The Energetics of Seasonal Reproductive Inhibition in White-Footed Mice

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THE ENERGETICS OF SEASONAL REPRODUCTIVE INHIBITION
IN WHITE-FOOTED MICE

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

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

by
Michelle E. Rightler
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APPROVAL SHEET

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

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Approved, May 2001

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ iv  
LIST OF TABLES .................................................................................................................. v  
LIST OF FIGURES ............................................................................................................... vi  
ABSTRACT ............................................................................................................................. viii  
INDIVIDUAL VARIATION IN THE ENERGETICS OF SEASONAL BREEDING IN \textit{PEROMYSCUS LEUCOPUS} ................................................................. 2  
A NOTE ON THE NEUROENDOCRINE MECHANISMS OF SEASONAL REPRODUCTIVE ENERGETICS .......................................................... 13  
METABOLISM, ACTIVITY AND FOOD CONSUMPTION ................................................. 21  
ACTIVITY PATTERNS, BODY COMPOSITION, AND DIGESTIVE EFFICIENCY .................. 48  
APPENDIX A ....................................................................................................................... 73  
APPENDIX B ....................................................................................................................... 76  
APPENDIX C ....................................................................................................................... 78  
LITERATURE CITED ......................................................................................................... 79  
VITA ..................................................................................................................................... 95
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LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Seminal Vesicle Weights (including fluid contents) for Castrate Males with Testosterone Implants (Mean ± SEM)</td>
<td>39</td>
</tr>
<tr>
<td>2. Comparison of the Seminal Vesicle Weights (including Fluid Contents) for Gonad-Intact Males and Castrate Males with Testosterone Implants (Mean ± SEM)</td>
<td>46</td>
</tr>
<tr>
<td>3. Mean Number of Fat Pads in Short Days and Long Days</td>
<td>58</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Interactions of Neuroendocrine Glands and Their Products</td>
</tr>
<tr>
<td>2</td>
<td>Body Weight of Gonad Intact Males</td>
</tr>
<tr>
<td>3</td>
<td>Mass-Specific Metabolic Rate of Gonad Intact Males</td>
</tr>
<tr>
<td>4</td>
<td>Food Consumption of Gonad-Intact Males Normalized with Respect to Body Weight</td>
</tr>
<tr>
<td>5</td>
<td>Time Spent Resting by Gonad-Intact Males</td>
</tr>
<tr>
<td>6</td>
<td>Body Weight of Castrate Males with Testosterone Implants</td>
</tr>
<tr>
<td>7</td>
<td>Mass-Specific Metabolic Rate of Castrate Males with Testosterone Implants</td>
</tr>
<tr>
<td>8</td>
<td>Food Consumption of Castrate Males with Testosterone Implants Normalized with Respect to Body Weight</td>
</tr>
<tr>
<td>9</td>
<td>Time Spent Resting by Castrate Males with Testosterone Implants</td>
</tr>
<tr>
<td>10</td>
<td>Videomex and Activity Data Collection Apparatus</td>
</tr>
<tr>
<td>11</td>
<td>Percent Body Water of Gonad-Intact Mice in Different Photoperiods</td>
</tr>
<tr>
<td>12</td>
<td>Body Weight of Gonad-Intact Mice in Different Photoperiods</td>
</tr>
<tr>
<td>13</td>
<td>Food Consumption of Gonad-Intact Mice</td>
</tr>
<tr>
<td>14</td>
<td>Time Spent Performing Stereotypic Movements</td>
</tr>
<tr>
<td>15</td>
<td>Time Spent Resting</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Total Ambulatory Time</td>
<td>62</td>
</tr>
<tr>
<td>17</td>
<td>Total Distance Traveled by Gonad-Intact Mice</td>
<td>63</td>
</tr>
<tr>
<td>18</td>
<td>Daily Feces Production by Gonad-Intact Mice</td>
<td>64</td>
</tr>
<tr>
<td>19</td>
<td>Fecal Pellet Size of Gonad-Intact Mice</td>
<td>65</td>
</tr>
<tr>
<td>20</td>
<td>Summary of Results</td>
<td>72</td>
</tr>
</tbody>
</table>
ABSTRACT

Small temperate-zone rodents use photoperiod as the primary predictor of the onset of winter. Variation in metabolic rate, body weight and food intake in response to photoperiod is common in temperate-zone mammals. These physiological modifications are presumed to prepare mammals for seasonal changes in temperature and food availability.

This study used white-footed mice, *Peromyscus leucopus*, to examine the differing metabolic responses to short and long photoperiods as they relate to their reproductive response to short photoperiods. Mice that do not maintain fertility in wintertime, Responsives, were found to eat less, run more, have a lower body weight and have a lower metabolic rate than mice that do maintain fertility in wintertime, Nonresponsives.

Exposure to short days did not affect Responsive mice. Their metabolic rates, body weights, activity levels, fat stores, and food intake remained the same. Nonresponsive mice, when exposed to short photoperiods, gained weight, ate more, lowered their activity levels and increased their metabolic rates. This study clarifies the increased energetic costs of maintaining fertility in wintertime and demonstrates the advantage of shutting down reproductive effort in wintertime for even a very short-lived mammal.
THE ENERGETICS OF SEASONAL REPRODUCTIVE INHIBITION
IN WHITE-FOOTED MICE
INDIVIDUAL VARIATION IN THE ENERGETICS OF
SEASONAL BREEDING IN *PEROMYSCUS LEUCOPUS*

**Introduction:**

Metabolic rates can vary from season to season in rodents. These seasonal changes in metabolism are often accompanied by other physiological modifications associated with winter declines in food availability and temperature. Winter conditions have been shown to cause fluctuations in the population structure and reproduction of some temperate zone rodents (Scheffer 1924, Boyd 1986). In winter months, most temperate zone rodent populations decline rapidly and many of the adults cease reproductive effort (Desjardins, et al. 1986). Even in winter, however, some young are born, although rarely during harsh winters (Moffatt, et al. 1993). Temperate zone rodents rely on photoperiod as the most reliable predictor of winter onset (Gaston and Menaker 1967, Lynch and Gendler 1980, Desjardins, et al. 1986, Blank, et al. 1994).

In addition to seasonal reproductive adjustments, temperate zone rodents make metabolic adjustments to prepare for winter conditions. Metabolic and associated thermoregulatory needs of small temperate zone rodents are increased in wintertime due to their high rate of heat loss because of their high surface-to-volume ratio. This increased wintertime metabolic cost can make up a large proportion of an individual rodent’s energy budget and, therefore, reproduction needs may be overridden (Blank and Ruf 1992).
This study will focus on these wintertime energy budgets by examining the individual variation in the metabolic characteristics of white-footed mice. This study will also examine how these characteristics change with season. The metabolic characteristics that will be examined are: 1) how reproductive responsiveness to short winter photoperiods relates to metabolic rate, 2) how food consumption is affected by photoperiod, 3) how body composition relates to season and reproductive responsiveness to short days, and 4) how digestive efficiency is affected by season and reproductive responsiveness.

Investigation of the question of how and why small mammals stop reproductive efforts during the winter began when the phenomenon of breeding cessation in populations of temperate zone rodents was first identified (Scheffer 1924), and has continued for approximately the last 70 years. Questions added to those on the mechanisms of seasonality have included the examination of the individual variation of seasonal response within a population (Reiter 1980). In order to better examine individual variation in the seasonal reproduction of temperate zone rodents, there has been a movement away from studying highly inbred strains of lab mice and rats and a greater emphasis has been placed on the study of wild, outbred species (Reiter 1980). Individual variation is an important factor when considering the evolutionary implications of a behavioral or physiological trait (Teskey, et al. 1998). The study of individual variation in a behavioral or physiological trait helps toward understanding how animals react to changes in their environment, such as sudden cold snaps or droughts. Even though these environmental changes may kill some individuals of a population, a large degree of individual variation may allow some individuals to survive and reproduce.
Energetics of Small Mammals:

Adult small mammals have two major types of energetic expenditures to which they may allocate energy brought in as food: maintenance of homeothermy and reproduction. Within each major type of energy use, there are several smaller metabolic allocation compartments that are interconnected. The amount of food energy allocated to each compartment may change with season because there may be seasonal variability in the amount of available food (Blank and Desjardins 1985). A dependable food supply is vital to small mammals during the winter. Even in moderately low winter ambient temperatures, fat stores aid a mouse’s survival only briefly (Bronson 1987). If adjustments are not made to an animal’s metabolic rates, activity, food caching behavior, and reproductive strategies, reduced food availability may result in death (Heldmaier and Steinlechner 1981, Bronson 1987, Stamper and Dark 1996, Zuercher, et al. 1999).

Stress Hormones and Behavior: Stressors can affect how a small mammal reacts to its environment. Low ambient temperature can act as a particularly severe stressor for a small, temperate zone rodent because of rapid heat loss due to their high surface-to-volume ratio. This heat loss can increase the need for food, which may increase behaviors such as food caching or torpor. Aggressive interactions with conspecifics can also act as a form of behavioral stressor; small rodents may suffer stress when they become aggressive as they seek mates. Reproductive activities are also a stressor. A female’s need for additional food and additional nesting material increase during pregnancy and lactation (Bronson, et al. 1991). All of these stressors
can stimulate production of the two major stress hormones, epinephrine and corticosterone (Ganong 1997).

Epinephrine is produced by the adrenal glands located cranial to the kidneys. This hormone increases heart rate and releases stored glycogen into the blood stream as glucose in order to provide quick energy. High epinephrine levels will also decrease blood flow to extremities and divert it to the body core (Sutherland 1972). This means that increased levels of epinephrine temporarily increase the metabolic rate of a rodent.

Corticosterone, a glucocorticoid produced in the adrenal cortex, can increase heart rate, reduce inflammation, delay healing, and affect nutrient absorption. In females, chronically high corticosterone levels may induce litter abortion. High corticosterone concentrations decrease metabolic rates by diverting blood away from the abdominal organs and increase circulation to the large muscles and the heart (Sutherland 1972). Consistently high levels of corticosterone may be caused by social stresses.

Behavioral Changes and Energy Savings: As winter approaches, temperate zone mice, such as *P. maniculatus*, change their behavior from being solitary to more social in nature (Blank and Ruf 1992). During the summer, most interactions among conspecifics are confined to breeding or rearing litters (Blank and Ruf 1992). As winter approaches, these normally aggressive mice will begin to huddle together in burrows or nests to conserve body heat (Blank and Ruf 1992, Vogt and Lynch 1982). Huddling to reduce heat loss requires lowered aggression among conspecific *P. leucopus* (Vogt and Lynch 1982). This huddling behavior may allow them to survive on smaller amounts of
food because less energy is required to maintain homeothermy (Vogt and Lynch 1982). Aggression is reduced during group huddling partly due to the reduction in testosterone titers in reproductively responsive *P. maniculatus* males (Blank and Ruf 1992). The presence of large numbers of conspecifics may also decrease or completely halt reproduction in *Peromyscus* species (Demas and Nelson 1998, Staubs and Bradley 1998).

**Individual Variation in Wintertime Fertility:** Many rodents halt all reproductive activities during the winter months. Winter daylengths rather than low ambient temperatures induce the cessation of reproductive activities in temperate zone rodents (Ellis and Turek 1979, Lynch and Gendler 1980, Wade and Bartness 1984, Nelson 1985, Vitale, et al. 1985, Feist and Feist 1986, Blank and Ruf 1992). However, low ambient temperatures do have some effect on reproductive activities of mice (as reviewed in Bronson 1989). In some populations of temperate zone rodents, there are small numbers of animals that do not halt their reproductive activities (henceforth termed "nonresponsive") (Scheffer 1924, Lynch and Gendler 1980, Zegers and Merritt 1988, Heideman and Bronson 1991, Heideman, et al. 1999b). This reproductive responsiveness to shortened daylengths has been demonstrated to be at least partly heritable in every species that has been tested for this response (Desjardins, et al 1986, Heideman and Bronson 1991, Kerbeshian, et al. 1994, Teskey, et al. 1998, Heideman, et al. 1999b).

**Seasonal Changes in Metabolism of Some Small Rodents**
Individual seasonal metabolic adjustments are necessary for the survival of temperate zone rodents in wintertime (Rosenmann, et al. 1975, Zegers and Merritt 1988, Blank, et al. 1994, Stamper and Dark 1996, Heldmaier, et al. 1999). The degree of wintertime metabolic adjustment made by a temperate zone rodent is associated with its reproductive status (Nelson, et al. 1992). Seasonal changes in reproductive status affect not only the maximum number of offspring that can be born in any one season, but also the degree of individual survivorship. Adaptations for wintertime survival for a temperate zone rodent should include metabolic adjustments in response to the shortened photoperiod cue. These metabolic adjustments would allow the individual to better cope with the effects of bad weather, colder temperatures, and potential lack of food.

The rest of this chapter will review the literature discussing variation in seasonal metabolic characteristics in three taxa of rodents. Deer mice, *Peromyscus maniculatus* and white-footed mice, *Peromyscus leucopus* will be examined together due to their close relationship and their employment of similar reproductive strategies. Voles, *Microtus* spp. and *Clethrionomys rutilus*, will be examined as a single group because the two genera are closely related in both their habitat preferences and responses to seasonal changes. Hamsters, *Phodopus sungorus* and *Mesocricetus auratus* will be examined as a single group because the two genera exhibit similar characteristics in seasonal physiological changes.

*Seasonal Changes in Fertility, Body Composition and Food Intake in Deer Mice, *P. maniculatus*, and White-footed Mice, *P. leucopus*: * Metabolic adjustments made by *P. maniculatus* and *P. leucopus*, in preparation for winter include the elevation
of the basal metabolic rate (BMR), increased non-shivering thermogenesis (NST), decreased body mass, and decreased circulating sex hormones caused by photoresponsive decrease in gonad size or cessation of their reproductive activity (Howard 1951, Barry 1976, Lynch and Gendler 1980, Millar and Schieck 1986, Blank, et al. 1988, Nelson, et al. 1992, Moffatt, et al. 1993, Blank, et al. 1994). The relationship among wintertime photoperiod, ambient temperature, and decreases in gonad size was investigated by Blank and Ruf (1992) in *P. maniculatus*. A distinct difference in absolute minimum cold tolerance was observed between nonresponsive and responsive phenotypes, with reproductively responsive males showing an increased tolerance for extreme cold by almost 20°C. Differing degrees of metabolic adjustments made by both phenotypes of *P. maniculatus* are triggered by the same factors that cause reduction of gonad size (Blank and Ruf 1992).

Daylength is the primary cue for seasonal adjustments in temperate zone rodents (Gaston and Menaker 1967, Lynch and Gendler 1980, Desjardins, et al. 1986, Blank, et al. 1994). Deer mice, *P. maniculatus*, decrease their body weight in response to short photoperiods; however, reproductively responsive males lost significantly more weight than reproductively nonresponsive males (Blank, et al. 1994). The body weight decreases seen in *Peromyscus* may be partly induced by decreased circulating testosterone levels, but are also partly dependent on melatonin secretion by the pineal gland (Vitale, et al. 1985). Weight loss also occurs prior to any significant decrease in food consumption (Dark, et al. 1983, Blank, et al 1994). Nelson, et al. (1992) demonstrated that in short days the caloric intake necessary to maintain reproductively active gonads increases, probably because of the increased costs of homeothermy in wintertime. Because reproductively responsive mice do not have the increased
metabolic cost of maintaining reproduction, they may exhibit increased cold tolerance than reproductively nonresponsive mice (Blank and Ruf 1992). If body weight is decreased prior to decreases in ambient temperature, it may confer several benefits to the individual animal. Decreased body weight requires less energy for survival and the maintenance of homeothermy (Stamper and Dark 1996). Fewer energy requirements may decrease the risk of both predation and death from exposure by reducing an animal's necessary foraging time.

An additional survival mechanism, non-shivering thermogenesis (NST), which relies on the readily metabolizable characteristics of brown adipose tissue (BAT), has not been shown to be induced by decreasing daylength (Feist, et al 1988). Cold ambient temperatures are the only proximate cues for inducing NST (Feist, et al. 1988). However, it has been suggested that both cold and decreased photoperiod may act synergistically to trigger mobilization of BAT. The mobilization of BAT enables an animal to use NST (Zegers and Merritt 1988). A synergistic action of cold and decreased photoperiod may be needed because the metabolic adjustments needed to mobilize BAT stores take less time than seasonal metabolic adjustments. Seasonal metabolic adjustments may be more important to the mouse than are the seasonal adaptations that cause molt and increased daily torpor.
Seasonal Changes in Reproduction and Locomotor Activity in Voles, *Microtus* spp. and *Clethrionomys rutilus*: Voles also demonstrate individual variation in the degree of reproductive photoresponsiveness to short daylengths (Hayward 1965, Clarke and Kennedy 1967). In short days, voles make metabolic preparations similar to those of *Peromyscus* for the winter months (Rosenmann, et al. 1975, Petterborg 1978, Moffatt, et al. 1993, Zuercher, et al. 1999). Voles also demonstrate a decreased locomotor activity pattern in response to short days (Kerbeshian and Bronson 1993). The short day-induced reduction in locomotor activity may be more than just a behavioral change to decrease foraging risks. In Alaskan red-backed voles, *C. rutilus*, muscle mass decline was a proportionally higher percentage of the overall body mass loss (Zuercher, et al. 1999). Total body fat, although low year round, decreased during the winter (Zuercher, et al. 1999).

Seasonal Changes in Substrate Metabolism and Torpor in Hamsters, *Phodopus sungorus* and *Mesocricetus auratus*: Many of the seasonal metabolic and physiological changes exhibited by mice and voles were first identified in hamsters. These physiological changes are often more extreme in hamsters, making them excellent research subjects (Blank, et al. 1994, Dark, et al. 1996). Restriction of carbohydrates can induce daily torpor in *P. sungorus* (Dark, et al. 1996). Thus, decreased food intake increases the rate at which metabolic and reproductive adjustments to daylength are made by many hamster species. Long bouts of daily torpor and reduction in testis size are usually exhibited by Djungarian (or Siberian) hamsters, *P. sungorus* (Elliott, et al. 1987). Restriction of food can also induce anestrus in female golden (or Syrian) hamsters, *M. auratus* (Schneider and Wade 1990). This substrate-based shift in fertility in *M. auratus* may be one of the underlying causes of
seasonal reproductive decreases in many wild populations of temperate zone rodents (Schneider and Wade 1990, Staubs and Bradley 1998). The seasonal decline in fertility is variable depending upon the body weight of the animal at the time of food restriction (Schneider and Wade 1990).

Metabolic shifts can also involve changes in the substrates metabolized. Stamper et al. (1996) recorded a shift away from a primarily carbohydrate-based metabolism to a lipid-based metabolism in *P. sungorus* during winter months. This winter substrate shift to lipids is consistent with the observation that Djungarian hamsters selectively metabolize fat during bouts of daily torpor (Heldmaier, et al. 1999).

An important indicator of seasonal adjustments made by hamsters is daily torpor (Heldmaier and Steinlechner 1981). Bouts of daily torpor confer significant metabolic savings for *P. sungorus* (Heldmaier, et al. 1999). In hamsters, daily torpor seems to be linked very strongly with short photoperiods (Reiter 1980, Vitale, et al. 1985). Short photoperiods can also induce hamsters to increase their metabolic rate and capacity to mobilize brown adipose tissue for non-shivering thermogenesis (Stamper, et al. 1999). Hamsters are remarkably sensitive to very slight changes in photoperiod (Heideman and Bronson 1993). This suggests that the hamsters can initiate the weeks-long metabolic preparations necessary for torpor and the mobilization of BAT at the first sign of shorter photoperiods.
Summary

Most temperate zone rodents anticipate seasonal changes by using daylength as their primary cue. However, temperate zone rodents exhibit considerable individual variation in their responses to seasonal daylength changes. Shortening photoperiods often induce metabolic changes as well as changes in body and pelage composition, substrate metabolism, reproductive state and behavior.

This thesis will describe how *P. leucopus*, the white-footed mouse, changes its metabolic behaviors in order to prepare for oncoming winter, as simulated by short photoperiods. I examine food consumption, metabolic rates, reproductive status, body composition and digestive efficiency as it relates to seasonal differences in photoperiod. There is a considerable amount of individual variation in the neuroendocrine pathway for seasonal fertility in the white-footed mouse (Heideman, et al. 1999a, Majoy and Heideman 2000). This individual variation has a heritable component (Heideman and Bronson 1991, Heideman, et al. 1999b). *P. leucopus* is an excellent model for this type of study because of its wide geographic distribution and its high level of individual variation in seasonal reproductive response. I will examine this variation in reproductive response as it relates to other aspects of seasonal response, such as metabolic rates, activity levels and body composition changes in white-footed mice.
A NOTE ON THE NEUROENDOCRINE MECHANISMS OF
SEASONAL REPRODUCTIVE ENERGETICS

GnRH, LH and FSH

GnRH stimulates gonadotrope cells to synthesize and release LH and FSH (Bronson 1989). GnRH is produced in the hypothalamus, released into the blood, and carried to the adenohypophysis via the hypothalamohypophyseal portal system (Ojeda and Urbanski 1994). GnRH receptors are heterotrimeric G-protein-coupled receptors in the adenohypophysis (Stojilkovic, et al. 1994). When GnRH binds to its receptor, several phospholipase pathways in the gonadotrope membrane are stimulated (Stojilkovic, et al. 1994). These phospholipase pathways result in the modification of the inositol 1,4,5-triphosphate and dicylglycerol signals. These two signals cause the cytoplasmic concentration of calcium ions to increase. The increase in cytoplasmic calcium causes the synthesis and release of LH and FSH (Stojilkovic, et al. 1994).

Serum GnRH concentrations are found to be significantly higher in reproductively inhibited hamsters, *M. auratus*, maintained in short photoperiods (Pickard and Silverman 1979). Exogenous administration of GnRH does not stimulate different responses in short and long day hamsters, however (Pickard and Silverman 1979). This lack of a difference indicates that LH and FSH are not directly part of a steroid-negative feedback loop, but is only affected by the downstream hormones produced by the gonads.
LH and FSH are released from the adenohypophysis. LH stimulates androgen release by the cells of Leydig in the testes. The two primary androgens are testosterone and dihydrotestosterone (Bronson 1989). FSH stimulates gametogenesis in the seminiferous tubules in the testes (Bronson 1989).

**Melatonin**

The pineal gland indirectly affects the secretion of hormones by the adenohypophysis (Turek and Campbell 1979). The duration of melatonin signals provide both a temporal cue to season as well as regulating fat deposition and food intake (Goldman and Darrow 1983 as reviewed by Ebling and Cronin 2000). Longer melatonin signals are indicative of short photoperiods. Melatonin that is administered at night will induce gonad regression in intact and pinealectomized hamsters, *M. auratus* maintained in stimulatory photoperiods, or long days (Stetson and Tate-Ostroff 1981). A diagram is included at the end of this section to outline the pathways that convert photic cues into neuroendocrine signals affecting the reproductive system (Tamarkin, et al 1985).

**Leptin**

The following review of leptin's origins and effects are summarized from a review by Ahima, et al. (2000). Leptin inhibits feeding, lowers body weight and decreases adipose tissue mass. Leptin helps to regulate lipid and glucose metabolism and energy partitioning, among other processes. Glucocorticoid treatment stimulates leptin synthesis. Leptin production is inhibited by the presence of testosterone. Thyroid hormones affect leptin as well, but the results of thyroid-leptin studies are conflicting.
Leptin not only regulates food intake and energy balance, but it also acts to regulate puberty and fertility in mice. Leptin acts to restore fertility or accelerate the onset of puberty in rodents. Leptin acts centrally to regulate the reproductive axis as well by stimulating GnRH secretion.

Leptin affects thyrotropin releasing hormones (TRH) indirectly through the expression of neuropeptide Y and pro-opiomelanocortins (POMC). Leptin may also regulate TRH neurons directly. POMC neurons are one of the primary targets of leptin. The effects of leptin on the POMC neurons are mediated by α-melanocortin stimulating hormone (α-MSH), which is a product of the POMC neurons themselves.

Cold exposure decreases leptin expression through the action of β-andrenergic receptors. Melatonin also decreases leptin levels.

**Thyroid Hormones**

Thyroid hormones, such as triiodothyronine, can also play an important part in reproductive inhibition. Supplementation of triiodothyronine caused partial recovery from reproductive inhibition in *P. maniculatus* (Hogg, et al. 1992). Reproductively inhibited *P. maniculatus* have been found to have lower mean serum thyroxine (T₄) and triiodothyronine (T₃) concentrations (Peebles, et al. 1984, Pitman and Bradley 1984). These lowered thyroid hormone concentrations were correlated with lower body weight and lower reproductive organ weights (Peebles, et al. 1984). The thyroid gland is also linked with circadian rhythms in humans (Martin, et al. 1963). Reduced secretion of
thyroxine and triiodothyronine has been shown to decrease serum LH concentrations in rats (Hwang, et al. 1974).

**Gonad Hormones (Male)**

Testosterone and dihydrotestosterone stimulate puberty and affect the maturation and expression of secondary sexual characteristics and behaviors, such as the production of pheromones and scent marking (Bronson 1989).

There is a carefully regulated steroid-negative feedback loop in which high levels of testosterone decrease the pulsatile release of GnRH which in turn decreases the serum concentrations of testosterone and dihydrotestosterone (Bronson 1989). However, implant studies have determined that a continually high level of testosterone is a more effective suppressor of LH secretion than a high level of testosterone produced in a pulsatile manner (Berndtson, et al. 1974 as reviewed by Desjardins 1981).

**Adrenal Hormones**

Inhibition of reproduction may occur through peripheral feedback loops involving adrenal androgens or through a short feedback loop involving glucocorticoids or adrenocorticotropic hormones (ACTH) inhibiting gonadotropin secretion (Christian 1975). However, there was no difference in serum ACTH concentrations in *P. maniculatus* between reproductively inhibited and non-inhibited mice (Bradley and Terman 1981, Coppes and Bradley 1984). Social interactions with the corresponding increases in serum corticosterone levels may also reduce reproductive efforts in small mammals (Christian 1971).
An increase in norepinephrine levels causes the activity levels of GnRH neurons to increase (He, et al. 1993). Norepinephrine is produced by the adrenal medulla and is under sympathetic neural control. Similarly, increases in dopamine levels also cause the activity levels of GnRH neurons to increase (Li and Pelletier 1992).

Other Hormones and their Effects

Prolactin and inhibin are two other hormones that signal certain reproductive behaviors, such as nesting. Prolactin serves as part of the neuroendocrine pathway that transduces photoperiod information to the reproductive axis (Reiter 1974). Increased circulating prolactin increases the number of LH receptors (Bartke 1976). The number of LH receptors is directly correlated with the serum levels of testosterone and dihydrotestosterone present. However, if the circulating titres of prolactin are too high, it can either lead to infertility or accelerate the onset of puberty in small mammals (McNeilly, et al. 1978, Advis and Ojeda 1978). High serum levels of inhibin can suppress FSH release, which will ultimately decrease the rate of gametogenesis.

N-methyl-D-asparate (NMDA) is an excitatory amino acid that stimulates gonadotropin secretion (Taj, et al. 1983). NMDA can counteract the effects of inhibitory photoperiods on the reproductive axis of *M. auratus* by maintaining large gonads and higher serum gonadotropin levels (Urbanski 1990). NMDA receptors are hypothesized to play a part in the beginning and end of the breeding season in photoperiodic rodents (Urbanski 1990).
Oxytocin has different effects in different species. Injection of oxytocin increases the foraging and feeding behavior in mice, but has an antagonistic effect on feeding behavior in rats (reviewed by de Wied, et al. 1993).

**The Effects of Short Days on Neuroendocrine Function**

Short days decrease serum LH, FSH, Prolactin and testosterone levels (Bronson 1989). GnRH release is affected by short photoperiods in *P. maniculatus* (Korytko, et al. 1995). Serum LH and FSH concentrations in male prairie deer mice (*P. maniculatus*) are elevated in mice that are reproductively inhibited when compared to non-inhibited mice (Bradley and Terman 1981b). Although GnRH is still being produced in the presence of short photoperiods, it is not released. This lack of GnRH release causes the GnRH neuron cell bodies to increase significantly in size (Korytko, et al. 1995). The increase in GnRH cell size is especially evident in reproductively responsive animals (Korytko, et al. 1995). However, the concentration of LH, FSH and Prl is lower in the peripheral circulation of hamsters, *M. auratus*, that are reproductively inhibited by short photoperiods (Stetson and Tate-Ostroff 1981).

Short days may also increase the sensitivity of GnRH neurons to steroid-negative feedback loops (Ellis 1979). An increased sensitivity of the steroid-negative feedback system has been demonstrated in male *M. auratus* in response to exogenous testosterone administration (Stetson and Tate-Ostroff 1981).
Food and the Neuroendocrine System

Food restriction can inhibit LH secretion (Sisk and Bronson 1986). Therefore, food restriction can result in lower serum testosterone levels. However, food restriction in rats does not affect the hypothalamic concentrations of POMC or their product, α-MSH (Harrold, et al. 1999).

Similarly, serum thyroxine concentrations are sensitive to both the quantity and quality of food available to a rodent (Earles 1988). The sensitivity of thyroxine to food quality and quantity correspond with the observation that the metabolic rate of small mammals is dependent upon food availability as well as food quality (McNab 1980). A decrease in food availability has been found to partially influence both serum thyroid hormone levels as well as reproductive effort in *P. maniculatus* (Cronin and Bradley 1988). Decreased thyroxine levels can also decrease food intake (Ganong 1997).
The Hypothalamohypophyseal-Gonadal Axis

Figure 1. Interactions of Neuroendocrine Glands and Their Products.
METABOLISM, ACTIVITY AND FOOD CONSUMPTION

Introduction

A number of demands are placed on the energy from food consumed by small, temperate zone rodents. The important energy demands for a small rodent include foraging activity costs, reproductive effort, cellular maintenance and thermoregulation (Bronson, 1989). The thermoregulatory and cellular maintenance demands may increase during the wintertime, with a corresponding decrease in the amount of energy that may be available for reproductive effort. This increased demand for energy causes strong selection favoring winter infertility. However, the short life expectancy of a typical temperate zone rodent causes a counter selection pressure favoring winter breeding attempts (Bronson 1985, Bronson 1989). The degree of latitude is roughly correlated with the severity of wintertime shortages of food and wintertime low ambient temperatures (Bronson 1989, Heideman and Bronson 1993, Demas and Nelson 1998). For that reason, the degree of a rodent population's wintertime infertility may vary roughly with latitude, and secondarily altitude (Hoffman 1984, Zegers and Merritt 1988, Bronson 1989, Heideman and Bronson 1993).
For a small, temperate zone rodent in the short photoperiods of winter, the inputs over which it may have most control are food consumption. To a lesser degree, a small, temperate zone rodent can control its energy demands through modification of its metabolic rate. In short photoperiods, voles decrease their food intake and inhibit reproductive function (Moffatt, et al. 1993). Reproductive inhibition in response to short photoperiods with concomitant decreased food consumption may be a response to the increased thermoregulatory costs of a non-tropical rodent in the winter. Winter reproductive quiescence also increases the capacity of a small rodent for cold tolerance (Blank and Ruf 1992). Decreased demands for food and decreased metabolic rate may also protect rodents from periods of glucoprivation (Bartness, et al. 1989, Stamper, et al. 1999) thus decreasing the need to leave the burrow in search of food during very harsh winter weather. This winter decrease in food intake can also reduce the rodent’s overall body size and would enable the rodent to survive harsher winter climate and reduced food supply (Blank, et al. 1985, Hansson 1990, Blank, et al. 1994).

The purpose of these experiments is to test the following hypotheses:

1. Because of the increased metabolic demands of low winter temperatures, all mice increase their metabolic rates in short winter photoperiods.
   a. Alternative 1: Reproductively nonresponsive mice will increase their metabolic rates in short winter photoperiods more than responsive mice because the maintenance of wintertime fertility results in higher energetic demands.
b. Alternative 2: Reproductively nonresponsive mice are unable to respond by increasing their metabolic rate in short winter photoperiods because the neuroendocrine pathway for responding to shortened photoperiods by controlling metabolic rates is nonfunctional in these mice.

c. Alternative 3: Both reproductively nonresponsive mice and reproductively responsive mice are unable to respond by increasing their metabolic rate in short winter photoperiods because fertility and metabolic rates are controlled by different neuroendocrine pathways.

2. Because higher metabolic rates require additional energy input, all mice will increase their food consumption in short winter photoperiods.

a. Alternative 1: Reproductively nonresponsive mice will increase their food consumption in short winter photoperiods more than responsive mice because the maintenance of wintertime fertility results in higher energetic demands.

b. Alternative 2: Reproductively nonresponsive mice are unable to respond by increasing their food consumption in short winter photoperiods because the neuroendocrine pathway for responding to shortened photoperiods by controlling food consumption is nonfunctional in these mice.

c. Alternative 3: Both reproductively nonresponsive mice and reproductively responsive mice are unable to respond by increasing their food consumption in short winter photoperiods because fertility and food consumption are controlled by different neuroendocrine pathways.
3. In order to decrease exposure to low temperatures that would challenge a mouse's ability to thermoregulate, all mice will decrease their activity levels in the presence of short winter photoperiods.

   a. Alternative 1: Reproductively nonresponsive mice will increase their activity levels in the presence of short winter photoperiods more than responsive mice because reproductively nonresponsive mice will need to forage for more food than reproductively responsive mice.

   b. Alternative 2: Reproductively nonresponsive mice are unable to respond by increasing their activity levels in short winter photoperiods because the neuroendocrine pathway for responding to shortened photoperiods by controlling activity levels is nonfunctional in these mice.

   c. Alternative 3: Both reproductively nonresponsive mice and reproductively responsive mice are unable to respond by increasing their activity levels in short winter photoperiods because fertility and activity are controlled by different neuroendocrine pathways.

**Materials and Methods**

**Animals:** Experimental mice were obtained from the Endocrinology and Population Ecology Laboratory at the College of William & Mary. The laboratory colony of Peromyscus leucopus is an outbred population of wild mice descended from founders trapped in the area adjacent to and within the campus of the College (Heideman, et al.)
There have been no new introductions to the colony since the founders were trapped in February 1995.

Three lines have been bred within the colony (Heideman, et al. 1999b). Each breeding line has contained at least 13 pairs of mice in each generation in order to maintain genetic diversity. The Responsive line was derived from mice whose gonads are small (pre-pubertal) in response to short day lengths. The Nonresponsive line was derived from mice with large gonads in short days. The Control line is an unselected line, in which breeders have been selected at random within each generation, and whose members still exhibit a full range of reproductive responsiveness to short day lengths.


Mice were weaned at age 21 ± 2 days and housed singly in polypropylene cages (29 x 18 x 12 cm) on pine bedding with a stainless steel wire cage top. Food (Harlan Teklad Rat and Mouse Chow) and filtered tap water were provided ad libitum. The sires and dams were exposed to long photoperiods (16L:8D, lights on at 0500 EST) so that successful breeding could occur. All gonad-intact experimental mice were exposed to a short day photoperiod (8L:16D, lights on 0900 EST) from their day of birth to the time of the first experimental test. Cages of experimental mice were cleaned and changed with new bedding every 21 ± 7 days. Handling and bedding changes were kept to a
minimum to reduce stress on the mice. All mice were maintained at 23 ± 3 degrees Celsius for the duration of the experiment.

**Metabolic Trials:** Metabolic data for each mouse were collected for 2 days at a rate of 5 samples per hour. My pilot data indicated that the first 24 hours are an adjustment period in which the mouse does not react normally; therefore, these data were not analyzed. Food was weighed before and after the metabolic trial and average daily food consumption was calculated. In order to prevent disturbance to the airtight metabolic chamber system that would require recalibration, the chamber was not opened to measure food consumption each day. If the mouse displayed signs of shredding its food and leaving it in its cage litter prior to the experiment, a cage cup was used to measure food consumption so that the mouse’s access to the food was limited to a smaller area in which the food could not be shredded into the bedding but remained instead within the cage cup.

The Oxymax system was calibrated at the beginning of each 48-hour experimental run with a certified gas standard of 20.50% oxygen and 0.500% carbon dioxide (Linde) and dry nitrogen (Linde). After calibration and initialization, the Oxymax automatically calculates oxygen consumption and carbon dioxide production as ml/kg/hr.

Cage air was analyzed by the Oxymax Analysis system and version 3.7 software package (Columbus Instruments, Ohio) on a 286 PC Clone. Cage air was sampled at 75% of the flow through an open circuit chamber at a rate of 0.25 L per minute. Oxygen consumption and carbon dioxide production measurements were taken at 12-minute intervals. Air was dried by a Drierite chamber system because the carbon dioxide
Experiment 1: Metabolic Rate Variability in Gonad-intact Males under Different Photoperiods

This experiment tested whether there was a difference in metabolic rate (oxygen consumption) in responsive and nonresponsive mice in either short or long photoperiods. This experiment was also used to obtain baseline data on food consumption for both reproductive responsiveness phenotypes in short and long photoperiods.

Animals: Ten mice from each breeding line (Responsive, Control, and Nonresponsive) were chosen for the experiment. Control line mice were added to this experiment when a shortage of nonresponsive line mice left the metabolic chamber unoccupied. Therefore, a group of control line mice that had testes that were “reproductively nonresponsive” in size were added to the experiment. To prevent loss of independence due to a high degree of relatedness, only one mouse from each pair of parents was used. Each breeding line group in the experiment was balanced with respect to age.

In long days, male mice from these lines reach their adult gonad size by 50 days of age (unpublished data). In short days, the nonresponsive line mice stabilize their testis size by 60 days (unpublished data). Therefore, 70 days of age was used as a conservative estimate for the age of onset of maturity for both lines of mice in the permissive conditions of abundant food, water and nesting material.
Metabolic tests of each mouse were conducted first in short day (SD) photoperiods for two days at age 70 – 112 days. Because reproductive response has been demonstrated in hamsters and *Peromyscus* species to have a refractory period beginning as early as age 16 weeks (112 days), mice older than 112 days were not used for this experiment (Reiter 1980, Young, et al. 1999). Our lab has unpublished data suggesting that in our population of *P. leucopus*, responsiveness persists for at least 8 weeks (56 days) past the 70 day gonad check, and so this limit of 112 days was conservative.

**Short Day Measurements:** Each mouse and its food were weighed prior to the beginning of the trial. The mouse and its cage were placed into an airtight Oxymax Rat Chamber 31.8 x 19.9 x 25.6 cm (Columbus Instruments, Ohio). Room air was pumped into the chamber at a flow of 0.56 L per minute and cage air was pushed out at the same rate. The chamber was placed in a modified low temperature incubator (Precision Low Temperature, Model #815) and maintained at 23 ± 2 degrees Celsius with a timer set for a short photoperiod (8L:16D, lights on 0900 EST).

**Long Day Measurements:** A second metabolic test was conducted after each of the above experimental subjects had spent 60 ± 5 days in a long day (16L:8D, lights on 0500 EST) photoperiod. A period of two months was used as a conservative estimate of the time for the necessary physiological adjustments to have been made to the new day length regime. This time estimate is based on previous findings in this species and other small non-tropical rodents (Urbanski 1990, Young, et al. 1999).

Each mouse and its food were weighed prior to the metabolic trial. Trials were conducted in the same manner as the short day metabolic trials, but the day length was
adjusted to the 16L:8D (lights on 0500 EST) period. Leftover food in the bin was weighed at the completion of the trial to collect food consumption information. If the mouse displayed any signs of shredding its food, food was provided in a cage cup in the method described above.

Data for body weight and food consumption were analyzed using a paired t-test with a 2x2 design (breeding line x photoperiod) as the individuals are the same in both short and long days. Data for oxygen consumption, carbon dioxide production, and the respiratory exchange rate (RER) (see equation 1) were also analyzed using a paired t-test. All statistics were analyzed using a standard significance level of $\alpha=0.05$.

$$ \text{Equation 1: } \text{RER} = \frac{\text{ml. CO}_2 \text{ produced}}{\text{ml. O}_2 \text{ consumed}} $$

Unfortunately, the CO$_2$ chopper in the Oxymax analyzer malfunctioned during the course of the experiment, and the respiratory quotient could not be accurately calculated.

Active metabolic rates (AMR) and resting metabolic rates (RMR) were calculated by identifying the hour of sampling that demonstrated the highest and the lowest average oxygen consumption for the 24-hour period, respectively (methods from Staub and Bradley 1998). Similar criteria have been applied to calculate the resting and active metabolic rates in *P. leucopus* and *P. maniculatus* by Zegers and Merritt (1988). The AMR and RMR were used instead of inducing a basal metabolic rate for two reasons: the AMR and RMR normalize the mouse’s daily activity and these two measurements
represent the naturally occurring daily maximums and minimums of a mouse's oxygen consumption. Artificially induced basal metabolic rate measurements were not of interest because this study focuses on the naturally-occurring variation in metabolic characteristics of white-footed mice.

Time spent resting was calculated as the number of 12-minute samples made by the Oxymax analyzer in which the metabolic rate was lower than the average metabolic rate for all 12-minute samples.

**Statistics:** Data for body weight collected at the beginning of the metabolic trial, food consumption, oxygen consumption, carbon dioxide production, and RER were analyzed with a standard significance level of $\alpha=0.05$. As each animal was tested in both short and long days, repeated measures ANOVAs were used. Active metabolic rates (AMR) and resting metabolic rates (RMR) were calculated by identifying the hour of sampling that demonstrated the highest and the lowest average oxygen consumption for the 24-hour period, respectively and were also analyzed using paired t-tests with a significance level of $\alpha = 0.05$. Food consumption was analyzed as a covariate of body weight using photoperiod and breeding line as factors in the ANCOVA analysis. Tukey's Honestly Significant Difference post hoc tests were also conducted.

**Experiment 2: Metabolic Rate Variability in Castrated Males with Testosterone Implants under Different Photoperiods**

The purpose of this experiment was to determine how much of a role testes play in the metabolic and feeding behaviors of individual mice. Castration with testosterone
replacement was chosen to remove variation in testis size and to retain a functionally normal adult male mouse. Previous work has shown that oxygen consumption increases dramatically at pubertal onset in male deer mice (Staubs and Bradley 1998). As previously defined, responsive-line mice should still be pre-pubertal at the age of 70 days, but nonresponsive-line mice have already achieved puberty by that age (Heideman, et al. 1999).

**Animals and Surgical Procedures:** Twelve mice from both the Responsive and Nonresponsive breeding lines were chosen at random for the experiment. To prevent loss of independence due to a high degree of relatedness, only one mouse from a litter was used. Each mouse was bilaterally castrated under Isoflurane (Pitman-Moore, Inc., Mundelein, IL) anesthesia at time of weaning (23 ± 2 days). During the same procedure, a Silastic implant (O.D. 0.1958 cm, I.D. 0.1475 cm) (Dow Corning) containing 10 mm of crystalline testosterone (Aldrich Chemical) sterilized in 0.1 M benzalkonium chloride was placed subcutaneously in the interscapular region of each mouse. Wound clips (BD Biosciences, Inc., Sparks, Maryland) were used for closure of both incisions. Each mouse was then assigned to either a short day or long day room (description above) and was later tested at age 80 ± 10 days.

**Statistics:** Data for body weight collected at the beginning of the metabolic trial, food consumption, oxygen consumption, carbon dioxide production, and RER were analyzed using a two-way ANOVA in a 2x2 design (line x photoperiod) with a standard significance level of α=0.05. As each animal was used only once, due to the continuous release of testosterone from the subcutaneous implants, analysis of variance tests were chosen. Active metabolic rates (AMR) and resting metabolic rates (RMR) were
calculated by identifying the hour of sampling that demonstrated the highest and the
lowest average oxygen consumption for the 24-hour period, respectively and were also
analyzed using a two-way ANOVA with a standard significance level. Food consumption
was analyzed as a covariate of body weight using photoperiod and breeding line as
factors in the ANCOVA analysis. Tukey’s Honestly Significant Difference post hoc tests
were also conducted.

Results for Experiment 1: Metabolic Rate Variability in Gonad-intact Males
under Different Photoperiods

Body Weight: There was no overall difference in body weight among breeding lines (F
= 1.012, P = 0.3784) or between photoperiods (F = 2.470, P = 0.1291). Body weight did
not differ for mice from each breeding line when transferred from short to long days
(Responsive: F = 0.052, P = 0.8251, Nonresponsive: F = 0.383, P = 0.5558, Control: F
= 2.533, P = 0.1501) (Fig 2).
Figure 2. **Body Weight of Gonad Intact Males.** Means and standard errors of body weight vs. photoperiod for gonad-intact males from the Responsive (n = 10), Nonresponsive (n = 8) and Control (n = 9) breeding lines from our colony. Groups marked with different letters were significantly different.

**Mass-Specific Metabolic Rate:** There was no significant difference in mass-specific metabolic rate among breeding lines in gonad-intact mice (F = 0.571, P = 0.5727). However, there was a trend (F = 4.120, P = 0.0536) for gonad-intact mice to have higher metabolic rates in short days (Fig. 3). Only nonresponsive mice increased their mass-specific metabolic rates significantly in the presence of short photoperiods (F = 13.095, P = 0.0085). The mass-specific metabolic rates of responsive- and control-line mice did
not change with photoperiod (Responsive: $F = 0.598$, $P = 0.4592$, Control: $F = 0.326$, $P = 0.5837$).

The mass-specific metabolic rates of nonresponsive mice were significantly higher than the metabolic rates of responsive mice in both photoperiods ($t = 12.898$, $P = 0.000$ in SD, $t = 21.934$, $P = 0.000$ in LD). The mass-specific metabolic rates of nonresponsive mice were also significantly higher than the metabolic rates of control line mice in both photoperiods ($t = 14.251$, $P = 0.000$ in SD, $t = 15.744$, $P = 0.000$ in LD).

Figure 3. Mass-Specific Metabolic Rate of Gonad Intact Males. Means and standard errors of metabolic rate vs. photoperiod for gonad-intact males from the Responsive ($n = 10$), Nonresponsive ($n = 8$) and Control ($n = 9$) breeding lines from our colony. Groups marked with different letters were significantly different.
**Normalized Food Consumption:** Food consumption was normalized for body weight because body weight differed among groups (see Eq. 2). Both photoperiod ($F = 9.631$, $P = 0.0050$) and breeding line ($F = 3.780$, $P = 0.0381$) had effects on food consumption (Fig. 4). Similar to the results for body weight and mass-specific metabolic rate, food consumption did not differ for responsive line mice between short and long days ($F = 0.112$, $P = 0.7468$). Nonresponsive gonad-intact males ate significantly more food in short days than they did in long days ($F = 13.758$, $P = 0.076$). There was also no significant difference between long day and short day food consumption for control line males ($F = 3.377$, $P = 0.1034$). In long days, normalized food consumption was the same for all lines ($F = 0.012$, $P = 0.988$).

**Equation 2:**

\[
\text{Normalized Food Consumption} = \frac{\text{grams food eaten per day}}{\text{gram body weight}}
\]
Figure 4. Food Consumption of Gonad-Intact Males Normalized with Respect to Body Weight. Means and standard errors of normalized food consumption vs. photoperiod for gonad-intact males from the Responsive (n = 9 in SD, n = 10 in LD), Nonresponsive (n = 8) and Control (n = 9) breeding lines from our colony. Groups marked with different letters were significantly different.
**Time Spent Resting:** Among gonad-intact males, the average time spent resting increased significantly in long days for each mouse line ($F = 3.799, P = 0.006$). The increase in time spent resting from short to long days is due to photoperiodic effects. There was no difference among lines for the amount of time spent resting in either short days ($F = 1.331, P = 0.283$) or long days ($F = 0.557, P = 0.580$) (Fig. 5).

![Figure 5. Time Spent Resting by Gonad-Intact Males.](image-url)

Figure 5. **Time Spent Resting by Gonad-Intact Males.** Means and standard errors of time spent resting vs. photoperiod for gonad-intact males from the Responsive ($n = 10$), Nonresponsive ($n = 8$) and Control ($n = 9$) breeding lines from our colony. Groups marked with different letters were significantly different. Time spent resting was calculated as the number of 12-minute samples made by the Oxymax analyzer in which the metabolic rate was lower than the average metabolic rate for all 12-minute samples.
Body Weight: There was no body weight difference among castrates with testosterone implants for either line or photoperiod regime ($F = 0.164, P = 0.920$). Castrate mice with testosterone implants were significantly heavier ($F = 27.208, P = 0.000$) than gonad-intact mice (Fig. 6). This difference was attributable to an increase in both body fat and muscle mass (see Table 1).

Figure 6. Body Weight of Castrate Males with Testosterone Implants. Means and standard errors of body weight vs. photoperiod for castrate males with testosterone implants from the Responsive (n = 10 in SD, n = 13 in LD) and Nonresponsive (n = 12 in SD, n = 11 in LD) breeding lines from our colony.
The 10 mm crystalline testosterone implants had a dramatic effect on the seminal vesicle weights (Table 1). Seminal vesicle weight is directly related to circulating testosterone titers (Bradley and Terman 1981). One possible result of testosterone supplementation may be the aromatization of testosterone into estradiol causing an estrogenization effect (Ganong 1997). Because there was such a large increase in seminal vesicle weights, the testosterone was apparently not aromatized into estradiol causing an “estrogenization” effect. If an “estrogenization” effect were to occur, the increase in body weight by castrated mice with testosterone implants would be more fat than muscle (Ganong 1997).

<table>
<thead>
<tr>
<th>Castrate Males + Testosterone</th>
<th>Mean Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short Day</td>
<td>864.7 ± 49.7</td>
</tr>
<tr>
<td>Long Day</td>
<td>807.3 ± 81.6</td>
</tr>
</tbody>
</table>

*Table 1. Seminal Vesicle Weights (including fluid contents) for Castrate Males with Testosterone Implants (Mean ± SEM).*
**Mass-Specific Metabolic Rate:** Castrate mice displayed significantly lower average oxygen consumption values than did gonad-intact mice ($F = 84.230, P = 0.000$). Among castrates, responsiveness to short day lengths does not have a significant impact on metabolic rate ($F = 0.728, P = 0.399$) (Fig. 7).

![Mass-Specific Metabolic Rate of Castrate Males with Testosterone Implants](image)

**Figure 7. Mass-Specific Metabolic Rate of Castrate Males with Testosterone Implants.** Means and standard errors of metabolic rate vs. photoperiod for castrate males with testosterone implants from the Responsive ($n = 10$ in SD, $n = 13$ in LD) and Nonresponsive ($n = 12$ in SD, $n = 11$ in LD) breeding lines from our colony. Groups marked with different letters were significantly different.
**Normalized Food Consumption:** There was a significant ($F = 2.953, P = 0.044$) difference in normalized food consumption between breeding lines and photoperiods. There was a tendency for responsive line mice to increase their food consumption from short to long days and for that increased consumption to be equivalent to the normalized food consumption among castrate nonresponsive mice in either photoperiod regime (Fig. 8). There was no significant difference in normalized food consumption values between the gonad-intact males and castrate males with testosterone implants ($F = 0.138, P = 0.711$).

![Figure 8. Food Consumption of Castrate Males with Testosterone Implants Normalized with Respect to Body Weight.](image)

Means and standard errors of normalized food consumption vs. photoperiod for castrate males with testosterone implants from the Responsive ($n = 10$ in SD, $n = 13$ in LD) and Nonresponsive ($n = 11$ in SD, $n = 11$ in LD) breeding lines from our colony. Groups marked with different letters were significantly different.
**Time Spent Resting:** Castrate males with testosterone implants in both lines spent significantly more time resting in short days than in long days (F = 8.731, P = 0.000). Here again, the nonresponsive line mice spent more time resting than did the responsive line mice (Fig. 9). There was no significant difference between gonad-intact males and castrate males with testosterone implants in the amount of time spent resting (F = 0.519, P = 0.473).

![Figure 9. Time Spent Resting by Castrate Males with Testosterone Implants.](image)

Means and standard errors of time spent resting vs. photoperiod for castrate males with testosterone implants from the Responsive (n = 10 in SD, n = 13 in LD) and Nonresponsive (n = 12 in SD, n = 11 in LD) breeding lines from our colony. Groups marked with different letters were significantly different. Time spent resting was calculated as the number of 12-minute samples made by the Oxymax analyzer in which the metabolic rate was lower than the average metabolic rate for all 12-minute samples.
Discussion: Experiment 1: Metabolic Rate Variability in Gonad-intact Males under Different Photoperiods

Responsive male mice were 10 - 12% lighter than nonresponsive males in both photoperiods. This body weight difference is not consistent with results reported from a much larger sample for male body weight between selected lines in Heideman et al. (1999b). The male mice used in this experiment were from later generations (F₄ and F₅) than those used in Heideman et al. (1999b) and this may account for the difference. Sampling error may have had an influence on the body weight difference observed in this experiment. However, a study using F₃, F₄ and F₅ mice by Majoy and Heideman (2000) also found a difference in body mass with nonresponsive males being heavier.

The smaller body size of short day responsive males may confer an advantage by reducing their daily energy requirements for thermoregulatory and cellular maintenance needs. These reduced demands may reduce the necessary foraging time, which would reduce exposure to predators or harsh climatic conditions. Smaller body size may also allow the responsive mice to tolerate colder temperatures by decreasing their energetic demands by entering torpor and needing less food than their nonresponsive counterparts (Blank and Ruf 1992, Blank, et al. 1994). Nonresponsive M. orchrogaster also failed to change in body weight despite a change in photoperiod (Moffatt, et al. 1993). Neither of our selected lines of our P. leucopus showed a significant difference in body weight between short and long days. However, our unselected control line gained weight in long days. This higher weight in long days of mice from the control line suggests that the body weight response to short photoperiods by Responsive and Nonresponsive mice may have been masked by the selection for their extremes in
gonad size in short days. This selection regime may have had a slight influence on overall body size in the responsive and nonresponsive lines.

Mass-specific metabolic rate was affected by both the degree of reproductive responsiveness and photoperiod. There was no difference between the metabolic rates of gonad-intact responsive mice in short days and in long days. However, nonresponsive-line mice increased their mass-specific metabolic rate in short days when compared with that of responsive-line mice in the same day length conditions (Fig. 3). This would suggest that in the wild a nonresponsive mouse might increase its metabolic rate in order to compensate for the increased demands of maintaining fertility in wintertime. In order to maintain this higher metabolic rate, a nonresponsive mouse would need to increase its food intake (Fig. 4). This increase in food intake may make a nonresponsive mouse more susceptible to possible wintertime food shortages. A similar susceptibility to food shortages is seen in Siberian hamsters that are reproductively nonresponsive to short daylengths (Stamper, et al. 1999). An increased metabolic rate without body weight change may also suggest that nonresponsive mice may only be successful in areas in which there was abundant food year-round, such as grain storage areas.

It has been hypothesized for *P. sungorus* that a shift from a more carbohydrate-based to a more fat-based metabolism should occur in short days because torpor should be induced by short photoperiods (Heldmaier, et al. 1999). However, no significant shift in the respiratory quotient was noted for either line of our *P. leucopus* in short days. Shifts in the respiratory quotient indicating a shift to fat-based metabolism have been reported during long bouts of torpor in deer mice, *Peromyscus maniculatus* (Stamper, et al.
1999). This suggests that long torpor bouts are probably not occurring in the *P. leucopus* in this study.

Nonresponsive mice ate 62% more food in short days than they consumed in long days. This short-day increase in normalized food consumption is consistent with their increased metabolic rate. Reproductively responsive mice in both short and long days had the same average food consumption per gram body weight as their nonresponsive conspecifics did in long days. Normalized food consumption patterns are similar for both selected lines of gonad-intact mice in short and long days. However, this pattern of food consumption is not seen in the control line mice. This difference in food consumption patterns suggests that metabolic rate and food consumption are at best loosely linked. The increased food consumption by nonresponsive mice in short days may be needed to allow the mice to maintain fertility in winter conditions (Blank, et al. 1994).

The average amount of time spent resting by nonresponsive mice is longer in short days than it is for responsive mice. The nonresponsive mice have significantly higher food consumption and a higher metabolic rate than responsive mice when exposed to short photoperiods. In combination, this suggests that the nonresponsive mice may be highly active in fewer "bursts" of activity and then resting for the rest of the day (Perrigo and Bronson 1983).

**Experiment 2: Metabolic Rate Variability in Castrated Males with Testosterone Implants under Different Photoperiods**
The castrate males were exposed to a high, pharmacological dose of testosterone from 10 mm implants of crystalline testosterone. Tests were done to ensure that an estrogenization effect was not occurring. An estrogenization effect would include a feedback loop in which the continuous release of testosterone would affect the mouse as if it were estrogen because of the aromatization of testosterone into estradiol (Ganong 1997). If an estrogenization effect were occurring, increased mass would not be muscle mass, but would be entirely fat (Ganong 1997). Also, the seminal vesicles would have remained small if an estrogenization effect was occurring because seminal vesicle weights have been shown to be intimately linked with testicular activity and testosterone concentrations (Bradley and Terman 1981). Seminal vesicles of castrate mice were weighed and their increased mass indicated that an estrogenization effect was not occurring in these mice. There was no difference in the seminal vesicle weight between lines or in either photoperiod regime (Table 2).

<table>
<thead>
<tr>
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</tbody>
</table>

Table 2. Comparison of the Seminal Vesicle Weights (including Fluid Contents) for Gonad-Intact Males and Castrate Males with Testosterone Implants (Mean ± SEM).

Castrate males with testosterone implants were as heavy as the nonresponsive males in long days. There was no difference in body mass in castrate males from either breeding line or in either photoperiod regime. The lack of a body weight difference may indicate
that the high, pharmacological dose of testosterone that was given may have masked any effect that photoperiod or phenotype have on these criteria.

Castrate males with testosterone implants had a significantly lower mass-specific metabolic rate in all photoperiods and across breeding lines when compared to gonad-intact mice. Castrate responsive mice displayed the same food consumption pattern as the gonad-intact responsive mice in both photoperiods. However, the nonresponsive castrate males ate more in long days, whereas the nonresponsive gonad-intact males ate more in short day. The presence of changes in food consumption across photoperiods and between lines may indicate that the mechanism for adjusting food consumption in short days may be influenced by testosterone titres.

High doses of testosterone caused a response in the activity levels in both lines in both photoperiods. There was a more dramatic difference in the activity levels of castrate mice from both breeding lines between short and long days. Castrate nonresponsive mice with implants spent significantly more time at rest in short days than did castrate responsive-line mice. The significantly lower average metabolic rate may be contributing to the large difference in activity levels between short and long days in castrate mice from both breeding lines.

The pharmacological dose of testosterone given to the castrate mice may have been so high that some normal photoperiodic behaviors or physiological characteristics may have been masked. Future research should be conducted using smaller doses of testosterone.
ACTIVITY PATTERNS, BODY COMPOSITION, AND DIGESTIVE EFFICIENCY

Introduction:

Body weight changes and seasonal reproductive inhibition in small, temperate zone rodents are not the only factors that change with the shift from summer to winter. With the onset of winter, the activity patterns of small temperate zone rodents change because their foraging needs also change (Perrigo and Bronson 1983). The foraging needs of a temperate zone rodent on a diet that is mixed folivorous-granivorous or strictly granivorous increase when plant life tends to die or go dormant with the colder temperatures of winter. These temperate zone rodents may decrease locomotor activity to conserve energy by saving calories as well as decreasing their potential exposure to colder winter temperatures outside their burrows (Bronson 1987). For example, in winter’s short daylengths, prairie voles (*Microtus orchrogaster*) decrease their wheel-running activity in the laboratory (Moffatt, et al. 1993).

Colder temperatures may be the ultimate reason that changes in body mass in response to winter-like short photoperiods have been observed in northern red-backed voles (*Clethrionomys rutilus*) (Zuercher, et al. 1999). Other small rodents decrease their weight when they are reproductively inhibited or in response to an anticipated decrease in the availability of food as indicated by decreasing daylengths (Hansson 1990).
Short winter photoperiods have a greater impact on body mass than does decreased food supply, as shown by evidence that voles begin to decrease their body mass before any changes in food availability are apparent (Dark and Zucker 1983). Even before the appearance of new vegetation, longer photoperiods induce increases in body mass (Dark, et al. 1983). Mass changes in individual body compartments in small rodents are affected differently by short photoperiods (Dark and Zucker 1983, Dark, et al. 1983, Dark, et al. 1985). The photoperiod-induced change in body mass is associated with the degree of reproductive responsiveness as well (Blank, et al. 1994).

There may be a seasonal shift in digestive efficiency by temperate zone rodents. In short days, a small, granivorous rodent might spend a longer period digesting each food bolus before excreting the rest as waste. A longer digestive time could increase the efficiency with which a small rodent extracts the nutrients from each bolus of food as it passes through its gut. A longer gut-passage time might allow a temperate zone rodent to reduce the time spent outside foraging during harsh winter conditions.

The purpose of these experiments is to test the following hypotheses:

1. Because low winter temperatures would increase the need for insulating fat, all mice will increase their fat stores in short winter photoperiods.
   a. Alternative 1: Reproductively nonresponsive mice will increase their fat stores in short winter photoperiods more than responsive mice.
   b. Alternative 2: Reproductively nonresponsive mice are unable to respond by increasing their fat stores in short winter photoperiods, because their neuroendocrine pathway controlling fertility and fat stores is nonfunctional.
c. Alternative 3: Both reproductively nonresponsive mice and reproductively responsive mice are unable to respond by increasing their fat stores in short winter photoperiods because fertility and fat stores are controlled by different neuroendocrine pathways.

2. Because mice require additional energy input to maintain homeothermy in low winter temperatures, all mice will increase their “foraging” activity levels in short winter photoperiods.

   a. Alternative 1: Because reproductively nonresponsive mice need more food to maintain fertility, they will increase their foraging activity in short winter photoperiods more than responsive mice.

   b. Alternative 2: Because reproductively nonresponsive mice may be using another behavior to reduce energetic demands, they will not respond by increasing their foraging activity in short winter photoperiods.

   c. Alternative 3: Both reproductively nonresponsive mice and reproductively responsive mice will not respond by increasing their foraging activity in short winter photoperiods because fertility and foraging activity may be controlled by different neuroendocrine pathways.

3. In order to cope with decreased wintertime food availability, all mice will increase their digestive efficiency in the presence of short winter photoperiods.

   a. Alternative 1: Because reproductively nonresponsive mice need more food to maintain fertility, they will increase their digestive efficiency in short winter photoperiods more than responsive mice.
b. Alternative 2: Because reproductively nonresponsive mice are unable to respond by increasing their digestive efficiency in short winter photoperiods, they will have the same digestive efficiency as reproductively responsive mice.

c. Alternative 3: Both reproductively nonresponsive mice and reproductively responsive mice are unable to respond by increasing their digestive efficiency in short winter photoperiods because fertility and digestion are controlled by different neuroendocrine pathways.

**Materials and Methods:**

**Body Composition:** All mice were maintained as described previously, singly housed in polypropylene shoebox-style cages (29 x 18 x 12 cm) on pine bedding with a stainless steel wire cage top. Food (Harlan Teklad Rat and Mouse Chow) and filtered tap water were provided ad libitum. Room temperatures were maintained at 23 ± 2 °C for the duration of the experiment. Each mouse was maintained in the photoperiod conditions to which it was randomly assigned from the age of weaning until time of testing, age 70 – 112 days. Photoperiod treatments were either short days (8L:16D, lights on at 0900 EST) or long days (16L:8D, lights on at 0500 EST).

At the beginning of the testing period, 7 – 10 days in duration, each mouse was placed in a clean cage with new pine bedding with food and water provided ad libitum. If any evidence of food shredding behavior was noted, a cage cup containing an excess of food was placed in the cage similar to the methods used for “shredders” in the metabolic
trials. The mouse was weighed at the beginning of the testing period and left undisturbed for a period of 7 to 10 days.

At the conclusion of the trial period, the mouse was removed from its cage, weighed and then immediately culled using carbon dioxide gas. Each mouse was then placed in its own Ziploc™ freezer bag, labeled and frozen at 0 °C for later processing. The cage bedding was removed in its entirety for later measurement of fecal production.

At the time of processing, the mouse was removed from the freezer, thawed and reweighed. The thawed body weight was noted and then the gut was removed from the body cavity. During dissection, the number of fat pads was noted on a 1 to 4 scale.

**Body Fat Scale**

1 = interscapular fat only (brown adipose tissue)

2 = interscapular fat + subcutaneous fat pad (white adipose tissue) over abdominal region

3 = interscapular fat + subcutaneous abdominal fat plus inguinal fat pads (white adipose tissue) inside abdominal cavity

4 = interscapular fat + subcutaneous abdominal fat + inguinal fat pads + retroperitoneal fat (white adipose tissue) near kidneys

Body fat was analyzed using a Mann-Whitney U test because the number of fat pads is discrete categories, rather than continuously distributed measures.
Following dissection, a weight of the mouse without its gut was measured and noted as the BW(t-g). A glass petri dish was used to hold the mouse during the drying period. The petri dish weight was noted and then a variable weight of paper towel (based on the number of fat pads) was added to the petri dish to prevent the dried carcass from sticking to the glass. The petri dish, eviscerated carcass and paper toweling was then weighed again and noted as Wt(g+BW+pt). In order to prevent added weight due to condensation, all measurements were taken while the glass and paper toweling were hot.

The petri dish, mouse carcass and paper towel were then placed in a 60 °C oven and dried for a period of 120 ± 8 hours, the time at which constant weight was achieved regardless of the amount of fat contained in each carcass. Following the drying period, the glass, carcass and paper towel were reweighed while hot and this weight was indicated as DW(g+BW+pt). The carcass and paper toweling were removed from the petri dish and weighed again to eliminate any error for fat residue left on the glass. That weight was indicated as DW(BW+pt). Each dried carcass was placed with its paper towel and an identification card in a plastic bag and frozen again at 0 °C for later ether extraction. All analyses were carried out blind with respect to breeding line and treatment.

**Fecal Production:** The cage bedding was collected at the end of the trial period for each mouse in the body content experiment and was air-dried for a period of at least 2 days. If the mouse’s water bottle had spilled into the bedding, the air-drying was continued until the bedding was dry to the touch.
The bedding was then gross-sifted through a wire mesh screen and then hand-sorted to isolate the feces from the bedding. Feces collected were weighed and indicated as FWt(wet). Feces were then frozen at 0 °C until the time of drying. Feces were dried in a 60 °C oven for a period of 48 ± 4 hours in pre-weighed glass petri dishes. The drying time was established using feces that reached a constant weight after 42 hours drying. For the purpose of this analysis, only dry weights are discussed. Calorimetric analysis of the feces will be completed later.

Fecal pellet size was rated as large or small, blind with respect to treatment and breeding line. Pellet size may indicate that the diameter of the intestine may be increasing to meet the increased energetic demands of maintaining reproduction in winter. For example, a study of maternal intestinal diameter showed that the intestine increased in size when large energetic demands of gestation and lactation were occurring (Hammond 1997).

**Activity Patterns:** For the activity pattern experiment, mice were initially tested in short days at age 70 – 112 days. Each mouse was placed in a modified rat cage (Fig. 1) with approximately 2 cm of pine bedding in the bottom and a cage cup was provided for food. Tap water and food were provided ad libitum throughout the course of the experiment. Analysis of activity was done remotely with a Videomex V system (Columbus Instruments, Columbus, Ohio) and results were printed on a dot matrix printer. Each testing session lasted 11 hours 18 minutes, due to the restrictions inherent in the Videomex monitoring system. Therefore, two back-to-back sessions were analyzed to comprise an approximate 24-hour period.
Figure 10. Videomex and Activity Data Collection Apparatus.
Results

Body Composition

Free Body Water: There was no significant difference (F = 0.0669, P = 0.7970) in the percentage of free body water (calculated as the difference between the BW(t-g) and the dried carcass weight DW(t-g)) between responsive and nonresponsive mice in short photoperiods (Fig. 11). There was no effect of photoperiod on the percent body water (F = 0.689, P = .4107). In this case, a Kruskal-Wallis nonparametric test was required because the variances were not homogeneous and the data were not normally distributed.

Figure 11. Percent Body Water of Gonad-Intact Mice in Different Photoperiods. Means ± SEM of percentage of body water of male responsive (n = 12) and nonresponsive (n = 10 in SD, n = 17 in LD) mice.
**Body Weight:** A two-way ANOVA demonstrated that there was a non-significant trend (F = 3.090, P = 0.084) for nonresponsive mice to be heavier than responsive mice in both photoperiod regimes. Photoperiod had no effect on body weight of mice from either breeding line photoperiods (F = 0.193, P = 0.6616) (Fig. 12).

![Body Weight Graph](image-url)

**Figure 12. Body Weight of Gonad-Intact Mice in Different Photoperiods.** Means ± SEM of body weight (grams) of male responsive (n = 12) and nonresponsive (n = 10 in SD, n = 17 in LD) mice.
**Fat Pads:** There was no difference in the number of fat pads among responsive mice and nonresponsive mice in both photoperiod regimes (Table 3). There was no significant difference in the number of fat pads among breeding lines ($Z = -0.691, P = 0.490$). Similarly, photoperiod had no significant effect on the number of fat pads ($Z = -0.059, P = 0.953$). Further analysis by ether extraction may yet yield significant differences in total body fat that could not be found by simply counting the number of fat pads.

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<th>Photoperiod</th>
<th># Fat Pads Mean ± S.E.M.</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
<td></td>
<td>Long Day</td>
<td>2.82 ± 0.05</td>
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<tr>
<td>Nonresponsive</td>
<td>Short Day</td>
<td>2.73 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Long Day</td>
<td>3.12 ± 0.04</td>
</tr>
</tbody>
</table>

**Table 3. Mean Number of Fat Pads in Short Days and Long Days.** Fat pads of gonad-intact male *P. leucopus* from the responsive line ($n = 13$ in SD, $n = 12$ in LD) and the nonresponsive line ($n = 15$ in SD, $n = 17$ in LD).
Consistent with previous experiments, there was a significant difference ($F = 3.592, P = 0.020$) in raw daily food intake between responsive and nonresponsive mice. Food intake was analyzed using a two-way ANOVA in a 2x2 design (line x photoperiod). There was also a significant difference between responsive and nonresponsive mice for their food consumption and body weight analyzed as covariates ($F = 5.990, P = 0.0193$). The interaction effect for breeding line and photoperiod was not significant ($F = 3.609, P = 0.0647$). In contrast to previous experiments, there was a dramatic shift in food consumption for nonresponsive mice with higher food consumption occurring in long days (Fig 13).

**Figure 13. Food Consumption of Gonad-Intact Mice.** Means ± SEM of food consumed (grams) by male responsive ($n = 11$ in SD, $n = 12$ in LD) and nonresponsive ($n = 18$ in SD, $n = 13$ in LD) mice.
**Activity Patterns**

**Stereotypic Movements:** Photoperiod had a significant effect on time spent performing stereotypic movements ($F = 4.228, P = 0.049$). Breeding line did not have a significant effect on stereotypic movements ($F = 2.563, P = 0.120$). However, nonresponsive mice spent significantly more time performing stereotypic movements (paired t-test: $t = 2.226$, $P = 0.042$) than responsive mice in both photoperiods (Fig 14).

![Figure 14. Time Spent Performing Stereotypic Movements.](image)

Figure 14. Time Spent Performing Stereotypic Movements. Mean ± SEM of hours spent performing stereotypic movements between responsive mice (n = 9) and nonresponsive mice (n = 8 in SD, n = 9 in LD).
Resting Time: There was no effect of either breeding line ($F = 2.397$, $P = 0.132$) or photoperiod treatment on total time spent resting ($F = 0.089$, $P = 0.767$) (Fig 15).

**Figure 15. Time Spent Resting.** Mean ± SEM of hours spent resting between responsive mice ($n = 8$) and nonresponsive mice ($n = 8$).
**Total Ambulatory Time:** Neither photoperiod (F = 1.994, P = 0.168) nor breeding line (F = 0.087, P = 0.770) had a significant effect on ambulatory time (Fig 16).

![Total Ambulatory Time Diagram](image)

**Figure 16. Total Ambulatory Time.** Mean ± SEM of hours spent moving between responsive mice (n = 8) and nonresponsive mice (n = 8).
**Distance Traveled:** There was a significant difference in the total distance traveled in long days between responsive and nonresponsive mice (paired t-test: \( t = 2.274, P = 0.038 \)) (Fig. 17). Short winter photoperiods appear to have a great impact on reducing the amount of foraging activity on both responsive and nonresponsive mice.

![Total Kilometers Traveled](image)

**Figure 17. Total Distance Traveled by Gonad-Intact Mice.** Mean ± SEM of total kilometers moved by responsive mice (\( n = 8 \)) and nonresponsive mice (\( n = 8 \)).
Digestive Efficiency

There was a trend for breeding line to affect feces production ($F = 3.522$, $P = 0.066$) as analyzed using a two-way ANOVA in a 2x2 design (line x photoperiod). Photoperiod had no effect on feces production ($F = 0.043$, $P = 0.836$) (Fig 18). However, Nonresponsive mice produced more feces per day than responsive mice ($F = 4.268$, $P = 0.044$).

![Feces Production](image)

**Figure 18.** Daily Feces Production by Gonad-Intact Mice. Mean ± SEM of feces produced per day by responsive mice ($n = 12$ in SD, $n = 11$ in LD) and nonresponsive mice ($n = 18$ in SD, $n = 13$ in LD).
Feces production did not covary with body weight (Two-factor ANCOVA: $F = 0.167, P = 0.6843$). The interaction of mouse line and photoperiod had a positive covariance with feces production (Two-factor ANCOVA: $F = 6.599, P = 0.0135$). As would be expected, food consumption was very strongly linked with feces production (Two-factor ANCOVA: $F = 64.952, P = 0.0001$).

Pellet size was significantly different for both responsive and nonresponsive mice in both short and long days (Two-way ANOVA: $F = 3.850, P = 0.015$). Both breeding line ($F = 6.487, P = 0.014$) and photoperiod ($F = 5.269, P = 0.026$) had significant effects on pellet size (Fig 19).

**Figure 19. Fecal Pellet Size of Gonad-Intact Mice.** Mean ± SEM of fecal pellet size produced by responsive mice ($n = 12$ in SD, $n = 11$ in LD) and nonresponsive mice ($n = 18$ in SD, $n = 13$ in LD). Please note that all fecal pellets for nonresponsive mice in long days were ranked as 2.
Discussion

Body water, expressed as a percent of the total body mass, did not differ among breeding lines nor did photoperiod have any apparent effect (Fig 11). Similarly, no body water change was seen in response to photoperiod in several *Peromyscus* species studied by Hayward (1965). Body water also did not change with photoperiod in the shrew, *Sorex araneus* (Myrcha 1969, Churchfield 1981). This lack of a percent body water change contrasts with the significant loss of body water from long to short days as seen in meadow voles, *M. pennsylvanicus* (Dark, et al. 1983) and red-backed voles, *C. rutilus* (Zuercher, et al. 1999).

In this experiment, there was no significant difference in body weight among breeding lines and photoperiods (Fig 12). This result confirms the need for greater sample sizes to see any subtle effects on body weight in a tremendously variable study species, *P. leucopus*. Body weight was significantly different between breeding lines in gonad-intact mice in both photoperiod regimes (Rightler, et al. unpublished data, Majoy and Heideman 2000), and did not differ in experiments using a much larger sample of mice from our laboratory population using mice in short photoperiods only (Heideman, et al. 1999b).

There was no significant difference in the number of fat pads between breeding lines and photoperiod treatments. Body water and fat pads were examined separately because it has been established that these two factors are controlled separately (Hayward 1965). Other studies in *P. leucopus* have shown no significant difference in fat stores in short or long days (Merson and Kirkpatrick 1983). Nonresponsive *P.
**maniculatus** have been found to have lower levels of cytochrome oxidase activity in their BAT than responsive *P. maniculatus* in short days (Blank, et al. 1988). High cytochrome oxidase activity would indicate that the responsive *P. maniculatus* are turning over fat stores in their BAT rapidly in order to maintain homeothermy. Nonresponsive male *P. maniculatus* have been found to have a higher total fat content than responsive mice in short days (Millar and Schieck, 1986).

Similar to our mice, there was no difference in body fat contents in red-backed voles, *C. rutilus*, in short or long days (Zuercher, et al. 1999). There was, however, a significant increase in total body fat of pine voles, *M. pinetorum*, in the winter months (Cengel, et al. 1978). But this winter increase in total body fat was not seen in short-tailed voles, *M. agrestis* (Evans, Body fat increased in short photoperiod in arctic ground squirrels, *Spermophilus parryii kennicottii* (Buck and Barnes 1999), but this may not be a fair comparison since these squirrels hibernate, whereas *P. leucopus* do not.

Food consumption was significantly different between responsive and nonresponsive mice in both photoperiods. Food consumption did not differ among responsive mice in short and long days, but was significantly different for nonresponsive mice in short and long days. In contrast to previous experiments, food consumption by nonresponsive mice decreased in short days rather than increasing in short days. As this is the first time that a decrease in short day food consumption by nonresponsive mice has been observed, it may be due to sampling error or the high degree of variability in these mice. Further studies on food consumption need to be conducted. Nonresponsive *P. maniculatus* did not change their food intake in a study by Blank, et al. (1994). Food consumption was reduced in responsive *P. maniculatus* after exposure to short days for
Meadow voles, *M. pennsylvanicus*, showed a similar food consumption increase by nonresponsive voles versus responsive voles in short photoperiods (Bronson and Kerbeshian 1995). Both responsive and nonresponsive voles ate the same amount in long days (Bronson and Kerbeshian 1995), similar to the results in chapter 2 (Fig 4). Another study in *M. pennsylvanicus* found that food consumption was higher in long days than in short days (Dark and Zucker 1983). Red-backed voles, *C. rutilus*, did not differ in short or long day food consumption when maintained at constant temperatures (Ure 1984). There is some evidence that maintenance of fertility does increase susceptibility to cold stress, as the red-backed voles maintained in long days ate significantly more during periods of low ambient temperatures (Ure 1984). For prairie voles, *M. orchrogaster*, both responsive and nonresponsive voles had lower food consumption rates in short days than in long days (Moffatt, et al. 1993). This short day decrease in food consumption is only temporary in *M. orchrogaster*, as this species has been found to spontaneously increase food intake after 15 weeks of short day exposure (Dark, et al. 1983).

Activity levels varied considerably between responsive and nonresponsive mice in both daylengths. In short days, nonresponsive mice spent 24% more time performing stereotypic activities, such as grooming, than responsive mice. Our responsive mice and nonresponsive mice did not differ in the distance traveled in short days. Short day wheel running data for *P. maniculatus* is similar to the short day activity patterns for our *P. leucopus* (Moffatt, et al. 1993). Responsive mice did travel 25% farther than nonresponsive mice in long days. The greater distance traveled by responsive mice may be due to a tendency to search more for potential mates during the relatively short
breeding season for responsive mice. This is consistent with the findings of Perrot-Sinal, et al. (1998) that indicated that the plasma testosterone levels of meadow voles, *M. pennsylvanicus*, are directly correlated with locomotor activity. Circadian timing of locomotor activity may also be controlled by the level of circulating plasma testosterone (Rowsemitt 1986). A strong effect of photoperiod can be seen in both the stereotypic activity levels (Fig 14) and the total kilometers traveled (Fig 17), with more movement seen in the shorter, summer-like photoperiods. There was not a strong photoperiod effect on the total time spent resting (Fig 15) or the total ambulatory time (Fig 16).

Nonresponsive mice also produced both greater amounts (Fig 18) and larger pellets (Fig 20) of feces than responsive mice. The total dry weight of feces produced per day is directly correlated to the food consumption rates, as might be expected. The possibility for higher assimilation efficiency in responsive mice cannot be tested until bomb calorimetric analysis is performed. Differences in pellet size may suggest a difference in intestine size in responsive and nonresponsive mice. A study on intestine size in our *P. leucopus* is currently being conducted by another student from our laboratory. Increased energy demands can increase the size of the intestine in pregnant and lactating female mice (Hammond 1997). Similarly, if our nonresponsive *P. leucopus* are increasing food intake to provide extra energy to maintain fertility in short photoperiods, their intestines may also increase in size.
Conclusion

In conclusion, our laboratory colony of *P. leucopus* exhibits a tremendous degree of variability in their responses to photoperiod. Many of the behaviors analyzed are loosely linked with reproductive response, at best. No significant difference was found in fat content or total body water. As a small, granivorous rodent, the fat stores of our mice are already very low. The high lability of these fat stores in rodents means that fat deposition is not very useful in maintaining homeothermy or fertility beyond the scope of a single day's foraging (Bronson 1987). Food consumption may be linked with reproductive response but, as can be seen with the conflicting results from chapter 3, our mice are extremely variable with respect to food consumption. Thyroxine is most likely involved in the link between reproductive response and food consumption. The variable food consumption rates would correspond with variable thyroxine levels. However, since the reproductively responsive mice are not changing their food intake or mass-specific metabolic rates, the serum thyroxine levels of responsive mice should remain at a stable, lower level. Thyroxine levels are probably fluctuating in nonresponsive mice and further studies should be conducted to examine this effect.

Similarly, the effects of leptin on energy balance and reproductive response to winter-like photoperiods should be studied. Leptin levels should be higher in reproductively responsive animals, since leptin production is inhibited by the presence of testosterone (Ahima, et al. 2000). Leptin levels should be higher in mice that are recovering from reproductive inhibition by nonstimulatory photoperiods (short days). Leptin is most likely involved in the regulation of the metabolic rates of *P. leucopus* either through direct action through the expression of pro-opiomelanocortins which affect thyrotropin releasing hormones downstream, or by leptin directly affecting the thyroid.
Locomotor and stereotypic activities show a strong photoperiodic influence, but may also directly reflect the testosterone levels of male *P. leucopus*. The degree of reproductive response seems to be closely linked with locomotor activity in long days, but not short days. This difference may be due to an overall decrease in short day (wintertime) activity seen in many temperate zone rodents or it may be due to differences in oxytocin levels. This behavioral modification in response to short days could decrease foraging times that could lower predation risk and exposure to cold. Huddling behavior may also increase in white-footed mice in response to short days (Vogt and Lynch 1982). A lower testosterone level, as indicated by decreased locomotor activity, may decrease aggression among conspecifics, which may allow huddling to occur.
Does Reproductive Response Affect Metabolic Characteristics?

Long or Short Days

Responsive phenotype

Eat less
Run more
Lose less energy through fecal pellets
Lower metabolic rate
Lower body weight

Yes

Short days cause reproductive inhibition?

Nonresponsive phenotype

Eat more
Run less
Lose more energy through fecal pellets
Higher metabolic rate
Higher body weight

No

Exposure to Short Days

Run less
Eat same
Maintain metabolic rate

Run less
Eat more
Increase metabolic rate
# APPENDIX A

## METABOLIC RATE AND FOOD CONSUMPTION DATA

### FOR GONAD – INTACT MALES

<table>
<thead>
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<th>Line</th>
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<th>Long Day Body Weight</th>
<th>Short Day Food Consumption</th>
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### APPENDIX A

**METABOLIC RATE AND FOOD CONSUMPTION DATA FOR GONAD – INTACT MALES**

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APPENDIX A

METABOLIC RATE AND FOOD CONSUMPTION DATA
FOR GONAD – INTACT MALES

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APPENDIX B

METABOLIC RATES AND FOOD CONSUMPTION OF
CASTRATE MALE MICE WITH TESTOSTERONE IMPLANTS

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APPENDIX B

METABOLIC RATES AND FOOD CONSUMPTION OF CASTRATE MALE MICE WITH TESTOSTERONE IMPLANTS

<table>
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<tr>
<th>Mouse #</th>
<th>Photo period</th>
<th>Line</th>
<th>Oxygen Consumed</th>
<th>Carbon Dioxide Produced</th>
<th>Active Metabolic Rate</th>
<th>Resting Metabolic Rate</th>
<th>Body Weight</th>
<th>Food Consumed per Day</th>
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APPENDIX C – FUTURE DIRECTIONS

A series of studies would be useful to follow these results.

<table>
<thead>
<tr>
<th>Study</th>
<th>Details</th>
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<tr>
<td>Bomb calorimetric analysis of fecal pellets</td>
<td>To examine whether digestive efficiency is changing with reproductive response to short daylengths.</td>
</tr>
<tr>
<td>Castration and metabolic rate experiment</td>
<td>To repeat with blank Silastic implants and implants approximating a normal physiological testosterone level.</td>
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<tr>
<td>Ether extraction of dried mice</td>
<td>To determine if there is a difference in total fat stores between breeding lines of our <em>P. leucopus</em> or photoperiods.</td>
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<tr>
<td>Effects of leptin on metabolic characteristics and food intake</td>
<td>To examine with respect to photoperiod and reproductive response.</td>
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<tr>
<td>Effects of thyroxine and reproductive response</td>
<td>To study with respect to body mass, food intake, activity levels, fat deposition and mass-specific metabolic rate.</td>
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<tr>
<td>Interaction between leptin and thyroxine</td>
<td>To conduct as they relate to metabolic characteristics of reproductive response to short days.</td>
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<tr>
<td>Other homeothermic behaviors/mechanisms</td>
<td>To examine with respect to mass-specific metabolic rate, e.g. pelage depth/weight, huddling versus non-huddling, and nesting behavior.</td>
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<tr>
<td>Metabolic characteristics of males and females</td>
<td>To compare with respect to reproductive responsiveness to inhibitory photoperiods.</td>
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</table>


Heideman, P. D., S. L. Kane and A. L. Goodnight. 1999a. Differences in the hypothalamic 2-[^125]I iodomelatonin binding in photoresponsive and
nonphotoresponsive white-footed mice, *Peromyscus leucopus*. Brain Research, 840: 56-64.


VITA

Michelle Elaine Rightler was born in Orlando, Florida on June 25, 1972. She participated in the International Baccalaureate Program and went on to complete her Bachelor’s Degree in Biology from the University of Central Florida in 1994. She moved to Virginia in 1997 and began attending the College of William & Mary in 1998. She has two children. Duncan Bayrd was born on November 1, 1994. Anastasia Laurel was born during her graduate study on November 19, 2000. She met and married her husband, Michael, in the summer of 1998. Michelle is currently a laboratory technician for the Biology Department at William & Mary as well as an adjunct professor of biology at Thomas Nelson Community College. She will begin her public high school teaching career in the fall of 2001 for Newport News Public Schools.