March 1981

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Photoperiod and nutrition are important variables affecting reproductive activity and growth in many rodents. Field and laboratory studies indicate that long photoperiod (spring-summer) cause increased growth while short photoperiods (fall-winter) inhibit these processes. In the montane vole (*Microtus montanus*) recently weaned animals gain weight at a much lower rate under short photoperiods or in total darkness than under long photoperiods (Vaughan et al., 1973; Peterborg, 1978). Adult *M. montanus* had more offspring and larger mean litter sizes under LD 18:6 than LD 6:18 (Pinter & Negus, 1965). Similarly, long (LD 16:8) or increasing photoperiods stimulated the onset of puberty in *M. arvalis*, while short (LD7:17) or decreasing photoperiods inhibited the onset of puberty (Lecyk, 1962). Short photoperiods caused reduced spermatogenesis and seminal vesicle weights in male *M. arvalis*, while long photoperiods induced increased ovulation in females. In contrast, photoperiod had no effect on the reproductive rates of *M. orchadensis* (Marshall & Wilkinson, 1956). *Dicrostonyx groenlandicus* reared on LD 6:18 grew faster than those on LD 20:4, but the latter group had larger testes (Hasler, 1975). In *M. agrestis*, long photoperiods stimulated male reproduction and caused greater body weight gain than did short photoperiod. Females produced fewer young, had lower ovarian and uterine weights, and fewer, smaller Graafian folicles under short photoperiods as compared to long. However, there was no effect on female body size (Clark & Kennedy, 1967; Breed & Clarke, 1970; Baker and Ranson, 1932). *Microtus pennsylvanicus* juveniles and adults had higher body weights under LD 18:6 than LD 6:18 (Pistole, 1980). *M. oregoni* reproductive activity is stimulated by long photoperiod, but due to fossorial habits this species appears to be less sensitive to light than more terrestrial forms (Cowan & Arsenault, 1958). There have been no reports of winter breeding in *Clethrionomys gapperi* possibly because of their behavioral avoidance of light during the winter (Evernden & Fuller, 1972).

Field reports of reproduction in pine voles vary greatly. Several studies report breeding all year (Rhoades, 1903; Linsdale 1928; Glass, 1949; Noffsinger, 1967; Paul, 1970; Goertz, 1971; Cengel et al., 1978) while others report the occurrence of breeding from February or March through November (Hamilton, 1938; Benton, 1955; Miller and Getz, 1969; Cengel et al., 1978). Noffsinger (1976) found significantly reduced body weights in males and females from January to May and significant differences in body length due to month. Molt to adult pelage began at 3 weeks of age and field data has shown that molting individuals can be found during any season of the year (Benton, 1955).

There are few laboratory studies characterizing growth, development, and reproductive activity in young and adult M. pinetorum. Hamilton (1938) found an increase of approximately 6g/wk in juvenile body weights whereas Paul (1970) reports an increase of 2-3g/wk. Growth rates of individuals from litters of 1 under LD 14:10 were only slightly less than those for individuals from litters of 2 or 3, with weight gain showing a sharp increase just prior to weaning (days 19-21) (Lockmiller, 1979).

Compounds present in plants which have antigonadotrophic effects may inhibit reproduction in many rodents. Two such compounds, Paracoumaric acid (PCA) and Ferulic acid (FA), are found at low concentrations in young plants but increase as plants reach senescence. These compounds caused significant reductions in uterine weight in M. montanus at a dose of 4 mg/g of chow when administered for 12 days (Berger et al., 1977) and in M. pennsylvanicus significant differences in uterine weights and mean number of follicles per female occurred with 12 mg FA/g of chow over a 21 day test period (Cranford et al., 1980; Pistole, 1980). There was no effect of FA at any reasonable dosage on uterine weights of juvenile M. pennsylvanicus.

Animals often use environmental cues for the initiation and termination of reproduction, such that production of offspring occurs during optimal seasons (Reynolds and Turkowski, 1972; Petterborg, 1978). Due to the fossorial nature of M. pinetorum this species may be less sensitive to photoperiodic cues than more terrestrial rodents. Since most evidence indicates year round breeding it is improbable that pine voles respond to green or dead vegetation as a reproductive cue.

This study investigated the effects of 3 photoperiodic regimes on growth and maturation in juvenile and adult M. pinetorum, while the effect of litter size on growth of juvenile pine voles was determined under two photoperiodic regimes. The role of the inhibitor compound FA on animals under different photoperiodic regimes was determined for juvenile M. pinetorum and adult and juvenile M. pennsylvanicus.

MATERIALS AND METHODS

To determine the effect of litter size and photoperiod on body weights of pine voles from birth to 50 days of age, five litters each, of size 4,3,2, and 1 young were maintained under LD 16:8, four litters of 2 young and five litters of 3 were maintained under LD 8:16. Individuals were weighed every 3 days using a Ohaus triple beam balance (± 0.01 g) and age of molt noted.

The effects of photoperiod on body weights of young adults (7 wks. of age) were determined using 43 animals (27♂, 19♀)

maintained under LD 16:8, and 28 animals (13♂, 15♀) under LD 12:12. At 11 weeks of age one third of the LD 16:8 group was switched to LD 12:12 and the other one-third to LD 8:16. One half of the LD 12:12 group was switched to LD 16:8. All animals were then weighed weekly on an electronic balance ($\pm$ 0.1g) for a total of 14 weeks. To determine the effect of photoperiod on reproductive development of juvenile M. pinetorum, groups of 4 to 7 individuals were raised under LD 8:16, LD 12:12, or LD 16:8 until 5 weeks of age. At this time all individuals were sacrificed and body, adrenal, uterine, and ovarian weight were determined for females, while body, adrenal, seminal vesicle, and testes weights were recorded for males.

All experimental pine voles were first or second generation offspring of wild-caught adults, and were housed as pairs after weaning. Water and food (Wayne lab blox) supplemented with sunflower seeds, apples, and oat sprouts were available ad libitum.

The inhibitor substance FA, obtained from Aldrich Chem. Corp., was used to determine the effects of plant inhibitory compounds on reproduction in juvenile M. pinetorum and adult and juvenile M. pennsylvanicus. The bioassays for effects on sexual maturation were wet uterine weights and testes weights. Additionally, body and adrenal weights were assayed as an indicator of the general condition of the test individuals. Previous tests have shown that total food consumption does not differ between control and experimental animals.

M. pennsylvanicus juveniles were selected randomly from a colony of lab animals at 16-21g and approximately 20 days of age, while adults were selected at 47-55g. All individuals were caged individually for the 21 day test periods, with food and water available ad libitum. The test chemical was dissolved in methanol, coated on to ground lab blox at a dose of 12 or 24 mg FA/g chow, and the chow air dryed for 24 hours. Control chow was treated in the same manner but without the test chemical. Tests were performed under LD 18:6, LD 16:8, and LD 12:12.

M. pinetorum juveniles were tested as described above but were randomly selected at 22 days of age (weaning). Due to their slower maturation rate, tests were run for 30 days rather than 21 days, under LD 16:8 and LD 12:12.

RESULTS

Among litters of 1,2,3 and 4 young in LD 16:8 there was a consistent trend for small litters to weigh more than large ones (1>2>3>4). Litters of 2 were significantly heavier than those of 4 (2 sample Z test, $p \leq 0.05$) at 11 and 17-23 days of age while litters of 3 and 4 were not statistically different. Under LD 12:12 litters of 2 were significantly heavier ($p \leq 0.05$) than those of 4 at 14 and 17 days of age. Though litters of 3 were consistently lighter than those of 2 under LD 8:16 no significant differences occurred. Growth rates of individuals from litters of

4 (0.45g/individual/day) were lower than those for individuals from litters of 1,2, or 3 (0.52g/individual/day). Under LD 16:8 this difference occurred from days 2-20 and under LD 12:12 from day 2-14.

Comparisons between litters of the same size but raised under different photoperiods show that photoperiod affects body weights and reproductive development of young animals. The average weight of pine voles just prior to weaning (day 20) were 13.77 $\pm$ 1.7 gms under LD 16:8, 14.72 $\pm$ 1.89 under LD 12:12, and 14.70 $\pm$ 2.04 under LD 8:16. Additionally in LD 8:16 animals from litters of 3 were significantly heavier (2 sample Z test, $p \leq 0.05$) than those raised under LD 16:8. Litters of 2 under LD 8:16 exhibited higher growth rates than those under LD 16:8 or LD 12:12 from day 29 to 44 postpartum. Other data show that under all 3 photoperiodic regimes juvenile to subadult molt began about 28 days postpartum.

The increased growth of M. pinetorum raised under LD 8:16 as compared to that of juveniles raised under LD 12:12 and LD 16:8 is correlated with increased reproductive development in females but not in males. Juvenile females at 5 weeks of age who were raised under LD 8:16 and LD 12:12 had significantly heavier uterine tracts (Wilcoxon rank sum test, $p \leq 0.015$) than those raised under LD 16:8. Juvenile females maintained under LD 12:12 had greater ovarian and adrenal weights ($p \leq 0.02$) than individuals tested under LD 8:16. Among juvenile males there were no significant differences in testes, seminal vesicle or adrenal weights for any photoperiod group. Photoperiod effects were principally limited to reproductive organs as neither males or females differed in body weight at 5 weeks of age.

Body weights of adult animals switched between photoperiodic regimes showed changes similar to juveniles. Switching from LD 12:12 to LD 16:8 (spring summer) or vice versa (summer fall) caused no change in body weights. However a switch from LD 16:8 to LD 8:16 (summer to winter) resulted in a sharp increase in body weights, but the sample is highly variable.

All experimental M. pennsylvanicus fed FA showed a decrease in reproductive organ weight though this effect was not significant due to high variances within experimental groups. No consistent trends occurred with respect to change in adrenal weights. Experimental and control adults maintained on LD 12:12 lost weight (6-8g) whereas those on LD 16:8 gained weight (2g) (significant effect of photoperiod, Wilcoxon rank sum test, $p \leq 0.005$); but there was no synergistic effect between the inhibitor substance and short photoperiod on reproductive organ weights. Juveniles showed no effect of photoperiod on body weight.

Male juvenile M. pinetorum showed a reduction in testes weight when fed FA under LD 16:8 and LD 12:12 while females showed a decrease in wet uterine weights under LD 16:8 but not LD 12:12. Body weights of pine voles under LD 12:12 did not differ from those tested under LD 16:8 at the beginning or end of experimentation.

DISCUSSION

Photoperiod has the opposite effect on growth in M. pinetorum as compared to M. pennsylvanicus where juveniles have higher body weights under long photoperiod (Pistole, 1980), and M. pinetorum juveniles have lower body weights. Higher growth rates under short photoperiods may be of adaptive value for M. pinetorum since they result in earlier reproductive development of females and probably earlier reproduction. Additional studies are being conducted to determine whether increases in body weight are correlated with increased reproductive development in subadult and adult M. pinetorum (i.e. 10, 15 and 20 weeks of age).

Average weight gains for M. pinetorum in this study were slightly higher than those reported by Paul (1970) and Lockmiller (1979) but much lower than those reported by Hamilton (1938). Though weight gains were similar for young from litters of 1, 2 and 3, young from litters of 4 had significantly lower weight gain rates before and after weaning which probably results from the increased energy drain on the adult lactating female (Hasler & Banks, 1975; Lockmiller, 1979).

No significant effect of FA occurred in M. pinetorum as expected because they appear to be continuous breeders. The results for M. pennsylvanicus do not show the significant effects reported by Pistole (1980) though there are consistent trends which support his data. In M. pennsylvanicus, photoperiod is an ultimate factor whereas plant compounds act as proximate factors affecting growth and reproduction. The variance among the response of individuals fed FA suggests that a population may be composed of responsive and nonresponsive individuals, indicating a genetic component. In M. pinetorum photoperiod may be a proximate factor whereas plant compounds are of no importance in cueing reproduction. The different responses of these 2 species may be due to differences in their habitats, as M. pennsylvanicus are more exposed to light (surface dweller) and its changes, than are M. pinetorum. Because photoperiod and plant compounds appear to play a minor role in cueing pine voles to environmental conditions, other factors such as nutrition may be of major importance for this species.

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This research was supported by a Dept. of Interior Fish and Wildlife Service Grant No. 3729491 to J. A. Cranford.