.

*Reproductive organ weights of suppressed males and females*

The mean paired testis weights and mean paired seminal vesicle weights of reproductively suppressed males were significantly (P < 0.05) less than the
corresponding weights in developed males. Similarly, the mean paired ovarian and mean paired uterine weights for suppressed females were significantly (P < 0.05) less than those for females that were reproductively developed. It is interesting to note that the range of variation in reproductive organ weights between suppressed and developed males was much greater than that between those of suppressed and developed females. For example, the mean paired testis weights and seminal vesicle weights for developed males were an average of nine times and thirty-seven times greater, respectively, than those of suppressed males. In developed females, the mean paired ovarian and uterine weights were an average of four times and seven times greater, respectively, than those of suppressed females. For both suppressed and developed males, a significant (P < 0.001; r = 0.8614) correlation was found between testis weight and seminal vesicle weight. A significant correlation (P = 0.001; r = 0.7538) was also shown between uterine and ovarian weights of both suppressed and developed females.

Although juveniles were excluded, as assessed by their pelage, the chronological age of the animals utilized in the current study is not known. Therefore, whether the significantly lower mean body and reproductive organ weights of suppressed animals from the summer are a result of loss of previously attained weight, perhaps due to various environmental conditions during the summer, or because young animals have not yet attained their full adult weight is not known. This information would be of
primary importance in understanding the mechanism of the observed reproductive suppression.

The results reported here for suppressed males and females are consistent with those from other studies of reproductive suppression in both natural (Terman, 1993) and laboratory populations of *P. leucopus* (Wolfe, 1981; Ransone and Bradley, 1992). It is interesting to note that the mean paired testis and seminal vesicle weights for reproductively suppressed males in this study were 40% less than the corresponding weights reported for suppressed animals from Ransone's and Bradley's study (1992) of laboratory populations of *P. leucopus*. Similarly, the suppressed animals from the current study had mean paired testis and seminal vesicle weights that were 54% and 78% less, respectively, than the corresponding weights in suppressed animals from Terman's (1993) seven year study of natural populations of *P. leucopus*.

Reproductively suppressed female *P. leucopus* from laboratory populations (Ransone and Bradley, 1992) also showed significantly smaller ovarian and uterine weights compared with their proven controls. Similarly, Terman (1993) showed a pattern of smaller ovarian and uterine weights in reproductively suppressed females from the field.

It is interesting to note that the mean paired ovarian and uterine weights of suppressed females from the current study were 59% and 33% less, respectively, than the corresponding weights reported by Terman (1993) for suppressed females. This
difference may be due to the fact that in the current study, only nine animals contributed to the mean paired ovarian weight and only eight animals contributed to the mean paired uterine weight. Whereas, in Terman's seven year study (1993), the mean paired ovarian weight was determined from fifty-six animals and thirty-five animals contributed to the mean uterine weight. Another factor that may account for this difference may be the time of collection. Although animals in both studies were collected during the same months, they may have been collected during different times in those particular months. For example, animals collected in early July still tend to be suppressed whereas those collected toward the middle or end of July tend to be reproductively developed.

Reproductively suppressed females from this study also had mean paired ovarian and uterine weights that were 58% and 39% less than the corresponding weights in suppressed population females from Ransone and Bradley's study (1992). The various environmental stresses and the different environmental conditions encountered by animals in the field compared with those in the laboratory may contribute to these differences. An additional factor that may contribute to this variability in reproductive organ weights between the current study and both Terman's (1993) study and Ransone and Bradley's (1992) study is the age of the animals collected. While in all three of these studies, the animals collected were classified as adults, the means of classification varied. Both, in the current study and Terman's (1993) study, adults were indicated
by pelage whereas in Ransone and Bradley's (1992) study, the date of birth was used to select adults. However, in the current study, whether an animal has just become an adult or has been one for some time is not known. Despite this variability, there is still a consistent pattern of lower reproductive organ weights in suppressed *P. leucopus* both from the laboratory and the field.

*Reproductive organ weights of developed males and females*

In the current study, a significant (*P* < 0.05) difference was also found between the mean paired testis and paired seminal vesicle weights of developed spring and autumn males. The mean paired testis weight of spring males was 32% less than that of autumn males (Table 1). Similarly, the mean paired seminal vesicle weight of spring males was 38% less than the corresponding weight in autumn males. The mean paired uterine weights for reproductively developed females from spring (*n* = 2) and autumn (*n* = 4) were also significantly different. However, there was no significant difference in the mean paired ovarian weight of females from spring (*n* = 9) compared with the corresponding weight in the autumn females (*n* = 9). This difference in uterine weights and the lack of a difference in ovarian weights between developed females could be due to differences in sample size.

The significant differences in reproductive organ weights between spring and autumn developed males are consistent with those reported by Terman (1993). In his
seven year study, developed males from the spring were shown to have a 45% lower mean paired testis weight compared to the same organ weight in autumn males. Males from the spring also had a mean paired seminal vesicle weight that was 54% lower than the corresponding weight in autumn males.

These variations in reproductive organ weights between spring and autumn males in natural populations of *P. leucopus* could be due to differences in food consumption and hence, metabolic differences that exist among animals from different seasons. However, no significant differences in body weight were found between spring and autumn males. It might be expected that differences in body weight would reflect possible differences in food consumption between animals collected from different seasons.

In addition, factors such as the level of activity and energy requirements, level of competition, predation, temperature, humidity and photoperiod could (both individually or together) influence the level of food consumption and subsequently, the growth of reproductive organs and the serum levels of both, corticosterone and thyroid hormone. It has been known for a long time that temperature has a direct influence on both thyroid activity and sexual development in male mice (Maqsood and Reineke, 1950). Therefore, assaying the thyroid levels and the percent body fat of animals from the three different seasons may provide more information about the seasonal variation in reproductive organ weights.
It is known that elevated levels of serum corticosterone cause decreases in serum thyroxine and T3 (Felig et al., 1995). Subnormal levels of T3 and consequently, lowered testosterone levels, both may cause a reduction in testis and seminal vesicle weights. Reduced thyroid hormone levels may also affect the ability of reproductive tissue to respond to gonadotropins (Turner and Bagnara, 1976).

**Testis histology in suppressed and developed males**

In the current study, histological evaluation of the testes from suppressed males revealed that the mean area of Leydig cells, mean seminiferous tubule cellular area and the mean number of spermatids with acrosome formation in suppressed males were significantly ($P < 0.05$) reduced compared with the corresponding values for reproductively developed males from spring and autumn. No significant differences were found between developed males with respect to the mean Leydig cell area, mean seminiferous tubule cellular area and mean number of spermatids with acrosome formation. For all males in this study, there is the expected significant positive correlation between each histological parameter and the respective animal's testis or seminal vesicle weights (Table 5).

The significantly reduced mean Leydig cell area of suppressed males suggests decreased levels of testosterone in the suppressed males. A reduction in testosterone as shown in reproductively suppressed population males of *P. maniculatus* (Bradley
and Terman, 1981b) may be partially responsible for reduced seminal vesicle and testis weights. The characteristic structure and endocrine function of the Leydig cells are sustained and controlled by the hypophyseal gonadotropic hormone LH, in the absence of which these cells undergo severe atrophy and stop producing testosterone. Leydig cells that have completely atrophied show reduced amounts of cytoplasm (Weiss and Greep, 1977). These findings suggest a possible absence or reduction of LH as an explanation for the atrophy of Leydig cells in reproductively suppressed males from this study. However, Bradley and Terman (1981c) reported no significant differences in mean serum LH concentrations between suppressed males from a laboratory population of *P. maniculatus* and the proven control males. This finding was in contrast to the significant differences between population males and their controls with respect to the weights and histological characteristics of their testes. In fact, their study indicated a significant negative correlation (P < 0.05; r = -0.661) between the testis weight of suppressed population males and the serum LH concentrations which may have been due to a lack of testosterone feedback inhibition on the pituitary (Badger et al, 1978). These results continue to suggest that low titers of LH are probably not the primary causal factor of this inhibition which may be due to the more direct involvement of other hormones.

The significantly reduced mean seminiferous tubule cellular area and mean number of spermatids with acrosome formation in suppressed males compared with
developed males indicates impaired spermatogenesis and spermiogenesis, which may, in part, be due to a decreased levels of testosterone. Decreased spermatogenesis was also found in reproductively suppressed male *P. maniculatus* from laboratory populations (Bradley and Terman, 1981c). These results, together with the results from the gravimetric analysis and the animals' nonscrotal testis condition, provide additional evidence for the drastic nature of reproductive suppression in summer males from natural populations of *P. leucopus*.

**Serum Corticosterone Concentration Differences between Animals from Different Seasons:**

The mean serum corticosterone concentration for reproductively developed males from autumn was significantly (P < 0.05) lower (less than half) than the corresponding value for developed males from spring as well as suppressed males from summer. There was no significant difference in mean serum corticosterone concentration between developed males from spring and suppressed males from summer. There were no significant differences in the mean serum corticosterone concentrations between developed and suppressed females in any comparison or between developed females from spring or autumn.
Serum corticosterone differences between laboratory and field animals

While reproductively developed males from laboratory populations have been shown to have a significantly lower mean corticosterone concentration compared with reproductively suppressed males (Ransone and Bradley, 1992), this was not observed in natural populations. In the current study, developed males from the spring had an even higher mean corticosterone concentration than that of suppressed males from the summer. It is interesting to note that the mean serum corticosterone concentration of developed spring males from this study was more than five times greater than that of proven control males from the laboratory (Ransone and Bradley, 1992). Likewise, the mean serum corticosterone concentration of developed males from autumn was more than twice the concentration of proven control males from the laboratory (Ransone and Bradley, 1992). In the current study, developed females from the spring had a mean serum corticosterone concentration that was more than four times greater than the corresponding value for proven control females from the laboratory (Ransone and Bradley, 1992). Similarly, the mean serum corticosterone concentration of developed females from autumn was more than twice the concentration of proven control females from Ransone and Bradley's (1992) study.

Ransone and Bradley (1992) found the mean serum corticosterone concentration of reproductively suppressed *P. leucopus* males to be significantly (P < 0.05) higher than
proven control males. In the current study, the mean serum corticosterone concentration of suppressed males (683.57 ng/ml) was more than three times greater than the corresponding concentration (201.9 ng/ml) for suppressed males from the laboratory. For females from this study, the mean serum corticosterone concentration (675.00 ng/ml) of suppressed females was also more than three times greater than the mean serum corticosterone concentration (168.5 ng/ml) of suppressed females from the laboratory population (Ransone and Bradley, 1992). These differences may be due to the larger absolute adrenal weights of suppressed field *P. leucopus* in contrast with those of suppressed animals from laboratory populations. These comparisons must be made in recognition of possible age differences between animals selected from laboratory populations compared with those captured at random from the field.

The differences between laboratory population and field animals with respect to corticosterone concentration could be attributed to varied stresses and environmental pressures in the field as opposed to those encountered in a laboratory environment. In Ransone and Bradley's (1992) study, all the animals used were reared in the laboratory and remained there for the duration of the study whereas in the current study, the animals were captured from the field and brought to the laboratory where they were subjected to a 30-40 hour quarantine period just prior to being sacrificed. Therefore, their exposure to a novel environment and the stress of captivity probably contributed to a higher level of serum corticosterone compared with animals raised in the
laboratory. It is important to note, however, that possible existing age differences between animals chosen from laboratory populations compared with those captured from the field may obscure the comparisons between laboratory and field animals. The various criteria for selection of animals, for example, the degree of reproductive inhibition, may also render these comparisons as inappropriate.

Under field conditions it is impossible to obtain basal levels of hormone concentration for each animal since the acute stress due to capture and handling may conceal possible actual differences in corticosterone concentration. In the current study, although the stress due to capture and handling was standardized (all animals were handled to the same extent) the significantly (P < 0.05) higher mean serum corticosterone concentrations for developed spring (793.2 ± 76.00) and suppressed summer males (683.6 ± 82.62) compared with developed autumn males (303.5 ± 65.73) may actually be due, in part, to differences in the original pre-capture basal level of serum corticosterone.

In the current study, the total corticosterone concentration was assayed. Circulating corticosteroids are firmly bound to a plasma carrier protein known as corticosteroid-binding globulin (CBG) and only about 5-10% of the corticosteroids are usually free and hence, biologically active (McDonald et al, 1986; McDonald et al 1988; Rosner 1990; Boonstra and Boag, 1992). Measuring the total corticosteroid concentration may not provide as accurate of an indication of the physiologically
effective hormone concentration as an estimate of the total and free corticosterone concentrations. If the reproductively suppressed males from the summer actually have a higher concentration of free corticosterone compared to the developed males from spring and autumn, that fact could account for their reproductive suppression. It is also possible that the developed males from the spring, which had an even higher mean total corticosteroid concentration than suppressed males, actually had a high concentration of CBG-bound corticosterone and hence a lower effective corticosterone concentration. This could explain why they are reproductively developed despite having high total corticosterone levels. The fact that a significant \((P < 0.05; r = -0.3635)\) negative correlation between corticosterone concentration and adrenal weight existed for all males in the current study may support the presence of a lower effective corticosterone concentration.

*Serum corticosterone concentration in relation to reproductive development*

For all males in the current study, a significant \((P = 0.007; r = -0.4581)\) negative correlation was found between paired seminal vesicle weight and corticosterone concentration. Similarly, paired testes weight and corticosterone concentration were significantly and negatively correlated \((P = 0.003; r = -0.5115)\). The significant negative correlation between serum corticosterone concentration and reproductive organ weights in males as opposed to the lack of correlation in females may suggest a greater
sensitivity of males to corticosterone concentration and perhaps, to stress-inducing factors in the field.

It has been shown that high levels of glucocorticoids are associated with a decreased synthesis and secretion of testosterone (Doerr and Pirke, 1975; Schaison et al., 1978; Nim et al., 1978; Magrini et al., 1978). It is not known exactly how glucocorticoids mediate their effects on the level of serum testosterone, however the effects of glucocorticoids on pituitary LH secretion have been investigated. Schaison et al. (1978) and McKenna et al. (1979) showed no reduction in plasma LH due to high levels of glucocorticoids. Reports of glucocorticoid interference with testicular DNA and protein synthesis (Evain et al., 1976; Saez et al., 1977) and with cholesterol side chain cleavage (Saez et al., 1977) provide evidence for a direct inhibiting effect on the testis by glucocorticoids.

This study does not provide evidence for an association between elevated levels of serum corticosterone and the reproductive suppression observed in natural populations of P. leucopus. Although both gravimetric and histological characteristics were markedly different between suppressed and developed males, an elevated serum corticosterone concentration did not prove to be unique to suppressed males. The results of this study do, however, provide further evidence for the trend of consistently elevated levels of serum corticosterone reported for reproductively suppressed P. leucopus.
Future Directions

The range of variation in mean serum corticosterone concentrations of laboratory versus field *P. leucopus* revealed in this study presents some uncertainty as to whether conditions in the field are very stressful and contribute to the higher mean corticosterone concentrations measured in *P. leucopus* from natural populations, versus the alternative that the stress of capture primarily accounts for the increased levels of serum corticosterone in field animals compared with those from the laboratory. Future investigations must be designed so that the animals from the field are sampled in a way so as to indicate their field versus capture state.

Further studies examining both the individual and combined contributions of food consumption, torpor, temperature, photoperiod and social interaction on reproductive curtailment in *P. leucopus* must be conducted. The role of corticosterone with respect to its effects on other hormone systems must also be investigated. Biochemical studies concerned with assaying levels of particular enzymes that may directly or indirectly be affected by serum corticosterone levels via metabolic inactivation could also be effective in eliminating the problems inherent in measuring the highly labile serum corticosterone concentration.

In addition, a long-term field study following the development of *P. leucopus*, perhaps through repeated capture, release and recapture of animals could help to
determine whether the suppressed animals from the summer are undergoing a loss of
development or a failure to develop.

A molecular-based study designed to explore a relatively more stable protein-
receptor expression as opposed to assaying the more labile serum hormone
concentrations may prove to be invaluable in understanding a possible HPA axis
process which may be contributing to the highly elevated serum corticosterone levels
reported for developed spring and suppressed summer male *P. leucopus* from the
current study.
## APPENDIX A

### MASTER DATA FILE

<table>
<thead>
<tr>
<th>Month/</th>
<th>Animal No.</th>
<th>Sex</th>
<th>Status</th>
<th>Body (g)</th>
<th>Adrenal (mg)</th>
<th>Testes (mg)</th>
<th>S.V. (mg)</th>
<th>U.H. (mg)</th>
<th>Ovaries (mg)</th>
<th>Conc. (mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRIL '92</td>
<td>219</td>
<td>F</td>
<td>perf.</td>
<td>21.4</td>
<td>13.45</td>
<td>scarred</td>
<td>6.45</td>
<td>415</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>F</td>
<td>?</td>
<td>17.4</td>
<td>8.75</td>
<td>scarred</td>
<td>7.30</td>
<td>640</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>373</td>
<td>F</td>
<td>?</td>
<td>18.8</td>
<td>15.70</td>
<td>scarred</td>
<td>6.20</td>
<td>1135</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>378</td>
<td>F</td>
<td>?</td>
<td>19.2</td>
<td>24.35</td>
<td>scarred</td>
<td>8.10</td>
<td>625</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>M</td>
<td>tns</td>
<td>18.5</td>
<td>11.70</td>
<td>150.95</td>
<td>24.35</td>
<td>1240</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>255</td>
<td>M</td>
<td>tns</td>
<td>24.4</td>
<td>10.10</td>
<td>348.75</td>
<td>223.57</td>
<td>1135</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>211</td>
<td>M</td>
<td>tns</td>
<td>20.4</td>
<td>11.35</td>
<td>336.30</td>
<td>199.65</td>
<td>640</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>264</td>
<td>M</td>
<td>tns</td>
<td>20.0</td>
<td>9.85</td>
<td>281.40</td>
<td>137.85</td>
<td>950</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>M</td>
<td>?</td>
<td>23.0</td>
<td>14.55</td>
<td>414.95</td>
<td>112.05</td>
<td>715</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>413</td>
<td>M</td>
<td>tns</td>
<td>19.6</td>
<td>13.30</td>
<td>415.00</td>
<td>215.50</td>
<td>850</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June '92</td>
<td>485</td>
<td>F</td>
<td>imp.</td>
<td>17.7</td>
<td>10.70</td>
<td></td>
<td>3.20</td>
<td>1.25</td>
<td>760</td>
<td></td>
</tr>
<tr>
<td></td>
<td>537</td>
<td>F</td>
<td>imp.</td>
<td>18.1</td>
<td>16.30</td>
<td></td>
<td>3.30</td>
<td>1.20</td>
<td>1015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>540</td>
<td>F</td>
<td>imp.</td>
<td>21.3</td>
<td>12.45</td>
<td></td>
<td>3.40</td>
<td>1.15</td>
<td>1090</td>
<td></td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>F</td>
<td>imp.</td>
<td>15.9</td>
<td>8.55</td>
<td></td>
<td>4.10</td>
<td>1.75</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>M</td>
<td>tns</td>
<td>15.6</td>
<td>12.50</td>
<td>54.25</td>
<td>8.90</td>
<td>885</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>576</td>
<td>M</td>
<td>tns</td>
<td>18.0</td>
<td>16.10</td>
<td>47.15</td>
<td>3.45</td>
<td>850</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>582</td>
<td>M</td>
<td>tns</td>
<td>16.4</td>
<td>14.60</td>
<td>38.80</td>
<td>4.10</td>
<td>540</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aug. '92</td>
<td>433</td>
<td>F</td>
<td>perf.</td>
<td>17.3</td>
<td>12.25</td>
<td></td>
<td>30.95</td>
<td>10.40</td>
<td>515</td>
<td></td>
</tr>
<tr>
<td></td>
<td>639</td>
<td>F</td>
<td>?</td>
<td>20.4</td>
<td>12.40</td>
<td></td>
<td>40.50</td>
<td>17.80</td>
<td>810</td>
<td></td>
</tr>
<tr>
<td></td>
<td>640</td>
<td>F</td>
<td>p/perf.</td>
<td>18.1</td>
<td>13.30</td>
<td></td>
<td>5.60</td>
<td>1090</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>614</td>
<td>F</td>
<td>p/perf.</td>
<td>17.8</td>
<td>12.30</td>
<td></td>
<td>5.90</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>M</td>
<td>?</td>
<td>22.6</td>
<td>20.20</td>
<td>581.60</td>
<td>230.20</td>
<td>460</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>634</td>
<td>M</td>
<td>?</td>
<td>21.1</td>
<td>10.60</td>
<td>435.40</td>
<td>159.00</td>
<td>285</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>635</td>
<td>M</td>
<td>?</td>
<td>21.0</td>
<td>12.10</td>
<td>588.60</td>
<td>506.90</td>
<td>540</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>588</td>
<td>M</td>
<td>tns</td>
<td>21.6</td>
<td>10.50</td>
<td>481.80</td>
<td>255.50</td>
<td>515</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>658</td>
<td>M</td>
<td>tns</td>
<td>21.6</td>
<td>10.40</td>
<td>488.90</td>
<td>242.30</td>
<td>575</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July '93</td>
<td>35</td>
<td>F</td>
<td>imp.</td>
<td>15.2</td>
<td>15.00</td>
<td></td>
<td>3.05</td>
<td>0.80</td>
<td>675</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>F</td>
<td>imp.</td>
<td>14.1</td>
<td>7.05</td>
<td></td>
<td>4.30</td>
<td>0.75</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>F</td>
<td>imp/sex</td>
<td>20.4</td>
<td>10.20</td>
<td></td>
<td>20.15</td>
<td>5.75</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>F</td>
<td>imp.</td>
<td>17.8</td>
<td>7.30</td>
<td></td>
<td>16.15</td>
<td>1.15</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>F</td>
<td>imp/ffis</td>
<td>13.8</td>
<td>11.25</td>
<td></td>
<td>3.60</td>
<td>0.65</td>
<td>575</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>M</td>
<td>tns</td>
<td>19.0</td>
<td>13.75</td>
<td>88.55</td>
<td>8.70</td>
<td>370</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>M</td>
<td>tns</td>
<td>20.0</td>
<td>9.05</td>
<td>30.95</td>
<td>5.75</td>
<td>950</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>M</td>
<td>tns</td>
<td>14.4</td>
<td>10.25</td>
<td>16.35</td>
<td>7.50</td>
<td>515</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>M</td>
<td>tns</td>
<td>15.2</td>
<td>7.10</td>
<td>77.95</td>
<td>7.50</td>
<td>675</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aug. '93</td>
<td>491</td>
<td>F</td>
<td>perf.</td>
<td>21.0</td>
<td>22.10</td>
<td></td>
<td>44.75</td>
<td>8.55</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td></td>
<td>549</td>
<td>F</td>
<td>imp/ip</td>
<td>25.5</td>
<td>13.75</td>
<td></td>
<td>7.45</td>
<td>485</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>761</td>
<td>F</td>
<td>p/perf/sex</td>
<td>25.3</td>
<td>18.70</td>
<td></td>
<td>29.35</td>
<td>8.05</td>
<td>605</td>
<td></td>
</tr>
<tr>
<td></td>
<td>850</td>
<td>F</td>
<td>perf.</td>
<td>16.1</td>
<td>11.55</td>
<td></td>
<td>4.95</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>849</td>
<td>F</td>
<td>perf/sex</td>
<td>19.0</td>
<td>15.65</td>
<td></td>
<td>265</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>807</td>
<td>M</td>
<td>tns</td>
<td>21.6</td>
<td>11.70</td>
<td>591.85</td>
<td>217.80</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>818</td>
<td>M</td>
<td>tns</td>
<td>22.4</td>
<td>17.80</td>
<td>526.00</td>
<td>241.75</td>
<td>850</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>822</td>
<td>M</td>
<td>tns</td>
<td>21.3</td>
<td>13.25</td>
<td>350.45</td>
<td>203.15</td>
<td>195</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>M</td>
<td>tns</td>
<td>20.6</td>
<td>16.85</td>
<td>455.50</td>
<td>248.95</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>tns</td>
<td>21.3</td>
<td>12.65</td>
<td>493.45</td>
<td>251.80</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>March '94</td>
<td>951</td>
<td>F</td>
<td>perf.</td>
<td>16.1</td>
<td>7.05</td>
<td></td>
<td>66.3</td>
<td>9.2</td>
<td>715</td>
<td></td>
</tr>
<tr>
<td></td>
<td>949</td>
<td>F</td>
<td>p/perf.</td>
<td>21.4</td>
<td>11.80</td>
<td></td>
<td>9.25</td>
<td>885</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>902</td>
<td>F</td>
<td>imp.</td>
<td>20.7</td>
<td>15.20</td>
<td></td>
<td>55.3</td>
<td>4.45</td>
<td>950</td>
<td></td>
</tr>
<tr>
<td></td>
<td>945</td>
<td>F</td>
<td>imp/sex</td>
<td>19.5</td>
<td>18.30</td>
<td></td>
<td>8.4</td>
<td>810</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>870</td>
<td>F</td>
<td>p/perf.</td>
<td>17.5</td>
<td>10.20</td>
<td></td>
<td>1.55</td>
<td>1135</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>918</td>
<td>M</td>
<td>tns</td>
<td>20.3</td>
<td>11.40</td>
<td>358.85</td>
<td>239.80</td>
<td>370</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>942</td>
<td>M</td>
<td>tns</td>
<td>21.6</td>
<td>14.85</td>
<td>475.25</td>
<td>233.90</td>
<td>760</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>943</td>
<td>M</td>
<td>tns</td>
<td>16.0</td>
<td>7.00</td>
<td>87.75</td>
<td>6.45</td>
<td>675</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>887</td>
<td>M</td>
<td>tns</td>
<td>19.9</td>
<td>8.45</td>
<td>316.15</td>
<td>129.55</td>
<td>850</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>905</td>
<td>M</td>
<td>tns</td>
<td>22.8</td>
<td>11.10</td>
<td>513.10</td>
<td>222.45</td>
<td>540</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
LITERATURE CITED


VITA

SAREENA KHOSLA