

\PHOTOPERIOD AND NUTRITIONAL INFLUENCES ON REPRODUCTION,
GROWTH AND PELAGE CHANGES IN THE PINE VOLE/

by

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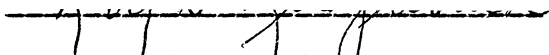
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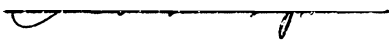
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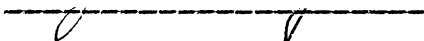
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TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGEMENTS.....	ii
TABLE OF CONTENTS.....	iii
LIST OF FIGURES.....	vi
LIST OF TABLES.....	vii
LIST OF APPENDIX TABLES.....	xiii
INTRODUCTION.....	1
Chapter 1. Effect of apple availability on natality, body weight and reproduction in pine voles during autumn.	
Introduction.....	5
Literature Review.....	6
Reproduction in pine voles.....	6
Litter size.....	6
Breeding season.....	8
Reproductive potential.....	9
Effects of nutrition on reproduction.....	10
Methods and Materials.....	16
Results.....	19
Natality.....	19
Body weight.....	21
Reproductive characteristics.....	24
Discussion.....	37

	<u>Page</u>
Chapter 2. Effect of photoperiod and nutrition on body weight and reproduction in adult pine voles.	
Introduction.....	43
Literature Review.....	44
Methods and Materials.....	48
Results.....	51
Food consumption.....	51
Body condition.....	54
Reproductive characteristics.....	63
Discussion.....	68
Chapter 3. Effect of photoperiod and nutrition on growth, molt and reproductive organs in young pine voles.	
Introduction.....	77
Literature Review.....	78
Methods and Materials.....	83
Results.....	86
Food consumption.....	86
Body condition.....	86
Molt.....	97
Reproductive characteristics.....	103
Discussion.....	106
GENERAL DISCUSSION.....	113
LITERATURE CITED.....	121

	<u>Page</u>
APPENDIX.....	128
APPENDIX A Effect of photoperiod and nutrition on reproduction in pine voles.....	131
APPENDIX B Effect of apple drops on reproduction in enclosed populations of pine voles.....	144
VITA.....	149
ABSTRACT	

LIST OF FIGURES

<u>Fig.</u>		<u>Page</u>
1	Mean kcal digestible energy consumed per vole per week in adult pine voles subjected to different nutritional and photoperiod regimes.....	52
2	Mean body weights in adult male pine voles subjected to different nutritional and photoperiod regimes.....	55
3	Mean body weights in adult female pine voles subjected to different nutritional and photoperiod regimes.....	56
4	Mean kcal digestible energy consumed per vole per week in young pine voles subjected to different nutritional and photoperiod regimes.....	87
5	Mean body weights in young male pine voles subjected to different nutritional and photoperiod regimes.....	89
6	Mean body weights in young female pine voles subjected to different nutritional and photoperiod regimes.....	90

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Number of immature pine voles and immature to adult ratio for pine voles trapped during autumn, 1980, in an orchard near Troutville, Virginia.....	20
2	Mean values ($\pm$ SE) and sample sizes by treatment group for body weight and change in body weight in adult male and female pine voles trapped in both October and November, 1980, or in both October and December, 1980, in an orchard near Troutville, Virginia.....	22
3	Mean squares for body weights and change in body weights in adult male and female pine voles trapped in both October and November, 1980, or in both October and December, 1980, in an orchard near Troutville, Virginia.....	23
4	Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in adult male and nonpregnant female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	25
5	Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in immature male and female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	26
6	Mean squares for final body weights and body lengths in adult male and nonpregnant female and in immature male and female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	27
7	Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	28

<u>Table</u>	<u>Page</u>
8 Mean squares for reproductive characteristics in adult male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	29
9 Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	31
10 Mean squares for reproductive characteristics in nonpregnant adult female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	32
11 Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in immature male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	33
12 Mean squares for reproductive characteristics in immature male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	34
13 Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in immature female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	35
14 Mean squares for reproductive characteristics in immature female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.....	36
15 Mean squares for kcal digestible energy consumed per vole on a weekly basis during the last half of the experiment in adult pine voles subjected to different nutritional and photoperiod regimes.....	53

<u>Table</u>	<u>Page</u>
16 Mean squares for body weights taken every 2 weeks during the last half of the experiment in adult male and female pine voles subjected to different nutritional and photoperiod regimes.....	57
17 Mean values ($\pm$ SE) and sample sizes by treatment group for change in body weight in adult male and female pine voles subjected to different nutritional and photoperiod regimes and surviving the last half of the experiment.....	59
18 Mean squares for change in body weights in adult male and female pine voles subjected to different nutritional and photoperiod regimes and surviving the last half of the experiment.....	60
19 Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in adult male and female pine voles subjected to different nutritional and photoperiod regimes.....	61
20 Mean squares for final body weights and body lengths in adult male and female pine voles subjected to different nutritional and photoperiod regimes.....	62
21 Mean values ($\pm$ SE) and sample sizes by treatment group for fat content (expressed as percent of dry body weight) in adult male and female pine voles subjected to different nutritional and photoperiod regimes.....	64
22 Mean squares for fat content in adult male and female pine voles subjected to different nutritional and photoperiod regimes.....	65
23 Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes.....	66

<u>Table</u>	<u>Page</u>
24 Mean squares for reproductive characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes.....	67
25 Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult female pine voles subjected to different nutritional and photoperiod regimes.....	69
26 Mean squares for reproductive characteristics in adult female pine voles subjected to different nutritional and photoperiod regimes.....	70
27 Mean squares for kcal digestible energy consumed per vole on a weekly basis in young pine voles subjected to different nutritional and photoperiod regimes.....	88
28 Mean squares for body weights taken every 4 or 7 days in young male and female pine voles subjected to different nutritional and photoperiod regimes.....	91
29 Mean values ($\pm$ SE) and sample sizes by treatment group for change in body weight and change in body length in young male and female pine voles subjected to different nutritional and photoperiod regimes.....	93
30 Mean squares for change in body weights and change in body lengths in young male and female pine voles subjected to different nutritional and photoperiod regimes.....	94
31 Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in young male and female pine voles subjected to different nutritional and photoperiod regimes.....	95

<u>Table</u>	<u>Page</u>
32 Mean squares for final body weights and body lengths in young male and female pine voles subjected to different nutritional and photoperiod regimes.....	96
33 Mean values ($\pm$ SE) and sample sizes by treatment group for fat content (expressed as percent of dry body weight) in young male and female pine voles subjected to different nutritional and photoperiod regimes.....	98
34 Mean squares for fat content in young male and female pine voles subjected to different nutritional and photoperiod regimes.....	99
35 Mean values ($\pm$ SE) and sample sizes by treatment group for age and body weight at initiation and completion of molt to adult pelage in young male pine voles subjected to different nutritional and photoperiod regimes.....	100
36 Mean values ($\pm$ SE) and sample sizes by treatment group for age and body weight at initiation and completion of molt to adult pelage in young female pine voles subjected to different nutritional and photoperiod regimes.....	101
37 Mean squares for ages and body weights at initiation and completion of molt to adult pelage in young pine voles subjected to different nutritional and photoperiod regimes.....	102
38 Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in young male pine voles subjected to different nutritional and photoperiod regimes.....	104
39 Mean squares for reproductive characteristics in young male pine voles subjected to different nutritional and photoperiod regimes.....	105

<u>Table</u>	<u>Page</u>
40 Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in young female pine voles subjected to different nutritional and photoperiod regimes.....	107
41 Mean squares for reproductive characteristics in young female pine voles subjected to different nutritional and photoperiod regimes.....	108

LIST OF APPENDIX TABLES

<u>Table</u>		<u>Page</u>
I	Mean values ($\pm$ SE) and sample sizes by treatment group for light intensity measured under different photoperiod regimes and sky conditions.....	129
II	Mean values ($\pm$ SE) and sample sizes by treatment group for mean body weight in young male and female pine voles 51-60, 61-70 and 71-80 days of age and subjected to different nutritional and photoperiod regimes.....	130
III	Mean values ($\pm$ SE) and sample sizes by treatment group for change in body weight in adult male and nonpregnant female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	134
IV	Mean squares for change in body weights and final body weights in adult male and female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	135
V	Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight in adult male and female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	136
VI	Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	138
VII	Mean squares for reproductive characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	139

<u>Table</u>	<u>Page</u>
VIII Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in nonpregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	140
IX Mean squares for reproductive characteristics in nonpregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	141
X Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in pregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	142
XI Mean squares for reproductive characteristics in pregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.....	143
XII Mean values ($\pm$ SE) and sample sizes by trapping period for body weight and body length in adult male and female pine voles in enclosures having apples present or apples removed during late autumn, 1980.....	146
XIII Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male and female pine voles in enclosures having apples present or apples removed during late autumn, 1980.....	147

INTRODUCTION

The pine vole (Microtus pinetorum) is a small, semifossorial rodent of major importance as a pest species in apple orchards. Orchards appear to be an excellent habitat for the species because voles attain higher densities in orchards than in their native habitat (Byers et al. 1976). Pine voles injure apple trees by girdling the roots and underground portions of the tree trunk. Damage has been estimated to reduce the potential apple production in Virginia 10% each year, resulting in an estimated economic loss of nearly \$7 million annually (Virginia Fruit 1971). Byers (1974) estimated a market value loss of about \$40 million annually throughout the vole's geographic range. Losses continue to occur even though orchardists spend an average of \$14.30 per acre to control pine voles (\$3.3 million annually).

Although pine voles have been an economic problem in orchards for many years, the U.S. Fish and Wildlife Service did not begin evaluating control measures until 1934 (LaVoie and Tietjen 1971). Early research attempts concentrated on finding chemicals to completely eliminate voles from orchards. Many growers attempt to control vole populations by spraying or hand baiting rodenticides, cultivating vegetation under the tree dripline and applying herbicides,

or a combination of both (Byers et al. 1976). LaVoie and Tietjen (1971) claim chemical treatments offer only temporary relief and suffer problems in time-cost economics, lack of trained operators, and use limitations. Davis (1976) notes the political overtones of getting chemicals approved by government agencies and formulating a management plan for adequate control of vole damage.

Hansson (1979) felt research on many pest species should take a broader approach, encompassing the ecology of the species within the community it inhabits. Research should be oriented towards finding weak points in the species' life history and devising control methods to manage their populations. The main objectives in pine vole research should be reducing and controlling tree damage, not complete eradication of vole populations (Horsfall 1972, Davis 1976). Ecological factors which control vole numbers will differ over the vole's geographic range (Paul 1970); thus, interactions between pine voles and their environment need to be studied.

Salmon and Marsh (1978) stated physiological processes differ with age and can affect rodent susceptibility to poisons and chemicals. Physiological processes and "physiological" age are in turn influenced by the environmental conditions under which rodents are born, grow and reproduce (Schwarz et al. 1964). The breeding season

and age at puberty in most mammals are highly variable and dependent on environmental factors such as photoperiod, nutrition, temperature and social factors (Sadlier 1969, Hasler 1975).

Nutrition is one factor which can be manipulated to aid in controlling herbivore populations. Outbreaks of herbivore populations occur in agricultural systems because a food source is readily available and often of good nutritional quality (White 1978). Myllymaki (1979) suggested altering the habitat, reducing refuge areas or influencing the food chain as possible methods for controlling pest species.

Cole and Batzli (1979), studying nutrition and population dynamics in Microtus ochrogaster, concluded quality of the food supply available in a habitat influenced population dynamics. Populations of Microtus townsendi supplemented with food had more immigration and reproduction, leading to a higher density than the control population (Taitt and Krebs 1981). Cengel et al. (1978) postulated quality of available food during late fall and winter influenced the partitioning of net food energy in pine voles into continued reproduction in a maintained apple orchard and deposition of body fat in an abandoned orchard. Noffsinger (1976) reported voles in a maintained orchard had a diet of higher nutritional value (i.e. more energy) which

contributed to the higher natality and lower mortality in the maintained orchard than in the abandoned orchard.

Reiter (1974) concluded many animal species cue reproduction to photoperiod as a way of increasing survival of offspring. Hasler (1975) noted that microtines on a poor diet or those exposed to constant short or declining photoperiods took longer to attain puberty. Pinter and Negus (1965) and Pinter (1968a,b) observed that M. montanus on an 18L:6D photoperiod and fed wheat sprouts every 3 days had higher levels of growth and reproduction than those on a 6L:18D regime or groups fed wheat sprouts every 15 days. Noffsinger (1976) suggested the interaction between energy intake and declining photoperiod of late autumn may determine length of the breeding season in pine voles.

Thus, nutrition and photoperiod are two environmental factors affecting reproduction and growth in microtines and may control population densities. The purpose of this study was to determine the influences of and interactions between photoperiod and nutrition on reproduction, growth and molt to adult pelage in the pine vole. Effects of different nutritional levels were determined in lab and field experiments and influences of photoperiod with diet were tested in 2 lab experiments.

Chapter 1. Effect of Apple Availability on Natality,

Body Weight and Reproduction in Pine Voles

During Autumn

Introduction

Previous studies have indicated a relationship between reproduction and nutrition in pine voles in apple orchards. Cengel et al. (1978), using stomach content analysis, discovered pine voles had greater availability of quality forage in a maintained orchard than in an abandoned orchard during winter, a period of critical nutritional intake. Lochmiller (1980) found higher levels of both biomass and gross energy of forages in a maintained orchard in summer, fall and winter. Cengel et al. (1978) and Noffsinger (1976) found a higher natality rate and a longer breeding season in maintained orchards than in abandoned orchards. In both studies the higher reproductive output in a maintained orchard was attributed to better nutritional intake, especially in late fall. This could result from the availability of apple drops or from better quality and quantity of available forage due to orchard management (i.e. mowing and fertilizing).

The objective of this experiment was to test the influence of a nutritional supplement, in the form of apple

drops, on body weight and reproduction in pine voles during autumn in an orchard environment.

Literature Review

Reproduction In the Pine Vole

Litter size

Pine voles have small litters relative to most other microtines (Hasler 1975). This could be an adaptation for allowing better mobility within the tight confines of a burrow system (Paul 1970). Horsfall (1963) and Schadler and Butterstein (1979) believed a small litter size may have evolved because of the species' nearly predator-free environment. Schadler and Butterstein (1979) felt the adaptation was also due to the species' relatively stable populations and sufficient food supplies.

In some field studies authors reported an average litter size of 2.0 with a range of 1 to 4 young per litter (Horsfall 1963, Boyette 1966, Gentry 1968). Schadler and Butterstein (1979), under laboratory conditions, observed an average of 3.1 young born per litter with 2.8 raised to weaning.

Valentine and Kirkpatrick (1970) found an average of 1.9 fetuses per pregnant female with a low of 1.0 in April and a high of 3.2 in June. Noffsinger (1976) discovered the average number of fetuses per pregnant female was 1.9 in a

maintained orchard and 1.5 in an abandoned orchard. Maximum values were 2.2 in September and 2.3 in November, and minimum values were 1.3 in May and 0.0 in March in maintained and abandoned orchards, respectively. No pregnant females were captured in the abandoned orchard in March. Cengel et al. (1978) also found a greater average number of fetuses per pregnant female in a maintained orchard. Highest mean number of fetuses was 2.2 in September in the maintained orchard and 2.0 in March in the abandoned orchard. Lowest means were recorded in November in the abandoned orchard and in January in both orchards when no pregnant females were captured. In North Carolina, Paul (1970) calculated an average litter size of 2.2 with lower averages in December and January and a peak in July.

Cengel (1975) found larger adult female voles were pregnant more often than smaller adult females. Paul (1970) found females under 129mm total length averaged 2.2 fetuses per pregnant female while females over 130mm averaged 2.5. Lochmiller (1980) reported size and weight of the litter were not significantly correlated with the initial body weight of the female. During the 21 days after parturition lactating females required an extra 105 kcal of metabolizable energy to support a litter of 2 young, as opposed to a litter of 1. This was greater than the difference in energy requirements (80 kcal ME) to support a

litter of 2 vs. 3 young.

Breeding season

Benton (1955), trapping in apple orchards in New York, found voles breeding from January to October. Reproduction occurred from mid-February to mid-November in southern New England (Miller and Getz 1969). Most of the voles captured were in upland hardwoods. Several authors have observed some reproduction throughout the year with seasonal variations in reproductive activity (Horsfall 1963, Boyette 1966, Goertz 1971, Noffsinger 1976).

In Virginia, Valentine and Kirkpatrick (1970) found voles reproducing in an orchard from April to October, with the peak occurring in July and August. Horsfall (1963) reported reproduction was highest from July to October and lowest from February to April. Goertz (1971), trapping in savanna edges, woods, and prairies in Oklahoma, observed high levels of breeding from December to April but depressed levels during summer. Reduced reproductive activity in summer was also reported in the Piedmont and Coastal Plain of North Carolina (Boyette 1966, Paul 1970). However, Paul (1970) noticed that in the mountains breeding peaked in July and was at minimum levels from October to January.

Breeding occurred year round in a maintained orchard (though at lower levels in January) but lasted from March to

November in an abandoned orchard (Cengel et al. 1978). Noffsinger (1976) had similar results in a different maintained orchard but in an abandoned orchard reproduction occurred from May to January. In both studies, peak levels of breeding in the maintained orchards were observed in July and September (trapping occurred bimonthly), and in Noffsinger's study extended into November. July was also the peak month of reproduction in the abandoned orchards. Cengel et al. (1978) did not trap their abandoned orchard in July but based their conclusion on the high percentage of immature voles trapped in September presumably born in July.

Reproductive potential

Gestation period in the pine vole is 24 days (Kirkpatrick and Valentine 1970). They reported a postpartum estrus in females and that implantation was not delayed by lactation. Schadler and Butterstein (1979) found 87% of the litters born in their lab were conceived within 3 days postpartum.

Goertz (1971) thought females could conceive 1 to 4 litters in a year. Gentry (1968) computed an annual average potential of only 1.7 litters per female, but cited 1 female in his enclosures which was pregnant 7 times over an 18-month time span. Paul (1970) calculated an adult female could produce 12.5 litters per year for a total annual

production of 29 young/female. Noffsinger (1976) calculated that the net reproductive rate (the number of times a population can multiply its numbers in a generation) was greater in a maintained orchard. He also found the reproductive rate for May-July and July-September cohorts in this orchard was greater than 1.0, indicating members of these cohorts can replace themselves numerically before all members of the cohort die. However, the reproductive potential in the pine vole is lower than most microtines because of their smaller litter sizes and older age at puberty (Schadler and Butterstein 1979).

Effects Of Nutrition On Reproduction

Gentry (1968) thought dispersal, predation and food were important factors controlling pine vole numbers. Food was most important, especially in winter, and the excess food available in baited traps may have allowed high densities to occur in winter.

Lochmiller (1980) determined energy requirements for maintenance and lactation in pine voles. Daily metabolic requirements showed strong seasonal trends reflecting thermoregulatory costs. Metabolic requirements increased from summer to winter but were moderated by a decrease in activity, an increase in body fat and a thick winter pelage. Lactating females consumed more food to meet their increased

energy demands. Increasing food consumption generally increases passage rate and decreases digestive efficiency; however, studies on other microtines (Clethrionomys glareolus and Microtus oeconomus) showed lactating females increased digestive efficiency by increasing the size of the alimentary tract (Myrcha 1964, Gebczynska and Gebczynski 1971). A female pine vole nursing an average litter of 2.2 young required 419 kcal of metabolizable energy over the entire 21 day lactation period (Lochmiller 1980). This energy demand was 135 kcal, or 48%, greater than maintenance costs for a nonreproducing female over the same time period. Energy demands on the female were higher with an increase in litter size and demands increased during the time from parturition to weaning. There was a greater effect of food restriction on maternal weight losses as well as growth and body composition of young in lactating females supporting 2 when compared to those supporting 1 young (Merson 1979).

Lactation appears to be the most energy-demanding portion of reproduction. When food availability and/or quality decrease, the demands of lactation put more stress on a female. Lochmiller (1980) concluded a combination of small litter size and high production efficiency may allow pine voles to breed even when food becomes scarce.

These findings agree with field studies on nutrition and reproduction in pine voles (Cengel et al. 1978,

Noffsinger 1976). Cengel (1975) felt pine vole food habits were based on selectivity and availability. Cengel et al. (1978) and Noffsinger (1976) found grasses were dominant in maintained orchards and forbs in abandoned orchards. Voles in a maintained orchard consumed mostly grasses from September to March but shifted to forbs in May and July (Cengel et al. 1978). In the abandoned orchard, forbs were the major dietary item year round. Roots were eaten in substantial amounts in both orchards in January and March, when other foods were unavailable or unacceptable. Cengel et al. (1978) found voles ate more apples in the maintained orchard and that most apple consumption occurred in fall and winter. Noffsinger (1976) stated gross energy of vegetation was lowest in both orchards in November and January. Lochmiller (1980) estimated total gross energy and dry biomass of forages were greater in the maintained orchard than the abandoned orchard, with values lowest for vegetation and highest for apples in winter.

Cengel (1975) felt the changes and differences in vegetation between the 2 orchard types may indicate the importance of nutrition on parameters of reproduction. In the maintained orchard, better food throughout the year accounted for higher reproductive output (Cengel 1975) and the availability of highly nutritious grasses and apple drops extended the breeding season (Estep 1975). Noffsinger

(1976) found a lower reproductive rate and a higher mortality rate in the abandoned orchard. He calculated the decline in reproduction in the abandoned orchard during fall and winter was due to females not consuming enough energy to meet energy requirements for pregnancy and lactation. Merson (1979) felt a higher mortality rate may be related to the lower quality and availability of food in this orchard type.

Body fat is influenced most by diet and reproductive condition (Estep 1975). Merson (1979) noted differences in body fat may be due to how the animal partitions its available food energy. Cengel et al. (1978) showed pine voles in an abandoned orchard had significantly greater body weights ($P < 0.01$) and more body fat ($P < 0.05$). However, Noffsinger (1976) reported fat content was higher for both sexes in the maintained orchard except during May for males and March and July in females, when values were greater in the abandoned orchard.

Hoffman (1958) discovered litter size in M. montanus and M. californicus was directly correlated with protein content of available forage. Sadlier et al. (1973) concluded Peromyscus maniculatus ceased reproducing during average winters because energy consumption and possibly protein quality of forage were not adequate to meet the demands of lactation. Flowerdrew (1972, 1973) found an

increased food supply lengthened the breeding season in 2 European rodents by advancing onset of breeding and extending reproduction into winter. In C. glareolus an extended breeding season into late fall and winter occurred because of favorable weather and food supply (Kubik 1965).

Cole and Batzli (1979) looked at the influence of nutrition on population dynamics in M. ochrogaster. Highest quality food was in an abandoned alfalfa field where voles had a longer breeding season, larger average litters, better survival, and higher densities than in the tall grass prairie or bluegrass pasture habitats. Voles fed an alfalfa diet grew faster and had higher rates of reproduction. They concluded quality of the food available to small herbivores has a major influence on reproduction, growth, survival and population density.

Krebs and DeLong (1965) supplemented the diet in a M. californicus population and found an increased growth rate and high reproduction rates but survival remained low. Zejda (1962) felt an increased food supply to C. glareolus had a more important effect on shifting the breeding season forward 2 months than in allowing reproduction to occur in winter. Andrzejewski (1975) showed a population of this species supplemented with food continued reproducing into winter and the young produced grew faster, attaining puberty and becoming sexually active by spring. He determined the

increase in food supply had no effect on winter survival. Supplemental food supplies were responsible for an earlier onset of breeding in P. leucopus (Hansen and Batzli 1979) and increased reproduction, body growth and rate of attaining puberty in both M. ochrogaster (Cole and Batzli 1978) and P. maniculatus (Taitt 1981).

Rodents with a good food supply may not only extend the breeding season but may also reproduce in winter. Zejda (1962) believed winter breeding in C. glareolus was due to a good mast crop in oak habitats and to favorable temperatures. Tast and Kaikusalo (1973) observed winter breeding in M. oeconomus and attributed it to the availability of high quality roots and vegetative shoots. Negus et al. (1977) found M. montanus breeding in February. They speculated that winter breeding before a population peak in a microtine cycle was logical because high numbers of voles from the previous year crop the vegetation. Cropping would stimulate vegetative growth, rhizome growth, and formation of new shoots which provides good winter nutrition.

Sealander (1972) studied circum-annual changes in C. rutilus and reported brown fat increased at the end of the breeding season, peaked in January, and then declined with onset of reproduction in spring. Andrzejewski (1975) found fat content in C. glareolus was highest and reproduction

lowest in winter in the grid not receiving additional food. Voles in areas with a supplemental food supply had lower fat content but were reproductively active in February. In contrast, Evans (1973) observed fat reserves in breeding female M. agrestis were significantly greater than in breeding males ($P < 0.01$) and females outside the breeding season ($P < 0.01$). Fat deposits were greatest during summer and very low in both sexes in winter.

In summary, the reproductive output of pine voles differs in various habitats. More specifically, both reproduction and nutritional quality are higher in maintained than in abandoned apple orchards. In other rodent species supplemental food supplies increase reproduction and growth. The following experiment was designed to test the influence of a supplemental food (apples) on reproduction in pine voles in an apple orchard.

Methods and Materials

This experiment was conducted during the autumn of 1980 in an apple orchard near Troutville, Virginia. Three treatment areas were established in the orchard. In 2 areas apple drops and apples were removed during early September. Apple drops were also picked up in the following weeks. No apples were removed from the remainder of the orchard.

A control grid in the area with apples present was

located near the middle of the orchard. This grid was 6 tree rows by 12 trees with a total of 64 trees. The areas with apples removed were on 1 side of the orchard, bordered by a pasture and a road at either end. These 2 areas were separated by an open space of missing trees. The smaller grid (Grid I) measured 4 rows by 4 trees; the larger grid (Grid II) was 4 rows by 11 trees.

All 3 grids were live-trapped for 3 consecutive days each month from August to October. Data on location of capture, sex, age, body weight (Pesola 100, $\pm 1g$) and length, and reproductive condition were recorded. Voles were classed as adults if they weighed 21g or more while those under 21g were considered immature. Captured animals were toe clipped for identification and released into a runway under the tree where they were captured.

In November Grid I and an equivalent number of trees chosen at random from the remainder of the orchard, but at least 2 trees away from the control grid, were snap-trapped. At this time Grid II and the control grid were live-trapped. In December Grid II and an equivalent number of trees from the remainder of the orchard were snap-trapped like the November sample.

After noting location of snap-trapped voles; age, body weight, length, and reproductive condition were recorded. The voles were placed on ice and returned to the laboratory

at VPI&SU. Testes were removed from all males, weighed, and frozen in a 0.09 percent saline solution containing Triton-X. Spermatozoa counts were done later using the technique of Sullivan and Scanlon (1976). Females were frozen immediately upon returning to VPI&SU; males were frozen after their testes were removed. Later, after thawing, adrenal glands were removed from all voles, seminal vesicles were removed from males, and uteri and ovaries were removed from females. During necropsy, uteri were examined for presence of visible embryos, and ovaries were examined for corpora lutea. Adrenal glands of both sexes, seminal vesicles of males and uteri of nonpregnant females were fixed for 2 weeks in a 5% formalin solution. Ovaries were fixed for 2 weeks in Bouins fluid. All organs were then stripped of excess tissue, blotted dry, and weighed (Cahn RTL electrobalance, $\pm 0.1\text{mg}$). The carcasses were used later for determination of crude body fat in a companion study (Servello 1981).

Body weights and lengths were analyzed by a 3 way analysis of variance and reproductive characteristics by a 2 way analysis of variance using the PROC GLM option of the Statistical Analysis System (Helwig and Council 1979).

Results

Natality

Eight pregnant females were captured in December in the area with apples present. These females had a mean of 1.9 fetuses per female. Pregnant females comprised 33% of all females captured in December in the area with apples present. No pregnant females were snap-trapped in November or December in the area with apples removed or in November in the area with apples present. Corpora lutea were found in 3 females in the area with no apples and in 2 females in the area with apples available in November. Only 1 female from the area with apples removed had corpora lutea in the December sample.

A ratio of immature to adult voles was calculated for both areas from September through December (Table 1). The ratio was similar between the 2 treatment areas in each month regardless of trap methodology. The proportion of young increased in both areas from September to October, continued increasing in the area with apples present into November, and declined in both areas from November to December. The decline was greater in the area with apples present. Both areas had nearly the same immature to adult ratio in December as in September.

Table 1. Number of immature pine voles and immature to adult ratio for pine voles trapped during autumn, 1980, in an orchard near Troutville, Virginia.

Month	N	Immature:Adult	Method of trapping
Apple Removed ¹			
September	13	24:76	live
October	45	37:63	live
November	53	37:63	live & snap
December	13	21:79	snap
Apple Present			
September	5	22:78	live
October	30	35:65	live
November ²	65	40:60	live & snap
December ²	10	19:81	snap

¹Combination of both areas with apples removed except December which are immatures from area snap-trapped in December.

²November figures combine live-trapped voles from grid and voles snap-trapped in surrounding area. December figures represent only those voles snap-trapped in December.

Body Weight

Mean body weight and mean change in body weight were determined for adult male and female pine voles trapped in both October and November or in both October and December (Tables 2 and 3). Voles snap-trapped in Grid I (November) or Grid II (December) which were live-trapped in October were compared to voles live-trapped in either October and November or October and December in the area with apples present.

Females had higher mean body weights in all groups (Table 2) and were significantly ($P < 0.09$) heavier than males in the October-December sample (Table 3). There were no significant differences due to presence or absence of apples ($P > 0.22$) or month ($P > 0.81$) and no significant ($P > 0.22$) differences in change in body weight.

Mean body weights did not change for males or females in either area from October to November or from October to December (Table 2). There were no trends in mean change in body weight because the mean changes were small and had relatively large standard errors. This would indicate that, even though there was no effect of treatment or month on body weight, weight dynamics were highly variable from individual to individual.

Mean final body weight and body length for all adult and immature voles snap-trapped in November and December are

Table 2. Mean values ($\pm$ SE) and sample sizes by treatment group for body weight and change in body weight in adult male and female pine voles trapped in both October and November, 1980, or in both October and December, 1980, in an orchard near Troutville, Virginia.

Group	Sex	N	Body weight (g)			Change in body weight
			October	November	December	
Grid I	M	8	25 $\pm$ 1	25 $\pm$ 1		-0.5 $\pm$ 0.9
Apple Removed	F	12	26 $\pm$ 1	26 $\pm$ 1		0.3 $\pm$ 0.9
Grid III	M	9	24 $\pm$ 1	24 $\pm$ 1		0.1 $\pm$ 0.9
Apple Present	F	7	26 $\pm$ 1	26 $\pm$ 1		0.0 $\pm$ 0.9
Grid II	M	6	25 $\pm$ 1		24 $\pm$ 1	-0.5 $\pm$ 1.2
Apple Removed	F	7	26 $\pm$ 1		25 $\pm$ 1	-1.0 $\pm$ 0.9
Grid III	M	11	24 $\pm$ 1		25 $\pm$ 1	1.1 $\pm$ 0.7
Apple Present	F	7	26 $\pm$ 2		26 $\pm$ 2	0.6 $\pm$ 1.0

Table 3. Mean squares (MS) for body weights and change in body weights in adult male and female pine voles trapped in both October and November, 1980, or in both October and December, 1980, in an orchard near Troutville, Virginia.

Source	df		Oct-Nov body weights	Oct-Dec body weights	Change in body weights
Sex (S)	1	MS	21.49	29.25	0.36
		P<	.14	.09*	.82
Treatment (T)	1	MS	10.71	1.56	10.20
		P<	.30	.69	.22
Month (M)	1	MS	0.01	0.58	0.40
		P<	.97	.81	.81
S X T	1	MS	0.66	0.01	1.31
		P<	.79	.97	.66
S X M	1	MS	0.67	2.12	5.29
		P<	.79	.64	.38
T X M	1	MS	0.07	9.23	8.18
		P<	.93	.33	.28
S X T X M	1	MS	0.96	0.00	0.85
		P<	.75	.99	.72
Error	64	MS	9.70	9.72 ¹	6.77 ²

¹Degrees of freedom for error term was 54.

²Degrees of freedom for error term was 59.

shown in Tables 4 and 5. Adults caught in November had significantly ($P < 0.03$) higher body weights than those trapped in December (Table 6). The difference was due primarily to changes in female body weight (Table 4). As a consequence there was a significant ($P < 0.01$) sex X treatment interaction (Table 6). There were no significant ($P > 0.39$) differences in adult body length.

Immature voles snap-trapped in December had significantly higher body weights ($P < 0.06$) and body lengths ($P < 0.003$) than those trapped in November (Table 6). Body weight increased from November to December in the area with apples removed and body length increased in all groups except females in the area with apples present (Table 5).

Reproductive Characteristics

Adult males in the area with apples present had significantly (Tables 7 and 8) higher values for adrenal glands weight ($P < 0.003$), seminal vesicles weight, paired testes weight and spermatozoa/mg testes ($P < 0.001$). All 4 characteristics decreased from November to December in the area with no apples and increased or remained unchanged in the area with apples present (Table 7); however, only paired testes weight showed a significant ($P < 0.04$) month X treatment interaction (Table 8). There were no significant ($P > 0.22$) differences due to month.

Table 4. Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in adult male and nonpregnant female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Group	<u>Male</u>				<u>Female</u>			
	Body weight		Body length		Body weight		Body length	
	(g $\pm$ SE)	(n)	(mm $\pm$ SE)	(n)	(g $\pm$ SE)	(n)	(mm $\pm$ SE)	(n)
November								
Apple Removed	25 $\pm$ 1	(17)	101 $\pm$ 1	(17)	26 $\pm$ 1	(24)	101 $\pm$ 1	(24)
Apple Present	25 $\pm$ 1	(17)	101 $\pm$ 1	(17)	24 $\pm$ 1	(17)	100 $\pm$ 1	(17)
December								
Apple Removed	24 $\pm$ 1	(20)	100 $\pm$ 1	(20)	24 $\pm$ 1	(18)	101 $\pm$ 1	(18)
Apple Present	26 $\pm$ 1	(21)	100 $\pm$ 1	(21)	23 $\pm$ 1	(16)	101 $\pm$ 1	(16)

Table 5. Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in immature male and female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Group	<u>Male</u>				<u>Female</u>			
	Body weight		Body length		Body weight		Body length	
	(g $\pm$ SE)	(n)	(mm $\pm$ SE)	(n)	(g $\pm$ SE)	(n)	(mm $\pm$ SE)	(n)
November								
Apple Removed	17 $\pm$ 1	(11)	88 $\pm$ 2	(11)	17 $\pm$ 1	(9)	87 $\pm$ 2	(9)
Apple Present	19 $\pm$ 1	(5)	87 $\pm$ 2	(5)	18 $\pm$ 1	(7)	92 $\pm$ 1	(7)
December								
Apple Removed	19 $\pm$ 1	(10)	94 $\pm$ 1	(10)	19 $\pm$ 1	(4)	93 $\pm$ 2	(4)
Apple Present	18 $\pm$ 1	(3)	94 $\pm$ 3	(3)	19 $\pm$ 1	(6)	93 $\pm$ 4	(6)

Table 6. Mean squares (MS) for final body weights and body lengths in adult male and nonpregnant female and in immature male and female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Source	df	<u>Adults</u>		<u>Immatures¹</u>	
		Body weights	Body lengths	Body weights	Body lengths
Sex (S)	1	MS	4.17	0.17	1.02
		P<	.46	.45	.61
Treatment (T)	1	MS	0.34	0.07	8.59
		P<	.83	.64	.14
Month (M)	1	MS	36.15	0.03	14.48
		P<	.03*	.75	.06*
S X T	1	MS	46.70	0.00	0.80
		P<	.01*	.98	.65
S X M	1	MS	7.93	0.23	0.88
		P<	.31	.39	.63
T X M	1	MS	17.14	0.03	6.56
		P<	.14	.77	.20
S X T X M	1	MS	3.56	0.01	0.44
		P<	.49	.87	.74
Error	142	MS	7.60	0.30	3.80

¹Degrees of freedom for error term was 47.

Table 7. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Group	Adrenal glands weight		Seminal vesicles weight		Paired testes weight		Spermatozoa sperm/mg testes wt $\times 10^3$	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	$\pm$ SE	(n)
November								
Apple Removed	9.8 $\pm$ 0.7	(15)	13.6 $\pm$ 3.1	(14)	33.5 $\pm$ 3.8	(17)	49.6 $\pm$ 8.2	(10)
Apple Present	11.8 $\pm$ 0.9	(17)	30.7 $\pm$ 6.6	(15)	57.7 $\pm$ 9.4	(17)	70.1 $\pm$ 14.1	(10)
December								
Apple Removed	9.6 $\pm$ 0.4	(21)	6.8 $\pm$ 0.8	(18)	27.9 $\pm$ 2.9	(20)	32.5 $\pm$ 5.8	(15)
Apple Present	11.5 $\pm$ 0.5	(19)	38.2 $\pm$ 5.6	(19)	76.0 $\pm$ 5.1	(21)	68.2 $\pm$ 7.3	(15)

Table 8. Mean squares (MS) for reproductive characteristics in adult male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Source	df	Adrenal glands weights	Seminal vesicles weights	Paired testes weights	Sperm/mg testes wts X 10 ³
Treatment (T)	1 MS	68.87	10434.02	26046.28	10947.56
	P<	.003*	.001*	.001*	.001*
Month (M)	1 MS	2.81	3.94	896.06	1084.52
	P<	.53	.92	.22	.28
T X M	1 MS	0.06	832.64	2669.33	689.17
	P<	.93	.13	.04*	.39
Error	68 MS	7.12	350.46 ¹	591.98 ²	911.27 ³

¹Degrees of freedom for error term was 62.

²Degrees of freedom for error term was 71.

³Degrees of freedom for error term was 46.

Mean values for adrenal glands and reproductive organ weights of adult females are shown in Table 9. There were no significant effects due to month ($P>0.48$) or treatment ($P>0.19$) in nonpregnant females (Table 10). Mean uterine and paired ovarian weights were the same in November between the 2 areas but were slightly higher in December for females in the area with apples present. Adrenal glands and paired ovarian weights in pregnant females were 131% and 205% higher, respectively, than in nonpregnant females trapped in December in the area with apples present (Table 9).

Immature male pine voles in the area with apples present had significantly (Tables 11 and 12) heavier seminal vesicles ($P<0.006$) and paired testes ($P<0.08$). Organ weights remained the same from November to December in the area with apples removed but increased slightly in the area with apples present (Table 11). However, as was seen in adult males, only paired testes weight showed a significant ($P<0.09$) month $\times$ treatment interaction (Table 12). There were no significant ($P>0.42$) differences due to month.

Young female voles in the area with apples present had significantly (Tables 13 and 14) higher values for adrenal glands weight ($P<0.04$), uterine weight ($P<0.01$), and paired ovarian weight ($P<0.02$). Females trapped in December had significantly heavier adrenal glands ($P<0.04$) and uteri ($P<0.05$). There was no change from November to December for

Table 9. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Group	<u>Nonpregnant Female</u>					
	Adrenal glands weight		Uterine weight		Paired ovarian weight	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)
November						
Apple Removed	9.2 $\pm$ 0.5	(22)	26.9 $\pm$ 2.9	(23)	3.3 $\pm$ 0.3	(19)
Apple Present	9.2 $\pm$ 0.6	(16)	25.6 $\pm$ 5.5	(13)	3.3 $\pm$ 0.3	(14)
December						
Apple Removed	9.2 $\pm$ 0.5	(16)	21.1 $\pm$ 3.0	(18)	2.8 $\pm$ 0.3	(18)
Apple Present	9.3 $\pm$ 0.5	(13)	27.3 $\pm$ 4.0	(14)	3.7 $\pm$ 0.5	(14)
Group	<u>Pregnant Female</u>					
	Body weight		Adrenal glands weight		Paired ovarian weight	
	(g $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)
December						
Apple Present	30.4 $\pm$ 1.4	(8)	12.2 $\pm$ 1.2	(7)	7.6 $\pm$ 0.5	(8)

Table 10. Mean squares (MS) for reproductive characteristics in nonpregnant adult female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Source	df		Adrenal glands weights	Uterine weights	Paired ovarian weights
Treatment (T)	1	MS	0.06	88.94	3.91
		F<	.91	.54	.19
Month (M)	1	MS	0.02	117.91	0.29
		F<	.96	.48	.72
T X M	1	MS	0.04	220.42	2.96
		F<	.93	.33	.26
Error	63	MS	4.85	229.04 ¹	2.27 ²

¹Degrees of freedom for error term was 64.

²Degrees of freedom for error term was 61.

Table 11. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in immature male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Group	Adrenal glands weight	Seminal vesicles weight	Paired testes weight
	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)
November			
Apple Removed	7.7 $\pm$ 0.5 (11)	3.3 $\pm$ 1.1 (6)	13.6 $\pm$ 2.2 (11)
Apple Present	8.0 $\pm$ 0.5 (4)	14.1 $\pm$ 1.7 (2)	14.5 $\pm$ 4.2 (4)
December			
Apple Removed	7.8 $\pm$ 0.4 (10)	3.2 $\pm$ 0.9 (8)	13.3 $\pm$ 2.8 (10)
Apple Present	9.4 $\pm$ 2.4 (3)	17.6 $\pm$ 11.8 (3)	27.0 $\pm$ 5.6 (3)

Table 12. Mean squares (MS) for reproductive characteristics in immature male pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Source	df		Adrenal glands weights	Seminal vesicles weights	Paired testes weights
Treatment (T)	1	MS	3.78	618.04	222.50
		P<	.35	.006*	.08*
Month (M)	1	MS	1.19	5.90	46.89
		P<	.60	.76	.42
T X M	1	MS	2.34	11.23	212.82
		P<	.46	.67	.09*
Error	24	MS	4.22	61.13 ¹	68.40

¹Degrees of freedom for error term was 15.

Table 13. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in immature female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Group	Adrenal glands weight	Uterine weight	Paired ovarian weight
	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)
November			
Apple Removed	5.3 $\pm$ 0.8 (8)	7.1 $\pm$ 1.0 (5)	1.9 $\pm$ 0.3 (4)
Apple Present	7.7 $\pm$ 1.0 (6)	8.5 $\pm$ 1.5 (7)	3.1 $\pm$ 0.6 (4)
December			
Apple Removed	7.3 $\pm$ 0.6 (4)	7.2 $\pm$ 0.7 (4)	1.3 $\pm$ 0.1 (3)
Apple Present	9.4 $\pm$ 1.4 (6)	13.1 $\pm$ 0.8 (5)	2.5 $\pm$ 0.3 (5)

Table 14. Mean squares (MS) for reproductive characteristics in immature female pine voles trapped in November and December, 1980, in an orchard near Troutville, Virginia.

Source	df		Adrenal glands weights	Uterine weights	Paired ovarian weights
Treatment (T)	1	MS	29.21	57.33	6.32
		P<	.04*	.01*	.02*
Month (M)	1	MS	29.57	33.37	0.48
		P<	.04*	.05*	.32
T X M	1	MS	0.09	26.06	0.00
		P<	.91	.08*	.89
Error	20	MS	6.34	7.72 ¹	0.71 ²

¹Degrees of freedom for error term was 17.

²Degrees of freedom for error term was 12.

uterine weight in the area with apples removed and an increase in the area with apples present, causing a significant ($P < 0.08$) month X treatment interaction. Females in both areas had an increase in adrenal glands weight from November to December.

Discussion

Natality

There was little difference in the percentage of young between the areas with apples present and apples removed. The percentage of young trapped in the area with apples removed was higher than in the abandoned orchards of Cengel et al. (1978) and Noffsinger (1976) during the fall. The September immature:adult ratio in both areas in this experiment was similar to that reported by Noffsinger (1976) in a maintained orchard and the November ratio was higher than the maintained orchards of Cengel et al. (1978) and Noffsinger (1976). However, the December ratio was similar to a maintained orchard in January (Noffsinger 1976).

The breeding season appeared to be longer in the area with apples present because 33% of the females captured in this area in December were pregnant. No pregnant females were trapped in the area with apples removed. Reproduction in November and December in both areas was more comparable to abandoned orchards than to the maintained orchards of

previous studies. In a maintained orchard Noffsinger (1976) found 83% and 50% of the females pregnant in November and January, respectively, while in the abandoned orchard only 35% were pregnant in each month. Cengel et al. (1978) reported 46% of the females pregnant in November in a maintained orchard and no pregnant females in an abandoned orchard.

There could be several reasons why the results of this experiment differ from those stated above. The condition of the different orchards and quality of the habitat probably created differences in reproduction, survival and trappability. In this study the immature:adult ratio was comparable to while reproductive activity was lower than those found in maintained orchards in previous field studies. If the rate of reproduction was higher in maintained orchards in the previous studies, survival or growth of young through November may have been higher in this experiment, increasing the percentage of young captured. Some discrepancies may have arisen because the previous studies used pelage as the criteria to differentiate age classes whereas body weight was used in this study.

Trappability could have influenced the results between the 2 areas under investigation here. Voles in the area with apples removed may have been more likely to enter a

trap baited with apple in November. The differences would have been reduced by December because most of the apple drops in the area with apples present had decomposed and were unavailable. There could have been some complications in trappability by using both snap-traps and live-traps. The live-traps captured a higher percentage of young in November than did snap-traps. In December the live-traps used in the control grid captured the same proportion of young as the snap-traps did in areas outside the grid.

Body Condition

The presence of apples appeared to have little effect on body weight. Voles lacking apples in fall were able to maintain body weight as well as those which had apples available. Body weights in November were similar to those of Cengel et al. (1978) and slightly higher (1-2g) than reported by Noffsinger (1976).

In a companion study Servello (1981) found a decrease in percent crude body fat from November to December in males in both areas and in females in the area with apples removed. However, he reported no difference in diet digestibility in either area in either month. The decline in fat between November and December could indicate environmental conditions were affecting maintenance (fat reserves) in voles except for females in the area with

apples present. In November body fat was higher in voles of the present study than for voles trapped by Cengel et al. (1978) and Noffsinger (1976). Voles trapped in the present study in November may have been in better condition than voles trapped in these previous studies.

Reproductive Characteristics

Adult males and juveniles of both sexes had heavier reproductive organs and adult males had higher sperm counts in the area with apples present. From November to December adults and juveniles in the area with apples had an increase in mean weight of reproductive organs. Voles in the area with no apples showed a decrease or no change in reproductive organ weights. These trends were most pronounced in adult males. In this study the reproductive organ weights and spermatozoa counts of adults in November were lower than those of Noffsinger (1976) and organ weights were similar to those of Cengel et al. (1978). In both of these studies voles in maintained orchards had an increase in reproductive organ weights from November to January while voles in abandoned orchards had a decrease in reproductive organ weights during this time. Merson (1979) found adult voles on a restricted food intake had lower gonad weights in both sexes and total spermatozoa and plasma testosterone levels in males than adults fed ad libitum amounts of food.

Weights for paired testes and ovaries of juveniles trapped in December in the area with apples available were comparable to those of adults trapped in the area with apples removed. It is likely either the juveniles with apples available had attained puberty or the adults with no apples available had become sexually quiescent. Lack of sexual activity in adults is more logical in light of 1) low sperm counts, 2) no pregnant females were captured in the area with apples removed, and 3) young born late in the year usually do not attain puberty until the following spring (Schwarz et al. 1964).

In summary, apple removal did not seem to affect body weight. Body fat decreased from November to December in males in both areas and in females in the area without apples (Servello 1981). Reproductive organs were heavier in the area with apples present and increased from November to December. There were pregnant females captured in December in the area with apples. However, there was no difference in the immature:adult ratio between the 2 areas. Females in the area with apples removed may have remained reproductively active until November but began losing energy reserves (body fat) from November to December. Males in the area with apples removed may have reduced reproductive activity before November and decreased reproductive organ weight and body fat from November to December in order to

survive. Voles in the area with apples available were able to both maintain themselves (though males did lose some body fat) and reproduce.

Thus, the availability of apples may have extended the breeding season into winter, although the evidence is not conclusive. It appears that adult males were more sensitive than females to the presence of apples because reproductive characteristics were significantly different in males. Higher reproductive organ weights could indicate the presence of apples allowed for greater reproductive output in late fall. However, if the reproductive output was greater in the area with apples, then 1) trappability of young was lower, 2) growth of young to adulthood was quicker, 3) juvenile mortality was higher, and/or 4) differences in the immature:adult ratio would not appear until January or February when young born in November and December would be more trappable.

Chapter 2. Effect of Photoperiod and Nutrition on Body Weight and Reproduction in Adult Pine Voles.

Introduction

Photoperiod and nutrition are 2 important factors affecting reproduction in small rodents. Baker and Ranson (1932a,b) reported field voles (Microtus agrestis) on a 9L:15D photoperiod had fewer litters and a smaller mean litter size relative to voles on a 15L:9D light regime. In a separate experiment voles were maintained on a 15L:9D photoperiod but were given different diets. Reducing the amount of growing grass in the diet did not significantly affect reproduction. Pinter and Negus (1965) observed M. montanus on an 18L:6D light regime had larger mean litter sizes than those on 6L:18D and groups fed sprouted wheat every 3 days produced more litters and more offspring than those fed wheat sprouts every 15 days. Both Noffsinger (1976) and Merson (1979) suggested studies be done to determine the interactive effects of diet and a declining photoperiod on reproduction in the pine vole.

This experiment was designed to test the influence of photoperiod and nutrition on body weight and reproduction in adult pine voles.

Literature Review

There have been few studies relating interactions between photoperiod and reproduction in pine voles. Valentine and Kirkpatrick (1970), discussing differences in length of the breeding season in various studies on the pine vole, felt photoperiod was not a causitive factor because it is relatively constant from year to year. Lack of winter breeding was suggested to be a result of changes in density, temperature or food supply. Cengel (1975) stated photoperiod could not account for differences in reproduction in an abandoned and a maintained orchard because it was similar in both orchards. Noffsinger (1976) thought a declining or short photcperiod reduced reproductive activity in a maintained orchard from November to January, even though there was an apparent increase in energy intake at this time.

Light intensity also affects reproductive success in pine voles. Geyer and Rogers (1979) reported reduced light intensity caused a decline in reproductive performance. Voles on the lower light intensity produced significantly ($P < 0.05$) fewer litters and had significantly ($P < 0.05$) fewer young born and weaned.

Lecyk (1962) found long or increasing amounts of light stimulated reproductive activity in Clethrionomys glareolus.

Breed and Clarke (1970) reported shorter photoperiods (<12L:12D) inhibited ovarian development in female Microtus agrestis but did not completely inhibit growth. Basler et al. (1976) found no significant difference in gonadal weights in either sex of the arctic species Dicrostonyx groenlandicus kept under 6L:18D and 20L:4D photoperiods.

Petterborg (1978) concluded M. montanus anticipated seasonal changes in their environment by using photoperiod as a cue, as did Pistole (1980) for M. pennsylvanicus. Other factors in conjunction with photoperiod also influence and may control reproductive cycles (Marshall and Wilkinson 1956, Khateeb and Johnson 1971). A combination of warmer temperatures and increasing daylight appeared to stimulate reproduction in M. oregoni (Cowan and Arsenault 1954). Negus et al. (1977) felt reproduction was more closely tied to availability of growing vegetation and that photoperiod was not a sensitive enough cue to time breeding in short-lived herbivores.

Hormones cued to photoperiod may affect weight changes in animals (Perrin 1972, Iverson and Turner 1974, Pistole 1980). Roth (1974) found significantly heavier testes ($P < 0.001$) and the most developed and active gonads in male C. gapperi on a 20L:4D photoperiod. M. agrestis exposed to a 16L:8D light regime had higher body weights and reproductive organ weights, and more sexual activity in both

sexes (Clarke and Kennedy 1967, Khateeb and Johnson 1971). Voles on 16L:8D could develop or maintain gonadal activity but a photoperiod of 6L:18D prevented development or functionality in the reproductive tract. Breed and Clarke (1970) indicated female M. agrestis on 14L:10D or greater exposure to light secreted enough estrogen to allow earlier perforation of the vagina. Animals under shorter periods may decrease secretions of LH and FSH causing a reduction in gonadal weight (Clarke and Kennedy 1967, Worth et al. 1973).

In studying the effects of diet on reproduction in pine voles, Merson (1979) found adult males and females were intolerant to restricted feed intake and did not store energy efficiently. On a restricted diet, males had a significant reduction ($P < 0.05$) in daily metabolic energy requirements but females showed no significant effect of restriction on either metabolic energy requirements or food assimilation. Some animals on restricted diets either did not reduce energy requirements or maintained high levels of activity and depleted their energy (fat) reserves, leading to death. Voles which survived on a restricted food intake had large initial losses in body weight due to reduction in body fat and loss of water. Feed restriction lowered the weights of gonads and some accessory sex organs in both sexes and total spermatozoa and plasma testosterone levels in males.

Merson and Kirkpatrick (1981) reported reproductive characteristics were more sensitive to feed restriction in female Peromyscus leucopus than body weight or body fat. The ability of the female to cease reproduction while maintaining body weight and body fat during periods of food shortage could enhance the chances of survival.

Howland (1971, 1972) and Piacsek and Meites (1967) found female rats fed 50% ad libitum amounts of chow had lower ovarian and pituitary glands weights than those fed an unrestricted diet. Food restriction did not influence LH production but did lower blood LH concentrations, possibly from reduced levels of LH-RH (Howland 1971, 1972). Piacsek and Meites (1967) reported production of FSH was unaffected by food restriction but synthesis of pituitary LH and hypothalamic LRF, and release of LH and FSH from the pituitary were reduced in restricted groups. However, underfed rats exposed to constant light overcame the reproductive inhibitive effects of nutrition. Moule (1963) stated inanition may reduce reproduction because of a 1) decrease in hormone production, 2) failure of hormone release to target organs, or 3) lack of sufficient substrates for target organs to function properly.

In summary, both photoperiod and nutrition affect the reproductive physiology of some rodents. Those on long photoperiods or on unrestricted feed levels are generally

more capable of producing offspring. This experiment investigated how photoperiod and nutrition affect reproduction in pine voles.

Methods and Materials

The experiment was conducted in a metal frame building of the Dept. of Fisheries and Wildlife Sciences. One to 2 inches of soil were placed in the bottom of 4 concrete troughs (built as fish raceways) inside the building. Each trough was divided into 4 equal sections 2.2 by 1.1m in area. Sunlight entered the building through skylight panels in the roof.

Pine voles were trapped from an orchard in late July and immediately placed in the troughs. In early September 2 males and 5 females were regrouped at random into each of 12 of the 16 sections. Half of these groups was fed an ad libitum amount of Purina Rabbit Chow (66% digestible energy, Servello 1981) ground in a Wiley mill. The other half was fed ad libitum amounts of both ground chow and apple. In mid-September fluorescent lights and black plastic were suspended from the rafters. This further subdivided the groups so that half was maintained on a constant 14L:10D photoperiod and the remaining half on a natural declining photoperiod. Thus, the 4 treatment groups were (1) 14L:10D photoperiod with apple and chow diet, (2) 14L:10D

photoperiod with chow diet, (3) natural declining photoperiod with apple and chow diet, and (4) natural declining photoperiod with chow diet. In subsequent discussion these groups are labeled LA (Long photoperiod-Apple), LC (Long photoperiod-Chow), SA (Short photoperiod-Apple), and SC (Short photoperiod-Chow), respectively.

Each day a known amount of chow and apple (in sections receiving apple) was given to the voles and unconsumed chow and apple collected. The chow was poured into bowls placed on 1 sq. ft. boards. Eight apple quarters were placed in each section by skewering each quarter onto nails driven into the boards. Unconsumed food was collected only from the boards. Very little chow was cached or spilled from the surface of the boards. The unconsumed food was collected for 7 days, dried in an oven at 100°C for 3 days, and weighed (Mettler P11N, $\pm 0.1g$). Apples became charred under this drying regime and components other than water were probably lost. Later, some apples were dried 3 days at 55°C and some at 100°C. The percentage difference in percent dry weight at these 2 temperatures was used to account for loss of these other components. Food consumption was then determined and converted to kcal digestible energy consumed per vole using data from Servello (1981). Water was provided ad libitum. Chow, apple and water were distributed evenly within a section to make it available to all

residents. Metal juice cans (17.6cm long by 10.7cm in diameter) and burlap were provided for nesting and bedding material.

The experiment ran 12 weeks. Natural photoperiod at the beginning of the experiment was about 12L:12D and at the end was about 9.5L:14.5D. Body weight (Ainsworth Model 200, $\pm 0.1g$), body length and reproductive condition were recorded every 2 weeks. Dead or missing voles were replaced during the first half of the experiment but no voles were added during the last 6 weeks. Voles used as replacements were wild caught and generally kept under lab conditions between 2 and 14 days.

Voles were sacrificed in mid-December and immediately placed on ice. Necropsy procedure was described previously in Chapter 1. After removal of the reproductive tracts, all remaining organs were removed from the carcass, stripped of any fat and the fat returned to the carcass. The carcasses were freeze dried and homogenized in a Waring blender. Body fat was determined from a sample of the carcass by ether extraction in a Soxhlet apparatus with duplicates run every 8 samples.

Final body weights and body fat were tested by a 3 way analysis of variance and reproductive characteristics by a 2 way analysis of variance using the Statistical Analysis System (Helwig and Council 1979). The variance between

sections (not variance between voles within a section) was used as the error term in these analyses. Body weights taken every 2 weeks were tested using vole nested within sex, photoperiod and diet as the error term for main effects. Differences due to week and interactions between week and the main effects were tested using week X vole nested within sex, photoperiod and diet as the error term. Since food consumption was measured for all voles in a section as a whole, it was tested using section nested within photoperiod and diet as the error term for main effects. Week and interactions between week and main effects were tested using week X section nested within photoperiod and diet as the error term. Because replacing voles during the first 6 weeks of the experiment created inconsistencies in the data, food consumption and body weights taken every 2 weeks were tested for the last half of the experiment only. All analyses were done using the PROC GLM procedure.

Results

Food Consumption

Food consumption on a weekly basis varied between 90-135 kcal digestible energy per vole (Fig. 1). During the last 6 weeks groups on a chow diet consumed significantly ($P < 0.001$) more digestible energy than those fed apple and

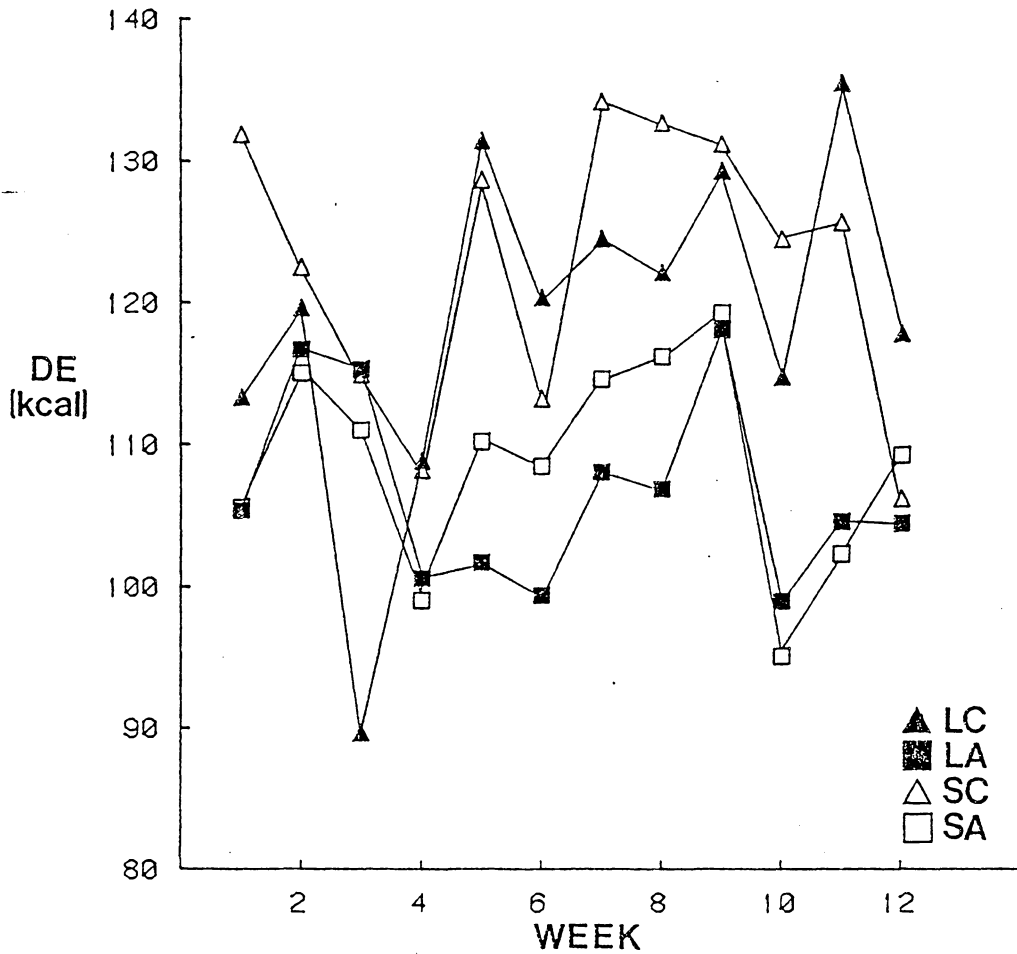


Fig. 1. Mean kcal digestible energy consumed per vole per week in adult pine voles subjected to different nutritional and photoperiod regimes.

Table 15. Mean squares for kcal digestible energy consumed per vole on a weekly basis during the last half of the experiment in adult pine voles subjected to different nutritional and photoperiod regimes.

Source	df	Kcal energy consumed
Photoperiod (P)	1	80.92
Diet (D)	1	5509.02**
P X D	1	51.27
Section (P X D) ¹	8	465.22
Week (W)	6	472.69**
W X P	6	98.13
W X D	6	159.87*
W X P X D	6	90.90
W X Section (P X D) ²	48	68.04
* P<0.05		
** P<0.001		

¹Error term used for testing P, D, P X D.

²Error term used for testing W, W X P, W X D, W X P X D.

chow (Table 15). During all weeks except week 3 groups fed chow consumed more energy than those with an apple supplement (Fig. 1). Apples usually comprised 50-65% of the energy consumed in groups receiving apple.

There were significant differences due to week ($P < 0.001$) and a week X diet interaction ($P < 0.05$). Consumption showed sharp declines in the LC group during week 3 and in both groups fed apple during week 10. In all 3 cases energy consumption increased the following 2 weeks. Both groups on the chow diet had a sharp decline in consumption during week 12. In all groups but the SC group energy consumption during the final week was within ± 5 kcal of consumption in the first week.

Body Condition

Mean body weights during the experiment were plotted for males and females by treatment group (Fig. 2 and 3). There were no significant ($P > 0.10$) differences due to sex or treatment during the last 6 weeks except a significant ($P < 0.03$) sex X photoperiod X diet interaction (Table 16). Males in the LC group and females in the IA group had the highest mean body weights during the experiment (Fig. 2 and 3). Conversely, males in the IA group and females in the LC group generally had the lowest mean weights.

There was a significant (Table 16) effect on body

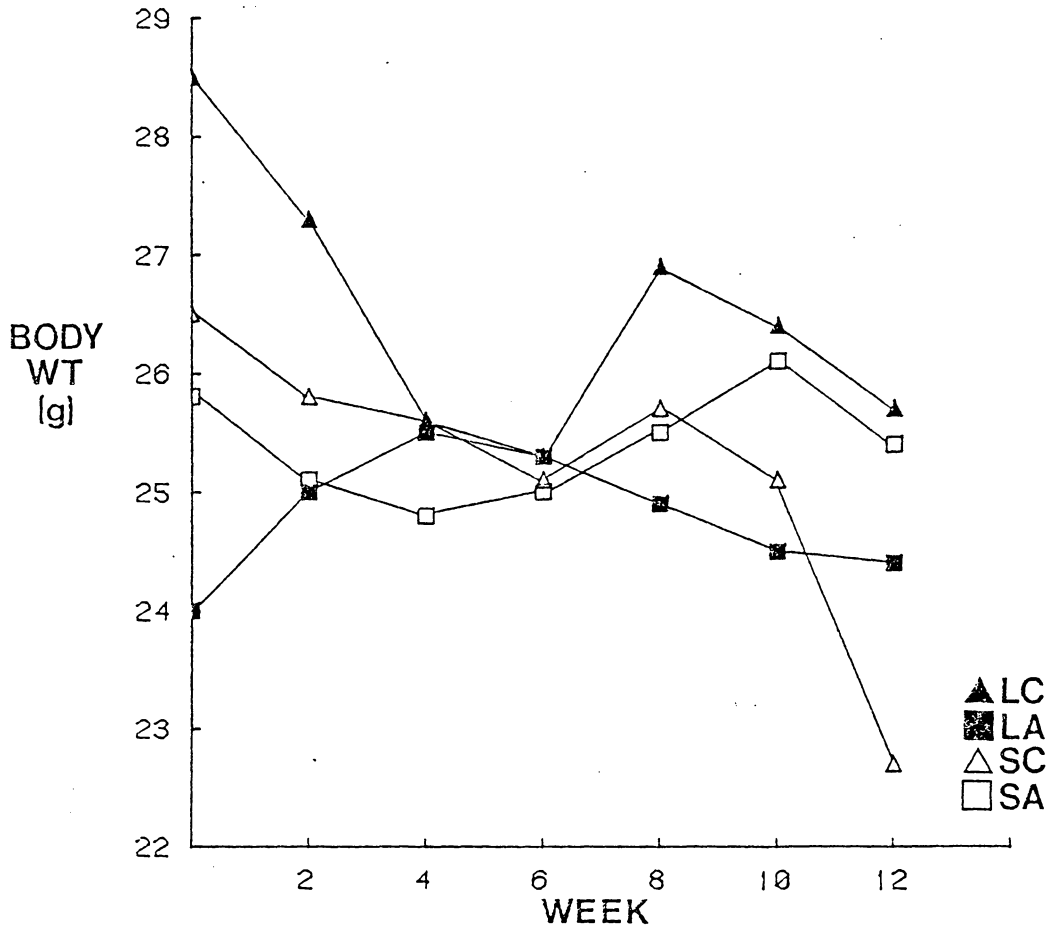


Fig. 2. Mean body weights in adult male pine voles subjected to different nutritional and photoperiod regimes.

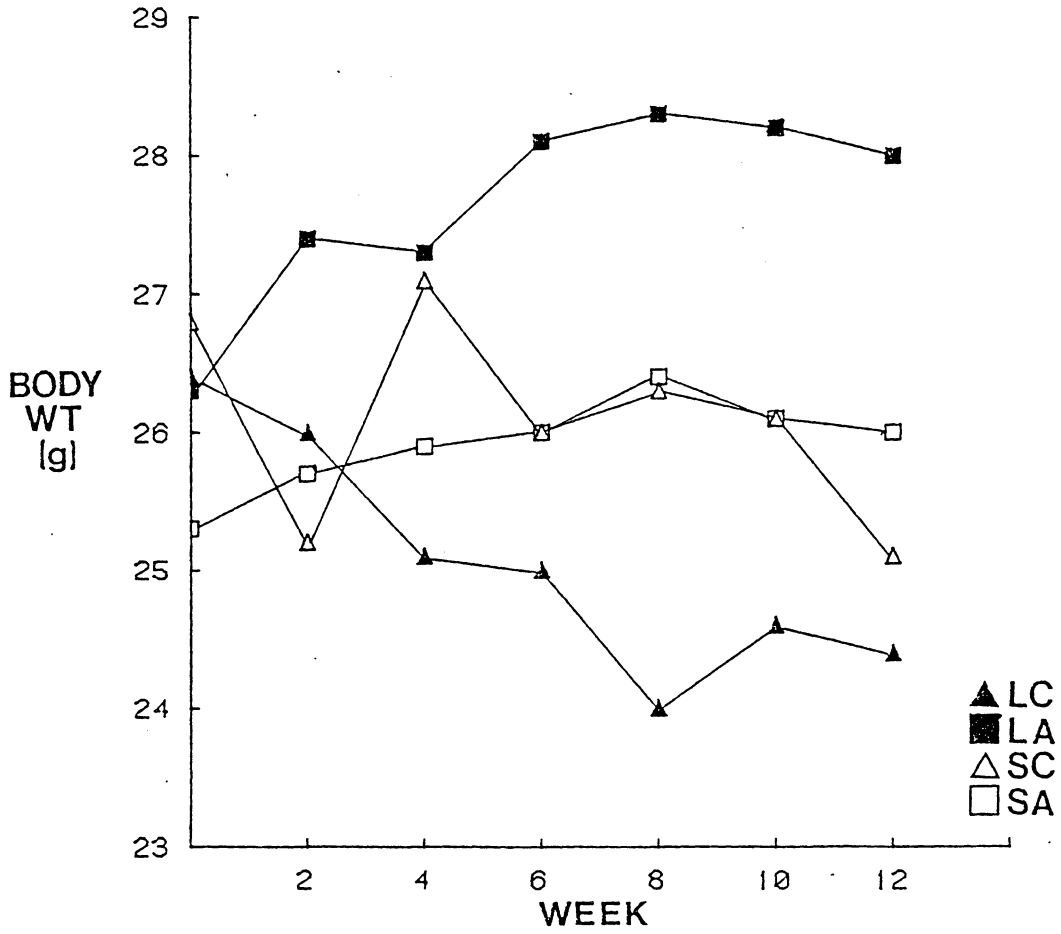


Fig. 3. Mean body weights in adult female pine voles subjected to different nutritional and photoperiod regimes.

Table 16. Mean squares for body weights taken every 2 weeks during the last half of the experiment in adult male and female pine voles subjected to different nutritional and photoperiod regimes.

Source	df	Body weights
Sex (S)	1	18.01
Photoperiod (P)	1	70.07
Diet (D)	1	2.15
S X P	1	24.72
S X D	1	71.04
P X D	1	19.32
S X P X D	1	227.86**
Vole (S X P X D) ¹	53	42.55
Week (W)	3	7.84***
W X S	3	1.10
W X P	3	1.34
W X D	3	2.21*
W X S X P	3	0.45
W X S X D	3	0.37
W X P X D	3	1.11
W X S X P X D	3	0.33
W X Vole (S X P X D) ²	159	1.27
* P<0.10		
** P<0.03		
*** P<0.005		

¹Error term used for testing S, P, D, S X P, S X D, P X D, S X P X D.

²Error term used for testing W, W X S, W X P, W X D, W X S X P, W X S X D, W X P X D, W X S X P X D.

weight due to week ($P < 0.005$) and a week X diet interaction ($P < 0.10$). Males and females fed only chow generally lost weight but groups given apple and chow did not change mean body weight by over + 1g between measurement periods.

In males all 4 groups had nearly identical mean body weights at week 6 (Fig. 2). Males fed chow gained weight from week 6 to week 8 but lost weight the last 4 weeks of the experiment. Males in the SA group gained weight from week 6 to week 10 but had a decline in mean body weight from week 10 to week 12. Females on a declining photoperiod had the same mean body weight at week 6 and, along with LA females, tended to maintain their mean weight the last 6 weeks (Fig. 3). Females in the LC group lost weight from week 6 to week 8 but regained most of this weight the next 2 weeks.

Mean change in body weight was determined for animals living the last 6 weeks of the experiment (Table 17). The variation in the data was great but there was a significant ($P < 0.03$) difference in change in body weight due to diet (Table 18). Voles fed chow lost weight but those fed apple and chow maintained body weight (Table 17). There was no significant effect due to photoperiod ($P > 0.87$) or sex ($P > 0.32$).

Final body weight was significantly ($P < 0.09$) higher in females than in males (Tables 19 and 20). There was a

Table 17. Mean values ($\pm$ SE) and sample sizes by treatment group for change in body weight in adult male and female pine voles subjected to different nutritional and photoperiod regimes and surviving the last half of the experiment.

	Male		Female	
	(g $\pm$ SE)	(n)	(g $\pm$ SE)	(n)
14L:10D				
Chow	-1.6 $\pm$ 0.3	(5)	-1.1 $\pm$ 0.7	(8)
Apple + Chow	-0.9 $\pm$ 0.3	(5)	0.0 $\pm$ 0.7	(13)
Natural Decline				
Chow	-1.4 $\pm$ 0.7	(5)	-1.6 $\pm$ 0.9	(10)
Apple + Chow	-0.6 $\pm$ 0.8	(5)	0.0 $\pm$ 0.3	(14)

Table 18. Mean squares (MS) for change in body weights in adult male and female pine voles subjected to different nutritional and photoperiod regimes and surviving the last half of the experiment.

Source	df		Change in body weights
Sex (S)	1	MS F<	4.32 .32
Photoperiod (P)	1	MS F<	0.11 .87
Diet (D)	1	MS F<	21.32 .03*
S X P	1	MS F<	1.12 .61
S X D	1	MS F<	1.24 .59
P X D	1	MS F<	0.83 .66
S X P X D	1	MS F<	0.19 .83
Error	57	MS	4.23

Table 19. Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in adult male and female pine voles subjected to different nutritional and photoperiod regimes.

Group	<u>Male</u>		<u>Female</u>	
	Body weight	Body length	Body weight	Body length
	(g $\pm$ SE) (n)	(mm $\pm$ SE) (n)	(g $\pm$ SE) (n)	(mm $\pm$ SE) (n)
14L:10D				
Chow	25.7 $\pm$ 1.8 (5)	104 $\pm$ 1 (5)	24.4 $\pm$ 1.0 (8)	104 $\pm$ 1 (8)
Apple + Chow	24.4 $\pm$ 0.8 (5)	102 $\pm$ 1 (5)	28.0 $\pm$ 1.3 (12)	105 $\pm$ 1 (12)
Natural Decline				
Chow	22.7 $\pm$ 1.3 (5)	102 $\pm$ 2 (5)	25.1 $\pm$ 1.1 (11)	103 $\pm$ 2 (11)
Apple + Chow	25.4 $\pm$ 1.0 (5)	104 $\pm$ 1 (5)	26.0 $\pm$ 0.8 (14)	103 $\pm$ 1 (14)

Table 20. Mean squares (MS) for final body weights and body lengths in adult male and female pine voles subjected to different nutritional and photoperiod regimes.

Source	df		Body weights	Body lengths
Sex (S)	1	MS	2.95	1.28
		F<	.09*	.26
Photoperiod (P)	1	MS	1.45	0.13
		F<	.23	.72
Diet (D)	1	MS	2.08	0.38
		F<	.16	.54
S X P	1	MS	0.00	0.44
		F<	.95	.51
S X D	1	MS	0.41	0.18
		F<	.52	.67
P X D	1	MS	0.01	0.05
		F<	.93	.82
S X P X D	1	MS	5.68	1.77
		F<	.02*	.19
Error	57	MS	12.30	15.36

significant ($P < 0.02$) sex X photoperiod X diet interaction. Body weight and body length were highest for males in the LC and SA groups and for females in the IA group. There were no significant ($P > 0.19$) differences in final body lengths.

Voles fed apple and chow had a significantly ($P < 0.01$) higher percentage of body fat (Tables 21 and 22). Females had significantly ($P < 0.04$) higher body fat levels than males. Females fed apple had mean values of about 40% body fat (Table 21).

Reproductive Characteristics

No litters were produced during the experiment. One female in the IA group had 2 small fetuses in her uterus when necropsied.

Males on the 14L:10D photoperiod had significantly heavier seminal vesicles ($P < 0.004$) and paired testes ($P < 0.008$), and significantly ($P < 0.06$) more spermatozoa/mg testes (Tables 23 and 24). Those fed an apple supplement had significantly ($P < 0.05$) heavier paired testes. Adrenal glands weight was not significantly ($P > 0.17$) different and was similar in all but the SC group (Table 23). Males fed apple and chow had higher mean values for reproductive characteristics within each photoperiod. Males in the IA group had the highest mean values for all 4 characteristics and those in the SC group had the lowest means.

Table 21. Mean values ($\pm$ SE) and sample sizes by treatment group for fat content (expressed as percent of dry body weight) in adult male and female pine voles subjected to different nutritional and photoperiod regimes.

Group	Male		Female	
	(% $\pm$ SE)	(n)	(% $\pm$ SE)	(n)
14L:10D				
Chow	31.1 $\pm$ 5.1	(5)	28.7 $\pm$ 4.1	(8)
Apple + Chow	32.2 $\pm$ 3.6	(5)	40.7 $\pm$ 3.2	(11)
Natural Decline				
Chow	25.6 $\pm$ 6.9	(5)	36.9 $\pm$ 4.8	(10)
Apple + Chow	36.8 $\pm$ 3.1	(5)	42.2 $\pm$ 2.3	(12)

Table 22. Mean squares (MS) for fat content in adult male and female pine voles subjected to different nutritional and photoperiod regimes.

Source	df		Body fat
Sex (S)	1	MS F<	553.35 .04*
Photoperiod (P)	1	MS F<	105.37 .37
Diet (D)	1	MS F<	880.83 .01*
S X P	1	MS F<	70.66 .46
S X D	1	MS F<	16.14 .72
P X D	1	MS F<	5.01 .84
S X P X D	1	MS F<	233.32 .18
Error	53	MS	128.59

Table 23. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes.

Group	Adrenal glands weight		Seminal vesicles weight		Paired testes weight		Spermatozoa sperm/mg testes wt X	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	10 ³ $\pm$ SE	(n)
14L:10D								
Chow	10.5 $\pm$ 0.7	(4)	30.0 $\pm$ 7.1	(5)	55.0 $\pm$ 6.5	(4)	62.5 $\pm$ 6.9	(5)
Apple +								
Chow	11.0 $\pm$ 1.0	(5)	38.7 $\pm$ 11.5	(5)	79.8 $\pm$ 11.7	(4)	87.3 $\pm$ 17.9	(5)
Natural Decline								
Chow	9.1 $\pm$ 0.5	(5)	6.0 $\pm$ 1.5	(5)	34.6 $\pm$ 7.1	(5)	40.5 $\pm$ 14.2	(5)
Apple +								
Chow	10.3 $\pm$ 0.5	(5)	12.8 $\pm$ 3.7	(4)	47.8 $\pm$ 8.3	(5)	53.8 $\pm$ 14.4	(5)

Table 24. Mean squares (MS) for reproductive characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes.

Source	df	Adrenal glands weights	Seminal vesicles weights	Paired testes weights	Sperm/mg testes wts X 10 ³
Photoperiod(P)	1 MS	5.21	3041.60	3045.03	3850.31
	P<	.17	.004*	.008*	.06*
Diet(D)	1 MS	3.66	286.78	1512.50	1805.00
	P<	.24	.31	.05*	.19
P X D	1 MS	0.54	4.18	143.23	165.31
	P<	.65	.90	.51	.69
Error	15 MS	2.49	258.99	324.63 ¹	971.68 ²

¹Degrees of freedom for error term was 14.

²Degrees of freedom for error term was 16.

Females on the 14L:10D photoperiod had significantly heavier uteri ($P<0.04$) and paired ovaries ($P<0.07$) than those on a declining photoperiod (Tables 25 and 26). Adrenal glands weight tended to be higher for females on a chow diet. There was a greater difference in uterine weight due to diet for females on the 14L:10D photoperiod than the declining photoperiod (Table 25).

Discussion

Food Consumption

Voies on a chow diet consumed more digestible energy than those fed apple and chow. Animals fed only chow consumed 4-5g of chow per animal per day. Those given apple and chow consumed 2g or less of chow per animal per day but, on a wet weight basis, generally ate over 20g of apple. Perhaps the energy intake was lower in the groups fed apple and chow because voles preferred apple and would fill their stomachs with it. They ate enough chow to get other required nutrients but consumption of apple, which is about 90% water, decreased total digestible energy intake. Lochmiller (1980) reported adult pine voles subjected to a 12L:12D photoperiod, a temperature of 14°C and given nesting material consumed an average of 18.17 kcal DE/vole/day (127 kcal DE/vole/week).

Availability of apples also affected water intake.

Table 25. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult female pine voles subjected to different nutritional and photoperiod regimes.

Group	Adrenal glands weight	Uterine weight	Paired ovarian weight
	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)
14L:10D			
Chow	10.9 $\pm$ 0.5 (8)	26.8 $\pm$ 1.5 (8)	3.3 $\pm$ 0.4 (8)
Apple + Chow	9.7 $\pm$ 0.5 (12)	38.2 $\pm$ 6.3 (12)	3.1 $\pm$ 0.5 (12)
Natural Decline			
Chow	11.1 $\pm$ 1.3 (10)	24.2 $\pm$ 3.1 (10)	2.3 $\pm$ 0.2 (10)
Apple + Chow	9.7 $\pm$ 0.6 (14)	25.0 $\pm$ 2.6 (14)	2.6 $\pm$ 0.3 (14)

Table 26. Mean squares (MS) for reproductive characteristics in adult female pine voles subjected to different nutritional and photoperiod regimes.

Source	df		Adrenal glands weights	Uterine weights	Paired ovarian weights
Photoperiod(P)	1	MS	0.10	873.36	5.07
		F<	.90	.04*	.07*
Diet(D)	1	MS	18.31	325.21	0.05
		F<	.11	.20	.85
P X D	1	MS	0.02	298.47	0.48
		F<	.96	.22	.57
Error	40	MS	6.89	188.39	1.44

An average of 17.14ml of water per animal per day was added to bottles in the SC group and 17.78ml water/animal/day in the LC group. Only 3.04ml/animal/day was added in the SA group and 3.55ml/animal/day in the LA group. However, apples provided 15.73ml/animal/day in the SA group and 16.95ml/animal/day in the LA group. It appears apples may be an important source of water for pine voles.

Body Condition

Voles on an apple and chow diet maintained or gained body weight and had higher levels of body fat even though they consumed less digestible energy. Groups on a chow diet lost weight. Thus, pine voles fed apple and chow were either more efficient in digesting or absorbing the food (energy) they consumed. However, the mechanism(s) by which this occurs is not understood.

Females had higher values than males for final body weight and body fat. There was no measure of difference in food consumption between males and females. Females may have been heavier and fatter because they ate more than males and/or because they inherently stored more body fat and were heavier.

Reproductive Characteristics

Voles on the 14L:10D photoperiod had higher values for

reproductive characteristics than those on the declining photoperiod. Voles on a long photoperiod during late spring and summer may be physiologically more fit to produce young than those on a short photoperiod in late fall or those below some critical daylength. This supports field data of Cengel et al. (1978) and Noffsinger (1976) who found the greatest percentage of pregnant females from May to September with the peak in July. Valentine and Kirkpatrick (1970) and Paul (1970) found a peak in reproduction during July and August. Other authors have found a decrease in reproduction during summer (Gcertz 1971, Boyette 1966, Paul 1970). This may occur due to factors other than photoperiod, such as high summer temperatures which may reduce activity and reproduction or dry up the vegetation and reduce nutritional quality and/or quantity.

Differences between the 2 photoperiods could have been influenced by differences in light intensity. Light intensity was measured after the experiment and was more variable but generally lower in the natural declining photoperiod than under the fluorescent lights in the 14L:10D groups (Appendix Table I). Light intensity in the natural declining photoperiod varied depending on time of day and sky conditions. The intensity ranged from less than 5 footcandles to 145 footcandles. Intensity in the 14L:10D regime ranged from 40 to 100 footcandles.

Within a photoperiod voles fed apple and chow had higher values for reproductive characteristics than those given chow. As with body weight and fat, voles on the apple and chow diet may have been more efficient in utilizing energy intake and increasing reproductive organ mass. Lochmiller (1980) found by proximate analysis that apples collected in the fall contained 43.6% total nonstructural carbohydrates. Perhaps the carbohydrates (especially simple sugars such as glucose) in apple could be readily absorbed into the vole's blood stream and stimulate the hypothalamic-pituitary axis, increasing the synthesis and/or release of gonadotrophic hormones. Female laboratory rats on an energy restricted diet had lower levels of circulating LH but restricted groups which had glucose added to their diet had plasma LH levels similar to control fed rats (Howland 1972). It is possible voles consuming apples may increase circulating levels of LH and perhaps FSH. This would explain the heavier reproductive organs in the apple and chow groups.

These effects of diet support previous field studies. Cengel et al. (1978) and Noffsinger (1976) found higher levels of reproduction in maintained orchards than in abandoned orchards. Voles in a maintained orchard may have a diet of higher nutritive value due to orchard management (i.e. mowing, fertilizing) and the presence of apple drops

(Cengel et al. 1978).

There appeared to be additive effects between photoperiod and diet. Voles on the 14L:10D photoperiod fed apple and chow had the highest values for reproductive characteristics while those on the declining photoperiod and given chow had the lowest values. Pinter and Negus (1965) also found additive effects between photoperiod and diet in M. montanus. Voles on an 18L:6D photoperiod and fed green wheat sprouts every 3 days recorded the most litters and the most offspring while voles on the 6L:18D photoperiod and fed sprouts every 15 days had the lowest productivity.

Field studies have found highest levels of reproduction in pine voles in mid to late summer in maintained orchards and lowest levels of reproduction in late fall to early spring in abandoned orchards (Cengel et al. 1978, Noffsinger 1976). The estimated energy intake was high during the summer and low during fall in the abandoned orchard (Noffsinger 1976). A possible explanation of these findings is that during summer, with a high intake of quality food and a long photoperiod, reproduction can occur at high levels. During fall, if food quality drops below some critical point, voles may reduce or cease reproduction and shunt production into maintainance. If food quality declines but remains above the critical point the declining photoperiod may cause a reduction in reproductive activity.

Lack of reproduction in this study was probably caused more by social and nonexperimental factors than differences in photoperiod or diet. Voles brought in from the wild usually require time to adjust to a strange environment before any will produce offspring. Merson (1979) felt captivity may have depressed reproductive condition in his adult pine voles on different diet intakes. The stress of capture, transport, being placed in the troughs and then being regrouped for this experiment surely affected reproduction. Second, adding strange voles as replacements during the first half of the experiment caused additional social stress. Adding a new vole likely disrupted the social hierarchy in that section. This lead to agonistic encounters and often death of the new vole. These social conflicts could have affected reproduction. Third, being handled once every 2 weeks and being disturbed when fed would put additional stress on the voles.

In summary, pine voles on the 14L:10D photoperiod were probably more capable of reproducing than those on the declining photoperiod. This agrees with studies showing rodents on a long or continuous light regime may have higher values for reproductive characteristics irrespective of diet (Negus and Pinter 1965, Piacsek and Meites 1967). Voles on the declining photoperiod may be reproductively suppressed due to low light intensity and/or activity of the pineal

gland on the pituitary. Some rodents on a short photoperiod either produce antigonadotrophic substances from the pineal which inhibit reproduction, or are more sensitive to the effects of these substances (Reiter 1980).

No groups in this experiment were on an energy deficient diet. Voles fed apple and chow consumed less digestible energy but had more body fat and males had heavier testes weights than those fed chow. It would appear apples may be more easy to digest and assimilate into body tissue or provide such a concentrated source of energy that it may influence the production and/or release of hormones affecting reproduction. However, the mechanisms by which such events may occur is unclear. Apples may also be an important source of water, especially in fall when the orchard flora becomes senescent. The results presented here generally support previous field studies.

Chapter 3. Effects of Photoperiod and Nutrition on Growth,
Molt and Reproductive Organs in Young Pine Voles.

Introduction

Two important components of population dynamics are 1) growth of young and 2) age of puberty. Both components can be affected by photoperiod, nutrition, temperature, social influences, and other factors (Hasler 1975). Perrin (1979) noted that increasing daylength in spring was a proximate factor regulating onset of growth in young red-backed voles (Clethrionomys gapperi), and seasonal changes in food quality influenced weaning success, growth and survival. Pinter (1968a,b) found both photoperiod and diet influenced growth rates, age at puberty and age at molt to adult pelage in young Microtus montanus. Young on an 18L:6D photoperiod and fed sprouted wheat every 3 days grew and matured more rapidly than young on a 6L:18D photoperiod or those fed wheat sprouts every 15 days. The objective of the present experiment was to determine the effect of photoperiod and nutrition on growth, molt to adult pelage and reproduction in juvenile pine voles.

Literature Review

Young pine voles are born naked and blind but show coordinated body movements by 3 to 4 days of age (Boyette 1966). At 5 to 6 days of age suckling juveniles moved their legs beneath them when the female moved. Young were not weaned until 18 to 28 days of age, but could survive without their mother's milk at 16 days of age.

Lochmiller (1980) found a good correlation between age of juvenile pine voles and body weight ($r=0.85$, $P<0.001$). He reported average weights of 1.9g at birth, 8.7g at 18 days of age, and 11.2g at 21 days of age. Goertz (1971) noted young averaged 2.3g after birth, about 8% of the female's body weight, and gained an average of 0.39g per day the first 14 days after initial capture as a newborn. Hamilton (1938) determined a growth rate of about 2g per week from 24 days of age until young reached mature weight. Lochmiller (1980) showed pine voles had an average growth rate of 0.41g/day from 22 to 46 days of age. Growth rate was greatest between the ages of 22-28 days and decreased with age. Before weaning, the metabolizable energy requirements of the lactating female were correlated with growth rate of the litter ($r=0.86$, $P<0.001$). Amount of energy deposited as growth by the young was related to litter size and day of lactation. After weaning, there was

no significant correlation between growth rate of juveniles and size of the litter or sex of the young.

Boyette (1966) felt immature pine voles reached puberty around 16 weeks of age. Schadler and Butterstein (1979) found average age at puberty to be 60 days in males and 105 days in females. In males measuring about 100mm total body length, Paul (1970) observed sperm development but saw no mature spermatids in males less than 117mm total length. Of the 85 voles captured between December and February, no females under 115mm total length were pregnant; however, 16 females 108-114mm length were pregnant in samples taken from April to November.

In field studies age classes have been determined by various means. Gentry (1968) classified groups by weight and pelage characteristics. Juveniles weighed from 8.0g to 16.5g and had a grayish pelage. Subadults weighed between 16.5-20.0g and adults were greater than 20.0g. Paul (1970) divided age groups by total length measurement but also considered reproductive condition and epiphyseal closure. Juveniles were under 105mm total body length and had a grayish-brown pelage. Subadults measured from 105mm to 115mm, with most having adult body proportions and pelage. Adults were greater than 115mm total length. The majority of adults were reproductively active.

Hamilton (1938) noted that young began losing their

juvenal pelage around 5 weeks of age and attained an adult pelage by 7 weeks of age. Benton (1955) felt immature voles molted into adult pelage between 7 and 10 weeks of age. Paul (1970) and Boyette (1966) also described the growth, development and pelage characteristics of juvenile pine voles.

Studies on other microtines have noted differences in cohorts born at different times of the year (Kubik 1965, Sealander 1972, Tast 1972, Brown 1973, Evans 1973, Fedyk 1974, Perrin 1979). In general, animals born early in the breeding season grow rapidly and attain puberty that same season. Individuals born late in the season grow slowly to some critical size, stop growth and development over winter, and initiate a second growth phase to attain adult body size and puberty the following spring. Schwarz et al. (1964) and Brown (1973) felt individuals born late in the season are physiologically the same age as young born in spring of the following year. Iverson and Turner (1974) found low weights recorded during fall and winter were partially caused by recruitment of young late in the year. Brown (1973) noted animals with a lower winter weight had lower energy expenditures and could consume less food to maintain themselves.

More specifically, Gliwicz et al. (1968) determined a cohort of Clethrionomys glareolus born between April 23 and

May 26 contributed the most to that year's population size (56%) and had the greatest input into reproduction (54%). Individuals born between May 27 and July 10 had a better overwinter survival and contributed most to the following year's reproduction (75%). Cowan and Arsenault (1954) found only the first 2 or 3 litters of Microtus oregoni produced in a breeding season reproduced that year. Negus et al. (1977) discovered M. montanus born in early spring matured in 3 to 4 weeks, but those born in late spring required 7 to 8 weeks before attaining puberty. Voles produced in the summer did not reach puberty until the following spring.

Fedyk (1974) felt the difference in seasonal generations in terms of physiology, morphology, and behavior may be an inherited trait or due to changes in the habitat. Lecyk (1962) and Breed and Clarke (1970) showed that increasing the exposure to light in animals born late in the season hastened onset of puberty. Petterborg (1978) thought the difference in seasonal generations was due to photoperiod and showed weight change response of M. montanus maintained under natural photoperiod and temperature was a function of age. Pinter (1968a) concluded both photoperiod and nutrition may cause seasonal differences in growth rate and puberty in young M. montanus. Laboratory mice on a restricted caloric intake had a longer life span and a delayed sexual maturation relative to controls (Ball et al.

1947).

Ecke and Kinney (1956) found a good correlation between age and pelage molting in laboratory-raised M. californicus between 17 and 60 days of age. In 88% of their specimens, they correctly aged the animal within 4 days of its known age. Pinter (1968b) reported giving young M. montanus fresh wheat sprouts every 3 days had no effect on progress of the molt of juvenal pelage but caused an earlier molt to subadult and adult pelages. Voles on an 18L:6D photoperiod completed their juvenal pelage, started and completed subadult molt, and began adult molt before groups exposed to 6L:18D. In Ochrotomys nuttali Linzey and Linzey (1967) discovered mice born after April 8 combined their post-juvenal and summer molt while those born after October 1 combined their post-juvenal molt and winter molt. Young produced after October 1 began the post-juvenal molt at a later age than those in spring and summer. Khateeb and Johnson (1971) believed changes in the endocrine system due to changes in the environment influenced molt and pelage characteristics.

In summary, environmental conditions under which young are raised have a strong influence on growth, molt and attaining puberty. This experiment investigated the effects of photoperiod and nutrition on growth, molt to adult pelage and reproductive organ weights in young pine voles.

Methods and Materials

Voies used in this experiment were born from 2 colonies maintained in labs at VPI&SU. Young from the colony in the Dept. of Biology were weaned at 21 days of age and those from the colony in the Dept. of Fisheries and Wildlife Sciences were weaned at 30 days of age. Some litters from the latter colony died when weaned at 21 days of age; therefore, litters from this colony were weaned at 30 days of age.

The experiment was conducted in 2 environmental chambers with young paired at random into male-female pairs. Because of an excess of males there were also 5 male-male pairs and 1 singly caged male. Young ranged from 21-43 days of age at the beginning of the experiment. The pairs were placed at random into 1 of the following treatment groups: (1) 15L:9D photoperiod with apple and chow diet, (2) 15L:9D photoperiod with chow diet, (3) 9L:15D photoperiod with apple and chow diet, and (4) 9L:15D photoperiod with chow diet. In subsequent discussion, these groups will be referred to as LA (Long photoperiod-Apple), LC (Long photoperiod-Chow), SA (Short photoperiod-Apple), and SC (Short photoperiod-Chow), respectively. The chow, Purina Horse Chow with 25% crude fiber (59.4% digestible energy, Servello 1981), was ground in a Wiley mill. The pairs were

housed in hanging wire cages and placed at random on a rack inside each of the chambers. Temperature was kept at $17\pm 1^{\circ}\text{C}$ and relative humidity was maintained at approximately 50%. Voles were given apple, chow and water ad libitum. Burlap was provided for nesting material.

Food consumption was monitored for 3 days every 2 weeks. A known amount of chow and apple (in pairs receiving apple) was fed to each pair daily. At the end of the third day all unconsumed chow and apple was collected. Kcal digestible energy consumed per vole per week was determined as described in Chapter 2.

The experiment was run in 2 phases. The initial phase began February 3, 1981, and ran for 8 weeks. Half way through phase 1 the chamber having the 9L:15D photoperiod heated up (due to a malfunction) over 22°C in an hour, killing most of the voles. A second phase began March 10 and ran 8 weeks, with most of the young being placed under a 9L:15D photoperiod.

In the initial phase voles were checked every 4 days for molt to adult pelage. Body weight (Ainsworth Model 200, $\pm 0.1\text{g}$), body length and reproductive condition were recorded every 7 days. In the second phase all measurements were taken every 4 days. It was determined from phase 1 that it was easier to relate molt and body weight with measurements taken every 4 days. The voles were sacrificed

and necropsied as described in Chapter 1. Body fat was determined by ether extraction in a Soxhlet apparatus as described in Chapter 2.

Body weight, body length, body fat and molt data were tested by 3 way analysis of variance using the Statistical Analysis System (Helwig and Council 1979). Reproductive characteristics were tested by a 2 way analysis of variance. Body weights taken during the experiment were tested using vole nested within sex, photoperiod and diet as the error term for main effects. Effects due to age group and interactions of the main effects with age group were tested using age group X vole nested within sex, photoperiod and diet as the error term. The analysis was run by pooling voles which were 51-60, 61-70 and 71-80 days of age into age groups. These groups were used because testing merely by age or by different age classes (i.e. 51-55 days of age, 56-60, 61-65, etc .) was not possible due to constraints in computer user disk storage space. The age groups represent a critical point because most of the voles 51-80 days of age began taking on adult characteristics. Food consumption was analyzed using pair nested within photoperiod and diet as the error term for main effects and week X pair nested within photoperiod and diet as the error term for effects due to week and interactions with week. The PROC GLM procedure was used for all analyses.

Results

Food Consumption

Young consumed between 127-182 kcal digestible energy per vole per week except the IA group during week 2 and the SA group during week 6 (Fig. 4). Groups on the 9L:15D photoperiod consumed significantly ($P < 0.10$) more digestible energy than those on the 15L:9D regime (Table 27). Groups on a chow diet generally consumed more energy each week than did groups given apple. Apples supplied 37-62% of the energy intake in apple-supplemented groups.

There was also a difference due to week ($P < 0.001$) and a significant ($P < 0.05$) week X diet interaction (Table 27). Consumption increased from week 1 to week 3 and decreased from week 3 to week 6 (Fig. 4). Groups fed apple and chow increased consumption during the last 2 weeks of the experiment. Groups fed only chow had little overall change in consumption during this time.

Body Condition

Mean body weights were plotted against age for both males and females (Fig. 5 and 6). Young at 51-80 days of age on an apple and chow diet had significantly ($P < 0.005$) higher body weights than those fed chow (Table 28 and Appendix Table II). Young 71-80 days of age were

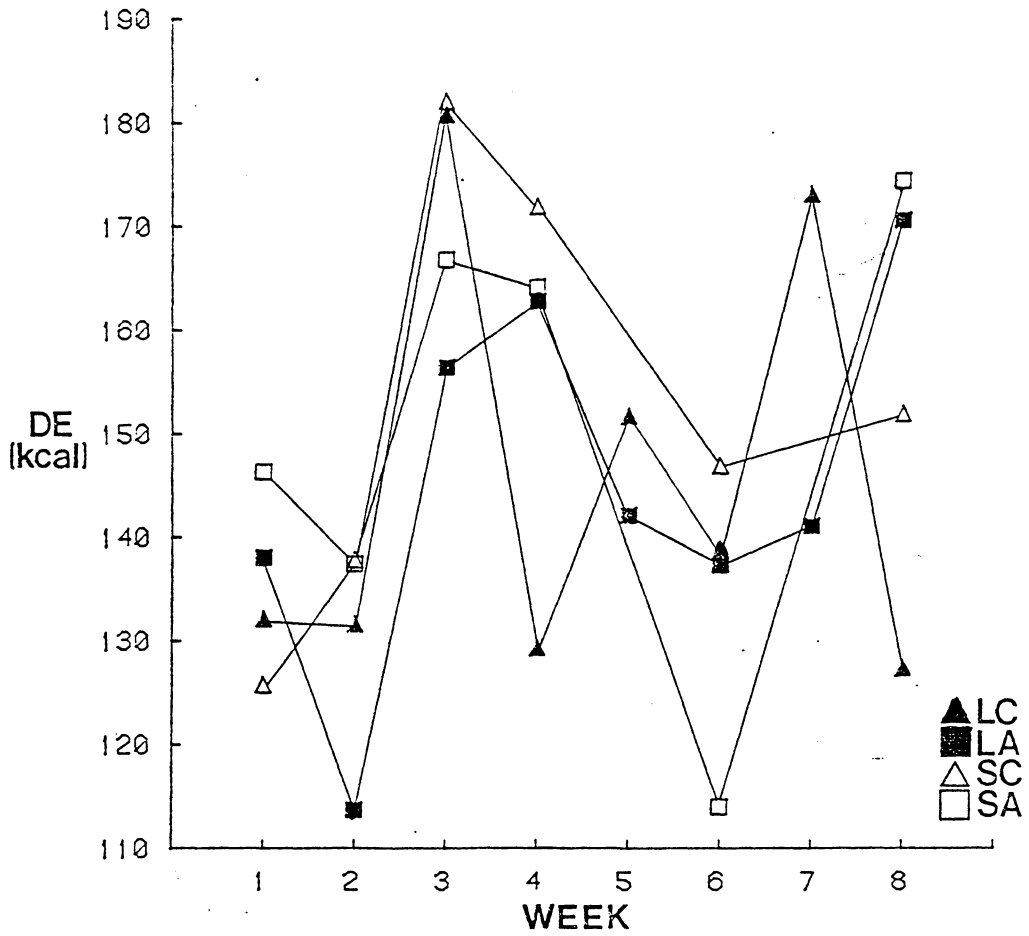


Fig. 4. Mean kcal digestible energy consumed per vole per week in young pine voles subjected to different nutritional and photoperiod regimes.

Table 27. Mean squares for kcal digestible energy consumed per vole on a weekly basis in young pine voles subjected to different nutritional and photoperiod regimes.

Source	df	Kcal energy consumed
Photoperiod (P)	1	2293.60*
Diet (D)	1	658.85
P X D	1	101.76
Section (P X D) ¹	27	547.30
Week (W)	7	3148.66***
W X P	5	610.36
W X D	7	1376.18**
W X P X D	5	453.18
W X Section (P X D) ²	61	527.12
* P<0.10		
** P<0.05		
*** P<0.001		

¹Error term used for testing P, D, P X D.

²Error term used for testing W, W X P, W X D, W X P X D.

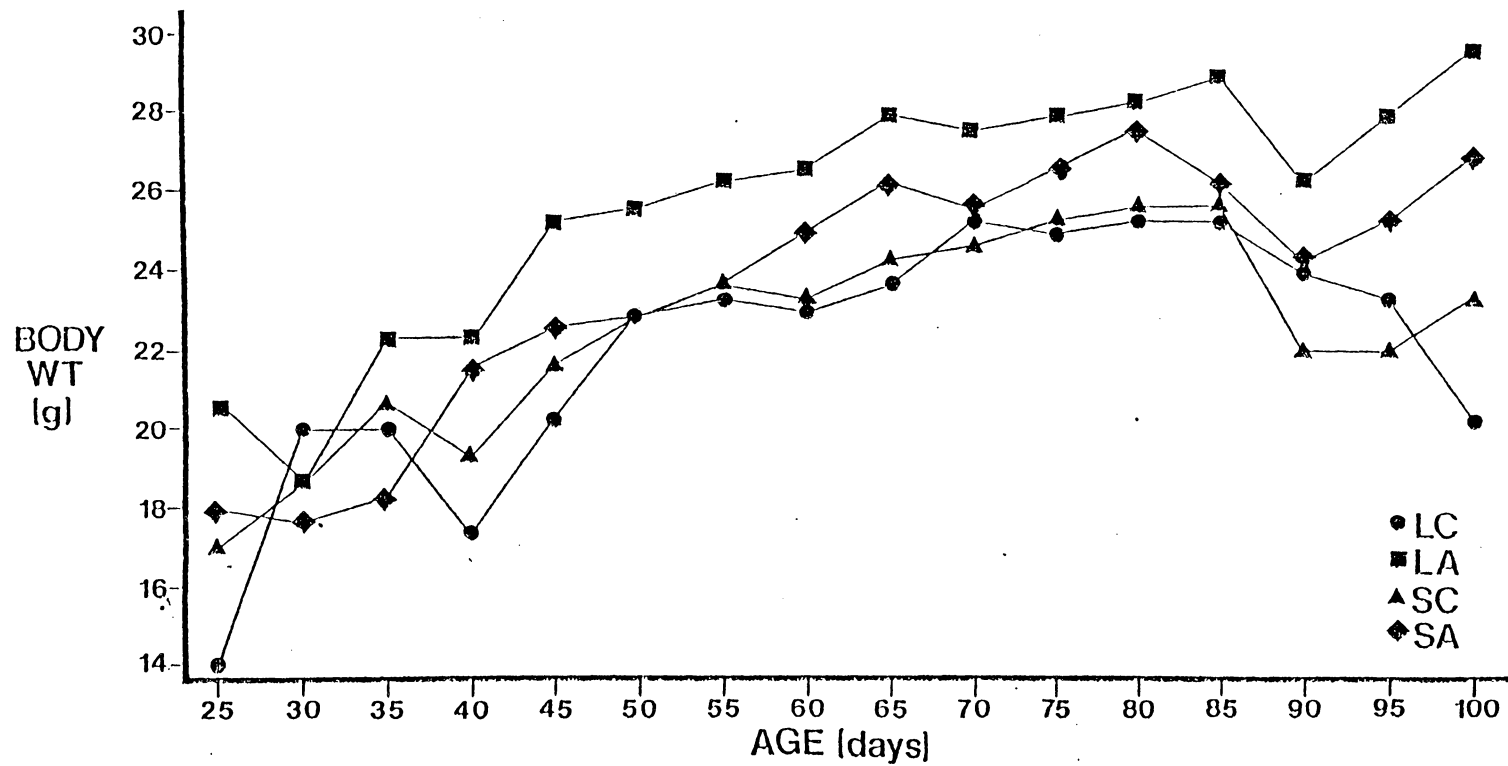


Fig. 5. Mean body weights in young male pine voles subjected to different nutritional and photoperiod regimes.

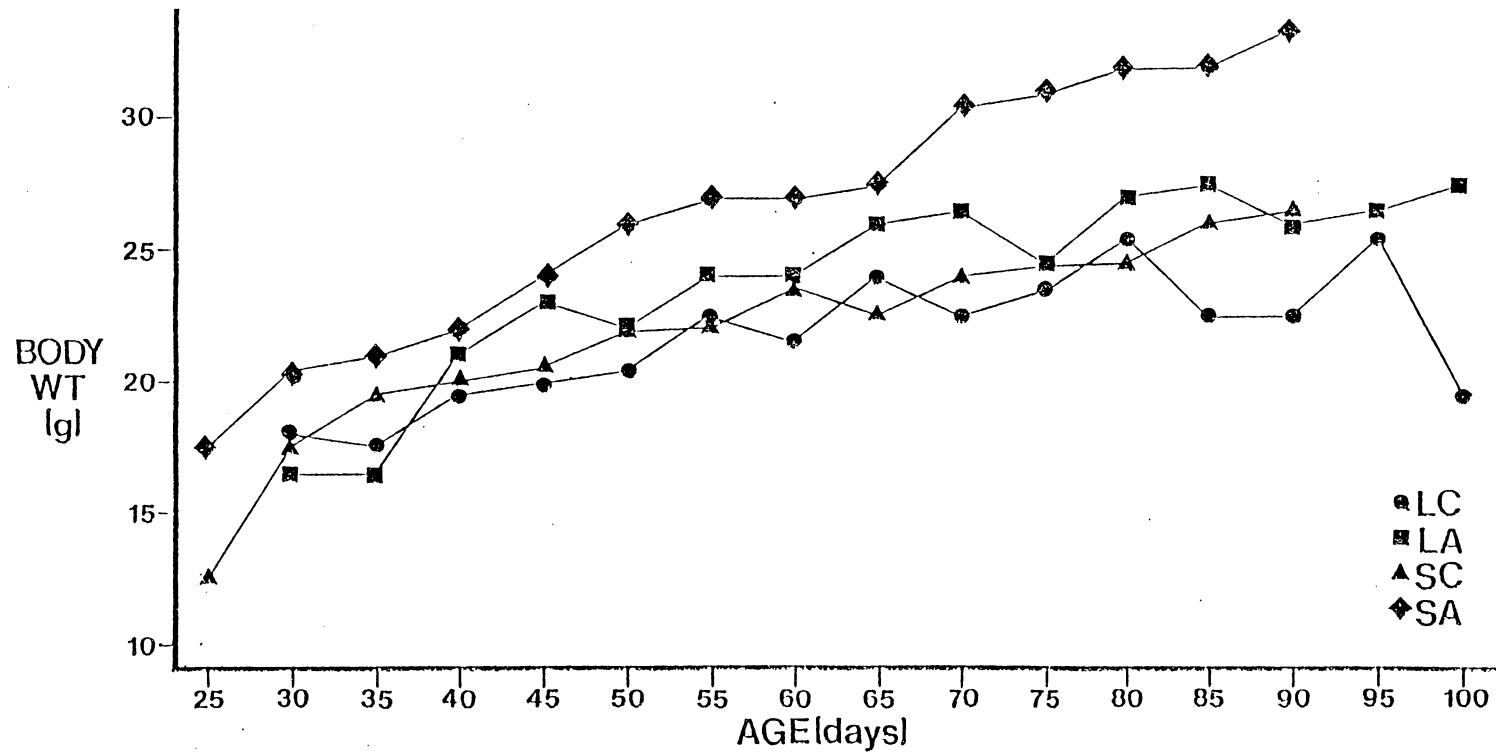


Fig. 6. Mean body weights in young female pine voles subjected to different nutritional and photoperiod regimes.

Table 28. Mean squares for body weights taken every 4 or 7 days in young male and female pine voles subjected to different nutritional and photoperiod regimes. Analysis done for voles 51-80 days of age.

Source	df	Body weights
Sex (S)	1	20.95
Photoperiod (P)	1	0.14
Diet (D)	1	325.52***
S X P	1	90.82
S X D	1	33.19
P X D	1	0.16
S X P X D	1	25.00
Vole(S X P X D) ¹	46	32.53
Age Class (A)	2	99.41**
A X S	2	2.03
A X P	2	4.58*
A X D	2	1.52
A X S X P	2	1.69
A X S X D	2	1.08
A X P X D	2	4.98
A X S X P X D	2	2.10
A X Vole(S X P X D) ²	73	1.24
* P<0.05		
** P<0.001		
*** P<0.005		

¹Error term used for testing S, P, D, S X P, S X D, P X D, S X P X D.

²Error term used for testing A, A X S, A X P, A X D, A X S X P, A X S X D, A X P X D, A X S X P X D.

significantly ($P < 0.001$) heavier than those of 51-60 days of age. There were significant interactions for age X photoperiod and age X photoperiod X diet ($P < 0.05$). These interactions make interpretation of main effects difficult and, in fact, may mask significant differences in main effects.

Males in all 4 groups increased in body weight until reaching 80-85 days of age (Fig. 5). From 85 to 90 days of age mean body weight decreased. Males fed apple and those in the SC group increased in weight from 90 to 100 days of age but LC males continued to lose weight. Males in the LA group had the highest mean body weights in all but 1 age class. Females gradually gained weight throughout the experiment (Fig. 6). Females in the SA group had the highest body weight for each age class.

Young on the 15L:9D photoperiod had a significantly ($P < 0.001$) greater increase in body length during the experiment than young on the 9L:15D photoperiod (Tables 29 and 30). Voles on an apple and chow diet gained significantly ($P < 0.03$) more body weight than those fed chow. Neither sex nor photoperiod had a significant ($P > 0.22$) effect on change in body weight.

Young on an apple and chow diet had significantly (Tables 31 and 32) higher mean final body weights ($P < 0.001$) and greater mean final body lengths ($P < 0.06$). There was a

Table 29. Mean values ($\pm$ SE) and sample sizes by treatment group for change in body weight and change in body length in young male and female pine voles subjected to different nutritional and photoperiod regimes.

Group	<u>Male</u>		<u>Female</u>	
	Change in body weight	Change in body length	Change in body weight	Change in body length
	(g $\pm$ SE) (n)	(mm $\pm$ SE) (n)	(g $\pm$ SE) (n)	(mm $\pm$ SE) (n)
15L:9D				
Chow	6.8 $\pm$ 1.1 (6)	24 $\pm$ 3 (6)	6.6 $\pm$ 1.6 (6)	22 $\pm$ 3 (6)
Apple + Chow	8.6 $\pm$ 1.8 (7)	22 $\pm$ 2 (7)	6.5 $\pm$ 1.5 (5)	22 $\pm$ 3 (5)
9L:15D				
Chow	7.2 $\pm$ 0.9 (9)	13 $\pm$ 1 (9)	6.6 $\pm$ 0.6 (5)	14 $\pm$ 2 (5)
Apple + Chow	9.4 $\pm$ 1.6 (7)	14 $\pm$ 2 (7)	11.8 $\pm$ 0.5 (4)	18 $\pm$ 2 (4)

Table 30. Mean squares (MS) for change in body weights and change in body lengths in young male and female pine voles subjected to different nutritional and photoperiod regimes.

Source	df		Change in body weights	Change in body lengths
Sex (S)	1	MS P<	18.26 .70	9.19 .58
Photoperiod (P)	1	MS P<	18.20 .22	786.66 .001*
Diet (D)	1	MS P<	55.74 .03*	5.82 .66
S X P	1	MS P<	11.48 .33	29.69 .32
S X D	1	MS P<	0.15 .91	15.38 .47
P X D	1	MS P<	17.12 .23	33.71 .29
S X P X D	1	MS P<	17.63 .23	1.73 .81
Error	41	MS	11.67	29.46

Table 31. Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight and body length in young male and female pine voles subjected to different nutritional and photoperiod regimes.

Group	<u>Male</u>		<u>Female</u>	
	Body weight	Body length	Body weight	Body length
	(g $\pm$ SE) (n)	(mm $\pm$ SE) (n)	(g $\pm$ SE) (n)	(mm $\pm$ SE) (n)
15L:9D				
Chow	25.4 $\pm$ 1.4 (6)	107 $\pm$ 1 (6)	23.6 $\pm$ 1.7 (6)	100 $\pm$ 1 (6)
Apple + Chow	28.8 $\pm$ 1.6 (6)	105 $\pm$ 1 (6)	27.5 $\pm$ 0.9 (4)	104 $\pm$ 1 (4)
9L:15D				
Chow	26.0 $\pm$ 1.1 (9)	102 $\pm$ 1 (9)	25.1 $\pm$ 1.4 (5)	102 $\pm$ 1 (5)
Apple + Chow	27.9 $\pm$ 1.9 (7)	103 $\pm$ 1 (7)	32.5 $\pm$ 0.8 (4)	108 $\pm$ 1 (4)

Table 32. Mean squares (MS) for final body weights and body lengths in young male and female pine voles subjected to different nutritional and photoperiod regimes.

Source	df		Body weights	Body lengths
Sex (S)	1	MS P<	0.79 .81	7.60 .40
Photoperiod (P)	1	MS P<	16.04 .28	13.44 .26
Diet (D)	1	MS P<	164.40 .001*	39.19 .06*
S X P	1	MS P<	34.44 .12	129.49 .001*
S X D	1	MS P<	24.65 .18	58.25 .03*
P X D	1	MS P<	0.88 .80	15.00 .24
S X P X D	1	MS P<	16.86 .27	0.20 .89
Error	39	MS	13.36	10.36

significant ($P < 0.001$) sex X photoperiod interaction for body length. Males on the 15L:9D photoperiod had greater body lengths than those on the 9L:15D regime but females had the opposite trend. There was also a significant ($P < 0.03$) sex X diet interaction for body length. Differences due to diet were small for males but females given apple were longer than those fed chow.

Voles given apple had a significantly ($P < 0.001$) higher percent body fat (Tables 33 and 34). There was a significant ($P < 0.08$) sex X diet interaction. Females in the SA group had more body fat than males while both sexes fed chow had similar fat levels (Table 33). Carcasses of voles with an apple supplement had 50% body fat.

Molt

Means for age and body weight at initiation and completion of molt to adult pelage were determined for males and females (Tables 35 and 36). There was a significant ($P < 0.05$) photoperiod X diet interaction for age at initiation of molt (Table 37). Young in the LA group began molt at a younger age than the LC group but the SC group started molting before the SA group.

Males completed molt at a significantly ($P < 0.05$) younger age than females. There was also a significant ($P < 0.08$) sex X photoperiod interaction. Both sexes

Table 33. Mean values ($\pm$ SE) and sample sizes by treatment group for fat content (expressed as percent of dry body weight) in young male and female pine voles subjected to different nutritional and photoperiod regimes.

Group	Male		Female	
	(% $\pm$ SE)	(n)	(% $\pm$ SE)	(n)
15L:9D				
Chow	46.6 $\pm$ 2.4	(6)	42.2 $\pm$ 3.4	(5)
Apple + Chow	52.4 $\pm$ 3.1	(7)	54.7 $\pm$ 1.4	(5)
9L:15D				
Chow	44.9 $\pm$ 3.3	(9)	44.0 $\pm$ 2.8	(6)
Apple + Chow	49.6 $\pm$ 4.0	(6)	58.5 $\pm$ 1.9	(4)

Table 34. Mean squares (MS) for fat content in young male and female pine voles subjected to different nutritional and photoperiod regimes.

Source	df		Body fat
Sex (S)	1	MS F<	11.02 .67
Photoperiod (P)	1	MS F<	16.27 .61
Diet (D)	1	MS F<	892.49 .001*
S X P	1	MS F<	53.47 .35
S X D	1	MS F<	195.15 .08*
P X D	1	MS F<	0.14 .96
S X P X D	1	MS F<	6.65 .74
Error	40	MS	60.44

Table 35. Mean values ($\pm$ SE) and sample sizes by treatment group for age and body weight at initiation and completion of molt to adult pelage in young male pine voles subjected to different nutritional and photoperiod regimes.

Group	Age at initiation	Age at completion	Body weight at initiation		Body weight at completion	
	($\bar{d} \pm$ SE) (n)	($\bar{d} \pm$ SE) (n)	($\bar{g} \pm$ SE)	(n)	($\bar{g} \pm$ SE)	(n)
15L:9D						
Chow	39 $\pm$ 1 (7)	56 $\pm$ 4 (5)	19.5 $\pm$ 1.3	(5)	23.5 $\pm$ 1.2	(5)
Apple + Chow	34 $\pm$ 1 (4)	56 $\pm$ 4 (4)	21.9 $\pm$ 1.1	(4)	26.8 $\pm$ 1.3	(4)
9L:15D						
Chow	37 $\pm$ 2 (10)	66 $\pm$ 5 (7)	21.2 $\pm$ 1.3	(10)	25.6 $\pm$ 1.4	(7)
Apple + Chow	40 $\pm$ 2 (8)	64 $\pm$ 5 (6)	22.0 $\pm$ 1.2	(8)	27.7 $\pm$ 1.5	(6)

Table 36. Mean values ($\pm$ SE) and sample sizes by treatment group for age and body weight at initiation and completion of molt to adult pelage in young female pine voles subjected to different nutritional and photoperiod regimes.

Group	Age at initiation		Age at completion		Body weight at initiation		Body weight at completion	
	($\bar{d} \pm$ SE)	(n)	($\bar{d} \pm$ SE)	(n)	($\bar{g} \pm$ SE)	(n)	($\bar{g} \pm$ SE)	(n)
15L:9D								
Chow	43 $\pm$ 2	(5)	74 $\pm$ 10	(4)	19.2 $\pm$ 1.7	(5)	22.6 $\pm$ 1.8	(4)
Apple + Chow	41 $\pm$ 3	(3)	79 $\pm$ 24	(2)	19.9 $\pm$ 1.0	(3)	25.5 $\pm$ 0.3	(2)
9L:15D								
Chow	38 $\pm$ 1	(8)	73 $\pm$ 4	(5)	20.3 $\pm$ 0.7	(8)	24.6 $\pm$ 1.5	(5)
Apple + Chow	40 $\pm$ 2	(7)	63 $\pm$ 7	(5)	22.8 $\pm$ 1.1	(7)	30.0 $\pm$ 1.0	(5)

Table 37. Mean squares (MS) for ages and body weights at initiation and completion of molt to adult pelage in young pine voles subjected to different nutritional and photoperiod regimes.

Source	df	Ages at initiation	Ages at completion	Body weights at initiation	Body weights at completion
Sex (S)	1 MS	49.40	876.48	2.41	0.00
	P<	.17	.05*	.63	.99
Photoeriod (P)	1 MS	7.02	55.60	24.05	55.94
	P<	.60	.60	.13	.03*
Diet (D)	1 MS	2.99	71.53	31.02	106.07
	P<	.73	.55	.09*	.003*
S X P	1 MS	64.39	666.50	3.12	9.98
	P<	.12	.08*	.58	.33
S X D	1 MS	0.61	32.31	1.03	7.51
	P<	.88	.69	.75	.40
P X D	1 MS	105.30	109.47	0.00	0.15
	P<	.05*	.47	.99	.90
S X P X D	1 MS	9.35	89.51	6.86	7.20
	P<	.54	.51	.41	.40
Error	44 MS	24.90	200.18 ¹	9.99 ²	10.09 ¹

¹Degrees of freedom for error term was 30.

²Degrees of freedom for error term was 42.

completed molt at similar ages on the 9L:15D photoperiod but males finished molting at a younger age than females on the 15L:9D photoperiod. There was little variation in age at initiation of molt but much variation in age at completion of molt. This is most obvious in LA females, where small sample size was also a problem.

Young fed apple and chow had significantly higher body weights at initiation ($P<0.09$) and completion ($P<0.003$) of molt than those given chow. Voles on 9L:15D began molting at a slightly higher body weight and completed molt at a significantly ($P<0.03$) higher weight. Body weight for both initiation and completion of molt in males and females was highest in the SA group and lowest in the LC group (Tables 35 and 36).

Reproductive Characteristics

Males on an apple and chow diet had significantly heavier seminal vesicles ($P<0.01$) and paired testes ($P<0.004$), and significantly ($P<0.05$) more spermatozoa/mg testes (Tables 38 and 39). Spermatozoa counts were significantly ($P<0.10$) higher for males on the 9L:15D photoperiod. There was a significant photoperiod X diet interaction for adrenal glands weight ($P<0.04$) and seminal vesicles weight ($P<0.09$). Adrenal glands weight was greatest in the LC and SA groups (Table 38).

Table 38. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in young male pine voles subjected to different nutritional and photoperiod regimes.

Group	Adrenal glands weight		Seminal vesicles weight		Paired testes weight		<u>Spermatozoa</u> sperm/mg testes wt X	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	10 ³ $\pm$ SE	(n)
15L:9D								
Chow	10.2 $\pm$ 1.1	(6)	25.6 $\pm$ 7.4	(6)	61.0 $\pm$ 3.3	(6)	47.5 $\pm$ 8.6	(6)
Apple + Chow	8.6 $\pm$ 0.6	(6)	59.0 $\pm$ 10.8	(6)	82.8 $\pm$ 5.5	(6)	59.0 $\pm$ 13.8	(6)
9L:15D								
Chow	8.6 $\pm$ 0.5	(9)	27.9 $\pm$ 3.4	(8)	54.8 $\pm$ 7.1	(9)	58.6 $\pm$ 9.9	(9)
Apple + Chow	10.0 $\pm$ 1.1	(6)	36.4 $\pm$ 6.5	(7)	77.3 $\pm$ 9.0	(7)	89.4 $\pm$ 10.9	(7)

Table 39. Mean squares (MS) for reproductive characteristics in young male pine voles subjected to different nutritional and photoperiod regimes.

Source	df	Adrenal glands weights	Seminal vesicles weights	Paired testes weights	Sperm/mg testes wts X 10 ³
Photoperiod (P)	1 MS	0.00	718.99	364.58	2434.29
	P<	.99	.15	.31	.10*
Diet (D)	1 MS	0.20	2581.32	3424.07	3485.92
	P<	.84	.01*	.004*	.05*
P X D	1 MS	20.43	1035.94	0.77	634.65
	P<	.04*	.09*	.96	.39
Error	23 MS	4.51	329.12	341.58 ¹	828.92 ¹

¹Degrees of freedom for error term was 24.

Females fed apple and chow had significantly ($P < 0.01$) heavier paired ovarian weights than those given chow (Tables 40 and 41). Uterine weights tended to be higher in females with an apple supplement, especially those in the SA group. Differences in organ weights due to diet were greater on the 9L:15D photoperiod.

Discussion

Food Consumption

As in the experiment described in Chapter 2, voles on a chow diet generally consumed more digestible energy than those given apple and chow. However, the differences in energy consumption were not as large as those for adults in Chapter 2. The groups fed apple and chow may have consumed less energy because they filled their stomachs with a more preferred food, apple, while supplementing their basic requirements with the commercial chow. Consuming apple would fill their stomach rapidly because of the high water content in apple.

Body Condition

Voles on the apple and chow diet grew faster than those fed chow. These young gained more body weight during the experiment, were generally heavier at each age class and had higher values for final body weight and body fat.

Table 40. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in young female pine voles subjected to different nutritional and photoperiod regimes.

Group	Adrenal glands weight	Uterine weight	Paired ovarian weight
	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)
15L:9D			
Chow	9.5 $\pm$ 1.3 (6)	17.7 $\pm$ 6.4 (6)	2.1 $\pm$ 0.2 (6)
Apple + Chow	8.5 $\pm$ 0.7 (4)	19.9 $\pm$ 3.9 (4)	3.0 $\pm$ 0.4 (4)
9L:15D			
Chow	7.3 $\pm$ 1.3 (5)	14.9 $\pm$ 2.8 (5)	1.9 $\pm$ 0.4 (5)
Apple + Chow	8.2 $\pm$ 1.9 (4)	28.5 $\pm$ 3.7 (4)	3.0 $\pm$ 0.4 (4)

Table 41. Mean squares (MS) for reproductive characteristics in young female pine voles subjected to different nutritional and photoperiod regimes.

Source	df		Adrenal glands weights	Uterine weights	Paired ovarian weights
Photoperiod (P)	1	MS	9.46	26.71	0.02
		P<	.33	.64	.84
Diet (D)	1	MS	0.03	274.84	4.56
		P<	.95	.14	.01*
P X D	1	MS	4.27	150.70	0.07
		P<	.51	.27	.73
Error	15	MS	138.88	114.29	0.55

These animals, though consuming less digestible energy, were seemingly more efficient in converting their food into body tissue and body fat.

Males may have grown slightly faster than females because they gained more weight in all but 1 treatment group. However, the data are not conclusive. Photoperiod did not affect growth or final body condition.

Molt

Neither photoperiod nor diet influenced age at initiation or completion of molt. Although males and females began molting at about the same age, males completed molt before females. Because females take longer to reach puberty (Schadler and Butterstein 1979), the physiological mechanisms governing maturation would extend the time it takes to reach adult pelage.

In this experiment young voles began molt to adult pelage at 5-6 weeks of age. Males and females completed molt at 8-9 weeks of age and 9-11 weeks of age, respectively. Previous studies found initiation of molt to adult pelage begins at about 5 weeks of age (Hamilton 1938, Benton 1955, Paul 1970). Reports of age at completion of molt range from 7 weeks of age (Hamilton 1938) to between 7-10 weeks of age (Benton 1955, Paul 1970). Benton (1955) noted completion of molt may take longer than 10 weeks.

This may help explain why there was little variation in this experiment for age at initiation of molt but a great deal of variation for age at completion of molt. Age at initiation of molt may be more inherently built into the animal's system and age at completion of molt more susceptible to environmental factors. This may be especially true for females, which had more variation in age to complete molt than males. Females take longer to mature and would be subject to more changes in the environment during growth and molt.

Young began molting at body weights ranging from 19-23g. Thus, using 21g body weight to separate immatures from adults in the field is fairly reasonable. Although it is difficult to compare lab and field results (Merson 1979), it would appear that animals classified as adults by weight in the field may not have attained puberty though they were beginning to take on adult characteristics.

Voies on the apple and chow diet were heavier at initiation and completion of molt than those fed chow. Because voles given apple and chow gained more weight and were heavier, it is logical they would molt at a higher body weight. The data do not allow conclusions as to whether age at initiation or completion of molt is regulated more by age, body weight or an interaction of the two.

Reproductive characteristics

Voies fed apple and chow had heavier reproductive organs than those given chow. It cannot be ascertained if the treatments affected age at puberty, but voles fed apple and chow probably had higher levels of gonadotrophic and sex steroid hormones. This would improve the chances of producing offspring at an earlier age than those fed only chow and increase their overall reproductive potential. Even though young on the chow diet consumed more digestible energy, it appears young fed apple and chow either produced more hormones or were more sensitive to the effects of hormones affecting reproductive organs.

The results of this experiment were complicated by many factors. The voles used in the experiment came from 2 different colonies with different environments. The voles were also weaned at different ages. Voles used in the second phase of the experiment were generally younger than those used in the first phase. Some voles may have been too old for the treatments to have enough of an impact to actually influence growth. Dietary effects should not have been influenced much by these factors, however, since voles of different ages were more evenly distributed among the diet groups. Effects of photoperiod may not have surfaced because of differences in age of the voles.

In summary, no groups were on an energy restricted

diet. Young fed a commercial chow diet consumed more energy but had less gain in body weight and lower mean values for body fat, reproductive organ weights, and body weight at initiation and completion of molt to adult pelage.

Therefore, voles on the apple and chow diet appeared to grow faster and were more capable of reproducing than those fed chow.

GENERAL DISCUSSION

Two hypotheses concerning reproduction and growth in pine voles were tested in the course of this research. The first hypothesis was that availability of apples increased reproduction and growth in pine voles. The second hypothesis was that a short or declining photoperiod would have a negative effect on reproduction and growth. Additive effects between photoperiod and nutrition were also noted.

In 3 experiments, groups having access to apples had higher reproductive organ weights than those with no apple. In the orchard experiment (Chapter 1) the differences were significant for adult males (organ weights and spermatozoa counts) and immatures of both sexes and also for young voles of both sexes under controlled environmental conditions (Chapter 3). Similar but nonsignificant ($P > 0.10$) trends were found in both adult male and female voles under semi-natural conditions in the raceway building (Chapter 2). These differences occurred even though voles with apples in their diet did not consume more digestible energy (Servello 1981, and Chapters 2 and 3). Howland (1972) found female laboratory rats on a restricted energy intake had lower levels of plasma LH and lower ovarian weights than control groups but rats on a restricted diet and supplemented with glucose had values of plasma LH and ovarian weights similar

to controls. The addition of glucose was theorized to have affected the hypothalamic-pituitary axis, stimulating the release of LH which in turn increased follicle growth and ovarian weight. Even though voles with apples available consumed less digestible energy, apples are highly digestible (Servello 1981) and contain readily available nonstructural carbohydrates (i.e. sugars). This may increase blood glucose levels which would affect the hypothalamus and/or pituitary gland, stimulating the production and release of gonadotrophic hormones. In apple orchards this could extend the breeding season or lead to year-round breeding if apples are available. Young produced between mid-summer and fall may grow rapidly enough to attain puberty that same year. In the orchard experiment, immature voles in areas with apples available had reproductive organ weights similar to those of adult voles in the areas with apples removed. These immature voles may have produced litters later that winter. Perhaps more importantly, they may have nearly mature gonads and a small body mass. A small body mass could be advantageous for overwinter survival because energy expenditures for maintenance and foraging may be lower than in larger voles (Brown 1973, Iverson and Turner 1974, Petterborg 1978). Greater gonadal development might allow young to produce litters earlier the following breeding season than young in

areas where apples were unavailable. The combined effect of a longer breeding season and more rapid maturation of young could result in higher population densities.

Photoperiod influenced reproductive organ weights in adult pine voles and spermatozoa counts in males. In the raceway experiment male and female voles exposed to a 14L:10D light regime had heavier reproductive organs and males had more spermatozoa/mg testes than those on a declining photoperiod (12L:12D to 9L:15D). Previous field studies have demonstrated that reproductive activity peaked during the long days of summer (Valentine and Kirkpatrick 1970, Paul 1970, Cengel et al. 1978). Thus, pine voles may use photoperiod as a cue for reproductive activity. Beiter (1980) felt animals exposed to short photoperiods either increase synthesis of or are more sensitive to antigonadotrophic substances released by the pineal gland while animals under long photoperiods become refractory to the inhibitory effects of these substances. Pine voles perhaps reduce levels of reproductive activity in the late fall and winter, increasing their own chances of survival, and increase reproduction in spring and summer when environmental conditions improve, increasing their offspring's chances of survival.

Photoperiod and nutrition may also have additive effects which influenced reproduction. Servello (1981),

using a more accurate technique for quantifying energy than used in previous studies, found little change in energy intake between seasons in different orchards. In the raceway experiment digestible energy consumption was higher in the chow only group on a declining photoperiod than in the declining photoperiod-apple and chow group. However, the apple-chow group tended to have higher values for reproductive characteristics with testes weights significantly ($P < 0.05$) heavier in the apple-chow group. It is possible voles on a declining or short photoperiod produce gonadotrophic hormones but the synthesis or release of releasing factors from the hypothalamus and/or hormones from the pituitary are inhibited by substances from the pineal gland. Conversely, voles consuming apples could have a lower intake of digestible energy but synthesis and release of gonadotrophic hormones may not be as inhibited by pineal gland activity. Therefore, pine voles may use both photoperiod and nutrition as cues for reproduction.

Noffsinger (1976) speculated an interaction of photoperiod and nutrition affected reproduction in pine voles in apple orchards. Voles in his maintained orchard were postulated to have had a diet of better quality in late fall and winter than those in the abandoned orchard. The low energy intake in the abandoned orchard and increased costs of thermoregulation in late fall and winter may have

caused the cessation of reproduction. In the maintained orchard reproduction could continue because of an apparent higher intake of energy, but at reduced levels because of the inhibitory effect of a declining or short photoperiod.

Availability of apples influenced body condition in both immature and adult pine voles. In the raceway experiment adults fed apple and chow maintained or gained body weight while those given only chow generally lost weight. Young voles given an apple supplement in the chamber experiment gained more weight, began and completed molt at higher body weights, and had higher final mean body weights than those fed only chow. Although voles fed apples had a lower digestible energy intake in both experiments, it appears that consuming apples allowed pine voles to maintain body weight and perhaps increase growth rates.

Body fat levels were usually higher in voles which had apples in their diet. In a companion study to the orchard experiment, Servello (1981) found body fat was higher in December for females in the area with apples present than for females in the area with apples removed. Body fat levels were the same in both areas for females in November and for males in November and December, though males had a 3-4% decline in body fat from November to December. In the adult and young voles in the raceway and chamber experiments, those fed apple and chow had significantly

($P < 0.01$) more body fat than those fed chow only.

Noffsinger (1976) and Servello (1981) theorized thermoregulatory costs in fall may affect partitioning of energy between reproduction (reproductive organ weight) and maintenance (body fat). Voles in the orchard and raceway experiments were subjected to declining ambient air temperatures which would increase thermoregulatory costs. However, voles with higher body fat levels also had heavier reproductive organs. This could indicate 1) availability of apples in the diet had a positive effect on energy assimilation and reproductive physiology in spite of increased thermoregulatory costs, and/or 2) body fat is a poor physiological index of nutrition (i.e. digestible energy intake) and reproductive status of a pine vole.

Adult male pine voles appear to be more sensitive to differences in nutrition than females. Males had significant differences in reproductive organ weights due to diet in both the orchard and raceway experiments and in spermatozoa counts in the orchard experiment. Females showed similar trends in reproductive organ weight but the results were not significant. It would appear that males react more rapidly to changes in the environment and adjust their reproductive status accordingly.

Comparisons can be made between reproductive organ weights in these 3 experiments. Adults subjected to a

declining photoperiod and fed apple and chow in the raceway experiment had lower reproductive organ weights than adults trapped in December in the area with apples present in the orchard experiment. This may indicate captivity and social stress were responsible for the lower weights (Merson 1979). However, adults fed chow only and on a declining photoperiod had similar organ weights as those trapped in December in the area with apples removed. Young voles in the chamber experiment had heavier reproductive organs than adults in both the orchard and raceway experiments. However, the young voles were exposed to the least harsh environmental conditions of the 3 experiments.

Effects of photoperiod and nutrition on reproduction and growth need further investigation. Adult pine voles were affected by different photoperiods. Future studies relating photoperiod to reproduction should be designed to test influences of light intensity at different levels of nutrition. It is not known if pine voles cue reproduction on a changing photoperiod or if some critical length of daylight causes a reduction in fall and, in spring, initiation of reproduction. The influence of availability of apples on digestible energy intake should be confirmed in both apple orchards and the laboratory. Experiments should be done to investigate the mechanisms of how photoperiod and availability of apples affect circulating levels of

gonadotrophins and steroid hormones. Effects of photoperiod on the pineal and how this organ in turn affects reproduction and growth should also be investigated. Future work is needed to determine how photoperiod and nutrition influence the physiological mechanisms governing growth, molt to adult pelage and age of attaining puberty in young voles of similar age.

In summary, availability of apples did not appear to increase the digestible energy intake in pine voles but voles with apple in their diet had more body fat, heavier reproductive organs, grew more rapidly and were heavier at initiation and completion of molt to adult pelage. Long photoperiod resulted in higher reproductive organ weights in adult pine voles and spermatozoa counts in males compared to those on a declining light regime. There may also be additive effects of photoperiod and nutrition. The availability of apples in the diet may hasten growth and attainment of puberty in growing voles regardless of photoperiod. It appears both photoperiod and nutrition probably act as cues regulating reproduction and growth in the pine vole.

Literature Cited

- Andrzejewski, R. 1975. Supplementary food and the winter dynamics of bank vole populations. *Acta Theriol.*, 20(2):23-40.
- Baker, J.R. and R.M. Ranson. 1932a. Factors affecting the breeding of the field mouse (Microtus agrestis). I. Light. *Proc. Roy. Soc. (London) Ser. B*, 11:313-322.
- _____. 1932b. Factors affecting the breeding of the field mouse (Microtus agrestis). II. Temperature and food. *Proc. Roy. Soc. (London) Ser. B*, 112:39-46.
- Ball, Z.B., R.H. Barnes and M.B. Visscher. 1947. The effects of dietary caloric restriction on maturity and senescence with particular reference to fertility and longevity. *Am. J. Physiol.* 150:511-519.
- Benton, A.H. 1955. Observations on the life history of the northern pine mouse. *J. Mamm.* 36(1):52-62.
- Boyette, J.G. 1965. A behavioral study of the pine mouse, Pitymus pinetorum pinetorum (Le Conte). Ph.D. dissertation, North Carolina State Univ. 134pp.
- Breed, W.G. and J.R. Clarke. 1970. Effect of photoperiod on ovarian function in the vole Microtus agrestis. *J. Reprod. Fert.* 23:189-192.
- Brown, E.B. 1973. Changes in patterns of seasonal growth of Microtus pennsylvanicus. *Ecology* 54(5):1103-1110.
- Byers, R.E. 1974. Pine mouse control in apple orchards. *The Mountaineer Grower* 335:3-13.
- Byers, R.E., R.S. Young and R.D. Neely. 1976. Review of cultural and other control methods for reducing pine vole populations in apple orchards. In *Proc. 7th Vert. Pest Conf., Monterey, CA*: 242-253.
- Cengel, D.J. 1975. Seasonal comparison of food habit patterns and reproductive attainment of the pine vole in two northern Virginia orchards. M.S. thesis, VPI & SU. 60pp.
- Cengel, D.J., J.E. Estep, and R.L. Kirkpatrick. 1978. Pine vole reproduction in relation to food habits and body fat. *J. Wildl. Manage.* 42(4):822-833.

- Clarke, J.R. and J.P. Kennedy. 1967. Effect of light and temperature upon gonad activity in the vole (Microtus agrestis). Gen. Comp. Endocrinol. 8:474-488.
- Cole, F.R. and G.O. Batzli. 1978. Influence of supplemental feeding on a vole population. J. Mamm. 59(4):809-819.
- Cole, F.R. and G.O. Batzli. 1979. Nutrition and population dynamics of the prairie vole, Microtus ochrogaster, in central Illinois. J. Anim. Ecol. 48:455-470.
- Cowan, I. Mct. and M.G. Arsenault. 1954. Reproduction and growth in the creeping vole, Microtus oregoni serpens Merriam. Can. J. Zool. 32:198-208.
- Davis, D.E. 1976. Management of pine voles. In Proc. 7th Vert. Pest Conf., Monterey, CA:270-275.
- Ecke, D.H. and A.R. Kinney. 1956. Aging meadow mice, Microtus californicus, by observation of molt progression. J. Mamm. 37(2):249-254.
- Estep, J.E. 1975. Seasonal variation in body fat and food habits of pine voles from two Virginia orchards. M.S. thesis, VPI & SU. 72pp.
- Evans, D.M. 1973. Seasonal variations in the body composition and nutrition of the vole Microtus agrestis. J. Anim. Ecol. 42(1):1-18.
- Fedyk, A. 1974. Gross body composition in postnatal development of the bank vole. II. Differentiation of seasonal generations. Acta Theriol. 19(27):403-427.
- Flowerdrew, J.R. 1972. The effect of supplementary food on a population of wood mice (Apodemus sylvaticus). J. Anim. Ecol. 41(3):553-566.
- _____. 1973. The effect of natural and artificial changes in food supply on breeding in woodland mice and voles. J. Reprod. Fert., Supp. 19:259-269.
- Gebczynska, Z. and M. Gebczynski. 1971. Length and weight of the alimentary tract of the root vole. Acta Theriol., 22:359-369.
- Gentry, J.B. 1968. Dynamics of an enclosed population of pine mice, Microtus pinetorum. Res. Pop. Ecol. 10:21-30.

- Geyer, L.A. and J.G. Rogers, Jr. 1979. The influence of light intensity on reproduction in pine voles, Microtus pinetorum. J. Mamm., 60(4):839-841.
- Gliwicz, J., R. Andrzejewski, G. Bujalska and K. Petruszewicz. 1968. Productivity investigation of an island population of Clethrionomys glareolus (Schreber, 1780). I. Dynamics of cohorts. Acta Theriol. 13(23):401-413.
- Goertz, J.W. 1971. An ecological study of Microtus pinetorum in Oklahoma. Amer. Midl. Natur. 86(1):1-12.
- Hamilton, W.J., Jr. 1938. Life history notes on the northern pine mouse. J. Mamm. 19(2):163-170.
- Hansen, L.P. and G.C. Batzli. 1979. Influence of supplemental food on local populations of Peromyscus leucopus. J. Mamm. 60(2):335-342.
- Hansson, L. 1979. Condition and diet in relation to habitat in bank voles Clethrionomys glareolus: population or community approach? Oikos 33:55-63.
- Hasler, J.F., A.E. Buhl, and E.M. Banks. 1976. The influence of photoperiod on growth and sexual function in male and female collared lemmings (Dicrostonyx groenlandicus). J. Reprod. Fert. 46:323-329.
- Hasler, J.F. 1975. A review of reproduction and sexual maturation in the microtine rodents. The Biol., 57(2):52-86.
- Helwig, J.T. and K.A. Council. 1979. SAS User's Guide, 1979 edition: SAS Institute, Inc., Raleigh, NC. 494pp.
- Hoffman, R.S. 1958. The role of reproduction and mortality in population fluctuations of voles (Microtus). Ecol. Mono. 28(1):79-109.
- Horsfall, F., Jr. 1963. Observations on fluctuating pregnancy rate of pine mice and mouse feed potential in Virginia orchards. Proc. Am. Soc. Hort. Sci. 83:276-279.
- _____. 1972. Pine mouse damage by a three-phase system. Va. Fruit 60(7):20-24.

- Howland, B.E. 1971. Gonadotrophin levels in female rats subjected to restricted feed intake. J. Repro. Fert., 27:467-470.
- . 1972. Effect of restricted feed intake of LH levels in female rats. J. Anim. Sci., 34:445-447.
- Iverson, S.L. and B.N. Turner. 1974. Winter weight dynamics in Microtus pennsylvanicus. Ecology 55(5):1030-1041.
- Khateeb, A. Al. and E. Johnson. 1971. Seasonal changes of pelage in the vole (Microtus agrestis). II. The effect of daylength. Gen. Comp. Endocrinol. 16:229-235.
- Kirkpatrick, R.L. and G.L. Valentine. 1970. Reproduction in captive pine voles, Microtus pinetorum. J. Mamm. 51(4):779-785.
- Krebs, C.J. and K.T. DeLong. 1965. A Microtus population with supplemental food. J. Mamm. 46(4):566-573.
- Kubik, J. 1965. Biomorphological variability of the population of Clethrionomys glareolus (Schreber, 1780). Acta Theriol. 10(10):117-179.
- LaVoie, G.K. and H.P. Tietjen. 1971. Pine vole research: a problem analysis. Denver Wildl. Res. Center. 17pp.
- Lecyk, M. 1962. The effect of the length of daylight on reproduction in the field vole (Microtus arvalis Pall.). Zool. Polon. 12(2):189-220.
- Linzey, D.W. and A.V. Linzey. 1967. Maturational and seasonal molts in the golden mouse, Ochrotomys nuttalli. J. Mamm. 48(2):236-241.
- Lochmiller, R.L. 1980. Bioenergetics and nutrition of the pine vole (Microtus pinetorum) in two Virginia apple orchards. M.S. thesis, VPI & SU. 185pp.
- Marshall, A.J. and O. Wilkinson. 1956. Reproduction in the Orkney vole (Microtus orcadensis) under a six-hour daylength and other conditions. Proc. Zool. Soc. (London) 126:391-395.
- Merson, M.H. 1979. Influence of restricted feed intake on the reproductive characteristics, energetics, and body composition of two species of rodents. Ph.D. dissertation, VPI & SU. 229pp.

- Merson, M.H. and R.L. Kirkpatrick. 1981. Relative sensitivity of reproductive activity and body-fat level of food restriction in white-footed mice. *Amer. Midl. Nat.*, 106(2):305-312.
- Miller, D.H. and L. L. Getz. 1969. Life-history notes on Microtus pinetorum in central Connecticut. *J. Mamm.* 50(4):777-784.
- Moule, G.R. 1963. Postpubertal nutrition and reproduction in the male. *Austral. Vet. J.* 39:299-304.
- Myrcha, A. 1964. Variations in the length and weight of the alimentary tract of Clethrionomys glareolus (Schreber 1780). *Acta Theriol.*, 9(10):139-148.
- Negus, N.C., P.J. Berger, and L. G. Forslund. 1977. Reproductive strategy of Microtus montanus. *J. Mamm.* 58(3):347-353.
- Noffsinger, R.E. 1976. Seasonal variation in the natality, mortality, and nutrition of the pine vole in two orchard types. M.S. thesis, VPI & SU. 126pp.
- Paul, J.R. 1970. Observations on the ecology, populations, and reproductive biology of the pine vole, Microtus pinetorum, in North Carolina. III. State Mus., Reports of Investigations No. 20. 28pp.
- Perrin, M.R. 1979. Seasonal variation in the growth, body composition, and diet of Clethrionomys gapperi in spruce forest. *Acta Therioil.* 24(23):299-318.
- Petterborg, L.J. 1978. Effect of photoperiod on body weight in the vole, Microtus montanus. *Can. J. Zool.* 56:431-435.
- Piacsek, B.E. and J. Meites. 1967. Reinitiation of gonadotropin release in underfed rats by constant light or epinephine. *Endocrinology* 81:535-541.
- Pinter, A.J. 1968a. Effects of diet and light on growth, maturation, and adrenal size of Microtus montanus. *Amer. J. Physiol.* 215(2):461-466.
- . 1968b. Hair growth responses to nutrition and photoperiod in the vole, Microtus montanus. *Amer. J. Physiol.* 215(4):828-832.

- _____, and N.C. Negus. 1965. Effects of nutrition and photoperiod on reproductive physiology of Microtus montanus. Amer. J. Physiol. 208(4):633-638.
- Pistole, D.H. 1980. Effects of environmental factors on reproduction, growth, and development in Microtus pennsylvanicus. M.S. thesis, VPI & SU. 66pp.
- Reiter, R.J. 1974. Circannual reproductive rhythms in mammals related to photoperiod and pineal function: A review. Chronobiologia, 1:365-395.
- Reiter, R.J. 1980. Photoperiod: Its importance as an impeller of pineal and seasonal reproductive rhythms. Int. J. Biometeor., 24(1):57-63.
- Roth, R.R. 1974. The effect of temperature and light combinations upon the gonads of male red-back voles. Biol. Reprod. 10:309-314.
- Sadlier, R.M.F.S. 1969. The ecology of reproduction in wild and domestic mammals. Methuen and Co., London. 321pp.
- Sadlier, R.M.F.S., K.D. Casperson, and J. Harling. 1973. Intake and requirements of energy and protein for the breeding of wild deermice, Peromyscus maniculatus. J. Reprod. Fert., Supp. 19:237-252.
- Salmon, T.P. and R.E. Marsh. 1978. Age as a factor in rodent susceptibility to rodenticides-A review. In Vertebrate Pest Control and Management Materials. J.R. Beck, ed., American Society for Testing and Materials. pp. 84-98.
- Schadler, M.H. and G.M. Butterstein. 1979. Reproduction in the pine vole, Microtus pinetorum. J. Mamm. 60(4):841-844.
- Schwarz, S.S., A.V. Pokrovski, V.G. Istchenki, V.G. Olenjev, N.A. Gutschinnikova, and C.A. Pjastolova. 1964. Biological peculiarities of seasonal generations of rodents, with special reference to the problem of senescence in mammals. Acta theriol., 8(2):11-43.
- Sealander, J.A. 1972. Circum-annual changes in age, pelage characteristics and adipose tissue in the northern red-backed vole in interior Alaska. Acta Theriol. 17(1):1-24.

- Servello, F.A. 1981. Nutritional ecology of the pine vole in relation to apple tree root damage. M.S. thesis, VPI & SU. 153pp.
- Sullivan, J.A. and P.F. Scanlon. 1976. Effects of grouping and fighting on the reproductive tracts of male white-footed mice (Peromyscus leucopus). Res. Pop. Ecol. 17:164-175.
- Taitt, M.J. 1981. The effect of extra food on small rodent populations. I. Deermice (Peromyscus maniculatus). J. Anim. Ecol., 50:111-124.
- _____ and C.J. Krebs. 1981. The effect of extra food on small rodent populations. II. Voles (Microtus townsendii). J. Anim. Ecol., 50:125-137.
- Tast, J. 1972. Annual variations in the weights of wintering root voles, Microtus oeconomus, in relation to their food conditions. Ann. Zool. Fennici 9:116-119.
- _____, and A. Kaikusalo. 1976. Winter breeding in the root vole, Microtus oeconomus, in 1972/73 at Kilpisjarvi, Finnish Lapland. Ann. Zool. Fennici 13:174-178.
- Valentine, G.L. and R.L. Kirkpatrick. 1970. Seasonal changes in reproductive and related organs in the pine vole, Microtus pinetorum, in southwestern Virginia. J. Mamm. 51(3):553-560.
- Virginia Fruit. 1971. 59(10):2. Virginia State Hort. Soc., Staunton, VA.
- White, T.C.R. 1978. The importance of a relative shortage of food in animal ecology. Cecologia (Berl.), 33:71-86.
- Worth, R.W., H.M. Charlton, and P.C.B. Mackinnon. 1973. Field and laboratory studies on the control of luteinizing hormone secretion and gonadal activity in the vole, Microtus agrestis. J. Reprod. Fert., Supp. 19:89-99.
- Zejda, J. 1962. Winter breeding in the bank vole, Clethrionomys glareolus Schreb. Zool. listy 25(4):309-321.

APPENDIX

Appendix Table I. Mean values ($\pm$ SE) and sample sizes by treatment group for light intensity measured under different photoperiod regimes and sky conditions during February, 1982.

Photoperiod	Sky Conditions	N	Light Intensity (footcandles)
Natural Decline	Cloudy	20	7 $\pm$ 2
	Partly Cloudy	6	34 $\pm$ 4
	Clear	15	62 $\pm$ 15
14L:10D	NA ¹	16	61 $\pm$ 5

¹Sky conditions assumed to have negligible effect on light intensity.

Appendix Table II. Mean values ($\pm$ SE) and sample sizes by treatment group for body weight in young male and female pine voles 51-60, 61-70 and 71-80 days of age and subjected to different nutritional and photoperiod regimes.

Group	Sex	51-60		61-70		71-80	
		(g $\pm$ SE)	(n)	(g $\pm$ SE)	(n)	(g $\pm$ SE)	(n)
15L:9D							
Chow	M	23.1 $\pm$ 1.3	(6)	24.8 $\pm$ 1.4	(6)	25.3 $\pm$ 1.4	(6)
	F	20.6 $\pm$ 1.6	(6)	22.5 $\pm$ 1.4	(6)	23.4 $\pm$ 1.3	(6)
Apple + Chow	M	26.3 $\pm$ 1.2	(7)	27.6 $\pm$ 1.2	(7)	28.2 $\pm$ 1.3	(7)
	F	24.2 $\pm$ 1.2	(5)	25.5 $\pm$ 1.0	(5)	26.2 $\pm$ 1.0	(5)
9L:15D							
Chow	M	22.8 $\pm$ 1.1	(12)	24.5 $\pm$ 1.1	(9)	25.6 $\pm$ 1.4	(8)
	F	22.8 $\pm$ 1.1	(8)	22.9 $\pm$ 1.7	(6)	24.6 $\pm$ 1.5	(5)
Apple + Chow	M	23.8 $\pm$ 1.6	(9)	26.0 $\pm$ 1.8	(7)	27.2 $\pm$ 2.0	(7)
	F	26.2 $\pm$ 1.9	(6)	27.8 $\pm$ 2.4	(5)	31.5 $\pm$ 0.8	(4)

APPENDIX AEffect Of Photoperiod and Nutrition
On Reproduction In Pine VolesIntroduction

This experiment was designed as a follow-up to the experiment in Chapter 2. The objective was to determine the effects of photoperiod and nutrition on reproduction in reproductively active pine voles under highly controlled environmental conditions. The experiment was not completed as planned and the data presented here are of a preliminary nature.

Methods and Materials

Voles used in this experiment were adults either wild-caught or first generation offspring of wild-caught voles. The animals were paired and placed in hanging wire cages. Pairs were given ad libitum amounts of water and a commercial rat chow. Burlap was provided as nesting material. The voles were maintained on a 15L:9D photoperiod.

Pairs which produced at least 1 litter in the 3 months prior to the beginning of the experiment were placed at

random into 1 of 4 treatment groups. Two groups were put on a 15L:9D photoperiod and the other 2 groups were put on a 9L:15D light regime. Within each photoperiod 1 group was fed ad libitum amounts of a commercial horse chow and the other group fed ad libitum amounts of both chow and apple. The chow, which contained 59.4% digestible energy (Servello 1981), was ground in a Wiley mill. All pairs were given water ad libitum.

The experiment was conducted in 2 environmental chambers in the Dept. of Fisheries and Wildlife Sciences at VPI&SU. Pairs were placed at random on the top 3 rows of a hanging wire cage rack in each chamber. The experiment began February 3, 1981, and was designed to run 8 weeks. However, on February 26 the chamber with the 9L:15D photoperiod overheated (due to a malfunction) to 47° C in an hour. Most of the voles in this chamber died due to the heat. Voles which survived the heat stress and those in the 15L:9D chamber were sacrificed on March 2. Therefore, the experiment lasted only 4 weeks.

Body weight was recorded at both the beginning and end of the experiment. Pairs were checked every 4 days for birth of litters. Size of any litters and approximate date of birth were recorded for each litter. Necropsy of the voles was described in Chapter 1.

Results and Discussion

Body Weight

Females had a significantly ($P < 0.001$) greater change in body weight than males (Appendix Tables III and IV).

Females had a mean weight loss of 4-5g in 3 of the 4 groups (Appendix Table III). Females on the 9L:15D and fed apple and chow were either pregnant at the beginning or end of the experiment. Therefore, no change in body weight could be calculated for this group and negated any sex X photoperiod X diet interaction.

Mean final body weight was determined for males, nonpregnant females and pregnant females (Appendix Table V). Males and nonpregnant females on a chow diet were significantly ($P < 0.01$) heavier than those fed apple and chow (Appendix Table IV). There was a significant photoperiod X diet interaction ($P < 0.003$) and a sex X photoperiod X diet interaction ($P < 0.002$). Males and nonpregnant females on the 15L:9D regime were heavier in chow groups than in the apple and chow groups. On the 9L:15D photoperiod females were heavier on the apple and chow diet while males on the chow diet weighed more than males given apple and chow.

Pregnant females were significantly ($P < 0.001$) heavier than nonpregnant females (Appendix Table IV). There were also significant interactions for photoperiod X diet

Appendix Table III. Mean values ($\pm$ SE) and sample sizes by treatment group for change in body weight in adult male and nonpregnant female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

	Male		Female	
	(g $\pm$ SE)	(n)	(g $\pm$ SE)	(n)
15L:9D				
Chow	-0.3 $\pm$ 0.8	(7)	-4.8 $\pm$ 5.4	(2)
Apple + Chow	0.7 $\pm$ 0.6	(3)	-4.4 $\pm$ 2.7	(3)
9L:15D				
Chow	2.3 $\pm$ 0.4	(6)	-4.1 $\pm$ 1.0	(4)
Apple + Chow	0.5 $\pm$ 1.0	(7)	0.0 ¹	

¹Females were pregnant at either beginning or end of the experiment.

Appendix Table IV. Mean values ($\pm$ SE) and sample sizes by treatment group for final body weight in adult male and female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Group	Male		Nonpregnant Female		Pregnant Female	
	(g $\pm$ SE)	(n)	(g $\pm$ SE)	(n)	(g $\pm$ SE)	(n)
15L:9D						
Chow	26.6 $\pm$ 1.3	(7)	30.8 $\pm$ 1.8	(3)	30.6 $\pm$ 1.9	(4)
Apple + Chow	23.4 $\pm$ 1.0	(3)	21.2 $\pm$ 1.3	(3)	29.4	(1)
9L:15D						
Chow	27.4 $\pm$ 0.3	(6)	26.1 $\pm$ 1.1	(5)	32.1 $\pm$ 3.0	(3)
Apple + Chow	25.2 $\pm$ 1.0	(7)	29.7 $\pm$ 1.8	(3)	33.8 $\pm$ 1.2	(5)

Appendix Table V. Mean squares (MS) for change in body weights and final body weights in adult male and female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Source	df		Change in body weights ¹	Male and Nonpregnant Female body weights	Pregnant and Nonpregnant Female body weights
State (S)	1	MS	179.26	6.00	188.25
		P<	.001*	.35	.001*
Photoperiod (P)	1	MS	4.59	7.95	28.11
		P<	.44	.29	.12
Diet (D)	1	MS	1.65	51.65	4.65
		P<	.64	.01*	.51
S X P	1	MS	0.38	1.51	2.79
		P<	.82	.64	.61
S X D	1	MS	1.07	0.20	14.28
		P<	.71	.86	.26
P X D	1	MS	10.51	70.95	115.55
		P<	.24	.003*	.004*
S X P X D	1	MS	0.00 ²	76.24	35.43
		P<	.99	.002*	.08*
Error	29	MS	7.33 ³	6.77	10.56 ⁴

¹Change in body weights tested for males and nonpregnant females.

²Degrees of freedom for S X P X D term was 0.

³Degrees of freedom for error term was 25.

⁴Degrees of freedom for error term was 19.
(P<0.004) and sex X photoperiod X diet (P<0.08).

Reproductive Characteristics

Seminal vesicles weight was significantly (Appendix Tables VI and VII) higher for males on the 15L:9D photoperiod ($P<0.001$) and for those fed only chow ($P<0.03$).

There was a significant photoperiod X diet interaction for uterine weight ($P<0.05$) and paired ovarian weight ($P<0.07$) in nonpregnant females (Appendix Tables VIII and IX). Females on the 9L:15D photoperiod with apple and chow diet and those on the 15L:9D regime with chow diet had the highest weights.

Pregnant females on the 9L:15D photoperiod had significantly ($P<0.008$) heavier adrenal glands (Appendix Tables X and XI).

Results from this experiment were complicated by several factors. Perhaps the most important factor was that the experiment ran only 4 weeks. This may have not been enough time for the treatments to exert an influence on body weight or reproduction. Final body weight and reproductive organ weights in nonpregnant females were not conclusive because some of these females had given birth to litters during the experiment.

Appendix Table VI. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Group	Adrenal glands weight	Seminal vesicles weight	Paired testes weight
	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)	(mg $\pm$ SE) (n)
15L:9D			
Chow	9.5 $\pm$ 1.1 (7)	56.2 $\pm$ 7.2 (7)	85.1 $\pm$ 1.6 (7)
Apple + Chow	10.4 $\pm$ 0.8 (3)	32.6 $\pm$ 9.2 (3)	91.3 $\pm$ 10.4 (3)
9L:15D			
Chow	9.4 $\pm$ 0.8 (6)	26.4 $\pm$ 4.7 (6)	82.8 $\pm$ 4.4 (6)
Apple + Chow	10.4 $\pm$ 1.0 (8)	18.4 $\pm$ 2.9 (8)	73.0 $\pm$ 8.5 (8)

Appendix Table VII. Mean squares for reproductive characteristics in adult male pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Source	df		Adrenal glands weights	Seminal vesicle weights	Paired testes weights
Photoperiod(P)	1	MS	0.02	22.87	2.06
		F<	.89	.001*	.17
Diet(D)	1	MS	0.74	5.67	0.29
		F<	.40	.03*	.60
P X D	1	MS	0.00	1.66	1.24
		F<	.99	.21	.28
Error	20	MS	6.18	189.64	270.62

Appendix Table VIII. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in nonpregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Group	Adrenal glands weight		Uterine weight		Paired ovarian weight	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)
15L:9D						
Chow	12.7 $\pm$ 3.2	(3)	45.0 $\pm$ 12.8	(3)	3.9 $\pm$ 0.3	(3)
Apple + Chow	8.1 $\pm$ 1.5	(3)	33.1 $\pm$ 2.0	(3)	2.5 $\pm$ 0.6	(3)
9L:15D						
Chow	10.8 $\pm$ 1.2	(3)	39.2 $\pm$ 2.5	(3)	3.3 $\pm$ 0.2	(3)
Apple + Chow	10.5 $\pm$ 1.8	(3)	61.1 $\pm$ 11.1	(3)	4.4 $\pm$ 1.2	(3)

Appendix Table IX. Mean squares (MS) for reproductive characteristics in nonpregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Source	df		Adrenal glands weights	Uterine weights	Paired ovarian weights
Photoperiod(P)	1	MS	0.02	1.30	0.65
		P<	.90	.28	.44
Diet(D)	1	MS	1.30	0.84	0.01
		P<	.28	.38	.94
P X D	1	MS	1.22	5.11	4.21
		P<	.30	.05*	.07*
Error	10	MS	12.30	186.27	1.24

Appendix Table X. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in pregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Group	Adrenal glands weight		Paired ovarian weight	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)
15L:9D				
Chow	9.7 $\pm$ 0.5	(4)	8.1 $\pm$ 2.3	(4)
Apple + Chow	8.6	(1)	3.0	(1)
9L:15D				
Chow	11.8 $\pm$ 0.9	(3)	6.2 $\pm$ 0.4	(3)
Apple + Chow	11.3 $\pm$ 0.3	(5)	9.0 $\pm$ 1.9	(5)

Appendix Table XI. Mean squares (MS) for reproductive characteristics in pregnant adult female pine voles subjected to different nutritional and photoperiod regimes in chamber experiment.

Source	df		Adrenal glands weights	Paired ovarian weights
Photoperiod(P)	1	MS	11.71	0.16
		P<	.008*	.70
Diet(D)	1	MS	1.35	0.03
		P<	.27	.87
P X D	1	MS	0.18	2.24
		P<	.68	.17
Error	9	MS	1.04	15.13

APPENDIX BEffect Of Apple Drops On Reproduction
In Enclosed Populations Of Pine VolesIntroduction

The objective of this experiment was to test the effect of a nutritional supplement, apple drops, on reproduction in enclosed populations of pine voles in an orchard environment.

Methods and Materials

The experiment was conducted at the VPI & SU Horticulture Farm. Galvanized steel sheet metal was placed around 4 apple trees such that half (.5m) was below and half was above the surface of the soil. The enclosures measured 5.5 X 5.5m in area.

Voles for the experiment were trapped from the Horticulture Farm and from orchards in Botetourt Co., Virginia. Two males and 4 females were placed in each enclosure. The voles were toe clipped for identification before being placed in an enclosure. Apples were removed from 2 of the enclosures and excess apples were dumped in the remaining enclosures.

The experiment was conducted from November 6 until December 31, 1980. The enclosures were live-trapped for 3 days every 10 days. There were 3 live trap periods followed by a final snap trap period. Body weight, body length and reproductive condition were recorded for each vole at first capture during a trap day. Necropsy for snap-trapped voles was described in Chapter 1.

Results and Discussion

Mean body weight at first capture for each trapping period are shown in Appendix Table XII. Mean values for selected reproductive characteristics were determined for both males and females (Appendix Table XIII). No conclusions could be drawn from these data due to the small samples and large degree of variation.

This experiment was beset with problems from the start. Voles came from 4-5 different orchards and were placed in the enclosures over a 2 month period of time. Therefore, some animals may have become dominant, causing reduced vigor or death of voles placed in the enclosures at a later date. Some voles became trap happy and others were not captured every trapping period. This created a problem of already small samples being reduced even further. The last trapping period, using snap traps, was planned to last 4-5 days. After 4 days very few voles had been trapped and it was

Appendix Table XII. Mean values ($\pm$ SE) and sample sizes by trapping period for body weight and body length in adult male and female pine voles in enclosures having apples present or apples removed during late autumn, 1980.

Group	<u>Male</u>				<u>Female</u>			
	Body weight		Body length		Body weight		Body length	
	(g $\pm$ SE)	(n)	(mm $\pm$ SE)	(n)	(g $\pm$ SE)	(n)	(mm $\pm$ SE)	(n)
Nov. 6-8								
Apple Removed	24 $\pm$ 3	(3)	103 $\pm$ 4	(3)	25 $\pm$ 7	(5)	101 $\pm$ 1	(5)
Apple Present	29	(1)	108 $\pm$ 2	(2)	25 $\pm$ 3	(3)	98 $\pm$ 3	(5)
Nov. 21-25								
Apple Removed	24 $\pm$ 3	(3)	105 $\pm$ 4	(3)	22 $\pm$ 1	(3)	100 $\pm$ 4	(3)
Apple Present	26 $\pm$ 0	(2)	106 $\pm$ 1	(2)	24 $\pm$ 3	(3)	101 $\pm$ 2	(3)
Dec. 5-8								
Apple Removed	23 $\pm$ 2	(4)	102 $\pm$ 4	(4)	23 $\pm$ 3	(2)	102 $\pm$ 1	(2)
Apple Present	26 $\pm$ 0	(2)	110 $\pm$ 0	(2)	24 $\pm$ 3	(3)	103 $\pm$ 3	(3)
Dec. 18-31								
Apple Removed	28 $\pm$ 1	(2)	109 $\pm$ 4	(2)	25 $\pm$ 1	(2)	105 $\pm$ 2	(2)
Apple Present	28 $\pm$ 2	(2)	105 $\pm$ 4	(2)	24 $\pm$ 3	(4)	102 $\pm$ 5	(4)

Appendix Table XIII. Mean values ($\pm$ SE) and sample sizes by treatment group for selected characteristics in adult male and female pine voles in enclosures having apples present or apples removed during late autumn, 1980.

Group	Adrenal glands weight		Seminal vesicles weight		<u>Male</u> Paired testes weight		<u>Spermatozoa</u> sperm/mg testes wt X	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	10 ³ $\pm$ SE	(n)
Apple Removed	9.9 $\pm$ 0.8	(2)	6.5	(1)	26.0	(1)	13.8	(1)
Apple Present	8.9 $\pm$ 0.6	(2)	7.5 $\pm$ 0.8	(2)	35.5 $\pm$ 15.5	(2)	24.4	(1)

Group	Adrenal glands weight		<u>Female</u> Uterine weight		Paired ovarian weight	
	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)	(mg $\pm$ SE)	(n)
Apple Removed	7.4 $\pm$ 0.6	(2)	10.7 $\pm$ 3.2	(2)	1.2 $\pm$ 0.1	(2)
Apple Present	7.8 $\pm$ 1.1	(4)	21.6 $\pm$ 8.4	(4)	2.4 $\pm$ 0.8	(4)

obvious voles still lived in the enclosures. Some traps were either snapped without a capture or soil had been pushed into the entrance of the runway. These remaining voles were snap-trapped the last week in December.

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EFFECT OF PHOTOPERIOD AND NUTRITION ON REPRODUCTION,
GROWTH, PELAGE CHANGES AND AGE OF PUBERTY IN THE
PINE VOLE

by

James J. Hashbrouck

(ABSTRACT)

Apple drops were removed from a portion of an apple orchard in September and apples left available in the remainder of the orchard. Presence of apples had little effect on the immature:adult ratio or on body weight. However, adult males and juveniles of both sexes had higher values for reproductive characteristics in November and December in the area with apples available.

In a laboratory experiment adult voles fed a rabbit chow diet supplemented with apple consumed less digestible energy but had higher body weights and more body fat, and males had heavier testes than those fed only chow. Adults on a 14L:10D photoperiod had higher values for reproductive characteristics than those on a declining photoperiod.

In a third experiment juveniles fed a horse chow diet supplemented with apples had higher body weights and more body fat, initiated and completed molt to adult pelage at

higher body weights and had heavier reproductive organs than those given only chow.

In these experiments voles with access to apples did not consume more digestible energy but they grew faster, were in better condition and were more likely to produce offspring. The consumption of apples possibly stimulated the hypothalamus and/or pituitary gland, increasing synthesis or release of gonadotrophins. Adult pine voles on a declining or short photoperiod may be inhibited reproductively because of antigonadotrophic substances produced by the pineal gland. Consuming apples could affect the degree of inhibition. It was hypothesized pine voles use both photoperiod and nutrition as cues for reproduction.