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Title: POPULATION DYNAMICS OF MICROTUS CANICAUDUS

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Four experimental groups were designed to examine the effects of a "greens" dietary supplement (wheat clippings), as well as individual versus group-housing, on the adrenal and reproductive physiology of Microtus canicaudus. Organ culture of adrenal glands, thin-layer chromatography, and fluorescence analysis of steroid-containing medium were used to quantitatively assess adrenal function. Gravimetric data were collected for adrenal glands, testes, and reproductive tracts, as additional indices of overall reproductive condition.

Males produced significantly more steroid than females for all experimental groups, probably indicative of their more aggressive nature. Group-housed males secreted more steroid than individually-housed males, in both greens and no-greens groups. This relationship held for measurements of total steroid produced, as well as for individual estimates of both corticosterone plus cortisol and adrenal progesterone. Housing did not appear to affect total steroid output in females, possibly due again to their less aggressive nature. Surprisingly, group-housed females secreted significantly less progesterone

than individually-housed females. Furthermore, fresh greens did not appear to affect adrenal secretion in males or females.

Analysis of adrenal weights indicated a significant sexual dimorphism with female adrenal glands being heavier than male glands. Adrenal weight was not greatly influenced by either diet or housing conditions.

With one exception, the supplementation of wheat clippings to a basal diet did not affect either testes or reproductive tract weights. Greens-fed females did, however, exhibit a greater percentage of individuals in an estrous condition than controls irrespective of housing conditions.

Breeding experiments with M. canicaudus demonstrated an increasing mean litter size from the first through the fifth litters, followed by a slight decrease in size of subsequent litters. Additionally, the gestation period in this species was determined to be 21 days with a mean litter size of 5.10.

Results of "greens" and "density-stress" experiments support the following tentative conclusions: first, the reproductive physiology of M. canicaudus does not appear to be as sensitive to stimulation by green vegetation as reported for similar species; secondly, male M. canicaudus are more sensitive to density-imposed stress than are females; thirdly, females appeared to be more "stressed" by individual-caging than by group-caging. Thus, altered endocrine balance may play a role in regulating high density populations of this species.

Population Dynamics of *Microtus canicaudus*

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POPULATION DYNAMICS OF *MICROTUS CANICAUDUS*

I. INTRODUCTION

Three to five year cyclic density changes in vole and lemming populations have been the subject of intensive research since Charles Elton's initial study of the phenomenon in 1924 (Elton, 1924). Prior to Elton's work, little was known about population changes in such animals. Most investigators believed that populations were stable in size and that any outbreaks in numbers of a species were due to man's interference with the "balance of nature" (Egerton, 1968). Elton's survey of the literature on the Norwegian lemming (*Lemmus lemmus*), and his examination of fur-trade statistics compiled by the Hudson Bay Company, led him to the conclusion that vole and lemming populations, as well as their predators, cycled with a period of from 3 to 4 years. In the late 1920's, he was able to substantiate his hypothesis using mark-recapture trapping techniques.

Since Elton's study, researchers have attempted to describe rodent population cycles in much greater detail, and to investigate possible regulatory mechanisms involved in these cycles. Reproduction, mortality, migration, nutrition, predation, and weather, as well as behavioral, physiological, and genetic factors have been considered as playing roles in these periodic fluctuations. Despite much progress in understanding these cycles, no single explanation has been found to account for the phenomenon.

Generalized Features of Microtine Cycles

Despite controversy among researchers concerning an explanation for the cycles, Krebs et al. (1973) point out some general facts that have emerged from various investigations. First, periodic fluctuations in numbers have been observed in a variety of different species and genera of microtine rodents. Secondly, the period of a cycle is variable, typically being 3 to 4 years between peak densities, although 2 and 5 year cycles have been noted (Krebs et al., 1973). Thirdly, the species that have been investigated and found to fluctuate are extremely widespread geographically and are present in a variety of ecological communities. These include lemmings in the arctic tundras of North America and Europe, meadow voles in New York, red-backed voles in the boreal forests of North America, and field voles in Indiana, California, and Great Britain (Elton, 1942; Chitty and Chitty, 1952; Fuller, 1969; Krebs et al., 1969; Andrews et al., 1975). Fourthly, the general pattern of an isolated cycle is relatively constant between various species and genera of microtines.

Chitty and Chitty (1962) have divided a generalized population cycle into three parts for descriptive purposes: an increase phase consisting of a large increase in numbers from the first spring through the first winter; a peak phase with little change in numbers extending from the first winter through the fall of the second year; and a decline phase which is often variable in length but inevitably results in a reduction in total number of animals.

The increase phase begins in the late spring or early summer in the first year of a generalized 3-year cycle (Chitty and Chitty, 1962). Population density at this time would be at a minimum level. Working with Microtus pennsylvanicus in southern Indiana, Krebs et al. (1971) found that the resident population increased from a low of approximately one animal per acre in the summer of 1967 to a peak density of approximately 100 animals per acre. Krebs (1964), studying the lemming cycle at Baker Lake in northeast Canada from 1959 through 1962, estimated a 28-fold increase in density from the summer of 1959 through the same period in 1960. Similarly, Andrews et al. (1975), investigating the cycle of brown lemmings (Lemmus trimucronatus) at Point Barrow, Alsaka from 1969 to 1972, found densities of zero to one animal per acre in the summer of 1970 compared with 60 to 80 animals per acre in the summer of 1971, a peak year.

The peak phase is typically characterized as a period of maximum density with little change in numbers (Chitty and Chitty, 1962). There are, however, a number of species in which numbers fluctuate markedly during the peak phase (Krebs et al., 1973). This fluctuation is most often characterized by a spring decline in numbers after the winter breeding period, and is typically followed by a return to the original, peak density by fall. This spring decline during the peak phase has been observed in M. pennsylvanicus (Krebs et al., 1969), L. trimucronatus (Thompson, 1955a, cited in Krebs, 1964; Krebs, 1964; Andrews et al., 1975), and in M. agrestris (Chitty and Chitty, 1962).

Krebs et al. (1971) noted that certain species including M. californicus and M. ochrogaster do not exhibit well-defined peak phases. Instead, their cycles are characterized by a gradual increase in numbers throughout the year preceding maximum density, and the length of the peak phase itself is noticeably curtailed.

The decline phase appears to be the most variable in structure (Krebs et al., 1973). Chitty (1955, quoted in Krebs and Myers, 1974) described three general types of decline. Type M declines or "crash declines" are characterized by a dramatic decrease in the number of animals during the winter and spring following a peak year. Type G declines are characterized by a gradual drop in numbers throughout the winter, spring, and summer following the peak year. Type H declines are characterized by a gradual decrease over a 2-year period with a brief recovery during the breeding season. Chitty and Chitty (1962) presented evidence that M. agrestis exhibited all three types of decline throughout an extended period of study (1932-1960). Krebs and Myers (1974) reviewed the literature on population fluctuations in microtines and noted that all three types of decline were documented by various investigators in both voles and lemmings.

Demographic Changes

Fluctuations in population density are necessarily the result of changes in reproduction, mortality, or migration. Each of these

parameters will be considered with respect to their possible contribution to cyclic population phenomena.

Any investigation into the population dynamics of a species must evaluate the reproductive characteristics of that species. Krebs (1964) describes reproduction in polyestrous mammals as a complex variable consisting of six components: litter size, pregnancy rate, length of breeding season, age at sexual maturity, sex ratio, and population size (Figure 1). Although each of the six components contribute to the overall reproductive rate, the length of the breeding season and the age at onset of sexual maturity appear to be the most important factors in microtine cycles (Krebs and Myers, 1974). A number of investigators have emphasized the role of extended winter breeding and recruitment during the increase phase in both lemmings, L. trimucronatus (Krebs, 1964; Andrews et al., 1975) and voles, M. ochrogaster and M. pennsylvanicus (Krebs et al., 1969). Advances in the onset of sexual maturity during the increase phase have been observed in some species of voles and lemmings (Krebs et al., 1973). Krebs (1964) found the peak year to be characterized by a shortened breeding season in his Baker Lake lemming study. In contrast, Mullen (1968) found reproduction in high density years to be more successful than in other years.

Mortality rate changes are an important component of the dynamics of population cycles. Hoffman (1958) and Chitty (1952) suggested that high juvenile mortality rates during the peak and decline phase of

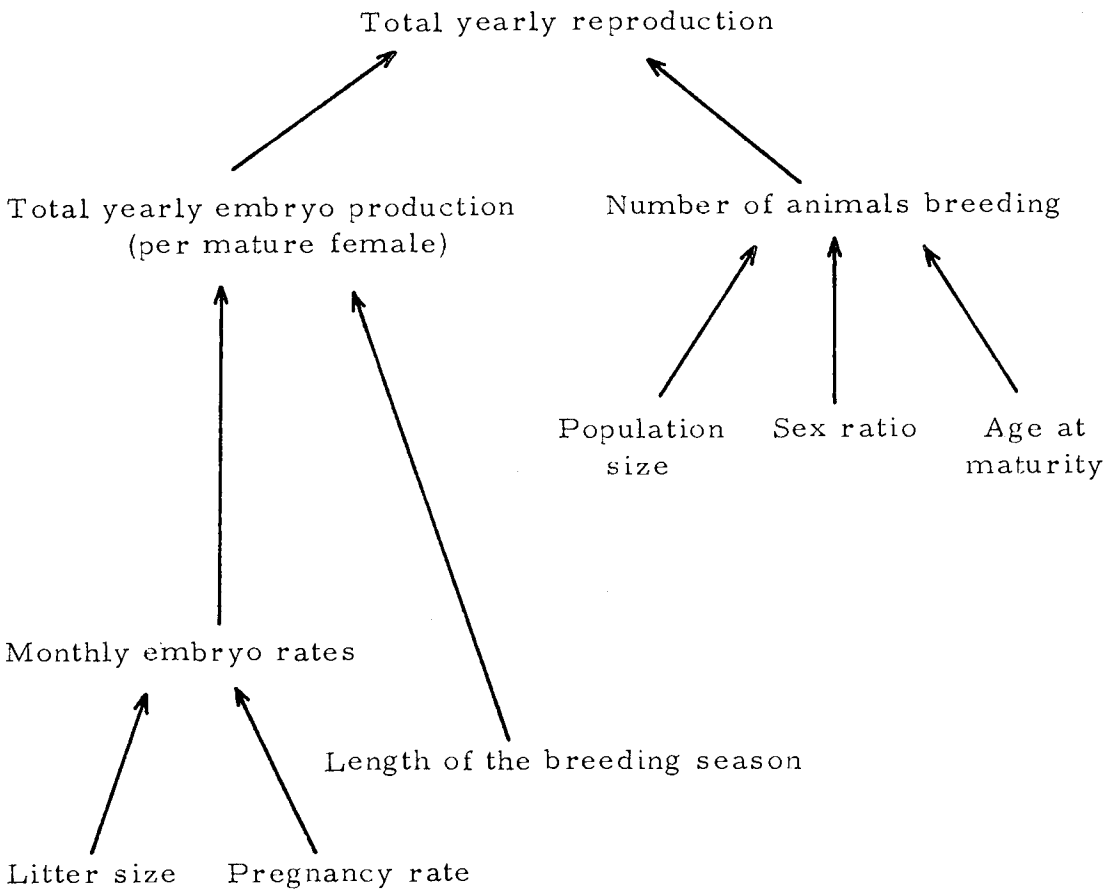

Figure 1. Components of reproduction in polyoestrous mammals (adapted from Krebs, 1968, p. 22).

M. montanus and M. agrestis, respectively, were directly associated with the population decline. However, Krebs (1964) found high juvenile mortality to be an important demographic feature only during the decline phase of L. trimucronatus. Chitty (1952), working with M. agrestis, suggested that adult mortality was low during the increase and peak phases, but high during the decline phase. Christian (1971) and Chitty and Phipps (1966) emphasized the role of intraspecific strife and mortality in relation to population density in M. pennsylvanicus and M. agrestis respectively.

Dispersal is the third component of population dynamics which could affect cyclic phenomena. Although early workers concentrated their investigative efforts on reproductive and mortality factors, evidence has been presented in the last 15 years, and particularly during the last 4 years, that dispersal may be an important component of cyclic density changes (Clarke, 1955; Louch, 1956; Krebs et al., 1969, 1971, 1973). Clarke (1955) showed that M. agrestis populations enclosed in large cement cages increased to numbers far in excess of those found in natural areas. Similar results were reported by Louch (1956) for M. pennsylvanicus and by Houlihan (1963) for M. californicus using laboratory enclosed populations. Krebs et al. (1969, 1973) presented the results of some interesting experiments performed with M. pennsylvanicus and M. ochrogaster in field situations. These investigators showed that like the laboratory populations, animals in enclosed plots reached abnormal numbers when compared to adjacent

unenclosed areas. Myers and Krebs (1971) measured dispersal rates of M. pennsylvanicus during a population cycle and found dispersion to be highest during the increase phase and lowest during the decline phase. They concluded that dispersal was a necessary component of population regulation in microtines. For a review of a behavioral-genetic hypothesis, and the implications of dispersal for this hypothesis, the reader is referred to Chitty (1967) and Krebs et al. (1973).

Hypotheses to Explain Microtine Cycles

Factors that could be involved in the regulation of population densities can be broadly classified as intrinsic or extrinsic factors. Extrinsic factors normally exert their effects from outside the population and include weather, disease, predation, and food. Intrinsic factors, in contrast, exert their effects from within the population and suggest behavioral or physiological changes within individual organisms. The following discussion will be limited to two currently popular hypotheses to explain microtine fluctuations: Pitelka's (1958) food supply hypothesis and Christian's (1950) behavioral-stress hypothesis (Figures 2 and 3). Kreb's et al. (1973) modified version of Chitty's (1967) behavioral-genetic hypothesis, mentioned above, also receives considerable support today. The extrinsic factors, predation and disease, have been largely discounted as control mechanisms in cycles (Krebs, 1964). However, Pitelka (1958) believes that predators may

by important in modifying the length and pattern of lemming cycles. Critical studies on the effects of disease on populations indicate local effects that do not occur with sufficient regularity to account for microtine cycles (Elton, 1942; Chitty, 1954). Similarly, severe weather conditions could affect animals in isolated populations, however, again would not account for regular fluctuations in numbers (Krebs, 1964).

Food Supply Hypothesis

Lack (1954) first suggested that the lemming cycle was due to extensive forage utilization by the lemmings, leading to destruction of both food and cover. Increased predator pressure due to the loss of protective vegetation, resulted in a decline of the population. Following this decline, the vegetation was allowed to recover and support an increase in the number of animals. Pitelka (1958) modified this hypothesis by suggesting that the depleted food supply led to malnutrition in the animals and a subsequent decline in the reproductive rate (Figure 2). Quantitative and/or qualitative changes in forage have been postulated to be limiting factors in the population dynamics of lemmings (Thompson, 1955b; Pitelka, 1957a, b) and voles (Bodenheimer, 1949; Kalela, 1962; Negus and Pinter, 1966; Batzli and Pitelka, 1971).

There is, however, considerable opposition to the food supply hypothesis. Rausch (1950) rejected malnutrition as a possible cause in the decline in numbers of lemmings in 1949 at Point Barrow, Alaska. Elton (1942) and Chitty (1952, 1960) presented reasons for rejecting the

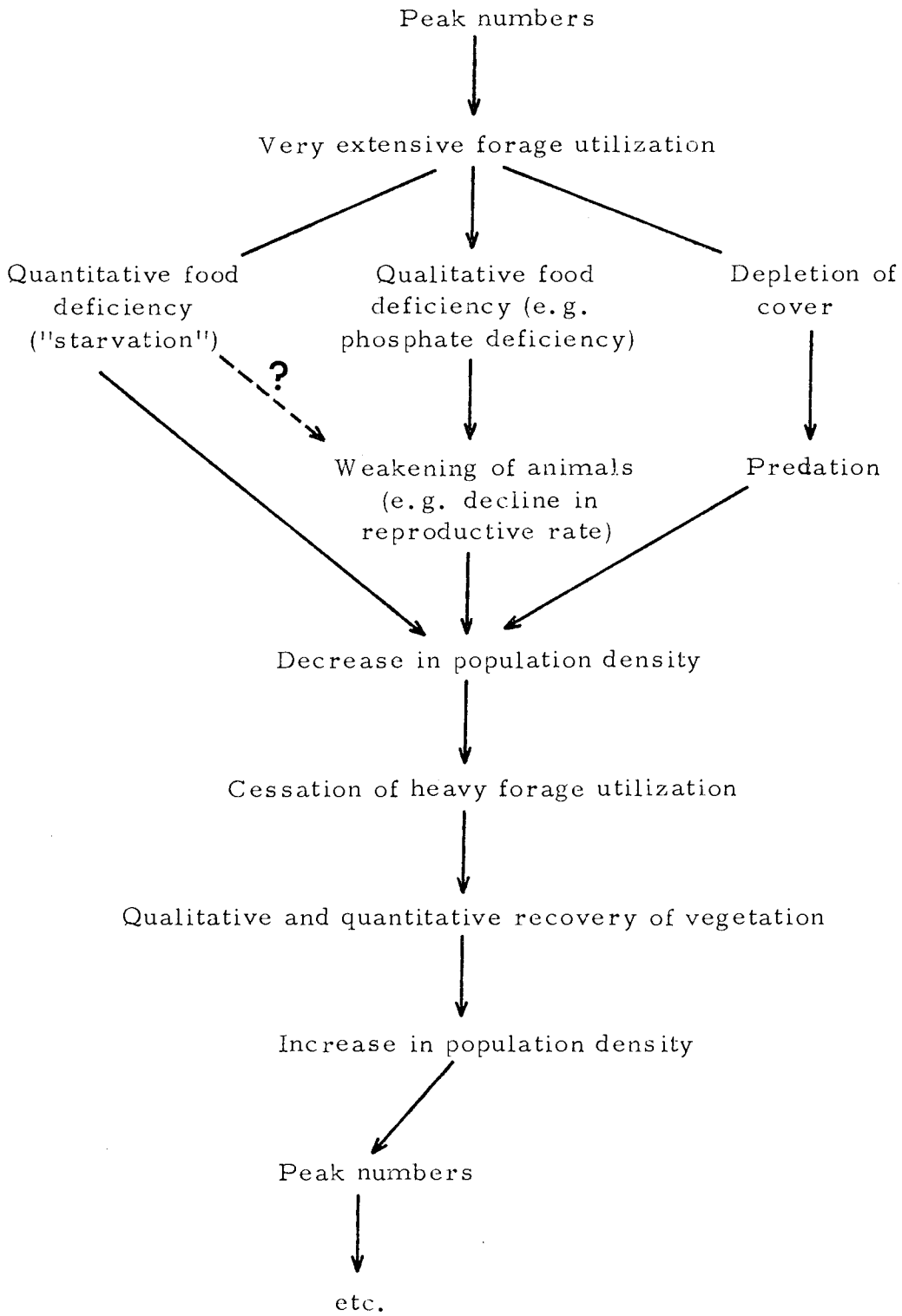

Figure 2. Pitelka's food supply hypothesis (adapted from Krebs, 1964, p. 62).

food supply hypothesis to explain cyclic fluctuations of M. agrestis. Likewise, Kalela (1957) postulated that food supply was not responsible for cycles in Clethrionomys rufocanus. Krebs (1964) rejected Pitelka's food supply hypothesis as an explanation of the Baker Lake lemming cycle for several reasons including lack of extensive forage utilization, no evidence of starvation, and no evidence of deficiency diseases among the young during the peak population.

Recently, some investigators have suggested that qualitative changes in vegetation may be much more important to population dynamics than overall quantitative changes. A number of reproductive parameters have been found to be affected by variations in nutrition. Greenwald (1957) found that M. californicus reached puberty much earlier during a season of good rainfall and plentiful irrigation when compared to a season of poor rainfall. Similarly, Batzli and Pitelka (1971), also working with M. californicus, demonstrated that seasonal changes in the animals' condition varied with dietary changes. The end of the breeding season in late spring was associated with low growth rates, low survival rates, and low fat reserves. At this time, vegetation was drying, and stomach analyses indicated that the diet was changing from one of green leaves to seeds. Bodenheimer (1949) showed a similar correlation between reproductive function and vegetation in M. guentheri, while working on irrigated and non-irrigated fields.

Negus and his co-workers have investigated the effects of green vegetation on the reproductive physiology of M. montanus in the laboratory (Pinter and Negus, 1965; Hinkley, 1966; Negus and Pinter, 1966; Negus and Berger, 1971). Using dietary supplements of both green vegetation and spinach extracts, they have demonstrated effects on a number of reproductive parameters in experimental versus control animals. Negus and Pinter (1966) provided evidence that a greens dietary supplement can: 1) increase the number of offspring in breeding pairs; 2) reduce litter loss and increase the frequency of postpartum mating in breeding pairs; 3) increase the uterine weight and the number of developing follicles in young females; 4) stimulate the onset of estrus in immature females as well as increase uterine and adrenal weights; and 5) cause wild adult M. montanus trapped in non-breeding condition to become reproductively active. In addition, Forslund (1972) found that the reproductive activity of wild M. montanus was closely associated with changes in vegetation. Hinkley (1966) investigating pituitary involvement in the response of M. montanus to green vegetation, demonstrated an increase in the number of gonadotroph cells in the anterior pituitary in greens-fed versus control animals. Initially, Pinter and Negus (1965) suggested estrogenic compounds in the plants were involved in these reproductive responses. Recent evidence, however, provided by Negus and Berger (1971) showed that green vegetation may affect the reproductive physiology of M. montanus by altering pineal gland function.

To date, there have been few studies on nutrient requirements or food preferences among wild microtines (Thompson, 1965). To accurately assess either the qualitative or quantitative aspects of the food supply hypothesis, and its possible role in regulating microtine cycles, it will be necessary to provide more conclusive evidence, particularly from field studies on cycling populations.

Behavioral-Stress Hypothesis

In 1950, Christian proposed a behavioral-endocrine feedback mechanism for regulating population cycles (Figure 3). His hypothesis was based on the previous work of Hans Selye (1946), who demonstrated that stress produced by various physical and chemical agents could cause altered pituitary-adrenal function in laboratory animals. Extreme imbalances of the endocrine system resulting from stress led to a condition Selye described as "shock disease." Typical symptoms included lethargy, convulsions, depletion of glycogen stores, and hypoglycemia leading to death (Christian, 1950). Christian postulated that stress resulting from increased "social pressure" in high density populations could lead to a population decline due to endocrine disturbances.

"Social pressure" as defined by Christian (1961, 1963) is a density-dependent phenomenon; the number of social interactions (e.g., agonistic behavior, intraspecific competition) being proportional to the number of animals in the population. Social pressure can thus operate throughout the population cycle, stimulating reproduction during periods

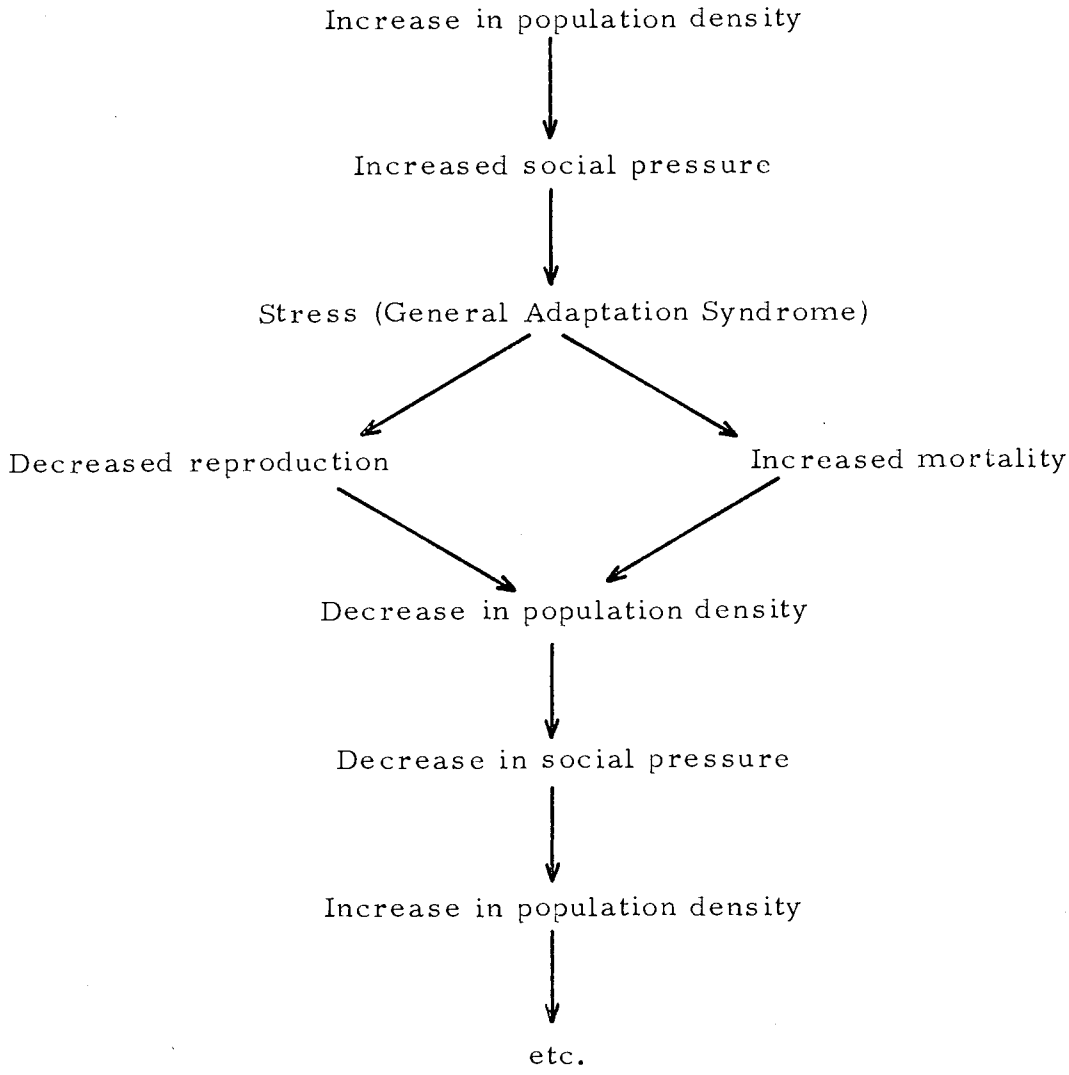

Figure 3. Christian's stress hypothesis. The system is purely phenotypic and operates through the General Adaptation Syndrome (adapted from Krebs, 1964, p. 64).

of low numbers and inhibiting reproduction and causing mortality during high density periods (Christian, 1963). Furthermore, Christian (1963) pointed out that the degree of stress in a population is also a function of environmental parameters, which could increase or decrease social interactions. For example, drought would cause a concentration of animals in areas of remaining water, resulting in increased social pressure due to competition for the water.

Christian et al. (1965) reviewed the evidence primarily from laboratory studies for endocrine involvement in mammalian population regulation. Increasing densities of laboratory populations of rodents showed progressive adrenocortical hypertrophy and thymic involution, delayed or total inhibition of reproductive function in both males and females, suppression of somatic growth, depression of inflammatory and immune responses, and a variety of inhibited reproductive functions. The combination of these responses suggested high circulating levels of adrenocorticotropic hormone (ACTH) and lowered levels of gonadotropic hormones, LH and FSH (Christian and Davis, 1964). Despite the large amount of data collected on the detrimental effects of high density in laboratory situations, considerable disagreement exists concerning the relevance of Christian's stress hypothesis to natural populations. Support for this hypothesis necessarily implies evidence in field situations of hyperadrenal activity, and/or some measure of decreased reproductive function.

Initial attempts at assessing adrenal function involved organ weight determination. Christian, himself, pointed out the problems inherent in using adrenal weight as an assay of adrenal function (Christian and Davis, 1964). Some of these difficulties include the presence of an immature X-zone, increased weight due to hypertrophy of the adrenal medulla rather than cortex, and increased weight due to lipid deposition resulting from sudden cessation of ACTH stimulation. Most importantly, sexual maturation and sexual condition must be considered in evaluating adrenal weight, as androgens involute the X-zone and decrease adrenal weight, while estrogens commonly increase adrenal cortical tissue. A significant dimorphism, presumably due to the effect of sex steroids, has been demonstrated in M. montanus (Negus and Pinter, 1966), M. agrestis (Chitty and Clarke, 1963), and M. pennsylvanicus (Louch, 1958). Krebs (1964) further pointed out that the relationship between adrenal weight and body weight is most likely not linear and that studies using adrenal weight (mg)/body weight (g) are subject to criticism.

Christian and Davis (1966) reviewed literature indicating a direct relationship between adrenal weight and population density for M. pennsylvanicus (Louch, 1958; Christian and Davis, 1966) and M. montanus (Adams et al., cited in Christian, 1961). Andrews (1970) demonstrated a similar relationship for Lemmus trimucronatus. In contrast, McKeever (1959) and Chitty (1961) failed to show a correlation between population density and adrenal weight in M. montanus and M. agrestis

respectively. Similarly, Krebs (1964) and Mullen (1968) did not find this relationship in their studies of L. trimucronatus. Forslund (1972) pointed out that discrepancies encountered in the literature on adrenal weights may be attributed to investigators' failure to properly separate data according to body size, sex, reproductive condition, and seasonal changes.

Recently, an improved method for assessing adrenal function in L. trimucronatus has been utilized by Andrews (1968a; Andrews and Strohbehn, 1971). Combining organ culture methods with chromatography and fluorometric assay, Andrews has been able to measure corticosteroid secretory rates, as well as respiratory activity of isolated adrenal glands. Using this technique, Andrews (1968a) presented evidence that secretory activity of adrenal glands, obtained from lemmings during a population high, was conspicuously higher than from adrenals obtained before or after a population "crisis." Furthermore, adrenals obtained during the crisis were refractory to exogenous stimulation by ACTH, while non-crisis glands showed typical dose-response curves. Working with the same population of L. trimucronatus during a decline phase in the summer of 1969, Andrews and Strohbehn (1971) found that animals emerging from under the snow showed marked adrenal hypertrophy, high basal levels of adrenal steroid secretion, high pituitary ACTH content and lowered sensitivity to exogenous ACTH. It is interesting to note that decreased reproductive

activity was not observed during the summer decline, and that gravid females were less responsive to stress factors. The investigators concluded that endocrine balance in wild lemming populations is a function of both physical and social environmental factors, and that the endocrine status of a population markedly affects its dynamics.

Based on results obtained from this lemming study, Andrews proposed a new mechanism for altered reproductive function resulting from high density stress (Andrews, 1968b; Andrews and Strohbehn, 1971; Andrews et al., 1972). In studying precursor utilization in lemming adrenal steroidogenesis, Andrews (1968b) found that moderate doses of exogenous ACTH increased the rate of adrenal progesterone synthesis. The investigators postulated that increased levels of progesterone might be responsible for the reduced fecundity often observed in crisis animals (Christian and Davis, 1964). Furthermore, Andrews et al. (1972) suggested that adrenal progestins and androgens might affect gonadotropin output directly.

This hypothesis is extremely interesting in light of recent research on the relation of the adrenal gland to reproduction in the laboratory rat. Resko (1969) showed that the adrenal gland of ovariectomized rats secretes progesterone and that exogenous ACTH induced mating behavior in estrogen-primed ovariectomized rats. Fajer et al. (1971) demonstrated that stressed rats showed similar adrenal and ovarian secretion rates of progesterone. Lisk (1969) found that exogenous progesterone administered to female rats facilitated or inhibited

ovulation and mating behavior depending at which point during the estrous cycle it was administered. Nequin and Schwartz (1971) presented evidence for direct adrenal involvement in mating and LH release in the rat. Stress (sham-ovariectomy) affected both the timing of lordosis and ovulation, again depending at which point during the estrous cycle the stress was superimposed. The authors concluded that under stress conditions the adrenal glands, presumably via progestin secretion, can affect the normal timing of events in the rat reproductive cycle. Perhaps most significantly, Firlit and Lawton (1974) have demonstrated that adrenalectomy at specific times during the development of the pre-puberal rat results in delayed onset of puberty and desynchronization of cyclic estrous events. Based on these findings, Andrew's hypothesis that the adrenal gland is involved in inhibiting reproduction in cycling microtines seems quite tenable.

Statement of Purpose

Very little is known about the ecology and reproductive biology of the grey-tailed vole, Microtus canicaudus. Originally thought to be a subspecies of M. montanus (Hall and Kelson, 1951; Anderson, 1959), Tyser (1975) presented evidence from breeding experiments followed by karyology, that M. canicaudus should be awarded species status. Some limited observations have been made on its natural history and distribution (Goertz, 1964; Maser and Storm, 1970; Pearson, 1972). Tyser (1975) provided the first definitive information on the

reproductive life history of this species. Weil (1975), investigating agonistic behavior in M. canicaudus, M. oregoni, and M. townsendii, suggested that aggressive behavior observed in M. canicaudus, particularly males, played a role in habitat segregation of these three species in the Willamette Valley. Evidence that M. canicaudus exhibits cyclic population fluctuations has been indicated by Maser and Storm (1970), and Forslund (1974).

To date, no studies have been performed on the population dynamics of M. canicaudus. The purpose of this investigation was to collect further information on the reproductive biology of M. canicaudus, and to test two hypotheses, the food supply hypothesis and the behavioral-stress hypothesis, as possible mechanisms involved in the cyclic fluctuations observed in this species.

Specifically, the effects of a dietary supplement of sprouted wheat clippings, and group versus individual-housing, were assessed in M. canicaudus in terms of adrenal and reproductive physiology. Organ culture of adrenal glands, chromatography, and fluorometric assay were used to assess adrenal secretory function. Gravimetric data were collected on adrenal glands, testes, and reproductive tracts as indices of general reproductive condition.

The response of M. canicaudus to high density and a dietary supplement of green vegetation was investigated in order to determine the role of these variables in cyclic fluctuations observed in this species.

II. MATERIALS AND METHODS

The methods used in this study can be conveniently described by dividing them into general laboratory procedures, specific laboratory procedures, and statistical procedures.

General Laboratory Procedures

Maintenance of Animals

Animals used in this study were offspring of a breeding colony of M. canicaudus housed in animal care facilities located in Weniger Hall #635 at Oregon State University. The original animals from which the colony was propagated were live-trapped in three areas of Benton County, Oregon from March to August, 1973 (Tyser, 1975). Present experimental animals were second to fourth generation offspring of the original wild stock.

All animals were housed in Bo-Kay fiberglass plastic boxes (60 cm x 15 cm x 15 cm) fitted with 1.2 cm wire mesh tops. Cage floors were covered with sawdust, and cotton batting was provided for cover and nesting material. All animals were provided with Purina Rat chow, Purina Rabbit chow, and tap water (ad libitum) and were maintained under standard conditions of temperature (20°-22°C) and photoperiod (16L:8D). In addition to the basal diet, breeding pairs received fresh lettuce clippings twice weekly.

Approximately 100 pairs of adult Microtus canicaudus (12 weeks old) were mated during the period from May 1974 to January 1975. In order to maintain outbred conditions in the colony, siblings and first cousins were not selected as breeding pairs. Once two animals were mated, they remained with each other continually. Accurate breeding records including parturition date for each litter, litter size, sex ratios, and records of litter loss were maintained for each mated pair. Daily inspection of breeding cages during the latter stages of pregnancy (predicted via periodic palpation) allowed for discovery of a litter with 24 hours after birth. Young were weaned at 18 days of age, toe-clipped for identification purposes, and housed with siblings (five animals per cage) for later experimental purposes.

Determination of Body and Tissue Weights

Body weights of experimental animals were determined to the nearest 0.5 g on an Ohaus triple beam balance. Adrenal glands were excised from donor animals, stripped of adipose tissue and fascia, and rinsed in a Petri dish containing 0.89% saline. The glands were then blotted on filter paper, weighed on a Roller-Smith rapid weight tissue balance to the nearest 0.1 mg, and stored in AFA (ethanol-formalin-acetic acid) fixative for later histological evaluation. Tissues to be fixed including testes and female reproductive tracts were similarly debrided of adipose tissue and fascia, and stored in AFA. Later, these organs were blotted on filter paper, and weighed to the nearest

0.1 mg on the Roller-Smith balance. Weights of fixed tissues were obtained as soon as possible after blotting to standardize loss of weight due to volatile liquids in the fixative. Female reproductive tract measurements were standardized by cutting the tract just in front of the cervix. Ovaries, oviducts, and uterine horns were subsequently weighed intact.

Specific Laboratory Procedures

Breeding Experiments

Information on the reproductive biology of M. canicaudus was obtained from breeding records for each mated pair. Mean litter sizes were calculated for consecutive litters from the first through as many as nine litters. To be consecutive, a litter had to be born within 25 days of the last parturition date. Tyser (1975) tentatively determined the gestation period of M. canicaudus to be 21 days. The mean number of litters and mean number of offspring produced per active female were determined for a 100-day breeding period. The day after first pairing was designated as Day 1. An active female was defined as one that had had at least one litter during the 100-day experiment. Overall mean litter size for M. canicaudus was also determined from the 100-day breeding experiment. Furthermore, the gestation period in M. canicaudus was estimated by determining the most frequently occurring interval between parturitions. Post-partum estrus and mating is

known to occur in several species of Microtus (Asdell, 1964) and was assumed to occur in this species.

Fresh Greens and Density-Stress Experiments

Experimental groups were designed to determine the effects of a "greens" (wheat clippings) dietary supplement as well as high density stress on the reproductive and adrenal physiology of M. canicaudus. Fresh wheat clippings were provided for the greens experiment by sprouting white wheat seeds in 90 cm x 15 cm x 5 cm soil boxes. Sprouted wheat was clipped when it reached a height of 13-20 cm, which entailed approximately 9 days growth under constant light and a temperature range of 26-30°C. A dietary supplement consisted of 5 grams of wheat clippings/animal given three times weekly. Artificial high density conditions were obtained by housing animals in groups of five (Christian, 1961); individually-housed animals acted as controls. All experimental animals were maintained under laboratory conditions previously described. At 4 weeks of age, animals were removed from sibling cages, and placed in one of the following experimental groups:

Group A: IH, NG, ♂; individually-housed, no greens, males.

Group B: IH, G, ♂; individually-housed, greens, males.

Group C: GH, NG, ♂; group-housed, no greens, males.

Group D: GH, G, ♂; group-housed, greens, males.

Group E: IH, NG, ♀; individually-housed, no greens, females.

Group F: IH, G, ♀; individually-housed, greens, females.

Group G: GH, NG, ♀ ; group-housed, no greens, females.

Group H: GH, G, ♀ ; group-housed, greens, females.

Determination of Body and Tissue Weights in Experimental Groups A-H

At 10 weeks of age, 25 animals in each experimental group were sacrificed by cervical dislocation. The following weight determinations were made using procedures previously described: body weight; paired adrenal weight (fresh); and fixed testes and reproductive tract weights. In addition, females were examined at the time of death for the presence or absence of a perforate vagina. A perforate or open vagina was taken to indicate that the animal was in an estrous condition.

Determination of Adrenal Secretory Function in Experimental Groups A-H

Adrenal secretory function was assessed in 15 animals for each experimental group. Animals were sacrificed by rapid decapitation within 30 seconds after initial handling. This was done to prevent rising endogenous levels of ACTH from altering basal adrenal secretory values. Body weights and the presence or absence of a perforate vagina in females were determined following decapitation. Incubation of adrenal glands and subsequent processing of steroid containing medium was carried out as described below.

Organ Culture Preparation. Adrenal glands were removed, cleansed of fascia and adipose tissue, halved, rinsed in sterile

Trowell's nutrient medium, and placed in 25 ml culture flasks containing 2.0 ml of Trowell's nutrient medium (Grand Island Biological Co.). The glands were allowed to incubate for 4 hours in a Labline metabolic shaker bath at 37°C and under an atmosphere of 95% O_2/5% CO_2 at 56.6 liters per hour. Following incubation adrenals were removed, blotted on filter paper, weighed to the nearest 0.1 mg on a Roller-Smith balance, and stored in AFA fixative. The steroid containing medium was immediately frozen and stored for later chemical analysis. During the incubation period reproductive tracts and testes were removed from donor animals and stored in AFA for future weight determinations.

Steroid Determination

The method for steroid determination was based on a procedure described by Andrews (1968c). The collected steroid-containing nutrient medium was processed by (1) solvent partitioning in peroxide-free ethyl acetate, (2) thin-layer chromatography in ethyl acetate: methanol (99:1) on silica gel plates (Merck), (3) elution of spots with 100% ethanol, and (4) quantitation of selected "spots" by acid-fluorescence. Specifically, one-half of each sample was used to obtain a value for total steroid, i.e., cholesterol, corticosterone, cortisol, etc. Following step (1), this part of the sample was fluoresced according to the methods of Silber et al. (1958) for corticosterone. From the other half of each sample (chromatogram spotted) the amounts of

corticosterone plus cortisol, and progesterone were estimated according to the procedure outlined above and the fluorescence methods of Silber et al. (1958) for corticoids and Short and Levitt (1962) for progesterone. Corticosterone fluorescence was carried out with an excitation band of 470-475 mμ, and a fluorescence band of 520-530 mμ. For progesterone, an exciting wavelength of 390 mμ and an emitted wavelength of 490 mμ were utilized. A Turner fluorometer (Model 110) and the following combination of Turner filters were used to detect fluorescence: for corticosterone - primary filter 47B, secondary filters 1-60 + 58; for progesterone - primary filters 2A + 47B, secondary filter 23A. For specific details of the isolation and quantification of the steroids, refer to the flow sheets Figures 4 and 5.

Statistical Procedures

Whenever possible, sample sizes, means, standard deviations, standard errors of the mean, and Student t-test values were calculated. Two-sided, 95% confidence limits were calculated using appropriate standardized table values to correct for sample size (Rohlf and Sokol, 1969).

III. RESULTS

Laboratory Reproduction of *Microtus canicaudus*

Consecutive Litter Size

The results of breeding experiments with *M. canicaudus* indicated a gradually increasing mean litter size from the first through the fifth litters, and thereafter a slight decrease in litter size through the eighth litter (Figure 6, p. 50 and Table 1, p. 60). The mean litter size of the first litter was significantly smaller than the succeeding second ($P < 0.001$), third ($P < 0.001$), fourth ($P < 0.001$), and fifth ($P < 0.001$) litters, respectively. Similarly, the second litter was found to be significantly smaller than the third ($P < 0.001$), fourth ($P < 0.001$), and fifth ($P < 0.001$) litters (Table 1). The mean sizes of the sixth, seventh, and eighth litters displayed a gradually decreasing trend; however, none of these litters were significantly ($P > 0.05$) less than the fifth litter. A slight increase in size of the ninth litter was observed, although this increase was probably due to the small sample size (Table 1).

100-Day Breeding Experiment

A number of reproductive parameters of *M. canicaudus* were measured during a 100-day breeding period. The mean numbers of litters and offspring per active female were demonstrated to be 3.19

and 16.26, respectively (Table 2). Data indicated an overall mean litter size of 5.10 for the 100-day period (Table 2).

Accurate breeding records yielded information on the time-intervals between consecutive litters during the 100-day breeding experiment and, thus, on the probable gestation period of M. canicaudus (see Figure 7). The most commonly occurring intervals between successive litters were 21 and 22 days, with 21 days being the mode (Figure 7). This would indicate a probable gestation period in M. canicaudus of between 21 and 22 days.

Results from Fresh-Greens and Density-Stress Experiments

For descriptive purposes, the following three sections on adrenal weight, fixed tissue weights, and adrenocortical secretory activity have each been sub-divided with respect to the two variables, housing and diet. This method allows for easier interpretation of the results in Section IV (Discussion).

Adrenal Weight Results

A significant sexual dimorphism in adrenal weight ($P < 0.001$) was demonstrated, female adrenal glands being heavier than male glands regardless of housing or diet (Table 3). Unexpectedly, separating adrenal weights of estrous from non-estrous females yielded a significant difference in only one group. Adrenal glands of Group E

females (individually-housed, no greens) in estrus weighed significantly more ($P < 0.001$) than similar females not in estrus, both before and after normalizing for body weight (Table 4).

Density Experiment. Group-housed males with no greens supplement (Group C) had significantly smaller ($P < 0.05$) adrenal glands than individually-housed males on a no-greens diet (Group A), although this relationship was not significant ($P > 0.05$) after normalizing adrenal weight for body weight (Table 3). Similarly, adrenals of group-housed males fed greens (Group D) weighed less than adrenals of individually-housed males fed greens, although not significantly ($P > 0.05$). After normalizing for body weight, the mean adrenal weight in Group D was slightly higher than that of Group B although not significantly ($P > 0.05$). No differences in adrenal weight ($P > 0.05$) were observed in comparing respective group-housed females (Groups G and H) with individually-housed females (Groups E and F) (see Table 3). Separation of estrous from non-estrous females yielded similar results (Table 4).

Greens Experiment. Adrenal weights of male voles fed fresh greens (Groups B and D) did not vary significantly ($P > 0.05$) from males with no greens supplement (Groups A and C) (see Table 3). Likewise, adrenals of group-housed females (Groups G and H) displayed no significant difference in weight ($P > 0.05$). However, females in Group E (individually-housed, no greens) had heavier ($P < 0.05$) adrenals than their counterparts in Group F, although after

normalizing for body weight this difference was not significant (Table 3).

After separation of estrous from non-estrous animals, only Group E females in estrus had significantly heavier ($P < 0.05$) adrenals than similar animals in Group F (Table 4). This same relationship held when normalizing adrenal weight for body weight (Table 4).

Testes and Reproductive Tract Weight Results

Density Experiment. No difference ($P > 0.05$) in testes weight was found between Group C and Group A males. However, group-housed males in Group D had slightly lighter ($P < 0.01$) testes than individually-housed males in Group B. This difference remained significant ($P < 0.05$) after normalizing for body weight (Table 5). Comparison of group-housed females (Groups G and H) with their counterparts in Groups E and F, respectively, yielded no differences ($P > 0.05$) in reproductive tract weights (Table 5). These relationships held after separating females who were in estrus from those not in estrus (Table 6).

Greens Experiment. Testes of individually-housed males in Group B (with greens) were heavier ($P < 0.001$) than testes of individually-housed males in Group A (no greens supplement)(Table 5). This relationship did not hold for group-housed males, as no significant differences in testes weight were observed between Groups C and

D. Comparison of greens-fed females (Groups E and G) with females not fed greens (Groups D and F) yielded no significant differences ($P > 0.05$) in reproductive tract weights (Table 5). This same relationship held after separating estrous from non-estrous animals (see Table 6). However, both the individually-housed females fed greens (Group F) and the group-housed females fed greens (Group H) exhibited a higher number of animals in estrus, based on a perforate vagina, when compared with Groups E and G respectively (Table 5). Forty-five percent of the females in Group F showed an estrous response in comparison to 30% in Group E. Similarly, 45% of the Group H females were found to be in estrus in comparison to 27.5% in Group G (Table 5).

Adrenocortical Secretory Function

A significant sexual dimorphism in total steroid secreted ($P < 0.001$) was displayed, males producing more steroid than females regardless of housing or diet (Figure 8 and Table 7). This relationship held after isolation and measurement of individual steroids, specifically corticosterone plus cortisol, and progesterone (Figures 9 and 10; Tables 8 and 9).

Density Experiment. Values for total corticoid production of group-housed males in Group C were higher ($P < 0.05$) than similar values of individually-housed males in Group A (Figure 8 and Table 7). Group-housed males in Group D also secreted more corticoid than

individually-housed males in Group B, although not significantly ($P > 0.05$). Comparable results were not observed in females. Specifically, no significant differences in total steroid secretion ($P > 0.05$) were observed in comparing group-housed females (Groups G and H) with individually-housed females (Groups E and F) (see Figure 8 and Table 7).

Group C males (group-housed, no greens) released greater amounts of corticosterone plus cortisol ($P < 0.05$) and progesterone ($P < 0.1$) than Group A males (individually-housed, no greens) (Figures 9 and 10; Tables 8 and 9). Groups B and D males did not show significant differences ($P > 0.05$) in either corticosterone plus cortisol or progesterone production. Similarly, no difference ($P > 0.05$) was observed in corticosterone plus cortisol production by females from Groups E and G. Surprisingly, individually-housed females in Group F secreted greater ($P < 0.01$) amounts of corticosterone plus cortisol than group-housed females in Group H (see Figure 9 and Table 8). Also of interest, group-housed females in Groups G and H released less progesterone than individually-housed females in Groups E ($P < 0.1$) and F ($P < 0.01$), respectively (see Figure 10 and Table 9).

Greens Experiment. Results based on total steroid production indicated that a dietary supplement of fresh greens had no effect on steroid production in M. canicaudus. No significant differences in adrenocortical production ($P > 0.05$) were observed in comparing

greens-fed males (Groups B and D) with respective males on the no-greens diet (Groups A and C) (see Figure 8 and Table 7). Similar results were observed in females, comparing Groups F and H with Groups E and G respectively (Figure 8 and Table 7).

Measurements of corticosterone plus cortisol yielded no significant differences ($P > 0.05$) between animals fed fresh greens and their counterparts fed no greens (see Figure 9 and Table 8). Group F females (individually-housed, greens) produced more corticosterone plus cortisol than Group E females (individually-housed, no greens), although not significantly ($P < 0.1$) (Figure 9 and Table 8). Similarly, comparison of progesterone levels indicated no significant differences ($P > 0.05$) between groups within the following pairs: Groups A and B, Groups C and D, Groups E and F, and Groups G and H (Figure 10 and Table 9).

IV. DISCUSSION

Laboratory Reproduction of *Microtus canicaudus*

Controlled laboratory breeding experiments with *M. canicaudus* demonstrated that mean litter size increased from the first through the fifth litters, and thereafter decreased through the eighth litter. Furthermore, the mean litter size of the first litter was significantly smaller than the second, third, fourth, and fifth litters. The results of this investigation are in agreement with preliminary data on litter sizes presented for *M. canicaudus* (Tyser, 1975) and with results reported for *M. montanus* (Negus and Pinter, 1965; Forslund, 1972). Negus and Pinter (1965) first noted and Forslund (1972) further emphasized the importance of considering the age and breeding history of females in examining population dynamics of microtines. The investigators pointed out that a decline in population numbers might be due to an increase in the number of subadult and/or primiparous females in the breeding population rather than other explanations such as the "social stress" theory. In the present investigation the results indicate that the reproductive history of female *M. canicaudus* should be determined as accurately as possible in future demographic studies of this species.

Twenty-one day gestation periods have been established for a number of species of *Microtus* including *M. montanus* (Negus and

Pinter, 1965), M. ochrogaster (Richmond and Conaway, 1969) and M. californicus and M. pennsylvanicus (as cited in Asdell, 1964). In addition, Tyser (1975) indicated a probable gestation period of 21 days for M. canicaudus. The present findings using a larger sample size substantiate his conclusion. In addition to gestation period, the following parameters were accurately assessed and documented for a 100-day breeding period: a mean number of litters and offspring per active female of 3.19 and 16.26, respectively, and an overall mean litter size of 5.10. This information on the reproductive biology of M. canicaudus, although based on a laboratory study, could prove valuable in estimating demographic changes of a natural population. Similar reproductive measurements in wild populations are extremely difficult due to problems encountered in determining age of breeding females, sizes of litters, and previous breeding history.

Physiological Responses to Fresh Greens and Density-Stress

Adrenal Weight

Results obtained in the present study indicate a significant sexual dimorphism in adrenal weight for adult M. canicaudus regardless of housing or diet. A number of investigators have reported similar results for a variety of species including M. montanus (Negus and Pinter, 1966), M. agrestis (Chitty and Clarke, 1963) and

M. montanus (Forslund, 1972). The difference in adrenal weight between sexes is likely due to the effects of sex steroids on adrenal tissue. As mentioned above, androgens involute the X-zone in maturing animals and decrease adrenal weight, while estrogens increase proliferation of cortical tissue (Chitty and Clarke, 1963; Christian and Davis, 1964).

Surprisingly, separation of estrous from non-estrous animals, based on the presence of an open vagina, within Groups E, F, G, and H, yielded significant differences in adrenal weight in Group E only. Due to the effects of estrogens, the more reproductively active animals would be expected to have heavier adrenals than their less active counterparts in comparable groups. A plausible explanation for the disparity is the inadequacy of this method of classifying estrous versus non-estrous animals. For example, animals sacrificed just after coming into estrus or upon entering anestrus might still have open vaginae, yet fail to show the effects of estrogen stimulation characteristic of full estrus. An alternative explanation that estrogens fail to increase adrenal weight in this species is less likely. A more accurate method of classification would be based on reproductive tract weights and/or histology.

With one exception, Group F females developing smaller adrenals than Group E females, the dietary supplement of fresh greens did not appear to affect adrenal weights in M. canicaudus. Previous research by Negus and his co-workers indicated that green vegetation stimulated reproduction in

M. montanus. Some of the observed effects attributed to greens were increased litter size, increased uterine weight, and stimulation of estrus. Increased levels of pituitary gonadotropins (FSH, LH) and subsequent increases in sex steroids were postulated to be responsible for the observed effects (Hinkley, 1966). The high levels of estrogens and androgens would be reflected by increased adrenal weight in females and decreased adrenal weights in males, respectively. In the present study with one exception, no significant differences in adrenal weight were observed when comparing greens-fed and no-greens groups under similar conditions. Based on adrenal weights alone, green vegetation does not appear to stimulate reproduction in this species. It is possible that group-housing inhibited gonadotropin production following Christian's (1963) predictions, thus masking any effects due to greens alone. However, this would not explain the lack of weight changes in individually-housed greens-fed animals.

According to Christian's hypothesis, evidence of increased social interaction among group-housed animals would be evidenced by increased adrenal weights in both males and females. In the present study there were no significant differences in adrenal weight when comparing group-housed females with individually-housed females. Separation of estrous from non-estrous females yielded similar results. Interestingly, individually-housed males actually had heavier adrenal weights than animals group-housed with similar diets, contrary to results predicted by Christian's hypothesis. Some of the problems

encountered in using adrenal weight as an index of adrenocortical function have been discussed above. These include increased weight due to lipid deposition resulting from cessation of ACTH stimulation and weight changes due to reproductive condition. In addition, the present data on adrenal weights were characterized by a high degree of variability. Forslund (1975) has indicated that extreme variability in adrenal weights is characteristic of wild populations of M. montanus and possibly of other species. Thus, the present group of experimental animals, only three generations removed from the wild, may closely approximate the genetic characteristics of a wild population. Another possible explanation for the observed results is that housing males individually may have acted as a "stressor" and caused adrenocortical hypertrophy; however, this was somewhat refuted by steroid secretory values. Establishment of social hierarchies among populations of small mammals is well documented (Christian, 1970). Such hierarchies operate to reduce agonistic encounters among all individuals and to favor reproduction by a few dominant members of the population. In the present investigation, establishment of social rank among individuals in group-housed cages would decrease the amount of social interaction and could account for the lack of adrenal weight differences. Of these explanations, the extreme variability of adrenal weights in a wild species seems the most plausible in accounting for the observed results.

Testes and Reproductive Tract Weight

Reproductive Organ Weight. Estimates of general reproductive condition based on reproductive organ weights were made. Greens appeared to improve reproductive condition in Group B males when compared to Group A males, as evidenced by an increase in testes weight. However, this pattern did not hold for group-housed males on a similar diet, there being no significant difference in testes weight between Groups C and D. A possible explanation for this lack of consistency is that "social stress" lowered circulating levels of gonadotropins in group-housed males and masked any stimulatory effects due to fresh greens.

Data on female reproductive condition were inconsistent. Negus' findings with M. montanus showed both an increase in the number of females in the estrous condition and an increase in uterine weight in greens-fed animals. In the present study female reproductive tract weights did not vary significantly between greens-fed and no-greens animals. Normal uterine weight changes due to estrous events of these polyestrous mammals presents one major difficulty in the use of uterine weight as an index of the effects of greens. An attempt to overcome this problem was made by separating estrous from non-estrous animals and re-evaluating uterine weight changes between the greens and no-greens groups. It was assumed that if greens were stimulating reproduction then the animals fed fresh wheat clippings from four

weeks of age would show an increase in uterine weight over animals fed the basal diet, even if both groups were in anestrus. Results of this further breakdown of reproductive tract data still indicated no significant differences between greens-fed and no-greens animals. In contrast to the gravimetric data, both individually-housed (Group F) and group-housed (Group H) females fed greens exhibited a higher percentage of animals in estrus when compared to similar groups receiving the basal diet only. Forty-five percent of Group F females showed an estrous response in contrast to 30% of Group E females, while 45% of Group H females were found to be in estrous in comparison to 27.5% in Group G. Based on these findings, it is difficult to make definite conclusions about the effects of fresh greens on the reproductive physiology of M. canicaudus. Although a higher percentage of animals appeared by external examination to be in estrus, a more exact criterion for separating estrous from non-estrous animals is needed. Separation of females on the basis of uterine histological differences would be appropriate. Furthermore, examination of ovarian histology, specifically the number of developing follicles, would provide another index with which to evaluate the effects of greens on this species.

According to Christian's hypothesis, increased social interaction and the resulting decrease in circulating gonadotropins would be reflected in lowered reproductive organ weights in both males and females. The present findings on reproductive organ weights suggest

low levels of social interaction in group-housed females and the possibility of social stress acting on group-housed males. Group-housed males (Group D) fed greens developed significantly lower testes weights than individually-housed males (Group B) fed greens. However, this relationship did not hold for comparable animals on the basal diet. There were no significant differences in female reproductive tract weights based on housing, even after separating estrous from non-estrous animals.

Adrenocortical Secretory Activity

Regardless of housing conditions or diet, males secreted significantly more adrenal steroid than females. This relationship held for measurements of "total steroid," as well as individual estimates of corticosterone plus cortisol and adrenal progesterone. These results are in accordance with data presented by Forslund (1972) for *M. montanus*. This author is in agreement with Forslund (1972) that some of this disparity in adrenal secretory activity between males and females is likely attributed to the significantly heavier adrenal glands in female *M. canicaudus*. Thus, after normalizing steroid output for 100 mg of adrenal weight, lower secretory values for females might be erroneously derived. Andrews (1970) reported only a slight difference in adrenal weight between sexes (females having heavier glands) in the arctic microtines (*Lemmus trimucronatus*, *Microtus oeconomus*, and *Clethrionomys rutilus*). Normalizing corticoid secretory values for

adrenal weight produced no significant differences between males and females (Andrews, 1970).

In the present study, steroid output in M. canicaudus was not affected by dietary fresh greens, in either sex, regardless of housing. These results complement data based solely on adrenal weights which indicated little response to dietary greens. These results taken together with weight determinations of reproductive organs and adrenal glands support an argument against increased reproductive sensitivity to resource signals present in green vegetation for this species.

Comparison of steroid output between group-housed and individually-housed animals yielded interesting results with respect to a social stress hypothesis for population regulation of this species. Of particular note, group-housed males secreted more total steroid than individually-housed males in both greens-fed and no-greens groups. However, these results were significant ($P < 0.05$) in the no-greens group only. These findings were complemented by the observation that group-housed males produced greater amounts of corticosterone and progesterone than individually-housed males. Again, these results were significant ($P < 0.05$) only in the case of animals not fed greens. The findings were in accordance with observations of Andrews (1970) who demonstrated that group-housed male Lemmus trimucronatus secreted greater amounts of corticoid than pair-housed males. Similarly, Christian and Davis (1964) reviewed a number of experiments in

which group-housed mice showed greater in vitro secretion rates of corticosteroids than singly-caged mice.

In the present study, increased steroid output by group-housed animals was not observed among females. In contrast, individually-housed females (Groups E and F) produced significantly more progesterone than comparable group-housed animals in Groups G (<0.1) and H ($P < 0.01$). These results suggest the possibility that male and female M. canicaudus respond differently to stress imposed by housing conditions. In males, group-housing may result in increased social interaction among animals and could account for the higher levels of corticoids observed. In females, however, individual-caging of animals may act as a "stressor," as evidenced by increased output of progesterone by singly-housed versus group-housed animals.

The observations in the present study that group-housed males produced greater amounts of steroid than individually-housed males, and that males in general produced more corticosteroid than females is most likely a function of greater aggressiveness in males. Intraspecific aggression is an important component of Christian's concept of social stress and has been shown to be species-, age-, and sex-dependent (Christian, 1970). A number of investigators have noted increased aggression, particularly among males, in high density populations of voles and lemmings (Krebs, 1964; Christian, 1971; Myers and Krebs, 1971). Of particular interest to the present investigation, Weil (1975) studying agonistic behavior among three species

of voles (M. canicaudus, M. oregoni, and M. townsendii) found that M. canicaudus males were most aggressive toward or intolerant of the other two species. This aggressive behavior in males is believed to be a direct function of circulating androgen levels (Sadleir, 1965; Christian, 1971).

In summary, the major findings of this study are as follows: 1) there was a significant sexual dimorphism in adrenal weight for adult M. canicaudus, female adrenal glands being heavier; 2) males secreted significantly more adrenal steroid than females regardless of housing or diet; 3) group-housed males secreted more corticosterone plus cortisol and progesterone than individually-housed males, and 4) individually-housed females secreted significantly more progesterone than group-housed females.

Finding #3 lends support to Christian's stress hypothesis for population density regulation in this species. Finding #4 suggests female M. canicaudus are more stressed by individual-caging than group-caging. Furthermore, it is speculated that an adrenal adaptive mechanism may have evolved in female M. canicaudus to encourage reproduction among colonies of animals as opposed to isolated individuals.

Based on adrenal weights, fixed tissue weights, and adrenocortical secretory values, it appears that the reproductive physiology of M. canicaudus is not as sensitive to stimulation by green vegetation as reported for similar species.

V. SUMMARY

Laboratory Reproduction

Breeding experiments indicated that mean litter size increased from the first through the fifth litters, and thereafter decreased through the eighth litter. Mean litter size of the first and second litters was significantly smaller than succeeding litters.

A gestation period of 21 days was determined for this species, confirming data presented by Tyser (1975). Additionally, an overall mean litter size of 5.10 was established for actively breeding females during a 100-day period. A mean number of litters (3.19) and offspring (16.20) were determined per active female for the same period.

Adrenal and Reproductive Physiology

A significant sexual dimorphism in adrenal weight for adults was documented with female adrenal glands being heavier.

With one exception (Group C males possessing smaller adrenals than Group A males), housing did not appear to affect adrenal weight in males or females. Greens-fed females (Group F) developed lighter adrenals than controls (Group E); however, no other groups displayed an adrenal weight response to dietary fresh greens.

Group-housed males (Group D) exhibited slightly lighter testes than individually-housed males (Group B). Also, fresh greens

increased testes weight in Group B males in comparison to Group A controls. With these two exceptions, housing and diet did not appear to affect testes or reproductive tract weights. Greens-fed females did, however, exhibit a greater percentage of individuals in an estrous condition than controls, irrespective of housing conditions.

Males secreted significantly more adrenal steroid than females regardless of housing or diet. Adrenocortical secretory values indicated that fresh greens had no effect on adrenal steroid production in this species. However, housing affected adrenal steroid output with group-housed males secreting more corticosterone plus cortisol and progesterone than individually-housed males. In contrast, individually-housed females secreted significantly more progesterone than group-housed females.

Adrenal weights, fixed tissue weights, and adrenocortical secretory values indicated that the reproductive physiology of M. canicaudus was not as sensitive to stimulation by dietary greens as reported for similar species. Despite inconsistencies in adrenal weight data, it was concluded on the basis of steroid production that M. canicaudus males are more sensitive to density-imposed stress than are females. In contrast, based on progesterone secretion, females appear to be more "stressed" by individual-caging than group-caging. Thus, adrenal adaptive mechanisms may play a role in the population regulation of this species.

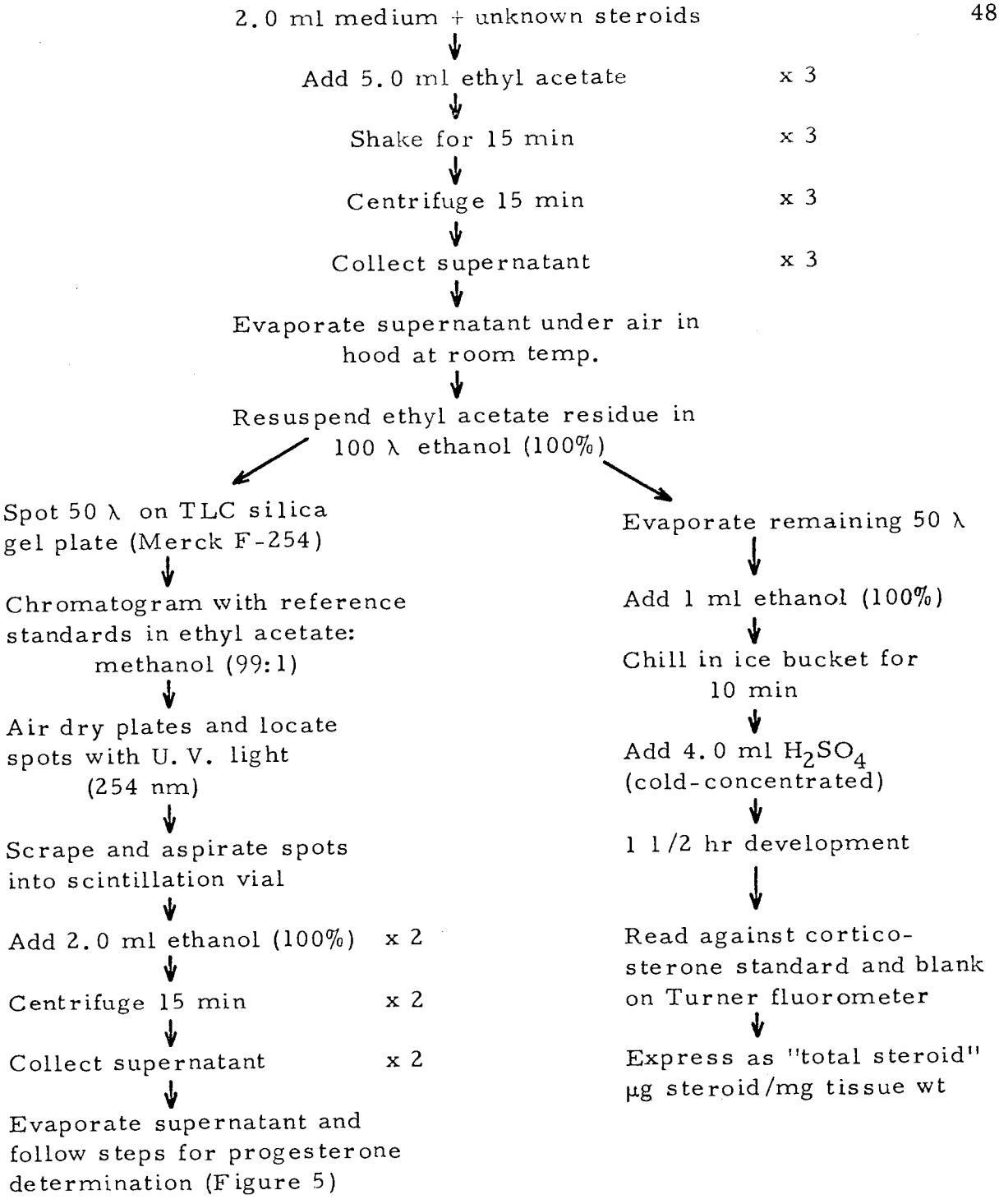

Figure 4. Flow-sheet for steroid determination.

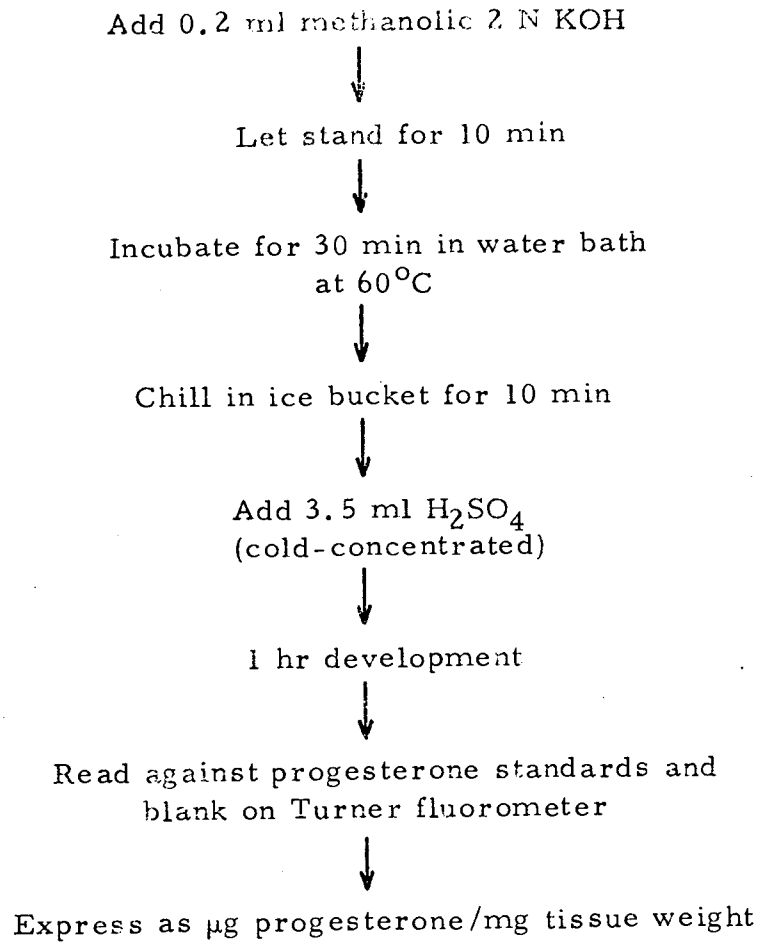

Figure 5. Flow-sheet for progesterone determination.

Figure 6. Consecutive mean litter sizes of adult Microtus canicaudus in the laboratory. Vertical lines represent standard errors of the mean. See Table 1 for N (sample size).

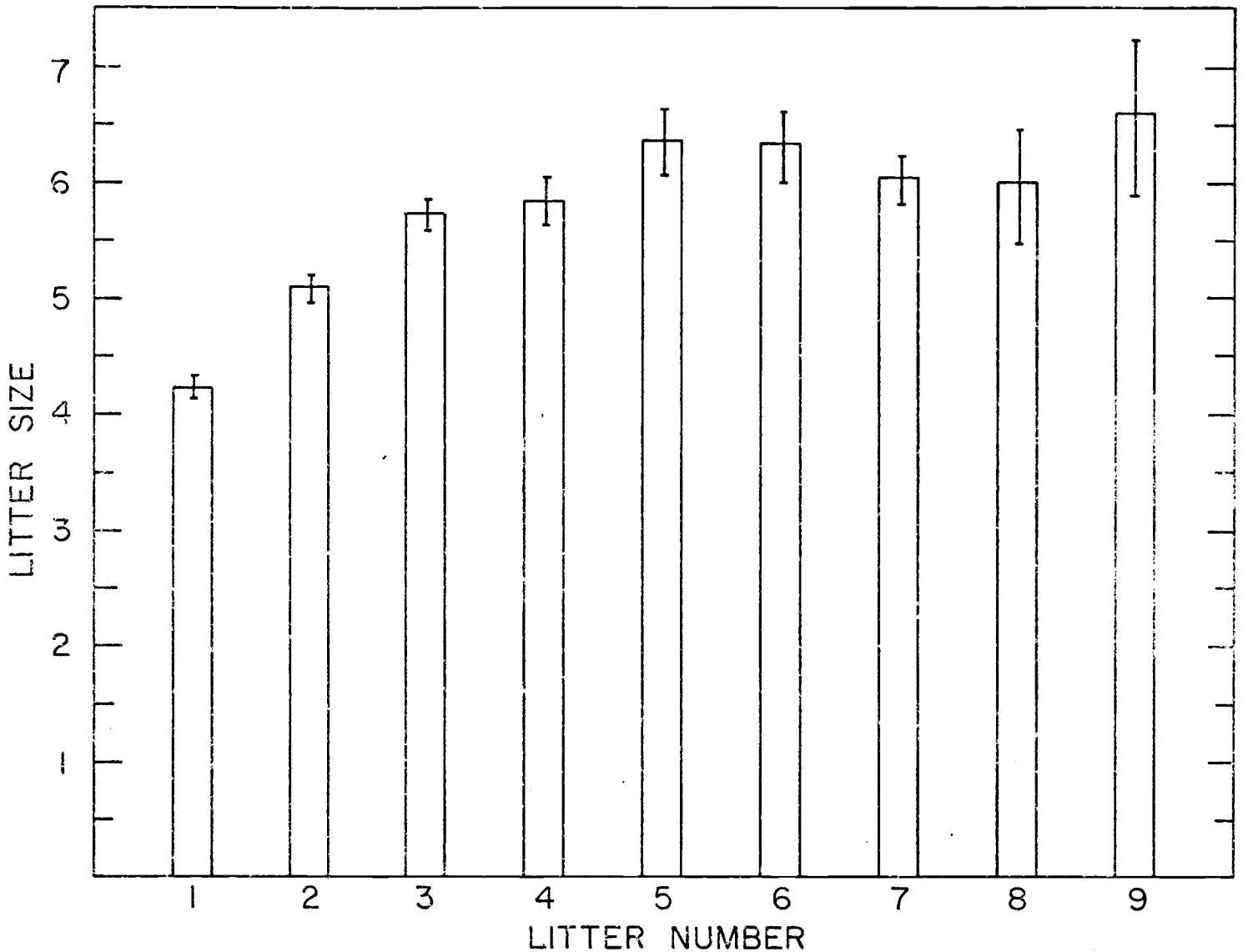
LITTER SIZE
7
6
5
4
3
2
1
1
2
3
4
5
6
7
8
9
LITTER NUMBER

Figure 7. Time-intervals between consecutive litters in Microtus canicaudus. Figures over bars indicate percent of total litters occurring at given time-intervals.

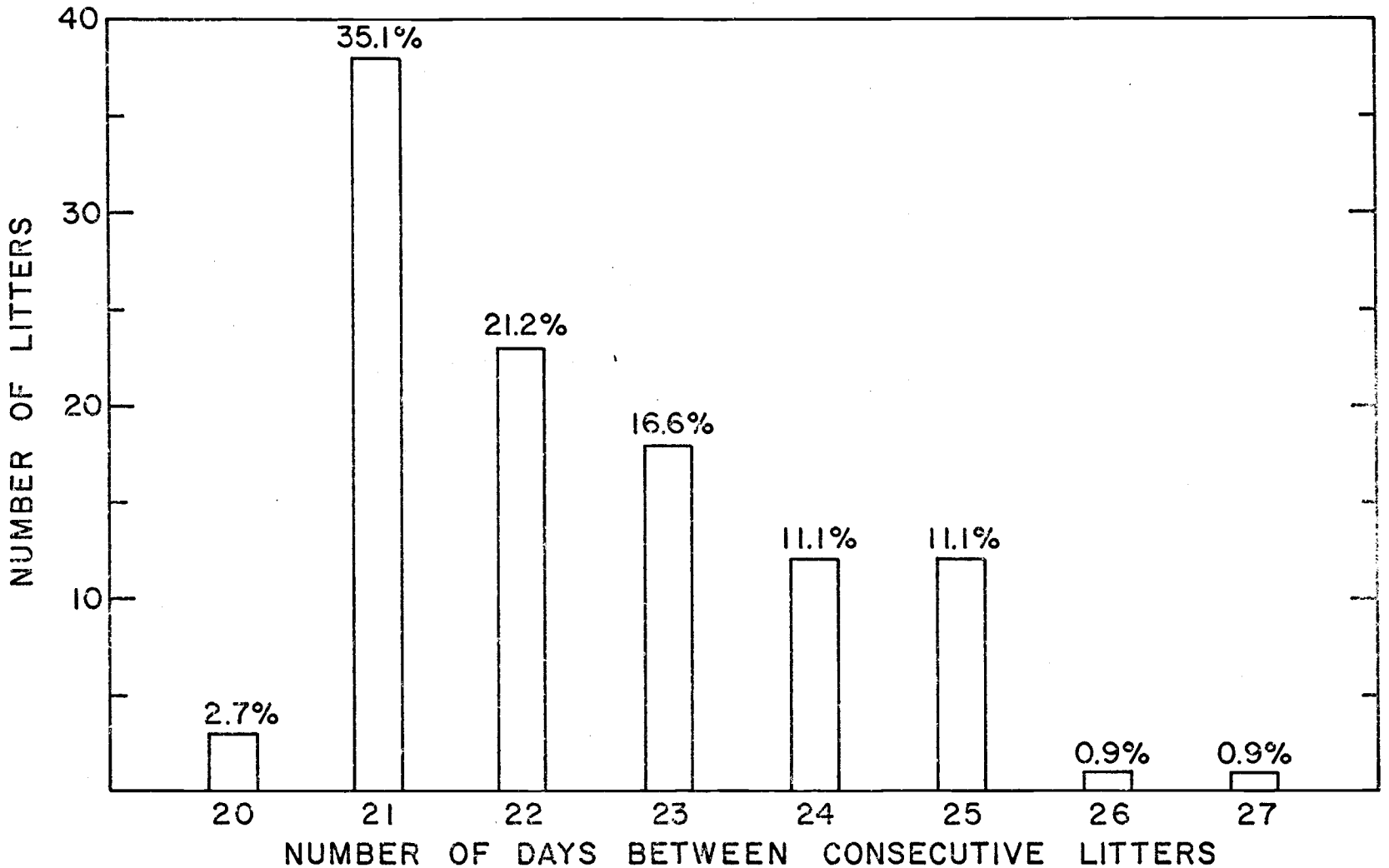
40
30
20
10
NUMBER OF LITTERS
35.1%
21.2%
16.6%
11.1%
11.1%
2.7%
0.9%
0.9%
20
21
22
23
24
25
26
27
NUMBER OF DAYS BETWEEN CONSECUTIVE LITTERS

Figure 8. Mean adrenocorticosteroid secretion of Microtus canicaudus in the laboratory (Groups A-H). Vertical lines represent standard errors of the mean.

Experimental Groups

Group A: IH, NG, ♂; individually-housed, no greens, adult males.

Group B: IH, G, ♂; individually-housed, greens, adult males.

Group C: GH, NG, ♂; group-housed, no greens, adult males.

Group D: GH, G, ♂; group-housed, greens, adult males.

Group E: IH, NG, ♀; individually-housed, no greens, adult females.

Group F: IH, G, ♀; individually-housed, greens, adult females.

Group G: GH, NG, ♀; group-housed, no greens, adult females.

Group H: GH, G, ♀; group-housed, greens, adult females.

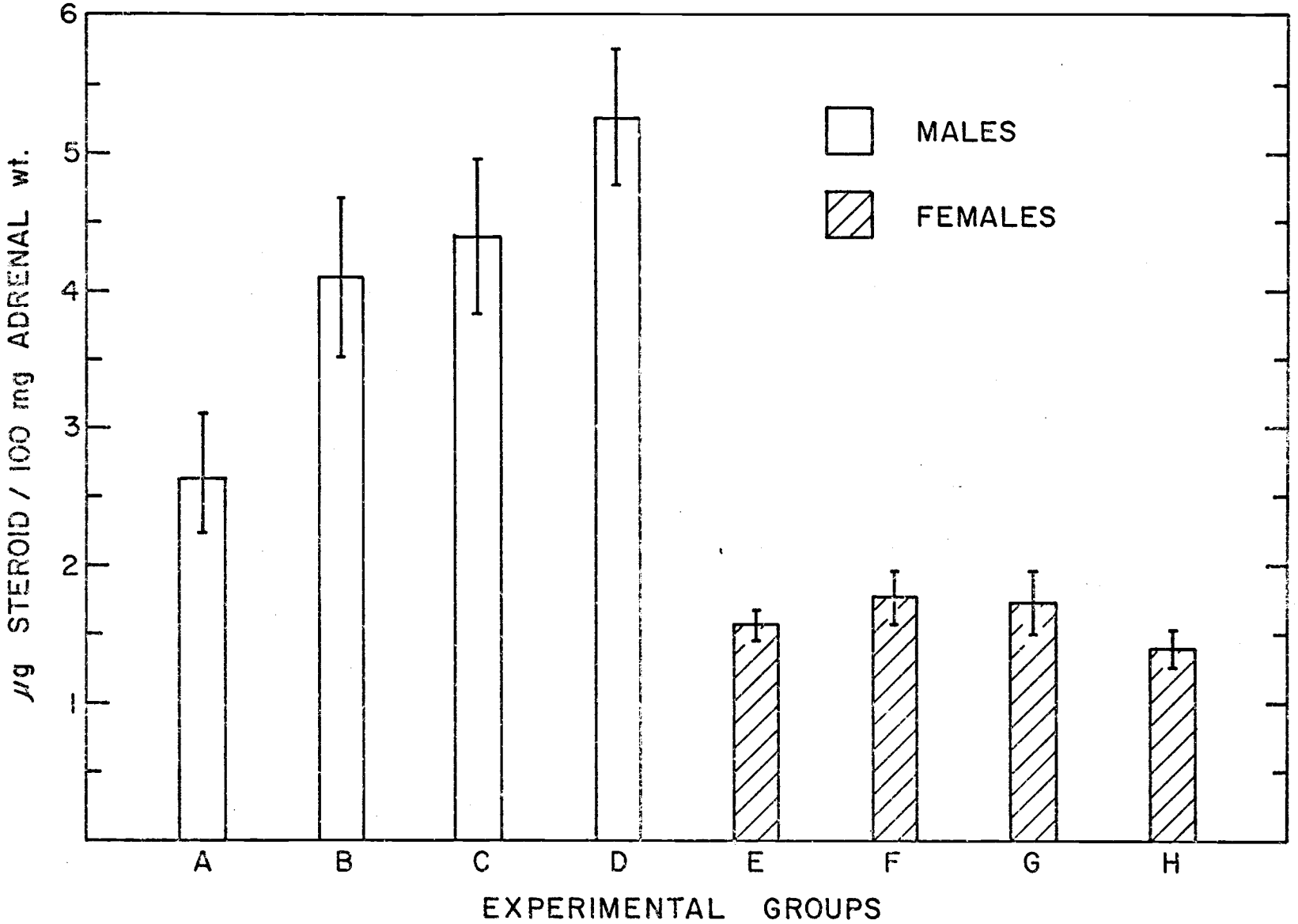
6
5
4
3
2
1
μg STEROID / 100 mg ADRENAL wt.
MALES
FEMALES
A
B
C
D
E
F
G
H
EXPERIMENTAL GROUPS

Figure 9. Mean corticosterone plus cortisol secretion of Microtus canicaudus in the laboratory (Groups A-H). Vertical lines represent standard errors of the mean.

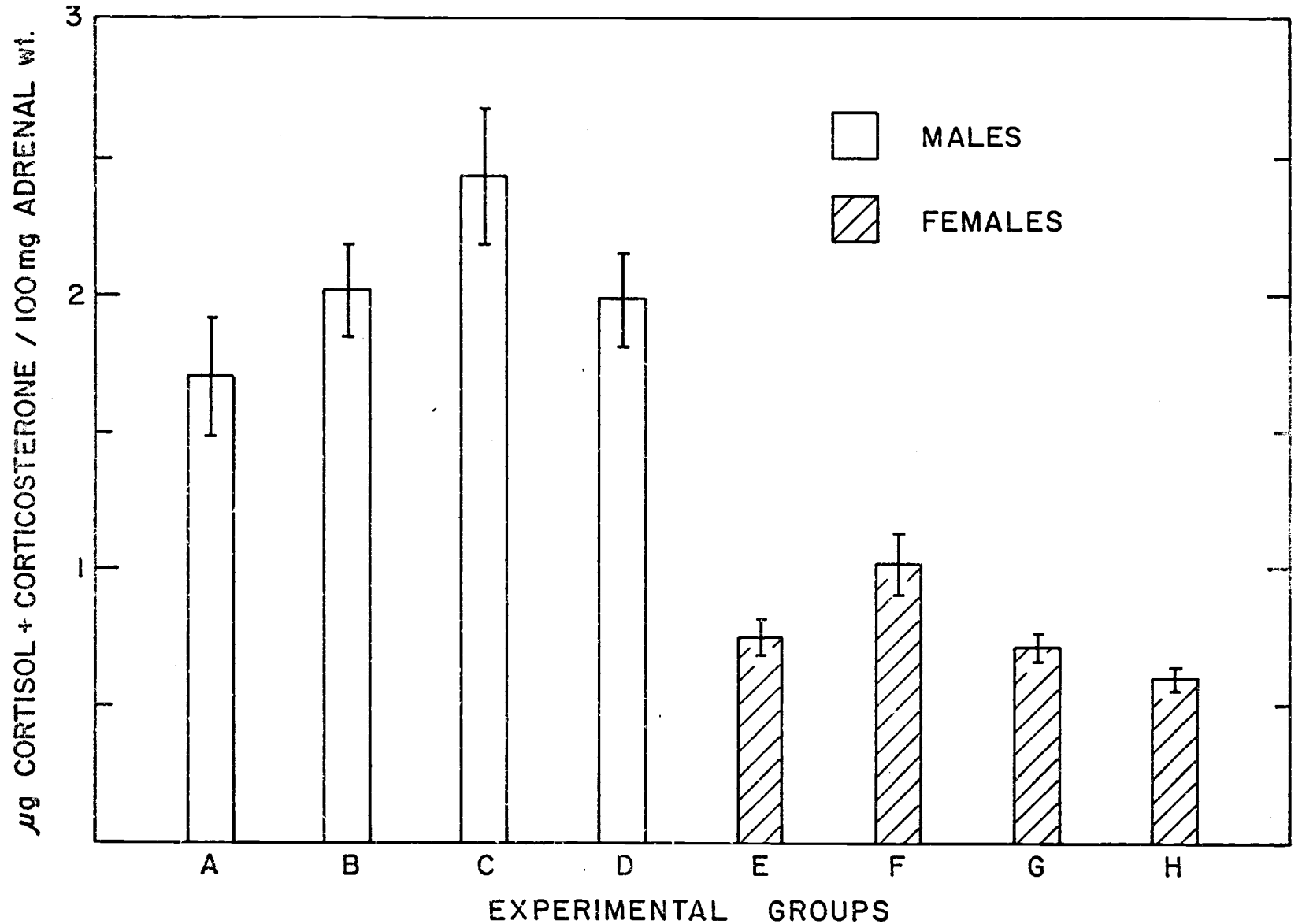
μg CORTISOL + CORTICOSTERONE / 100 mg ADRENAL wt.
3
2
1
MALES
FEMALES
A
B
C
D
E
F
G
H
EXPERIMENTAL GROUPS

Figure 10. Mean progesterone secretion of Microtus canicaudus in in the laboratory (Groups A-H). Vertical lines represent standard errors of the mean.

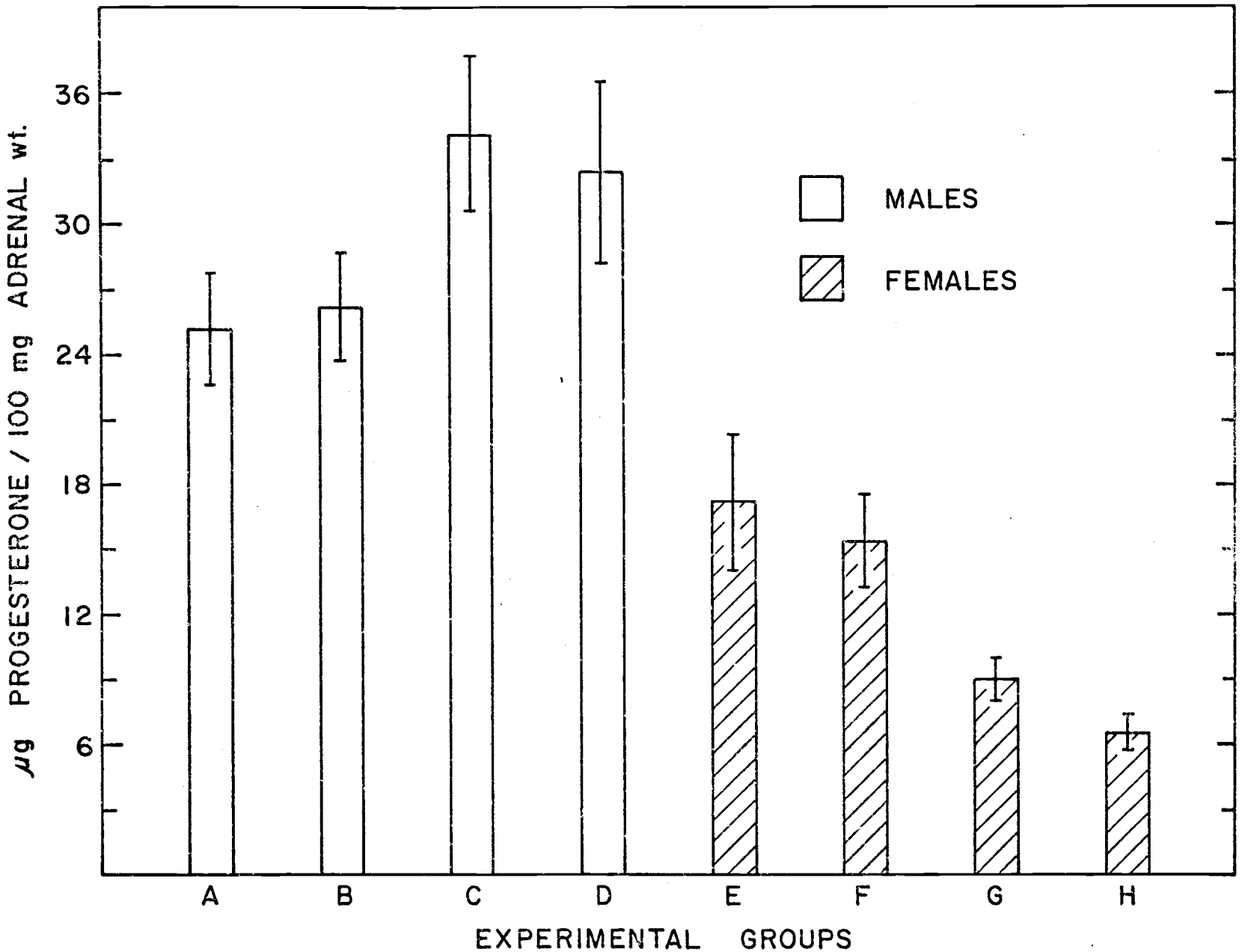
µg PROGESTERONE / 100 mg ADRENAL wt.
36
30
24
18
12
6
MALES
FEMALES
A
B
C
D
E
F
G
H
EXPERIMENTAL GROUPS

Table 1. Consecutive mean litter sizes of adult Microtus canicaudus in the laboratory.

Litters	N	Litter Size*	Range
1	81	4.23 ± 0.20	2-6
2	60	5.10 ± 0.20	4-7
3	51	5.73 ± 0.26	4-8
4	33	5.85 ± 0.40	4-8
5	28	6.35 ± 0.61	3-11
6	24	6.33 ± 0.63	3-9
7	17	6.05 ± 0.46	5-8
8	11	6.00 ± 1.08	3-9
9	3	6.66 ± 2.87	6-8

*All values = $\bar{x}$ ± 95% confidence limits.

Table 2. Mean 100-day reproductive performance of Microtus canicaudus in the laboratory.

N	Active ♀ (100 days of mated life)		
	No. of Litters*	No. of Offspring*	Litter Size*
60	3.19 ± 0.20	16.26 ± 1.31	5.10 ± 0.28

* $\bar{x} \pm$ 95% confidence limits.

Table 3. Summary of adrenal weights of Microtus canicaudus (Groups A-H).

Experimental Group	N	Body Wt.* (g)	Paired Ad. Wt.* (mg - wet)	Ad. Wt. / Body Wt. (mg)* (100 g)
Group A (IH, NG, ♂)	25	33.15 ± 1.47	6.44 ± 0.76	19.92 ± 2.99
Group B (IH, G, ♂)	25	33.41 ± 1.58	6.30 ± 1.08	18.39 ± 2.84
Group C (GH, NG, ♂)	25	33.61 ± 1.47	5.37 ± 0.64	16.58 ± 2.74
Group D (GH, G, ♂)	25	33.08 ± 1.39	5.92 ± 1.09	19.75 ± 4.55
Group E (IH, NG, ♀)	25	24.33 ± 0.87	15.50 ± 1.86	62.63 ± 6.52
Group F (IH, G, ♀)	25	23.36 ± 1.24	13.34 ± 0.99	57.67 ± 6.69
Group G (GH, NG, ♀)	25	23.76 ± 0.75	15.18 ± 1.78	64.73 ± 8.20
Group H (GH, G, ♀)	25	23.96 ± 1.05	14.61 ± 1.96	63.05 ± 10.54

*All values = $\bar{x}$ ± 95% confidence limits.

Table 4. Summary of adrenal weights of female Microtus canicaudus after separating estrous[a] and non-estrous animals.

Reproductive Condition	Experimental Group	N	Body Wt.* (g)	Paired Ad. Wt.* (mg - fixed)	Ad. Wt. (mg) / Body Wt. (100 g)
Estrous	Group E (IH, NG, ♀)	6	24.75 ± 3.14	18.63 ± 5.12	74.77 ± 15.78
	Group F (IH, G ♀)	10	25.45 ± 3.19	13.38 ± 1.38	54.03 ± 8.71
	Group G (GH, NG, ♀)	9	24.55 ± 2.35	14.11 ± 3.09	58.52 ± 14.94
	Group H (GH, G, ♀)	12	24.20 ± 2.20	14.35 ± 2.05	61.34 ± 12.37
Non-estrous	Group E (IH, G, ♀)	19	24.78 ± 1.28	14.51 ± 1.45	58.79 ± 5.29
	Group F (IH, NG, ♀)	15	23.00 ± 2.55	13.32 ± 1.20	60.09 ± 8.30
	Group G (GH, NG, ♀)	16	22.96 ± 0.98	15.78 ± 1.87	68.99 ± 8.25
	Group H (GH, G, ♀)	13	23.42 ± 1.85	14.86 ± 2.94	64.64 ± 15.01

[a] Estrous condition determined by presence or absence of perforate vagina.

* All values = $\bar{x}$ ± 95% confidence limits.

Table 5. Summary of testes and reproductive tract weights of Microtus canicaudus (Groups A-H).

Experimental Group	N	Body Wt.* (g)	Testes-Rep. Tract Wt.* (mg - fixed)	Testes or R.T.*(mg) / Body Wt. (100 g)	% Estrous
Group A (IH, NG, ♂)	40	33.15 ± 1.47	214.47 ± 12.29	651.03 ± 33.38	-
Group B (IH, G, ♂)	40	33.41 ± 1.58	241.54 ± 10.95	732.70 ± 38.43	-
Group C (GH, NG, ♂)	40	33.61 ± 1.47	226.20 ± 12.67	681.65 ± 41.51	-
Group D (GH, G, ♂)	40	33.08 ± 1.39	222.01 ± 9.82	676.75 ± 29.68	-
Group E (IH, NG, ♀)	40	24.33 ± 0.87	28.50 ± 5.01	117.07 ± 19.76	30.0
Group F (IH, G, ♀)	40	23.36 ± 1.24	29.80 ± 4.71	127.63 ± 19.79	45.0
Group G (GH, NG, ♀)	40	23.76 ± 0.75	29.24 ± 4.26	124.10 ± 18.69	27.5
Group H (GH, G, ♀)	40	23.96 ± 1.05	25.60 ± 3.67	108.53 ± 14.91	45.0

*All values = $\bar{x}$ ± 95% confidence limits.

Table 6. Summary of reproductive tract weights of female Microtus canicaudus after separating estrous[a] and non-estrous animals.

Reproductive Condition	Experimental Group	N	Body Wt.* (g)	Rep. Tract Wt.* (mg - fixed)	Rep. Tract Wt.* / Body Wt. (mg) (100 g)
Estrous	Group E (IH, NG, ♀)	6	24.75 ± 3.14	42.81 ± 24.65	169.22 ± 90.61
	Group F (IH, G, ♀)	10	24.45 ± 3.19	31.93 ± 7.44	126.92 ± 30.58
	Group G (GH, NG, ♀)	9	24.55 ± 2.35	35.91 ± 13.05	144.71 ± 50.71
	Group H (GH, G, ♀)	12	24.20 ± 2.20	26.61 ± 6.29	111.02 ± 24.15
Non-estrous	Group E (IH, NG, ♀)	19	24.78 ± 1.28	22.24 ± 2.81	90.94 ± 11.93
	Group F (IH, G, ♀)	15	23.00 ± 2.55	22.90 ± 6.11	99.07 ± 24.10
	Group G (GH, NG, ♀)	16	22.96 ± 0.98	26.02 ± 3.03	115.48 ± 31.60
	Group H (GH, G, ♀)	13	23.42 ± 1.85	22.89 ± 2.55	101.25 ± 29.59

[a]Estrous condition determined by presence or absence of perforate vagina.

*All values = $\bar{x}$ ± 95% confidence limits.

Table 7. Summary of total[a] adrenocorticosteroid production of Microtus canicaudus (Groups A-H).

Experimental Group	N	Body Wt.* (g)	Paired Ad. Wt.* (mg - wet)	Corticoid* / Ad. Wt. (μg / 100 mg)
Group A (IH, NG, ♂)	13	32.76 ± 3.07	5.46 ± 1.26	2.65 ± 0.85
Group B (IH, G, ♂)	14	31.85 ± 3.05	4.84 ± 0.65	4.09 ± 1.27
Group C (GH, NG, ♂)	15	34.06 ± 2.85	3.86 ± 0.60	4.39 ± 1.20
Group D (GH, G, ♂)	14	35.39 ± 1.64	3.72 ± 0.43	5.26 ± 1.08
Group E (IH, NG, ♀)	14	23.11 ± 1.04	12.77 ± 1.47	1.58 ± 0.24
Group F (IH, G, ♀)	13	22.19 ± 1.13	12.91 ± 3.16	1.77 ± 0.44
Group G (GH, NG, ♀)	15	24.13 ± 1.20	13.58 ± 1.54	1.75 ± 0.49
Group H (GH, G, ♀)	15	24.23 ± 1.89	14.06 ± 1.29	1.38 ± 0.32

[a]Total steroid includes corticosterone, cortisol, and cholesterol, etc.

*All values = $\bar{x}$ ± 95% confidence limits.

Table 8. Summary of corticosterone plus cortisol secretion of Microtus canicaudus (Groups A-H).

Experimental Group	N	Body Wt.* (g)	Ad. Wt.* (mg - wet)	Corticosterone + Cortisol* (μg) Ad. Wt.	(100 mg)
Group A (IH, NG, ♂)	15	32.90 ± 2.62	5.23 ± 1.09	1.70 ± 0.47	
Group B (IH, G, ♂)	14	32.46 ± 2.89	5.05 ± 0.73	2.02 ± 0.36	
Group C (GH, NG, ♂)	15	34.06 ± 2.85	3.86 ± 0.60	2.44 ± 0.53	
Group D (GH, G, ♂)	14	35.39 ± 1.64	3.72 ± 0.43	1.99 ± 0.37	
Group E (IH, NG, ♀)	13	23.76 ± 1.61	12.83 ± 1.48	0.75 ± 0.15	
Group F (IH, G, ♀)	14	22.17 ± 1.04	12.89 ± 2.89	1.02 ± 0.24	
Group G (GH, NG, ♀)	15	24.13 ± 1.20	13.58 ± 1.54	0.71 ± 0.10	
Group H (GH, G, ♀)	15	24.23 ± 1.89	14.06 ± 1.29	0.60 ± 0.08	

*All values = $\bar{x}$ ± 95% confidence limits.

Table 9. Summary of progesterone secretion of Microtus canicaudus (Groups A-H).

Experimental Group	N	Body Wt.* (g)	Ad. Wt.* (mg - wet)	Progesterone* (μg) Ad. Wt.	(100 mg)
Group A (IH, NG, ♂)	11	34.68 ± 3.19	5.16 ± 1.45	25.24 ± 5.88	
Group B (IH, G, ♂)	13	31.73 ± 3.15	5.10 ± 0.78	26.21 ± 5.51	
Group C (GH, NG, ♂)	15	34.06 ± 2.85	3.86 ± 0.60	34.14 ± 8.02	
Group D (GH, G, ♂)	8	35.37 ± 2.31	3.76 ± 0.66	32.50 ± 9.81	
Group E (IH, NG, ♀)	8	25.12 ± 2.06	13.26 ± 1.37	17.27 ± 7.61	
Group F (IH, G, ♀)	14	22.42 ± 1.08	13.08 ± 2.94	15.44 ± 4.02	
Group G (GH, NG, ♀)	13	23.76 ± 1.24	13.39 ± 1.76	8.97 ± 7.58	
Group H (GH, G, ♀)	14	24.39 ± 2.01	14.21 ± 1.34	6.60 ± 1.75	

*All values = $\bar{x}$ ± 95% confidence limits.

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