THE UNIVERSITY OF MANITOBA

SEASONAL CHANGES IN MASS AND COMPOSITION OF BROWN AND WHITE ADIPOSE TISSUES AND IN THE PATTERN OF GROWTH OF *CLETHRIONOMYS GAPPERI* AND *MICROTUS PENNSYLVANICUS*

BY

MARIA JOSEFINA PRIETO ANDERSON

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ABSTRACT

Total mass and gross composition of brown and white adipose tissues of C. gapperi and M. pennsylvanicus were determined for one year from samples of animals trapped in their natural habitat. Mean body weight, total length, and skeletal muscle mass were also obtained.

Winter-acclimatization in both species was characterized by a marked increase in mass of brown and white adipose tissues. The increase in brown fat mass started in late summer; tissue mass reached a maximum in winter and declined in spring. White fat showed a similar trend, except that the increase in mass did not start until early winter.

Changes in gross composition of adipose tissues (percentages of lipid, water, and protein) did not show a marked seasonal trend and were caused mainly by variations in lipid and water content.

White fat of pregnant or lactating females had a lower lipid and higher water and protein percentages than non-pregnant, non-lactating females. Gestation and lactation did not alter the percentage composition of brown fat.

Lipid reserves in both species were higher in winter than in summer. C. gapperi had more lipids stored in brown than in white fat for most of the year, whereas M. pennsylvanicus had similar amounts in summer and fall, but had more

white than brown fat lipids during the early part of the year.

Mean body weight, total length, and skeletal muscle mass of monthly samples of both species were lower in fall and winter than in spring and summer. The smaller average size during the cold seasons was caused by an increased number of young animals in the monthly samples. Mean total length and skeletal muscle mass of young voles remained relatively unchanged in fall and winter, indicating a reduction in growth during the cold seasons.

Increased mass and lipid reserves of brown and white adipose tissues, as well as a decrease in the energy expenditure for growth, are likely to be important factors in the survival of C. gapperi and M. pennsylvanicus in winter.

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INTRODUCTION

Small mammals incapable of hibernation make various behavioral and physiological adjustments to survive severe winters in northern regions. These adjustments reduce energy expenditure for certain activities, thus providing additional energy for thermoregulation.

A common strategy employed by small mammals in regions with a permanent snow cover in winter is utilization of the subnivean microclimate. The snow cover insulates against severe cold, providing a warmer, more stable environment than that found above the snow (Formozov, 1969).

Despite the stability of their microclimate, subnivean mammals contend with periods of thermal stress. One such period occurs in autumn, after ambient temperature has started to decline and before the snow cover reaches a depth of 15-20 cm. During this interval, the fall critical period, mammals in the subnivean environment may be subjected to relatively low, widely fluctuating temperatures (Pruitt, 1957). Once the snow cover has reached a depth of 15-20 cm, temperatures in the subnivean microclimate vary over a much narrower range than on the surface of the snow (Pruitt, 1957). Similarly, a spring critical period, corresponding to the transition between annual snow-cover and snow-free periods, has been recognized by Fuller (1967). During this interval, the insulating

effect of the snow cover may be comparatively low when melting occurs during the day and refreezing at night. Consequently, subnivean animals may be exposed to great thermal stress until the snow cover has completely disappeared and the soil begins to warm up. Moreover, melting snow may flood nests, runways, and burrows, forcing animals to abandon their microhabitat, exposing themselves to fluctuating surface temperatures (Fuller, 1967).

Although fall and spring critical periods are the most likely to impose thermal stress on small mammals, winter conditions are also potentially damaging since subnivean temperatures can never be entirely independent of the thermal gradient through the snow (Fuller et al., 1969). Thus, conditions in the subnivean environment are far from favorable at all times, so that the utilization of a microhabitat must be complemented by other adjustments if the animals are to survive harsh winter conditions.

Adaptive changes in mammals in cold climates are of two principal types: those which result in a decrease in heat loss to the environment, and those which result in an increased ability for heat production (Hart, 1964).

Behavioral adjustments such as the utilization of nests are significant in decreasing heat loss, thus providing for an economy in energy expenditure. This has been demonstrated in warm-acclimated northern white-footed mice, Peromyscus leucopus noveboracensis, in which the O_2 consumption at $5^{o}C$ was 35% lower in animals supplied with nests as compared to those

without nests (Glaser and Lustik, 1957). Moreover, it has also been shown that whereas winter-acclimatized P. l. noveboracensis immediately start building nests when placed in the cold, summer-acclimatized animals do not respond as rapidly, and construct relatively poor nests when compared to their winter-acclimatized counterparts (Sealander, 1952).

A decrease in heat loss and hence in energy expenditure for thermoregulation can also be achieved by huddling. The metabolic rate of the harvest mouse, Reithrodontomys megalotis, at a temperature of 1°C is 28% lower when three mice are huddled than in single individuals (Pearson, 1960). In warm-acclimated P. leucopus, and in two subspecies of deer mice, P. maniculatus bairdii, and P. maniculatus austerus, the survival time of individual mice at -23°C is approximately doubled when two animals are huddled (Sealander, 1952). Similar effects of social aggregation on heat production and resistance to cold have been shown in the bank vole, Clethrionomys glareolus (Gebczynski, 1969), and in the yellow-necked field mouse, Apodemus flavicolis (Fedyk, 1971).

Behavioral responses such as nest-building and huddling may considerably protect small mammals against the cold, but there are times when they must leave their nests and burrows in search of food. During these times they become exposed to unfavorable conditions, but such exposure may be lessened by seasonal changes in activity patterns. Such is the case in the field vole, Microtus agrestis, which exhibits a reversal from mostly nocturnal activity in summer to diurnal activity

in winter (Erkinaro, 1961). Some species adjust to cold by decreasing the total amount of daily activity. This has been observed in red-backed voles, Clethrionomys gapperi, which at low ambient temperatures (-8 to -18°C) reduce both their diurnal and nocturnal activity (Getz, 1968). Similarly, daily activity in P. maniculatus, and in the northern red-backed vole, C. rutilus, is lower in winter than in summer (Stebbins, 1971; 1972).

In addition to these behavioral adjustments, structural changes such as increased fur insulation also result in decreased heat loss. Seasonal changes in fur insulation in voles have been reported for C. rutilus (Sealander, 1972), and for the prairie vole, Microtus ochrogaster (Cherry and Verner, 1975). Reduced heat loss due to increased insulating value of the fur is relatively more important in large than in small mammals since, due to their large surface area : volume ratio; the latter are not able to increase their pelage to the same extent as the former (Scholander et al., 1950).

The fall-winter decrease in growth rate, which has been observed in many northern populations of small mammals, is believed to be another winter survival strategy whereby the economy in energy expenditure for cell proliferation and growth may result in greater availability of energy for thermoregulation (Sealander, 1966; Fuller, 1969; Iverson and Turner, 1974; Stebbins, 1976, 1978). In addition, cold-acclimated laboratory rats have a lower body weight than control animals of the same age, and this is mainly due to a lower muscle mass (Heroux,

1958). Reduction in muscle growth due to cold has been further evidenced by a decrease in DNA synthesis in skeletal muscle of cold-acclimated laboratory rats, when compared to warm-acclimated rats (Nusetti and Aleksiuk, 1975), and in winter-acclimatized meadow voles, Microtus pennsylvanicus, when compared to summer-acclimatized voles (Narayansingh and Aleksiuk, 1971).

In addition to the energy-saving mechanisms so far described, cold-acclimation and cold-acclimatization in small non-hibernators also involve an increased ability for metabolic heat production. Thus the maximum metabolic rate at low ambient temperatures is higher in laboratory rats acclimated to 6°C than in those acclimated to 30°C (Héroux et al., 1959). Similarly, the metabolic capacity of C. rutilus increases markedly during the cold seasons of the year (Rosenmann et al., 1975).

Increased heat production in response to low ambient temperatures occurs either by shivering or non-shivering thermogenesis. The former process consists of an increase in metabolic rate produced by muscular contractions; the latter involves an increase in metabolic rate produced by mechanisms other than muscular contractions (Jansky, 1973). Shivering is regarded as an emergency mechanism which, due to its high energy cost, cannot be sustained for long periods (Hemingway, 1963). Laboratory experiments have shown that suppression of shivering by drugs such as curare produce a 60% reduction in cold-induced O_2-uptake of warm-acclimated rats; no such reduction occurs in cold-acclimated rats (Davis et al., 1960). The process of cold-acclimation, therefore, involves the

substitution of shivering by non-shivering thermogenesis (Jansky, 1973).

Increased metabolic heat production is associated with an increase in lipid utilization. The annual cycle of lipid deposition and depletion exhibited by several species of small nonhibernators has been regarded as a physiological adjustment whereby increased lipid reserves in winter may serve to support increased metabolic heat production. Early studies on the body composition of P. leucopus have shown that animals have a higher fat content in winter than in summer (Sealander, 1951). This pattern of increased energy reserves in winter was also demonstrated in A. flavicolis (Sawicka-Kapusta, 1968), and in C. glareolus (Fedyk, 1977). Not all species investigated conform to this general pattern, however, and factors other than increased energy requirements imposed by low ambient temperatures may also influence the seasonal pattern of lipid deposition and depletion in small nonhibernators. In an investigation of lipid reserves in four species of rodents with overlapping geographic ranges, Fleharty et al. (1973) have shown that, whereas P. maniculatus and R. megalotis exhibited the usual pattern of high lipid reserves in winter and low reserves in summer, the hispid cotton rat, Sigmodon hispidus, differed in that the lowest reserves were attained in spring; no seasonal pattern of fat deposition was evident in M. ochrogaster, although their fat content fluctuated on a monthly basis. These interspecific differences were attributed by the investigators to differences in feeding habits and in the zoogeographical

history of the four species. Hayward (1965) has also shown that five out of six races of P. maniculatus from different geographic regions had more fat in winter than in summer; only the desert race, P. m. sonoriensis, accumulated more fat in summer than in winter. Other factors which have been shown to influence the lipid content on small nonhibernators are breeding activity (Caldwell and Connell, 1968), age (Sawicka-Kapusta, 1974), age and season of birth (Fedyk, 1974), and physiological and reproductive condition of the individuals (Evans, 1973).

Lipid storage in small mammals occurs mainly in brown and white adipose tissues. White fat serves essentially as a fuel reserve and its lipids are drawn upon whenever energy expenditure exceeds energy intake (Masoro, 1968). This fuel-supplying role of white fat is of particular significance during shivering, a process which requires rapid mobilization of free-fatty acids to support the increase in metabolic rate (Himms-Hagen, 1972). The role of brown fat, on the other hand, is essentially that of heat production (Smith and Horowitz, 1969), and its lipid reserves are used locally to support the rise in metabolic rate associated with thermogenesis in this tissue (Himms-Hagen, 1972).

It is reasonable to assume that seasonal changes in the total lipid content of small nonhibernators would involve changes in either one or in both of the two adipose tissues. This assumption is partly substantiated by the finding that brown fat mass increases as the need for extra heat production becomes greater with declining ambient temperatures in autumn

and winter. Such an inverse relationship between environmental temperature and the mass of the interscapular brown fat depot has been demonstrated in the common shrew, Sorex araneus (Buchalkzyk and Korybska, 1964; Hissa and Tarkkonen, 1969), in C. rutilus (Sealander, 1972), in P. leucopus (Lynch, 1973), and in the muskrat, Ondatra zybethica (Aleksiuk and Frohlinger, 1971). A similar inverse relationship has been demonstrated for the axillary brown fat depot of the red squirrel, Tamiasciurus hudsonicus (Aleksiuk, 1971), and for the total brown fat mass of M. pennsylvanicus (Didow and Hayward, 1969).

An investigation of the composition of brown fat in M. pennsylvanicus did not reveal significant seasonal changes in the percentages of lipid, water, and protein (Didow and Hayward, 1969), indicating that changes in mass were due to equivalent changes in these three basic components. Fall and winter increases in the percentage of lipids in brown fat have been demonstrated, however, in T. hudsonicus (Aleksiuk, 1971), and in O. zybethica (Aleksiuk and Frohlinger, 1971).

While seasonal changes in mass and composition of brown adipose tissue in small nonhibernators are well documented, similar investigations on white adipose tissue have not been conducted. The one notable exception is the study by Sealander (1972) on C. rutilus in which seasonal variations in the amounts of both types of adipose tissues were reported. However, whereas amounts of brown fat in C. rutilus were determined by removing and weighing this tissue, amounts of white fat were estimated visually, by assigning index numbers accord-

ing to the relative abundance of subcutaneous and visceral white fat (Sealander, 1972). Therefore, no quantitative information is available on seasonal variations in mass of white adipose tissue.

The study reported in this thesis was undertaken for the following purposes:

1. To describe and compare seasonal changes in total mass of brown and white adipose tissues in two species of rodents which remain active throughout the year, the red-backed vole, Clethrionomys gapperi, and the meadow vole, Microtus pennsylvanicus.

2. To determine whether changes in mass of these lipid-storing tissues are accompanied by changes in the proportions of the three basic components: water, lipid, and protein.

3. To describe and compare seasonal changes in the amount of lipids stored in brown and white adipose tissues, and to relate differences in the annual pattern of change in the lipid reserves of brown and white fat to the different physiological roles of these tissues.

4. To describe the pattern of seasonal growth in both species by determination of body weight, total length, and total skeletal muscle mass, and to determine whether seasonal changes in muscle growth are associated with changes in the gross composition of this tissue.

MATERIALS AND METHODS

1. Trapping and Care of Animals

Trapping was conducted from May, 1976 to May, 1977, within a radius of approximately 5 miles of the University of Manitoba campus in Winnipeg (50°10'N; 97°07'W). As there were very few captures in this area during the winter, additional animals were collected approximately 70 miles west of Winnipeg, at the University of Manitoba Field Station, Delta Marsh (50°11'N; 98°19'W), from January to May, 1977.

A total of 148 C. gapperi and 101 M. pennsylvanicus were live-trapped in Sherman traps, baited with a mixture of peanut butter and oatmeal, and provided with nesting material (Terylene, 100% polyester fiber). Because live captures in Sherman traps were very few in December, snap-trapping with Museum Special traps was also used that month. To improve chances of capture, snap-traps were set 24 hours a day and checked in the evening and in the morning. Seven C. gapperi were snap-trapped in December, but no M. pennsylvanicus were captured by this method. Snap-trapping was not used at any other time during this study.

In late spring and summer, traps were set at about 5 P.M. and checked at 8 and 10 P.M. If there were no captures during that period, traps were left open overnight and checked again at about 6 A.M. Usually enough animals were trapped in

the evening and overnight during this time of the year, and no trapping was necessary during the hot hours of the day, thus eliminating the possibility of animals being subjected to heat-stress in the traps.

Trapping was conducted between 8 A.M. and 5 P.M. in the fall, winter, and early spring, but when no captures occurred during this interval, traps were left open as late as 11 P.M. During this time of the year the traps were checked at intervals of approximately 1 hour to minimize the danger of cold-exposure and depletion of fat stores while the animals were in the traps. Care was taken to ensure that traps had plenty of nesting material and food.

In general, 2-4 animals were collected daily and brought to the laboratory. This criterion was adopted to ensure that required dissections could be performed within 24 hours of capture, thus avoiding animals becoming acclimated to indoor conditions. Captures in excess of 4 were released in the trapping area. Changes from this routine were necessary when trapping was conducted at the Delta Marsh Field Station. On those occasions as many animals as possible were collected in 2-3 days, and transported to the laboratory in Winnipeg for dissection and tissue analysis. The length of captivity in the laboratory did not usually exceed 12 hours for animals collected at the University of Manitoba area, while it varied from 1 to 4 days for those captured at the Field Station at Delta Marsh.

In the laboratory the animals were housed singly in plastic cages, at room temperature, and provided with water and food (Wayne Lab Blox-F6, Allied Mills Inc.) ad libitum.

As it was established in the beginning of the study that 20-30 animals could be conveniently dissected and analyzed each month, a monthly sample size of 10-15 animals of each of the two species was decided upon. Captures were infrequent in late fall and winter, however, and even intensive efforts yielded only a few animals. No M. pennsylvanicus were captured in December, and only 3 and 4 in January and February, respectively. Only 3 C. gapperi were live-trapped in December, in addition to 7 which were snap-trapped that month. M. pennsylvanicus were less numerous than C. gapperi in both trapping areas, and monthly samples of the former were smaller than those of the latter in all months, except in May, 1976, when equal numbers (7) of each species were caught.

2. Dissections

Dissections were performed to isolate tissues for mass determinations, and to obtain samples for determination of the gross composition (water, lipid, and protein content) of brown fat, white fat, and skeletal muscle.

All dissections were performed with the aid of a standard dissecting microscope. With the exception of the pelt, carcass (muscle and bone), gastrointestinal tract, and tissue samples used for composition analysis (water, lipid, and protein content determination), all excised tissues and organs

were placed in pre-weighed vials containing paraffin oil to prevent drying, and the vials were re-weighed at the end of dissection. The increase in weight was recorded as weight of tissues and organs. The tissue samples which were to be used for composition analysis were placed in pre-weighed containers and weighed immediately after excision.

A. Preparation of animals for dissection

Animals were killed with excess ether and weighed immediately after death to obtain total body weight (TBW). Linear dimensions (tail, hind foot, and total length) were then measured and recorded. The thoracic cavity was cut open, and approximately 2 ml of an aqueous heparin solution (1,000 I.U. /1 ℓ) was injected into the heart to prevent blood clotting. The heart and lungs were excised, and the blood which drained into the thoracic cavity was removed with a syringe. The specimens were re-weighed immediately after this, and the decrease in weight was recorded as the weight of excised organs and blood. Draining of the blood was performed to prevent weight loss due to probable loss of blood during dissection, and also to facilitate dissection of tissues.

Sexual maturity of individuals was assessed by examination of reproductive organs during dissection. The presence of placental scars, mature follicles, scrotal testis, and tubules in the epididymis were taken as indicative of sexual maturity.

B. Weight of gastrointestinal contents

The weight of ingesta was determined and subtracted from TBW to eliminate variations in body weight due to varying amounts of chyme in the gastrointestinal tract. This was done during dissection, by removing and immediately weighing the stomach and intestines, cutting these organs open, removing their contents, and re-weighing the empty organs. The weight of ingesta was recorded as the difference between the full and empty organs.

The term body weight (BW) in this thesis refers to TBW minus the weight of the gastrointestinal contents.

C. Tissue mass determination

Total mass of brown adipose tissue was determined by dissecting and weighing all brown fat depots, which were located as described for P. maniculatus (Rauch and Hayward, 1969). An estimated 98% of the total brown fat present was dissected as complete removal from the intercostal site was not feasible.

Total mass of white adipose tissue was determined by dissecting and weighing all visible white fat (subcutaneous, visceral, and fat layers between muscles). Layers of white fat were often found overlying brown fat depots, and in these cases care was taken to separate the two tissues as completely as possible.

When all adipose tissues had been excised, the pelt and remaining organs, including brain, were removed and weighed.

The carcass, consisting of muscle and bones only, was weighed, placed in a plastic bag, and stored at -65°C for later determination of total muscle mass.

Total muscle mass was recorded as the difference between carcass wet weight and skeleton wet weight, after the muscle had been digested in an enzyme bath (procedure given in Appendix 1). Approximately one-third of the total number of carcasses of each species (33 M. pennsylvanicus and 44 C. gapperi), with body weights spanning the entire range of body weights, were randomly selected and digested in this manner. Simple linear regression of muscle mass (Y) on carcass weight (X) was then calculated for each species, and the total muscle mass of animals for which muscle digestion was not performed was calculated from the regression equation (Appendices 1A and 1B).

3. Tissue Composition Analysis

Water, lipid, and protein content of tissue samples were determined for each animal dissected from June, 1976 to May, 1977. Tissues used were the interscapular brown fat depot, subcutaneous white fat, and superficial muscles from both thighs. Procedures were tested and standardized using six liver tissue samples from a laboratory mouse. Results of standard determinations are shown in Appendix 2.

A. Water content

Fresh tissue samples were placed in a drying oven (Labline Inc.) at 45-50°C and dried to constant weight (approximately 8 hours). Water content was recorded as the difference between wet and dry tissue weights.

B. Lipid content

Dry tissue samples were placed in micro-extraction thimbles (Fisher Scientific Co.) and extracted with petroleum ether (boiling range 30-60°C) in a Soxhlet apparatus (Fisher Scientific Co.) for 24 hours. As petroleum ether is a neutral fat solvent, only neutral lipids were extracted by this method. Therefore, the term lipids in this thesis refers only to neutral lipids (triglycerides), the main form of fat storage in mammals.

After extraction the tissue samples were placed under a fume hood for 1 hour to allow ether to evaporate. They were then transferred to a drying oven for 2 hours to ensure evaporation of moisture which might have been absorbed from the atmosphere. Tissue samples were then quickly removed from thimbles and weighed, and the decrease in weight was recorded as the lipid content of the sample.

C. Protein content

The amount of protein in each tissue sample was calculated by multiplying its nitrogen content by 6.25, assuming

that mixed proteins contain 16% nitrogen (Munro and Fleck, 1969).

The nitrogen content of the tissue samples was determined by the micro-Kjeldahl method (Association of Official Agricultural Chemists, 1965), with two minor modifications: (1) A ratio of 0.8 g K_2SO_4 : 1 ml 36 N H_2SO_4 was used, as recommended by Munro and Fleck (1969). This permitted using a minimum amount of distilled water to dissolve the salts which formed upon cooling of the digest, while still giving a boiling temperature high enough to allow complete breakdown of nitrogenous compounds in the sample. (2) The mercury catalyst (HgO) was added to the digestion mixture in the form of a solution of 10 g HgO/100 ml 4N H_2SO_4 (MacKenzie and Wallace, 1969), rather than in the less convenient powder form recommended by the A.O.A.C. (1965).

Blank nitrogen determinations (all reagents minus tissue) were run; the mean nitrogen content of the blanks was calculated and subtracted from that of the tissue samples in the final calculations.

Details of the complete micro-Kjeldahl procedure are given in Appendix 3A.

Recovery of nitrogen by the micro-Kjeldahl procedure was tested using a standard urea solution (4.258 g urea/1 ℓ of solution). This solution contained 2 mg of nitrogen per ml. Recovery was 97.56% ± 1.43 (Appendix 3B).

4. Treatment of Data

A. Brown and white adipose tissue mass

The total mass of brown and white adipose tissues is expressed as absolute weights and as percentages of body weight. The latter expression gives a better indication of adjustments made in response to changing requirements for heat production and lipid reserves, respectively; the former serves to indicate whether changes in tissue mass are related to changes in body size.

Monthly mean mass of the interscapular brown fat depot is shown for comparison with previous studies. In addition, the ratio of interscapular depot : total brown fat mass is shown for each month to assess the validity of that depot as an index of total brown fat mass.

B. Lipid reserves

Absolute lipid content of adipose tissues was calculated for each animal from total tissue mass and percentage of lipids in samples analyzed. These calculations assume homogeneous composition of the tissue.

Tissue lipid content is expressed in mg of tissue lipid/g of body weight.

C. Comparison between voles trapped at Winnipeg and at the Delta Field Station

Comparisons between voles captured at these two sites were possible only for March, April and May, 1977, when trap-

ping was successful at both locations.

D. Comparisons between live-trapped and snap-trapped C. gapperi

Data from seven C. gapperi snap-trapped in December were compared with those of three red-backed voles live-trapped during that month (Appendix 4). Significant differences between voles captured by the two methods were found only for brown fat. Therefore, mass and gross composition of brown fat of snap-trapped voles were excluded from the data.

5. Statistical Tests

All data were grouped according to the month in which animals were captured. Monthly means were tested for significant differences by the Student's t-test for unpaired means after homogeneity of variances had been verified by the variance ratio test (Zar, 1974).

When sample sizes are equal or nearly equal, the Student's t-test is robust enough to withstand marked departures from the basic assumption of equality of variances. With unequal sample sizes, however, as was the case in this study, it tends to give too few significant results when the larger sample has the larger variance, and too many when the larger sample has the smaller variance (Boneau, 1960). Thus, to test for significant differences between means when variances were significantly different, the method used in this thesis was Cochran's approximation to the Behrens-

Fisher solution (Snedecor and Cochran, 1967), in which the ordinary t is replaced by t', the prime indicating that the test-statistic does not follow the Student's t-distribution. Cochran's approximation is convenient because it uses the standard t-table, but it is slightly more conservative than the Behrens-Fisher solution in that slightly larger values of t' are required for significance (Snedecor and Cochran, 1967).

The criterion for statistical significance adopted in this thesis is $P < 0.05$.

6. Meteorological Data

Mean monthly temperatures and depth of snow cover were calculated from daily weather records taken at the Winnipeg International Airport. These records were contained in the Monthly Meteorological Summaries supplied by Weather Information, Environment Canada. Comparisons of weather conditions during the study period with long-term normals were also made using information contained in the Meteorological Summaries.

RESULTS

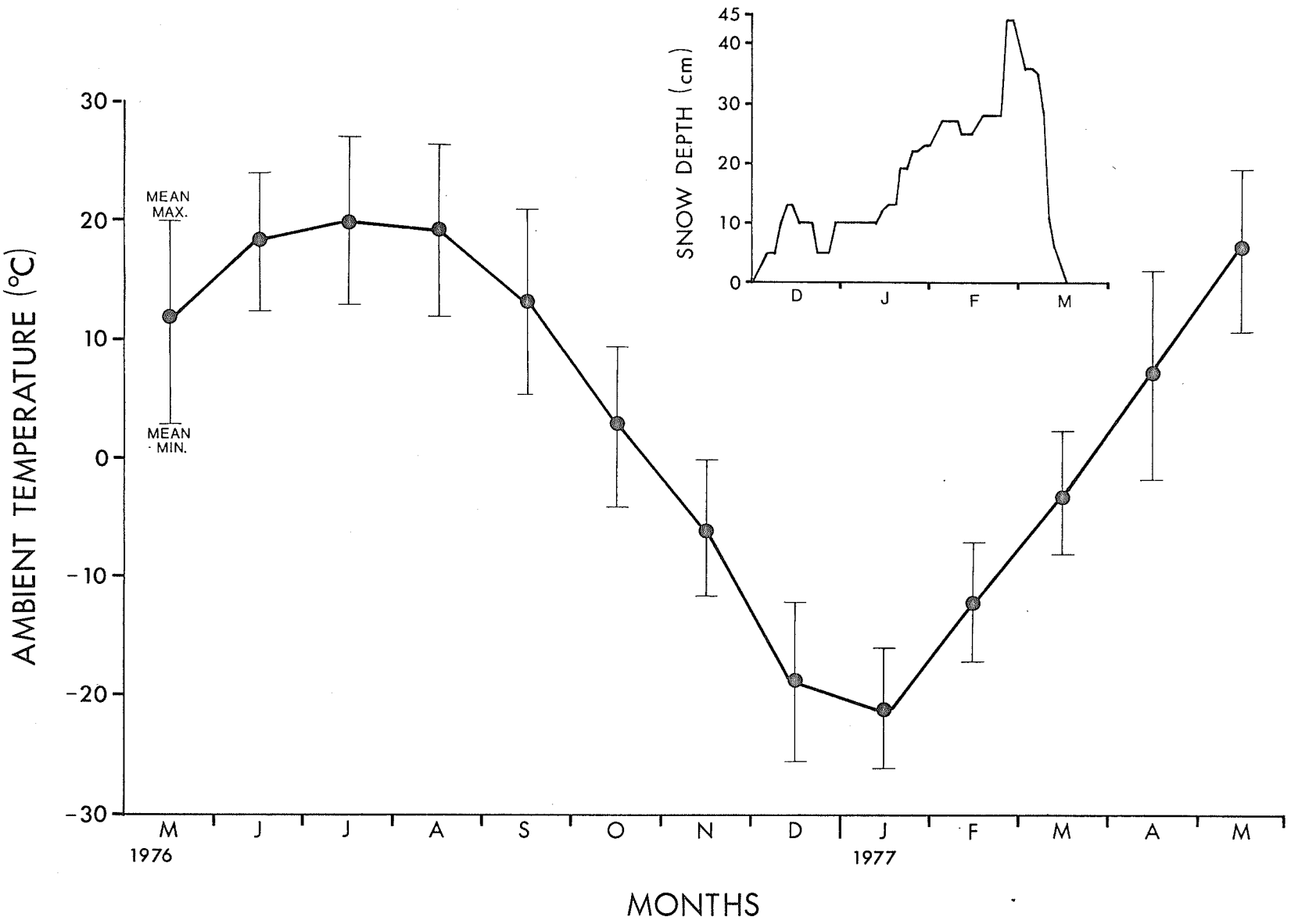
1. Seasonal Changes in Environmental Temperature

The 1976-1977 fall and winter seasons in the Winnipeg area were characterized by lower than average precipitation. The months of October and November were the driest on record, and only one day with measurable snow precipitation (0.8 mm) occurred in November. December had colder than usual nights, and below average snowfall. Overnight low temperatures in December averaged -24.9°C, which was the lowest average since 1933 (Fig. 1). Daily mean temperatures in December were also below average.

Because of lower than normal snowfall in late autumn and early winter, the critical snow depth of 15 cm (hiemal threshold) necessary for effective insulation and stabilization of the subnivean microclimate (Pruitt, 1957) was not achieved until mid-January (Fig. 1). Therefore, it can be said that the fall critical period of 1976-1977 in this area was longer than usual. These conditions, coupled with below average temperatures in December, were likely to impose severe stress on subnivean animals.

In contrast with the harsh weather conditions of early winter, late winter and spring of 1977 were milder than usual. Temperatures in February were, on the average, 4°C higher than the long-term normal for that month, with four

Figure 1. Monthly mean temperatures and depth of snow cover (inset) in Winnipeg, from May, 1976 to May, 1977. Vertical lines indicate monthly mean maximum and minimum temperatures.


AMBIENT TEMPERATURE (°C)
30
20
10
0
-10
-20
-30
MEAN MAX.
MEAN MIN.
SNOW DEPTH (cm)
45
40
30
20
10
0
D
J
F
M
M
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977
MONTHS

days of above-freezing daily maxima. Similarly, March and April monthly mean temperatures were 5 and 7°C warmer than usual, respectively. May temperatures in 1977 were the warmest on record, with a monthly mean 7°C above average, and 2°C higher than the previous warmest May of 1922. By March 15 only a trace of snow remained on the ground, and the snow cover had completely disappeared by March 27.

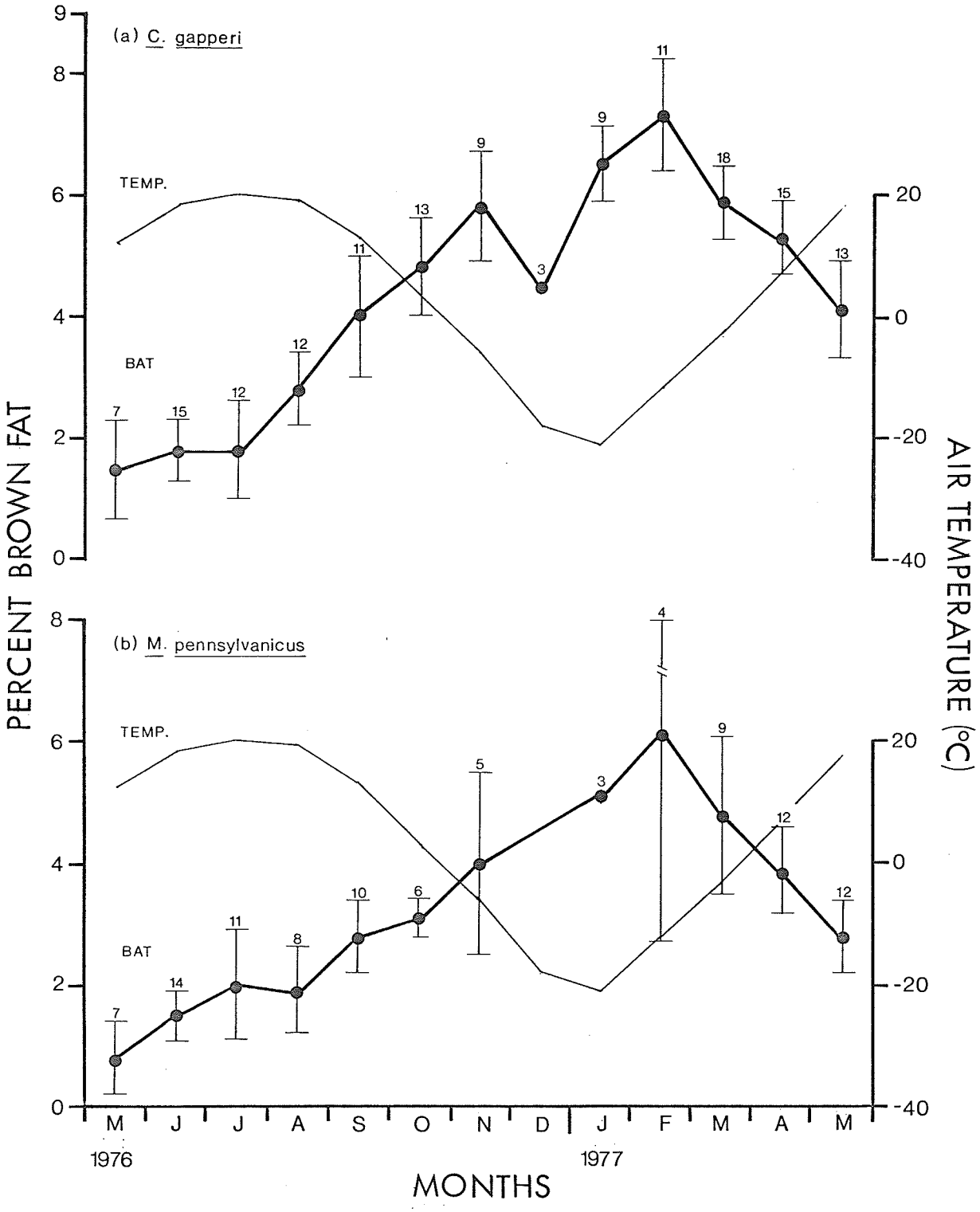
2. Seasonal Changes in Brown Adipose Tissue

A. Changes in mass

Both species of voles exhibited a pattern of seasonal change in total brown fat mass relative to body weight which was inversely related to seasonal changes in ambient temperatures (Fig. 2a and 2b). The pattern was strikingly similar in both species, with the percentage of this tissue being low in the summer, rising throughout the fall and early winter to a maximum in February, and declining from then on.

Monthly mean percentage of brown fat in *C. gapperi* (Fig. 2a) remained at approximately 1.5-1.8% of the body weight from May, 1976 through July, rising to 7% between July and February. This fourfold increase in relative brown fat mass of red-backed voles occurred in a progressive manner throughout the fall and early winter, except for a slight decline in December. From February until the following May the percentage of brown adipose tissue in red-backed voles declined progressively, but it did not reach the low level observed in May of the previous year.

Figure 2. Seasonal changes in brown fat mass as a percentage of body weight in C. gapperi and M. pennsylvanicus. Vertical lines represent the 95% confidence intervals of the means for all samples of 4 or more voles. Sample sizes are shown above the brackets. Lighter curve represents monthly mean ambient temperatures.

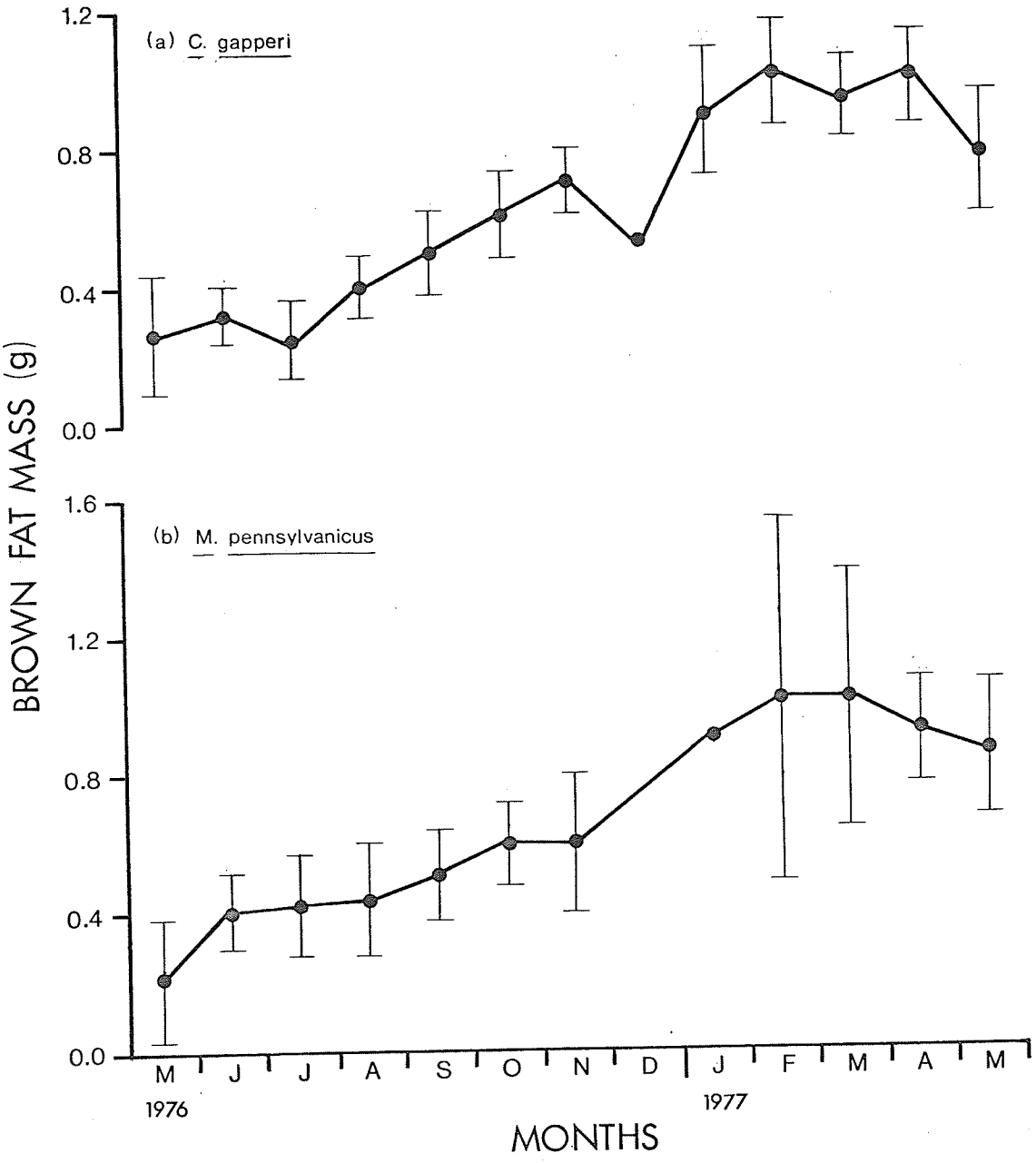

(a) C. gapperi
TEMP.
BAT
(b) M. pennsylvanicus
TEMP.
BAT
PERCENT BROWN FAT
AIR TEMPERATURE (°C)
MONTHS
1976
1977
M J J A S O N D J F M A M
0 2 4 6 8 9
20 0 -20 -40
7 15 12 12 11 13 9 3 9 11 18 15 13
7 14 11 8 10 6 5 3 4 9 12 12

Changes in monthly mean percentage of brown fat in M. pennsylvanicus (Fig. 2b) followed the same general pattern, except that the tissue mass increased from approximately 0.8% to 2% of body weight between May and July, 1976, and showed no changes between July and August. From August to February the percentage of brown adipose tissue in meadow voles increased threefold, from 2 to 6% of body weight. As in C. gapperi (Fig. 2a), there was a decline in the relative mass of this tissue in M. pennsylvanicus from February to May, 1977, but it did not fall to the same level observed in May of the previous year.

Changes in absolute mass of brown adipose tissue in C. gapperi and M. pennsylvanicus are shown in Figures 3a and 3b, respectively. The fall-winter increase in relative mass of this tissue (Fig. 2a and 2b) resulted from increases in absolute mass. However, the progressive decline in mean percentage brown fat from February to May, 1977 (Fig. 2a and 2b) was not accompanied by similarly pronounced decreases in absolute mass (Fig. 3a and 3b), and can be largely attributed to the comparatively large mean body weight of both species trapped in spring (Fig. 9 and 10).

There were no significant differences in mean percentage of brown fat between males and females of either species (Appendices 5A and 6A). Similarly, no significant differences in mean absolute brown fat mass were found between male and female M. pennsylvanicus (Appendix 6B), but male C. gapperi had significantly higher ($P < 0.05$) absolute brown fat mass

Figure 3. Seasonal changes in the absolute mass of brown fat in C. gapperi and M. pennsylvanicus. Vertical lines represent the 95% confidence intervals of the means for all samples of 4 or more voles. Sample sizes are as shown in Figure 2.


(a) C. gapperi
(b) M. pennsylvanicus
BROWN FAT MASS (g)
1.2
0.8
0.4
0.0
1.6
1.2
0.8
0.4
0.0
M J J A S O N D J F M A M
1976
1977
MONTHS

than females in October (Appendix 5B). Nevertheless, data from males and females were pooled when calculating the October mean value shown in Figure 3a, since this did not alter the general pattern of increase in tissue mass over the fall.

The only significant difference between voles captured at Winnipeg and those captured at the Delta Field Station was found in absolute brown fat mass of M. pennsylvanicus in May, 1977 (Table I). It is unlikely, therefore, that differences in January and February, if they existed, would be of such an extent as to alter the pattern of seasonal change in brown fat mass seen in Figures 2 and 3.

Results shown in Table II suggest a decreasing trend in the fraction — mean percent interscapular brown fat/mean percent total brown fat — during the period of intense growth of this tissue in fall and early winter. This may indicate that changes in mass of the interscapular depot are not as pronounced as changes at other sites. This is in agreement with the observation made during this study that whereas animals of both species had a discreet interscapular depot at all times of the year, brown fat in the summer was almost completely absent from sites such as the perirenal and suprarenal areas. Also, both species had a small amount of brown fat in the inguinal region in winter, but not in summer.

Table I. Comparison of total absolute and relative brown fat (BF) mass between voles captured in Winnipeg (WPG), and those captured at the Delta Field Station (DFS), from March to May, 1977.

Species	Month	Area	n	BF Weight (g) $\bar{X}$ (SE)	% BF $\bar{X}$ (SE)
C. gapperi	March	WPG	9	0.94 (0.09)	5.88 (0.41)
		DFS	9	0.96 (0.04)	5.99 (0.27)
	April	WPG	5	0.92 (0.10)	5.04 (0.47)
		DFS	10	1.05 (0.06)	5.35 (0.22)
	May	WPG	6	0.83 (0.07)	4.45 (0.27)
		DFS	7	0.74 (0.12)	3.74 (0.54)
M. pennsylvanicus	March	WPG	2	0.75 (0.14)	3.49 (0.27)
		DFS	7	1.17 (0.18)	5.19 (0.56)
	April	WPG	7	0.84 (0.09)	3.82 (0.36)
		DFS	5	1.03 (0.05)	3.89 (0.43)
	May	WPG	5	0.64 (0.08)	2.37 (0.39)
		DFS	7	1.04 (0.08)**	3.12 (0.28)

** Indicates significant differences ($P < 0.01$) between WPG and DFS voles.

Table II. Seasonal changes in the relative mass of the interscapular brown fat depot (% IBF), and in the ratio — interscapular brown fat : total brown fat mass (% IBF/% TBF) — in C. gapperi and M. pennsylvanicus.

		C. gapperi			M. pennsylvanicus		
		% IBF			% IBF		
Year	Month	n	$\bar{X}$ (SE)	$\frac{\% \text{ IBF}}{\% \text{ TBF}}$	n	$\bar{X}$ (SE)	$\frac{\% \text{ IBF}}{\% \text{ TBF}}$
1976	May	7	0.36 (0.07)	0.25	7	0.20 (0.05)	0.25
1976	Jun	15	0.45 (0.05)	0.25	14	0.39 (0.03)	0.25
1976	Jul	12	0.42 (0.08)	0.23	11	0.43 (0.08)	0.21
1976	Aug	12	0.49 (0.04)	0.18	8	0.46 (0.06)	0.24
1976	Sep	11	0.72 (0.07)	0.18	10	0.58 (0.04)	0.20
1976	Oct	13	0.85 (0.06)	0.18	6	0.51 (0.05)	0.17
1976	Nov	9	0.95 (0.07)	0.16	5	0.77 (0.08)	0.19
1976	Dec	3	0.72 (0.09)	0.16			
1977	Jan	9	1.23 (0.06)	0.19	3	1.01 (0.02)	0.20
1977	Feb	11	1.41 (0.08)	0.19	4	1.10 (0.22)	0.18
1977	Mar	18	0.97 (0.06)	0.16	9	0.86 (0.09)	0.18
1977	Apr	15	0.92 (0.06)	0.18	12	0.68 (0.06)	0.18
1977	May	13	0.66 (0.06)	0.16	12	0.60 (0.06)	0.21

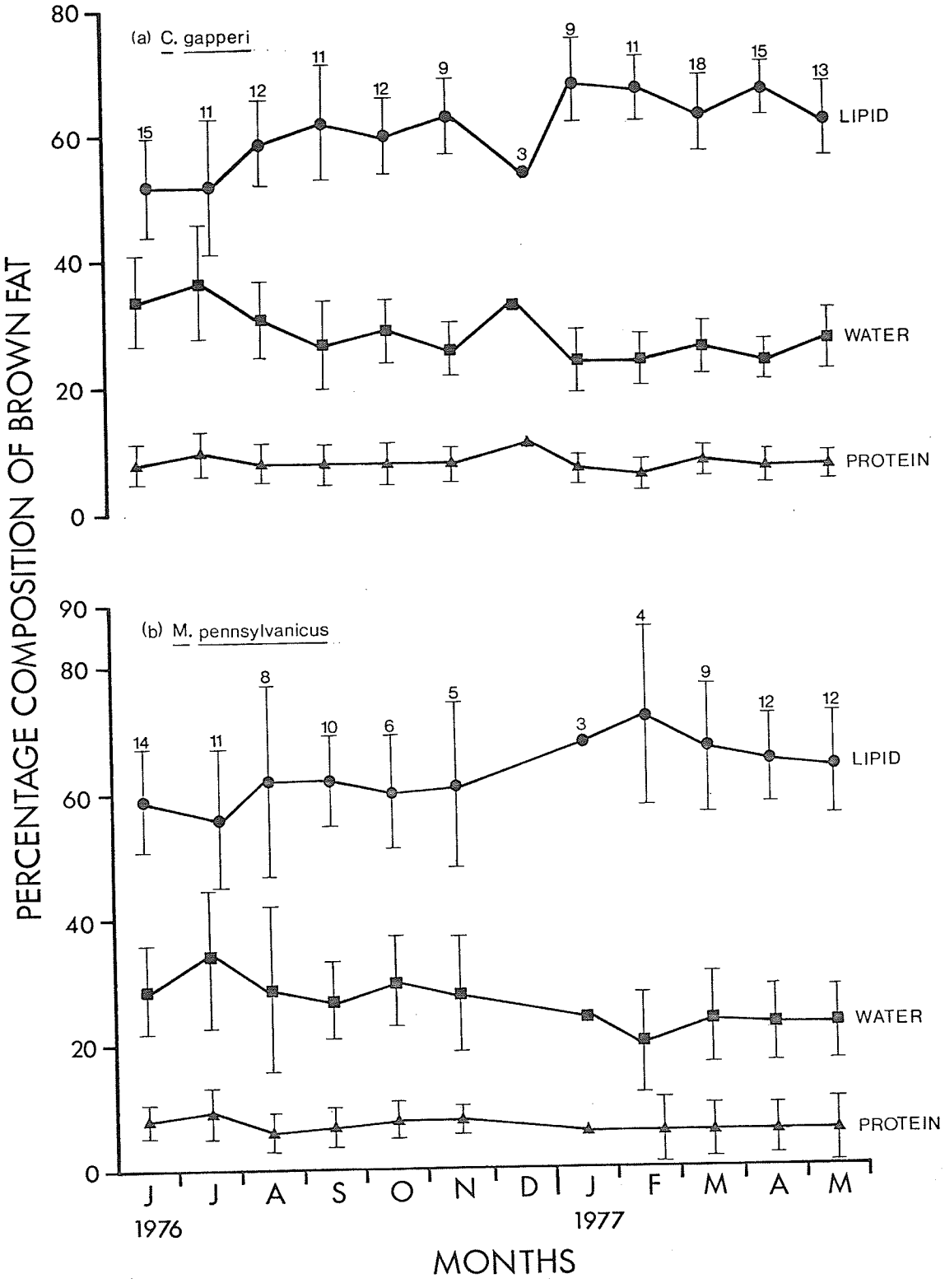
B. Changes in composition

Compositional changes in brown fat of both species of voles were due mainly to variations in the lipid and water components, while the protein component remained essentially unchanged throughout the year (Fig. 4a and 4b). There was a clear inverse relationship between the percentages of lipid and water, while the slight changes in the percentage of protein, whenever they occurred, were directly related to water, and inversely related to lipid.

Although there were variations in the proportion of lipid and water throughout the year, there was not a distinct seasonal pattern of change like that seen in tissue mass (Fig. 2 and 3). In general, the lipid content of both species fluctuated between 60 and 70%. Exceptions to this general pattern were seen in C. gapperi in June, July, and December, when mean percentage of lipid in brown fat was 50-55% (Fig. 4a), and in M. pennsylvanicus in July and February, when lipid percentage in brown fat was 56 and 72%, respectively (Fig. 4b).

Similarly, the proportion of water in brown fat of C. gapperi remained between 24 and 30% for most of the year, except for June, July, and December, when it was 34, 37, and 33%, respectively (Fig. 4a). Brown fat in M. pennsylvanicus had approximately 23-30% water for most of the year, with the exception of July and February, when water content was 34 and 20%, respectively.

Figure 4. Monthly mean percentages of lipid, water, and protein in brown fat of C. gapperi and M. pennsylvanicus. Vertical lines represent the 95% confidence intervals of the means of all samples of 4 or more voles. Sample sizes are shown above mean percentages of lipids and are the same for the other two components, with a few exceptions (Appendices 5D, 5E, 6D, and 6E).


(a) C. gapperi
(b) M. pennsylvanicus
PERCENTAGE COMPOSITION OF BROWN FAT
LIPID
WATER
PROTEIN
0
20
40
60
80
90
15
11
12
11
12
9
3
9
11
18
15
13
14
11
8
10
6
5
3
4
9
12
12
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977
MONTHS

The protein content in brown adipose tissue of both species of voles was relatively constant in comparison with the other two components. With the exception of rises to 10 and 11% in C. gapperi in July and December, respectively (Fig. 4a), the protein content in brown fat of both species fluctuated between 6 and 9% throughout the year (Fig. 4 a and 4b).

The relatively constant percentage composition of brown fat in these voles, particularly during the periods of intensive tissue growth in the fall, indicates that increases in tissue mass were due to increases in all three basic components. On the other hand, the decline in percentage of lipids in brown fat of C. gapperi in December (Fig. 4a) corresponds to a loss of 0.16 g of lipid in the total tissue mass. This lipid depletion accounts for approximately 89% of the decrease in mean absolute brown fat mass of C. gapperi between November and December (Fig. 3a).

Although the proportions of lipid, water, and protein in brown fat of C. gapperi and M. pennsylvanicus were fairly constant for most of the year, data shown in Figures 4a and 4b suggest a slight elevation in the percentage of lipids, and a decline in the percentage of water in winter, particularly in meadow voles (Fig. 4b). Examination of Table III, however, indicates that voles captured at the Delta Field Station from March to May, 1977 had a higher proportion of lipids, and lower proportions of water and protein in brown fat than those captured in Winnipeg, although only a few

Table III. Comparison of percentage composition of brown fat between voles captured in Winnipeg (WPG) and at the Delta Field Station (DFS), from March to May, 1977.

Species	Month	Area	n	% Lipid $\bar{X}$ (SE)	% Water $\bar{X}$ (SE)	% Protein $\bar{X}$ (SE)
C. gapperi	March	WPG	9	58.76 (3.08)	29.64 (1.94)	8.88 (0.83)
		DFS	9	67.24 (2.99)	22.66 (2.11)*	6.15 (0.41)**
	April	WPG	5	63.59 (2.83)	25.89 (2.08)	8.01 (0.83)
		DFS	10	68.56 (1.62)	22.72 (1.01)	5.94 (0.24)
	May	WPG	6	61.04 (1.79)	27.90 (1.25)	8.09 (0.42)
		DFS	7	63.56 (4.61)	26.49 (3.17)	6.50 (0.63)
M. pennsylvanicus	March	WPG	2[1]	53.30 (4.23)	33.44 (1.80)	7.02 ———
		DFS	7	71.35 (3.28)*	20.67 (2.43)*	6.11 (0.45)
	April	WPG	7	60.13 (3.55)	27.85 (2.52)	6.96 (0.57)
		DFS	5	71.68 (1.62)	17.06 (1.28)	5.47 (0.45)
	May	WPG	5	59.19 (4.66)	28.95 (3.87)	8.67 (1.05)
		DFS	7	70.13 (2.03)	18.10 (1.07)	5.43 (0.63)

[1] Except for % Protein, where n = 1.

Significant differences between WPG and DFS voles are indicated by * ($P < 0.05$), and ** ($P < 0.01$).

differences were statistically significant. It appears, therefore, that the increase in lipid and the decrease in water content in brown fat of these voles in winter can be partly attributed to minor differences between the two populations.

Monthly means shown in Figures 4a and 4b represent combined data from male and female voles. Segregation of brown fat composition data according to sex (Appendices 5C-5E, 6C-6E) did not reveal significant differences between the sexes, except for significantly higher lipid content in brown fat of male C. gapperi in March and April (Appendix 5C).

3. Seasonal Changes in White Adipose Tissue

A. Changes in mass

The pattern of seasonal change in white fat mass relative to body weight in C. gapperi (Fig. 5a) differed from the pattern seen in brown fat (Fig. 2a) in that tissue mass did not rise above summer levels in the fall. Instead, mean percent white fat in red-backed voles declined in late summer, returned to summer levels in November, but declined again in December, after which it showed a greater than twofold increase to a maximum in February (Fig. 5a). A similar pattern of change was seen in mean absolute white fat mass of C. gapperi (Fig. 6a), except that maximum mass was reached in April, rather than February.

Figure 5. Seasonal changes in white fat mass as a percentage of body weight in C. gapperi and M. pennsylvanicus. Means for males (solid squares) and females (solid triangles) are shown separately where they differ significantly. The broken line was drawn through the mid point between significantly different mean values for males and females. Vertical lines represent the 95% confidence intervals of the means for all samples of 4 or more voles. Sample sizes are shown above the brackets.

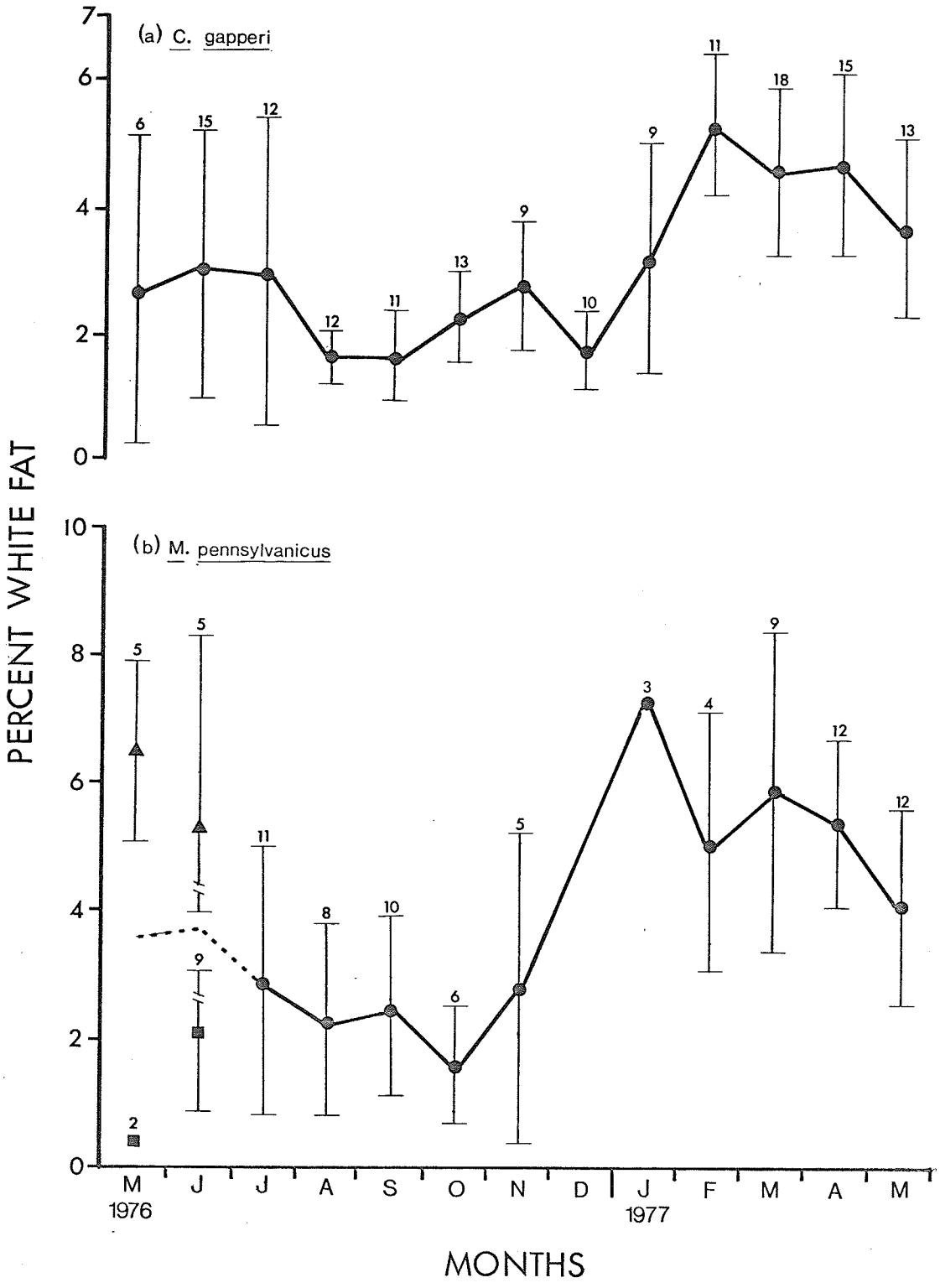
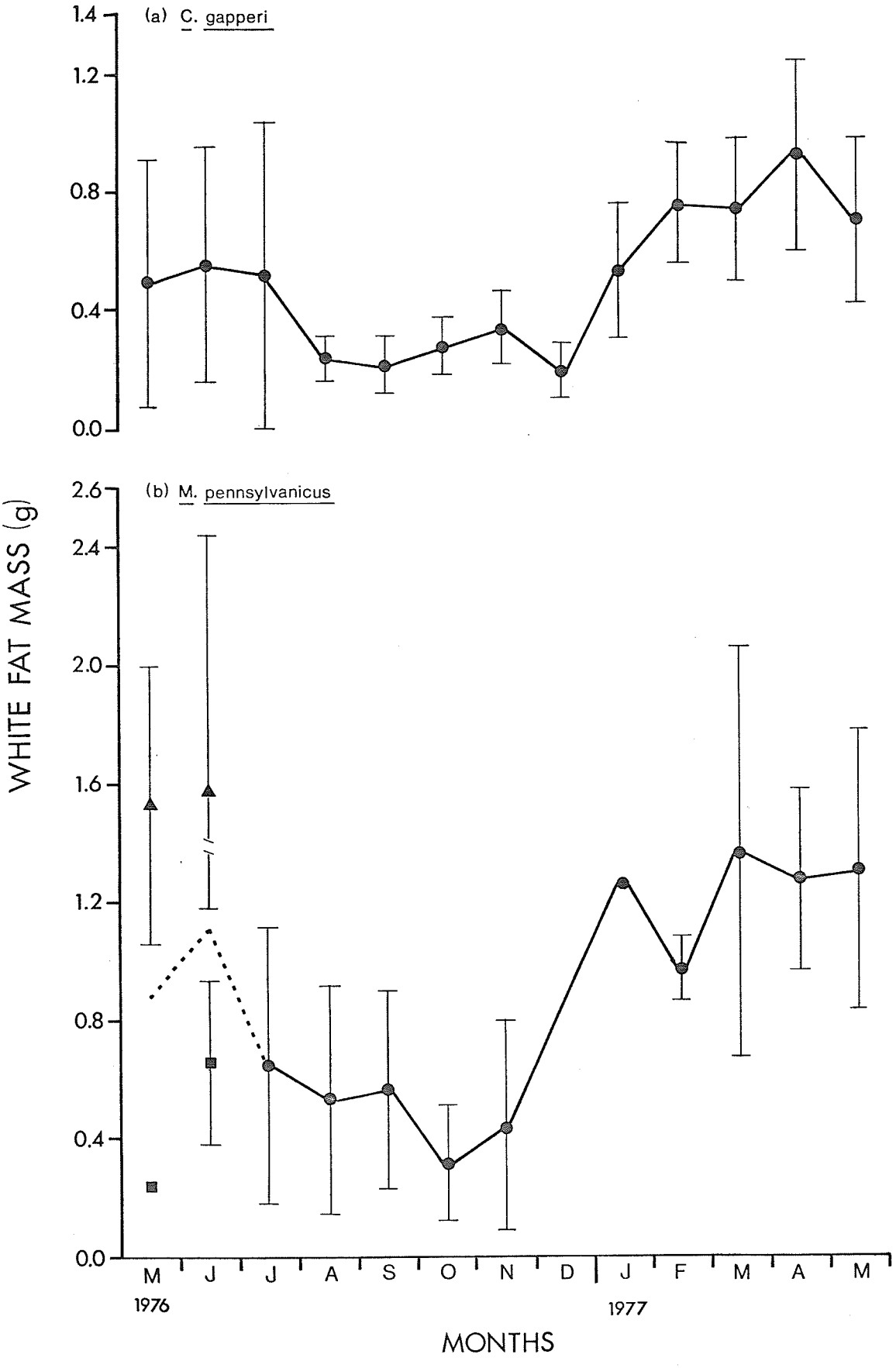

(a) C. gapperi
(b) M. pennsylvanicus
PERCENT WHITE FAT
MONTHS
M
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977

Figure 6. Seasonal changes in the absolute mass of white fat of C. gapperi and M. pennsylvanicus. Means for males (solid squares) and females (solid triangles) are shown separately where they differ significantly. The broken line was drawn through the mid point between significantly different mean values for males and females. Vertical lines represent the 95% confidence intervals of the means for all samples of 4 or more voles. Sample sizes are as shown in Figure 5.


(a) C. gapperi
1.4
1.2
0.8
0.4
0.0
(b) M. pennsylvanicus
2.6
2.4
2.0
1.6
1.2
0.8
0.4
0.0
WHITE FAT MASS (g)
M
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977
MONTHS

High mean values for C. gapperi from May to July, 1976 (Fig. 5a and 6a) were due to a large white fat mass in females, although differences between the two sexes were not statistically significant (Appendices 7A and 7B). The annual pattern of change in mass of this tissue differed slightly between the two sexes. Although both sexes of C. gapperi had a greater white fat mass in winter than in fall, males also had more white fat in winter than in summer, whereas females had similar amounts both seasons (Appendices 7A and 7B).

White fat mass in M. pennsylvanicus also showed the greatest rise in early winter rather than in the fall, increasing over twofold between November and January (Fig. 5b and 6b). However, maximum mean percent white fat in meadow voles was reached in January (Fig. 5b), one month prior to red-backed voles (Fig. 5a). In addition, differences between males and females were more pronounced in M. pennsylvanicus. Females of this species had significantly higher percentages of white fat than males in May, 1976 ($P < 0.001$), and June ($P < 0.01$) (Fig. 5b, Appendix 8A). Similarly, females had significantly higher mean absolute white fat mass in May, 1976 ($P < 0.005$), and June ($P < 0.005$) (Fig. 6b, Appendix 8B). Differences between male and female meadow voles were not statistically significant in any other month, except for mean absolute tissue mass in February (Appendix 8B). However, the sample of M. pennsylvanicus for that month consisted of only two males and two females, a sample too small to allow definite conclusions with regards to significant differences between the two

sexes, particularly since the calculated t-value (4.340) was only slightly higher than the critical value (4.303) at the 5% level of significance.

Although differences between male and female meadow voles were statistically significant in only two months (Fig. 5b and 6B), females tended to have more white fat than males at all times, except March and May, 1976 (Appendices 8A and 8B).

Differences in white fat mass between males and females in the summer appear to be related to breeding activity. In general, pregnant and/or lactating females had a greater white fat mass than males, but variability among females was very high when compared to males (Appendices 7A, 7B, 8A, and 8B). The reasons for the great variability in white fat mass among females during the breeding season were unclear, but may be related to differences in the stage of gestation in individual females.

Table IV shows a comparison between mean white fat mass of animals captured at Winnipeg and those captured at the Delta Field Station. White fat mass of C. gapperi collected at the Field Station was higher than in animals collected in Winnipeg from March to May, 1977, but differences were significant only in March (Table IV). Similarly, M. pennsylvanicus captured at the Delta Field Station also had more white fat than those captured in Winnipeg, but differences were statistically significant only in May (Table IV).

Table IV. Comparison of total absolute and relative white fat (WF) mass between voles captured in Winnipeg (WPG), and those captured at the Delta Field Station (DFS), from March to May, 1977.

Species	Month	Area	n	WF Weight (g)	%WF
				$\bar{X}$ (SE)	$\bar{X}$ (SE)
C. gapperi	March	WPG	9	0.51 (0.09)	3.11 (0.48)
		DFS	9	1.00 (0.14)*	6.13 (0.80)**
	April	WPG	5	0.61 (0.13)	3.18 (0.54)
		DFS	10	1.08 (0.18)	5.40 (0.77)
	May	WPG	6	0.64 (0.09)	3.42 (0.46)
		DFS	7	0.78 (0.21)	3.97 (1.03)
M. pennsylvanicus	March	WPG	2	0.86 (0.48)	3.85 (1.83)
		DFS	7	1.52 (0.34)	6.48 (1.21)
	April	WPG	7	1.16 (0.17)	5.20 (0.62)
		DFS	5	1.46 (0.21)	5.61 (1.07)
	May	WPG	5	0.63 (0.11)	2.31 (0.50)
		DFS	7	1.70 (0.20)**	5.34 (0.68)*

Significant differences between WPG and DFS voles are indicated by * ($P < 0.05$), and ** ($P < 0.01$).

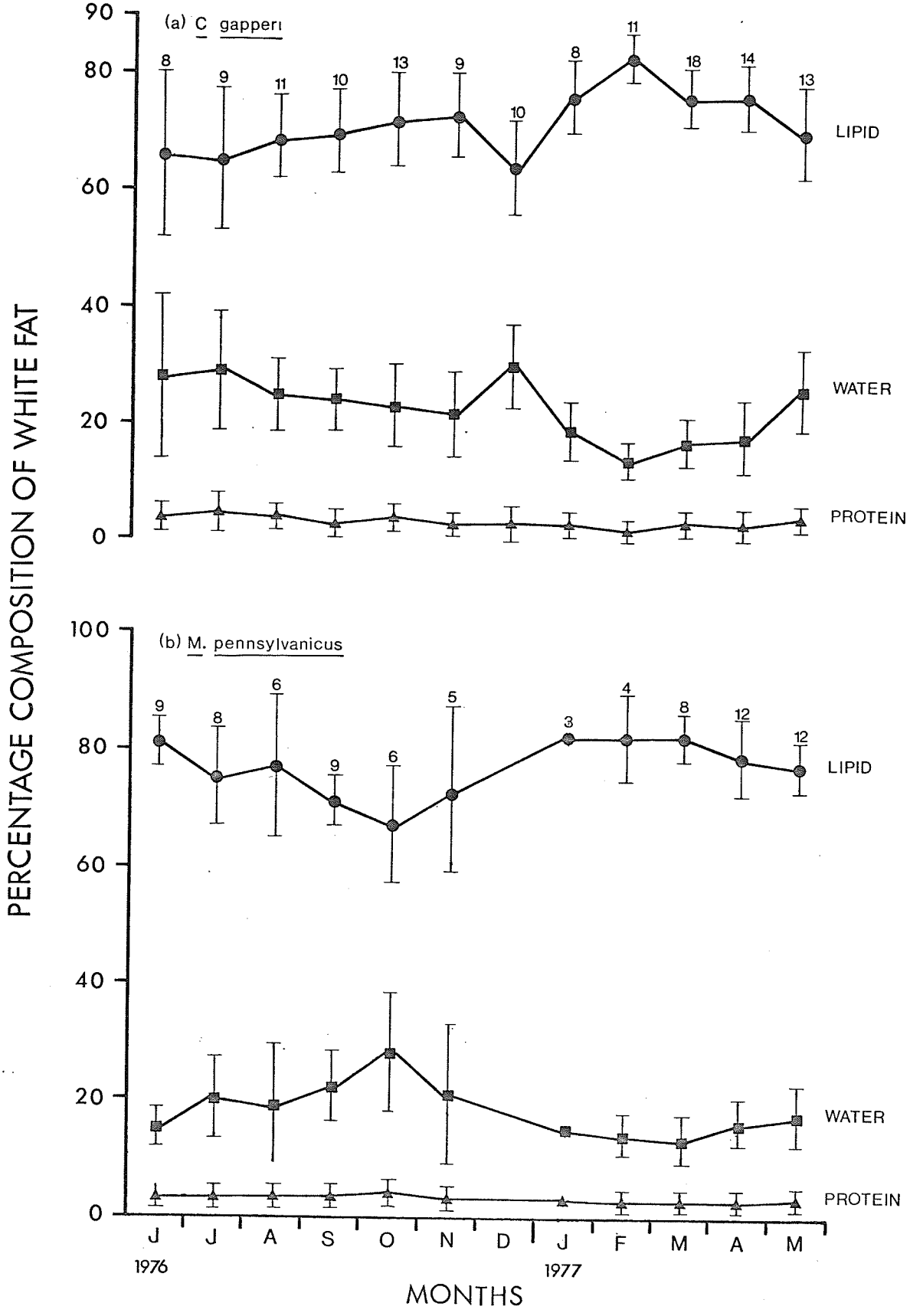
Although differences between voles from the two locations were statistically significant in only one out of three months tested, Table IV indicates that voles captured at the Field Station had a consistently higher white fat mass. It is possible, therefore, that such a difference also existed in January and February, in which case at least part of the rise in the mass of this tissue in Winter (Figs. 5a, 5b, 6a, and 6b) might be attributed to differences between populations from the two trapping areas. A seasonal effect, however, was also present, since C. gapperi and M. pennsylvanicus captured in Winnipeg from March to May, 1977 (Table IV) had a higher mean percentage of white fat, and a higher mean absolute white fat mass, than voles captured in Winnipeg during the previous fall (Figs. 5a, 5b, 6a and 6b).

B. Changes in composition

Annual variations in the percentages of lipid, water and protein in white fat of both species are shown in Figures 7a and 7b. As in brown fat (Fig. 4a and 4b), compositional changes in white fat were due to fluctuations in the proportions of lipid and water, while the percentage of protein remained relatively constant.

The percentage of lipids in white fat of red-backed voles (Fig. 7a) and meadow voles (Fig. 7b) varied from approximately 65 to 82% throughout the year, but the two species differed with regards to the seasonal pattern of change. In C. gapperi the percentage of lipids in white fat

Figure 7. Monthly mean percentages of lipids, water, and protein in white fat of C. gapperi and M. pennsylvanicus. Means do not include pregnant or lactating females. Vertical lines represent the 95% confidence intervals of the means for all samples of 4 or more voles. Sample sizes are shown above mean percentages of lipids and are the same for the other two components, with a few exceptions (Appendices 7D, 7E, 8D, and 8E).


(a) C gapperi
90
80
60
40
20
0
8
9
11
10
13
9
10
8
11
18
14
13
LIPID
WATER
PROTEIN
(b) M. pennsylvanicus
100
80
60
40
20
0
9
8
6
9
6
5
3
4
8
12
12
LIPID
WATER
PROTEIN
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977
MONTHS
PERCENTAGE COMPOSITION OF WHITE FAT

increased during the fall and early winter, from 65% in July to a maximum of 82% in February (Fig. 7a). The increase occurred progressively during that period, except for a decline to summer levels in December. M. pennsylvanicus, on the other hand, showed a decline in white fat lipid percentage between summer and fall from 82% in June to approximately 65% in October, and a subsequent increase to a maximum of 82% in winter (Fig. 7b). Thus, although both species had a similar proportion of lipids in white adipose tissue in winter, the percentage of lipids in white fat of C. gapperi in summer was low when compared to M. pennsylvanicus.

The decline in the percentage of lipid in white fat of C. gapperi in December (Fig. 7a) corresponds to a loss of 0.16 g in the total absolute lipid content of this tissue. This loss accounts for 89% of the 0.18 g decrease in mean absolute white fat mass of red-backed voles in December (Fig. 6a).

Changes in the proportion of water in white fat of both species were the reverse of changes in the proportion of lipids (Fig. 7a and 7b), a relationship also seen in brown fat (Fig. 4a and 4b). The percentage of water in white fat fluctuated between 14 and 30%, approximately. However, since changes in the water component were the reverse of changes in the lipid component, the pattern of seasonal change in mean percent water in white fat differed between the two species, as did the pattern of seasonal change in the percentage of lipid.

Of the three basic components, protein showed the least amount of change throughout the year. Although the percentage of protein in white fat of both species fluctuated from 2.5 to 5.0%, no distinct seasonal pattern of change was evident (Fig. 7a and 7b).

Gestation and lactation also altered the percentage composition of white adipose tissue. White fat of pregnant and/or lactating females had significantly lower proportions of lipid, and significantly higher proportions of water and protein, than white fat of non-pregnant, non-lactating females (Table V). For this reason, samples from pregnant and lactating females were not included in the means shown in Figures 7a and 7b. No significant differences in mean percentage composition of white fat were found between males and females, when pregnant and/or lactating females were excluded from monthly means (Appendices 7C-7E, and 8C-8E).

When monthly samples from March to May, 1977 were segregated according to area of capture, significant differences were found in C. gapperi in March, with red-backed voles captured at the Delta Field Station having significantly lower proportions of water and protein in white fat than those captured in Winnipeg (Table VI). No significant differences were found between M. pennsylvanicus captured in Winnipeg and those captured at the Delta Field Station (Table VI).

Table V. Comparison of percentage composition of white fat between pregnant and/or lactating females (P/L), and non-pregnant, non-lactating females (NP/NL) of C. gapperi and M. pennsylvanicus.

Species	% Component	P/L			NP/NL		
		n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
C. gapperi[1]	Lipid	10	15.33	(1.49)	7	69.19	(3.11)***
	Water	10	69.27	(1.08)	7	25.62	(2.56)***
	Protein	10	11.81	(0.84)	7	3.91	(0.40)***
M. pennsylvanicus[2]	Lipid	10	22.14	(2.62)	10	77.42	(2.55)***
	Water	10	62.21	(1.88)	11	17.42	(2.11)***
	Protein	9	12.54	(0.91)	10	2.69	(0.42)***

[1] Samples comprise females captured from June through August, 1976.

[2] Samples comprise females captured from June through September, 1976.

*** Indicates significant differences ($P < 0.001$) between the two groups of females.

Table VI. Comparison of percentage composition of white fat between voles captured in Winnipeg (WPG) and at the Delta Field Station (DFS), from March to May, 1977.

Species	Month	Area	n	% Lipid $\bar{X}$	% Lipid (SE)	% Water $\bar{X}$	% Water (SE)	% Protein $\bar{X}$	% Protein (SE)
C. gapperi	March	WPG	9	72.98	(1.90)	22.06	(1.48)	3.93	(0.40)
		DFS	9[1]	79.53	(2.63)	12.55	(0.87)**	2.14	(0.20)**
	April	WPG	5	77.33	(2.52)	17.50	(3.35)	3.14	(0.32)
		DFS	9[2]	75.94	(3.28)	18.57	(2.78)	3.20	(0.49)
	May	WPG	6	72.58	(2.01)	22.97	(1.84)	3.65	(0.18)
		DFS	7	66.78	(5.64)	28.11	(4.83)	3.91	(0.54)
M. pennsylvanicus	March	WPG	1	85.53	———	11.76	———	2.11	———
		DFS	7	81.56	(1.35)	13.70	(1.48)	2.41	(0.19)
	April	WPG	7	81.89	(1.91)	15.03	(1.67)	2.32	(0.17)
		DFS	5	73.75	(3.66)	16.59	(1.82)	2.30	(0.17)
	May	WPG	5	75.24	(2.29)	18.98	(3.10)	2.58	(0.33)
		DFS	7	78.44	(1.17)	15.61	(1.66)	2.46	(0.25)

[1]Except for % protein, where n = 8.
[2]Except for % water, where n = 10, and % protein, where n = 8.
Significant differences between WPG and DFS voles are indicated by ** ($P < 0.01$).

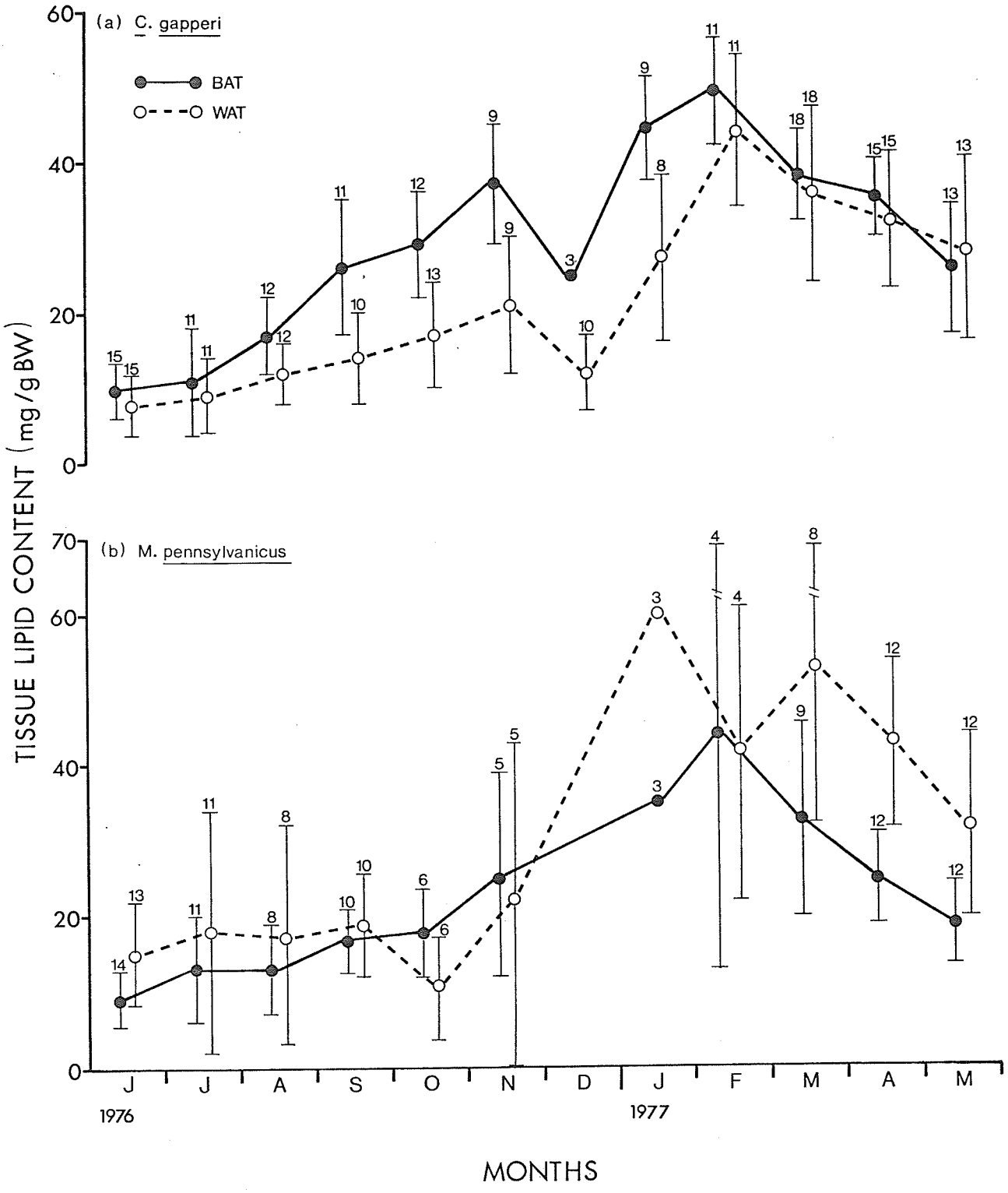
4. Seasonal Changes in Lipid Reserves

Both species of voles had larger lipid reserves in winter than in summer (Fig. 8a and 8b). Brown fat lipid in these voles increased approximately threefold from August to a maximum in February. Similarly, white fat lipid in C. gapperi showed a greater than threefold increase between August and Febraury (Fig. 8a). In M. pennsylvanicus there was also a greater than threefold increase in white fat lipids between summer and winter, but maximum lipid content in white fat of this species occurred in January (Fig. 8b).

The seasonal pattern of change in lipid content of brown fat in red-backed voles (Fig. 8a) and meadow voles (Fig. 8b) closely resembles the pattern of change in the mass of this tissue relative to body weight (Fig. 2a and 2b). This is to be expected, since changes in the percentage of lipids in brown fat were slight (Fig. 4a and 4b) when compared to changes in mass. The amount of lipid in brown fat throughout the year did not differ significantly between males and females of either species (Appendices 9A and 10A).

The seasonal pattern of change in white fat lipid reserves, however, differed from the pattern seen in relative tissue mass. The amount of lipids stored in white adipose tissue of red-backed voles was not higher in summer than in fall (Fig. 8a), as was the mass of this tissue relative to body weight (Fig. 5a). This is due to the fact that females during reproduction had a significantly lower percentage of lipids in white fat than non-reproducing females (Table V),

Figure 8. Seasonal changes in the lipid content of brown and white adipose tissues relative to body weight in C. gapperi and M. pennsylvanicus. Vertical lines represent the 95% confidence intervals of the means for all samples of 4 or more voles. Sample sizes are shown above the brackets.


(a) C. gapperi
BAT
WAT
(b) M. pennsylvanicus
TISSUE LIPID CONTENT (mg/g BW)
0
20
40
60
70
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977
MONTHS

and since the majority of females caught in the summer were either pregnant or lactating, the mean lipid content in white fat of this species was not higher in the summer than in the fall, despite the great white fat mass of female red-backed voles in the summer (Appendices 7A and 7B). In fact, when monthly mean lipid content in female C. gapperi was calculated by including data obtained from reproducing females, significant differences between males and females were found only in July (Appendix 9B).

A similar situation exists with regards to lipid reserves in white fat of M. pennsylvanicus, in which mean lipid content of white fat remained approximately at the same level during the summer and fall seasons (Fig. 8b). There were no significant differences in the amount of lipid stored in white fat between males and females (Appendix 10B), despite the higher mass of white fat of females during the breeding season (Fig. 5b, Appendices 8A and 8B). Again, this is due to reproducing females having a significantly lower percentage of lipids in white fat than non-reproducing females (Table V).

Although both species of voles had greater lipid reserves in winter than in summer, they differed with regards to the amounts stored in each of the two types of adipose tissues. C. gapperi had more lipids stored in brown than in white fat for most of the year (Fig. 8a). Lipid reserves in brown fat of red-backed voles were significantly higher than white fat lipid reserves from August to January (Appendix 9C).

M. pennsylvanicus had similar amounts of lipids in brown and white fat during the summer and fall, but had more white than brown fat lipids in January, March, April and May, 1977 (Fig. 8b, Appendix 10C).

Segregation of the samples for March, April, and May, 1977 according to the location in which animals were captured revealed no significant differences in the amount of lipids in brown fat (Table III). Significantly higher white fat lipid reserves, however, were found in C. gapperi and M. pennsylvanicus captured at the Delta Field Station in March and May, respectively, when compared with voles captured in Winnipeg during the corresponding months (Table VII).

5. Seasonal Changes in Growth Pattern

Voles of both species captured in fall and winter were smaller than those captured in spring and summer, as evidenced by lower mean body weight, total length, and total skeletal muscle mass of C. gapperi and M. pennsylvanicus during the cold seasons (Figs. 9a-9c, 10a-10c).

Mean body weight of monthly samples of C. gapperi became progressively lower during the summer, but remained essentially unchanged during the fall and early winter, except for a slight decline in December (Fig. 9a). From January on, mean body weights became gradually higher; the greatest increase occurred in early spring between March and April (Fig. 9a). Mean body weights of male red-backed voles were significantly higher than those of females in April and May,

Table VII. Comparison of lipid content (mg of tissue lipid[1]/g of body weight) in brown fat (BF) and white fat (WF) of voles captured in Winnipeg (WPG) and at the Delta Field Station (DFS), from March to May, 1977.

Species	Month	Area	BF Lipid Content			WF Lipid Content		
			n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
C. gapperi	March	WPG	9	35.38	(4.07)	9	23.21	(3.94)
		DFS	9	40.52	(3.01)	9	49.13	(6.94)*
	April	WPG	5	32.23	(3.74)	5	24.98	(3.64)
		DFS	10	36.68	(1.72)	9	36.94	(4.78)
	May	WPG	6	27.29	(2.25)	6	25.24	(4.12)
		DFS	7	25.09	(5.39)	7	29.47	(9.18)
M. pennsylvanicus	March	WPG	2	18.48	(0.03)	1	48.58	———
		DFS	7	37.62	(5.41)	7	53.01	(9.94)
	April	WPG	7	23.52	(3.02)	7	43.23	(5.85)
		DFS	5	28.02	(3.32)	5	41.82	(8.71)
	May	WPG	5	14.43	(3.09)	5	17.37	(3.91)
		DFS	7	21.65	(1.72)	7	41.87	(5.15)*

[1] $\frac{\text{Total tissue mass (mg)} \times \text{Percentage of lipid}}{100}$ = mg of tissue lipid

* Indicates significant differences ($P < 0.01$).

1977 ($P < 0.05$, Appendix 11B).

Mean total length (Fig. 9b) and mean total skeletal muscle mass (Fig. 9c) of monthly samples of C. gapperi paralled mean body weight. There were no significant differences between sexes in total length (Appendix 11C), but male red-backed voles had a significantly higher mean total muscle mass than females in April ($P < 0.05$) and May, 1977 ($P < 0.01$, Appendix 11D).

The apparent pattern of change in body size in monthly samples of M. pennsylvanicus was similar to that seen in C. gapperi. Mean body weight of meadow voles (Fig. 10a) became progresively lower during summer and early fall, reaching the lowest mean value in November. From January until spring it became progressively higher with the most rapid change occurring between April and May, 1977 (Fig. 10a).

Mean total length (Fig. 10b), and mean total skeletal muscle mass (Fig. 10c) of M. pennsylvanicus paralleled mean body weight (Fig. 10a). Male meadow voles had significantly higher mean body weight than females in May, 1976 ($P < 0.01$, Appendix 12B), and significantly higher mean total skeletal muscle mass in May, 1976 ($P < 0.01$), April ($P < 0.05$), and May, 1977 ($P < 0.01$, Appendix 12D).

Female M. pennsylvanicus had significantly higher mean body weight, total length, and total skeletal muscle mass than males in August (Appendices 12B and 12C). There were only two males in the sample of meadow voles for August, however, and both were young animals which had not yet reached

Figure 9. Monthly mean body weight, total length, and absolute skeletal muscle mass of C. gapperi. Vertical lines represent the 95% confidence intervals of the means of all samples of 4 or more voles. Sample sizes are shown above the means for body weight and are the same for the other two measurements, except for total length in March, where n = 17.

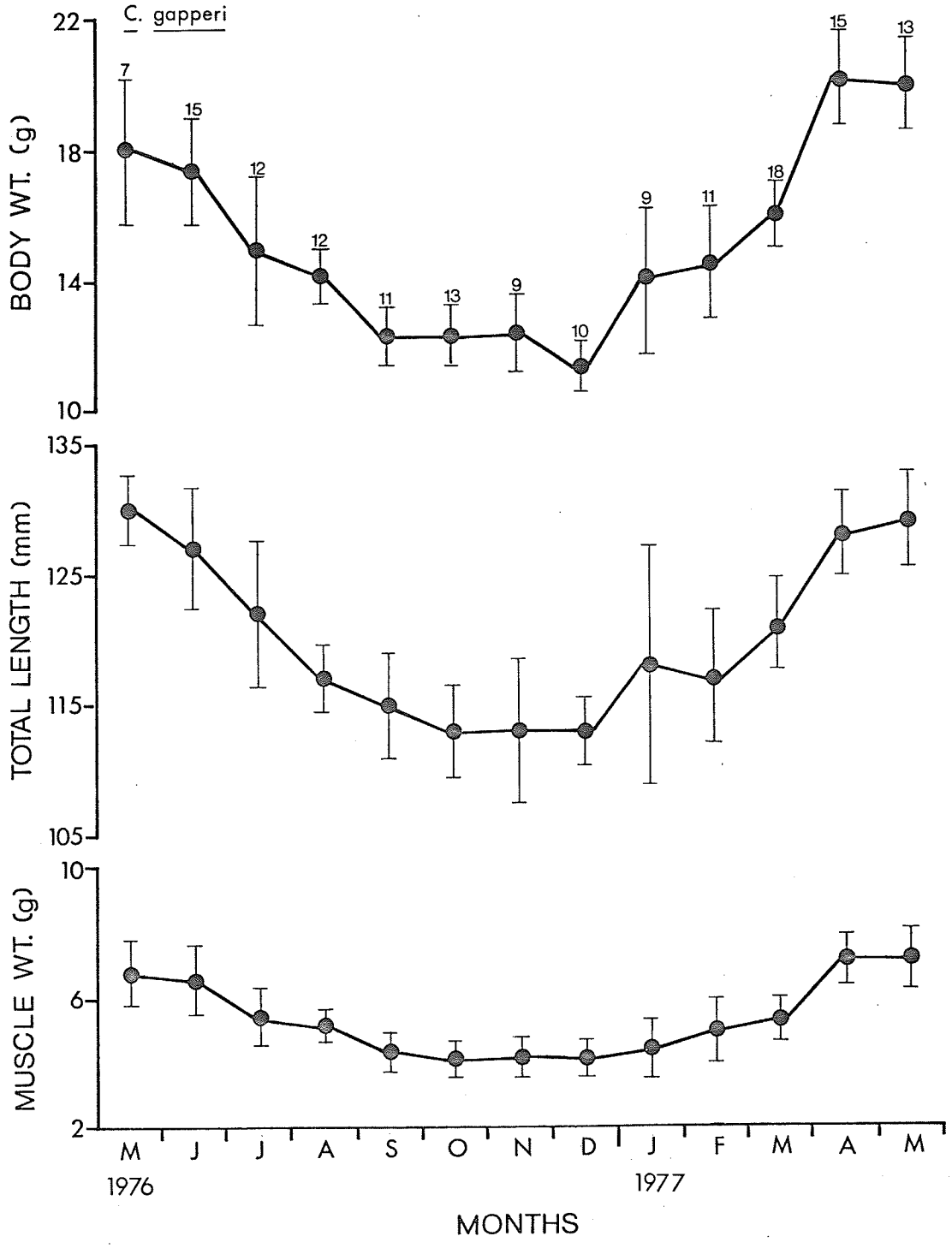
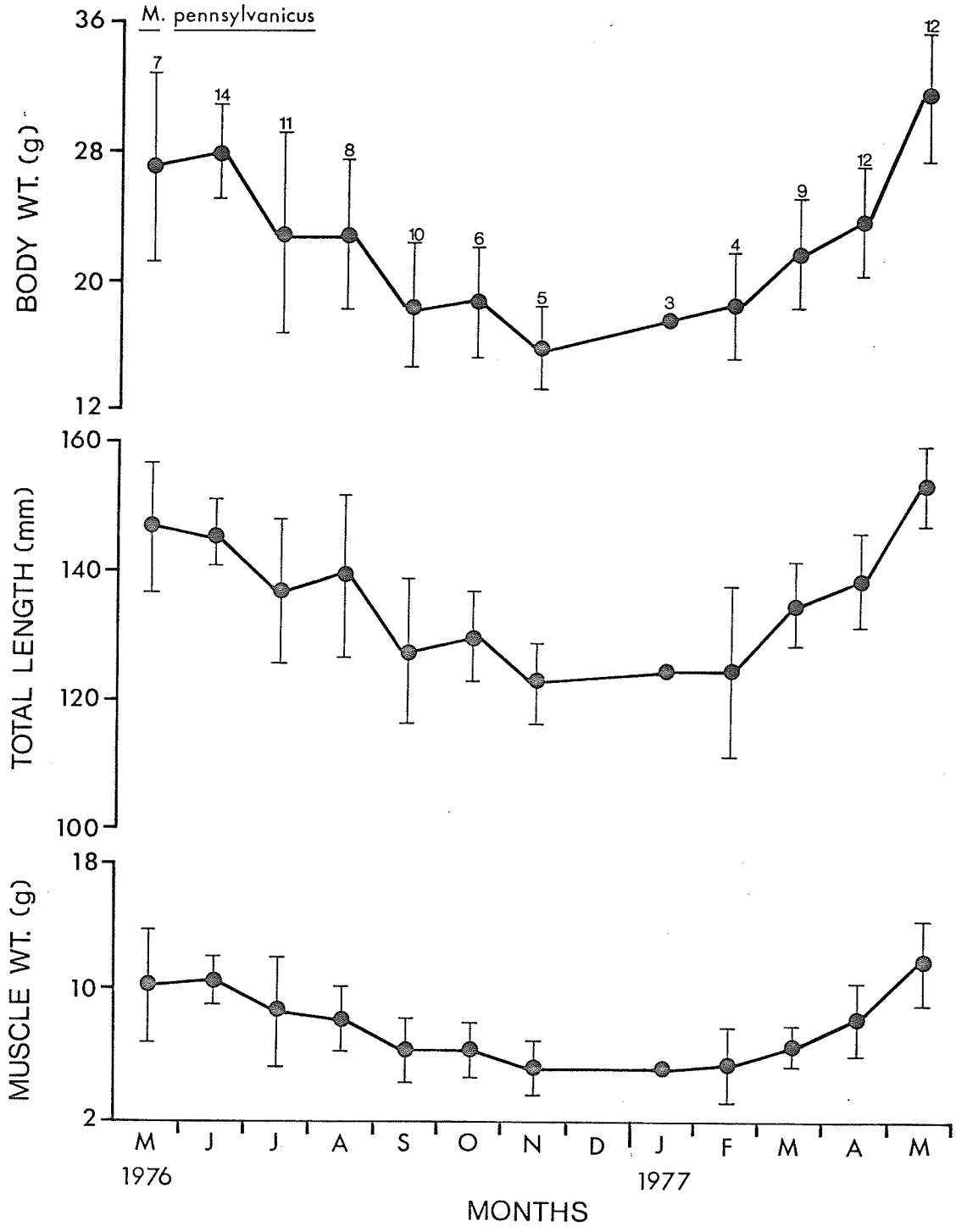

C. gapperi
BODY WT. (g)
22
18
14
10
7
15
12
12
11
13
9
10
9
11
18
15
13
TOTAL LENGTH (mm)
135
125
115
105
MUSCLE WT. (g)
10
6
2
M
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977
MONTHS

Figure 10. Monthly mean body weight, total length, and absolute skeletal muscle mass of M. pennsylvanicus. Vertical lines represent the 95% confidence intervals of the means of all samples of 4 or more voles. Sample sizes are shown above the means for body weight and are the same for the other two measurements.


M. pennsylvanicus
BODY WT. (g)
36
28
20
12
7
14
11
8
10
6
5
3
4
9
12
12
TOTAL LENGTH (mm)
160
140
120
100
MUSCLE WT. (g)
18
10
2
M
J
J
A
S
O
N
D
J
F
M
A
M
1976
1977
MONTHS

sexual maturity. The six females in the sample, on the other hand, were sexually mature animals, one of them being pregnant, and the other five having already had litters, as evidenced by placental scars. Thus, differences between male and female meadow voles in August are probably related to differences in age, rather than sex.

No significant differences in mean body weight, total length, and total muscle mass were found between voles captured in the Winnipeg area and those captured at the Delta Field Station (Table VIII).

The apparent summer to fall decline in size of both species of voles (Fig. 9 and 10) was due to an increasing number of young voles in the samples as the breeding season progressed. This can be seen when monthly samples are separated into young and mature voles, and their total length and muscle mass are compared, as shown in Tables IX and X for C. gapperi and M. pennsylvanicus, respectively. Total length and muscle mass, rather than body weight, are shown in these tables, because these measurements are uncomplicated by changes in fat deposition, thus giving a better indication of growth. Mean body weights of mature and immature C. gapperi and M. pennsylvanicus are shown in Appendices 11A and 12 A, respectively.

Sexually immature animals were shorter and had a smaller muscle mass than mature animals (Tables IX and X), and the predominance of young voles in the samples for fall and winter was the cause of the apparent decrease in monthly mean

Table VIII. Comparison of mean body weight, total length, and total skeletal muscle mass between voles captured in Winnipeg (WPG) and voles captured at the Delta Field Station (DFS), from March to May, 1977.

Species	Month	Area	n	Body Weight (g) $\bar{X}$	(SE)	Total Length (mm) $\bar{X}$	(SE)	Muscle Mass (g) $\bar{X}$	(SE)
C. gapperi	March	WPG	9	15.91	(0.68)	122.00	(1.83)	5.70	(0.26)
		DFS	9[1]	16.04	(0.46)	119.00	(1.74)	5.07	(0.18)
	April	WPG	5	18.40	(1.24)	130.00	(1.76)	7.10	(0.59)
		DFS	10	19.67	(0.56)	127.20	(1.45)	7.20	(0.25)
	May	WPG	6	18.67	(0.86)	129.50	(2.29)	7.15	(0.55)
		DFS	7	19.44	(0.70)	127.93	(1.30)	7.20	(0.40)
M. pennsylvanicus	March	WPG	2	21.21	(2.31)	135.50	(4.50)	6.95	(0.30)
		DFS	7	21.10	(1.59)	135.50	(2.46)	6.68	(0.40)
	April	WPG	7	21.98	(1.14)	134.90	(2.68)	7.49	(0.48)
		DFS	5	27.29	(2.43)	144.40	(4.87)	9.89	(1.40)
	May	WPG	5	28.43	(2.37)	151.40	(4.90)	10.82	(1.76)
		DFS	7	34.00	(1.85)	154.64	(2.49)	12.58	(1.16)

[1] Except for Total Length, where n = 8.

Table IX. Monthly mean total length and skeletal muscle mass of sexually mature and immature C. gapperi, from May, 1976 to May, 1977.

	TOTAL LENGTH (mm)						MUSCLE MASS (g)					
	Mature			Immature			Mature			Immature		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	129.64	(0.70)	0	———		7	6.77	(0.34)	0	———	
Jun	13	128.77	(1.44)	2	116.50	(2.04)	13	6.94	(0.35)	2	4.41	(0.38)
Jul	7	126.00	(2.72)	5	115.90	(1.66)	7	6.19	(0.39)	5	4.50	(0.13)
Aug	3	121.83	(0.44)	9	115.72	(0.84)	3	5.59	(0.00)[1]	9	5.25	(0.13)
Sep	1	124.00	———	10	113.95	(1.46)	1	4.92	———	10	4.38	(0.16)
Oct	1	123.50	———	12	112.54	(1.34)	1	5.63	———	12	4.02	(0.10)
Nov	1	131.00	———	8	111.13	(0.76)	1	5.00	———	8	4.08	(0.15)
Dec	0	———		10	112.85	(0.73)	0	———		10	4.23	(0.14)
Jan	2	135.50	(3.50)	7	112.79	(1.92)	2	5.98	(0.54)	7	4.13	(0.15)
Feb	1	134.00	———	10	114.75	(1.12)	1	7.62	———	10	4.64	(0.14)
Mar	1	129.00	———	16	120.03	(1.25)	1	5.53	———	17	5.38	(0.18)
Apr	15	128.13	(1.15)	0	———		15	7.16	(0.25)	0	———	
May	13	128.65	(1.23)	0	———		13	7.18	(0.32)	0	———	

[1] Standard deviation = 0.006.

Table X. Monthly mean total length and skeletal muscle mass of sexually mature and immature M. pennsylvanicus, from May, 1976 to May, 1977.

	TOTAL LENGTH (mm)						MUSCLE MASS (g)					
	Mature			Immature			Mature			Immature		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	147.14	(3.52)	0	———		7	10.38	(1.22)	0	———	
Jun	14	145.93	(1.97)	0	———		14	10.81	(0.47)	0	———	
Jul	8	143.56	(3.94)	3	120.50	(2.25)	8	10.36	(1.55)	3	5.36	(0.31)
Aug	6	146.08	(3.69)	2	122.50	(3.50)	6	9.21	(0.38)	2	5.90	(0.99)
Sep	3	145.33	(6.39)	7	120.78	(2.48)	3	9.14	(0.77)	7	5.23	(0.31)
Oct	3	136.67	(1.86)	3	126.83	(2.46)	3	7.14	(0.54)	3	6.02	(0.56)
Nov	0	———		5	123.00	(1.67)	0	———		5	5.35	(0.48)
Jan	1	133.50	———	2	120.00	(1.00)	1	6.20	———	2	5.02	(0.18)
Feb	1	136.00	———	3	121.33	(1.20)	1	7.32	———	3	5.37	(0.37)
Mar	8	136.94	(1.62)	1	124.00	———	8	7.04	(0.11)	1	4.37	———
Apr	12	138.88	(2.80)	0	———		12	8.49	(0.70)	0	———	
May	12	153.29	(2.42)	0	———		12	11.85	(0.99)	0	———	

total length and skeletal muscle mass during the cold seasons (Figs. 9b, 9c, 10b and 10c).

Although some samples in fall and winter were small, particularly of meadow voles (Table X), there is no reason to believe they were not representative of the populations. It appears, therefore, that overwintering populations of C. gapperi and M. pennsylvanicus consist largely of animals born during the previous summer, and which do not reach sexual maturity until the following spring. Moreover, these young voles show an arrest in growth during the fall and winter, and rapid growth in spring, as indicated by relatively stable total length and muscle mass from September to February, followed by an increase over the following three months (Tables IX and X).

The apparent decrease in mean skeletal muscle mass of voles during fall and winter coincided with an increase in the percentage of lipids of this tissue. In C. gapperi it rose from approximately 2.2 to 4% between September and February (Table XI), and in M. pennsylvanicus from approximately 2 to 5.6% during the same interval (Table XII). In both species the percentage of lipids in skeletal muscle tissue declined again in spring.

Changes in the percentage of water in skeletal muscle were the reverse of changes in the percentage of lipids, decreasing in both species from approximately 75% in September to approximately 72% in February, and increasing from then on (Tables XI and XII). There was no indication of a seasonal

Table XI. Monthly mean percentage composition of skeletal muscle of C. gapperi, from June, 1976 to May, 1977.

Month	n	% Lipid		% Water		% Protein	
		$\bar{X}$	(SE)	$\bar{X}$	(SE)	$\bar{X}$	(SE)
Jun	14	1.47	(0.21)	74.49	(0.25)	21.50	(0.46)
Jul	12	1.49	(0.20)	74.66	(0.39)	21.40	(0.32)
Aug	11	1.71	(0.13)	74.02	(0.23)	21.72	(0.18)
Sep	11	2.24	(0.38)	74.50	(0.38)	21.23	(0.19)
Oct	13	2.39	(0.33)	73.83	(0.46)	21.16	(0.28)
Nov	9	3.05	(0.49)	73.11	(0.45)	21.05	(0.26)
Dec	10	2.81	(0.19)	73.82	(0.72)	20.32	(0.26)
Jan	9	3.71	(0.52)	72.97	(0.47)	21.21	(0.28)
Feb	11	4.10	(0.24)	71.94	(0.26)	21.22	(0.25)
Mar	18[1]	3.20	(0.32)	73.18	(0.33)	21.23	(0.12)
Apr	15	2.51	(0.17)	74.11	(0.24)	20.82	(0.20)
May	13	2.48	(0.27)	73.51	(0.35)	20.99	(0.31)

[1]Except for % Protein, where n = 17.

Table XII. Monthly mean percentage composition of skeletal muscle of M. pennsylvanicus, from June, 1976 to May, 1977.

Month	n	% Lipid		% Water		% Protein	
		$\bar{X}$	(SE)	$\bar{X}$	(SE)	$\bar{X}$	(SE)
Jun	14[1]	1.46	(0.24)	74.65	(0.22)	21.41	(0.33)
Jul	11	1.84	(0.43)	75.12	(0.97)	19.99	(0.76)
Aug	8	1.28	(0.26)	75.05	(0.30)	20.90	(0.31)
Sep	9	2.13	(0.27)	74.57	(0.33)	20.90	(0.25)
Oct	5	1.75	(0.37)	74.14	(0.35)	21.15	(0.23)
Nov	5	2.48	(0.39)	73.79	(0.35)	20.56	(0.24)
Jan	3	3.67	(0.65)	73.04	(0.40)	20.78	(0.85)
Feb	4	5.55	(0.64)	71.47	(0.18)	20.11	(0.63)
Mar	9[2]	4.49	(0.79)	71.89	(0.66)	20.48	(0.33)
Apr	12	3.32	(0.53)	73.11	(0.48)	20.83	(0.26)
May	12	2.25	(0.21)	74.03	(0.24)	20.83	(0.35)

[1]Except for % Protein, where n = 13.

[2]Except for % Protein, where n = 8.

pattern of change in the proportion of protein in skeletal muscle of these voles. The percentage of this component fluctuated only slightly, remaining at approximately 20-21% for most of the year (Tables XI and XII). Comparison of skeletal muscle composition between male and female C. gapperi and M. pennsylvanicus are shown in Appendices 13 and 14, respectively.

DISCUSSION

C. gapperi and M. pennsylvanicus showed marked seasonal changes in mass of brown and white adipose tissues which led to distinctly higher lipid reserves in winter than in summer. These changes in lipid reserves, coupled with a reduction in growth during the fall and early winter, are likely to play a major role in the survival of these animals in winter.

Previous studies on the gross body composition of small nonhibernators have shown that increased lipid reserves during the cold seasons of the year is a common seasonal adjustment in these animals (Sealander, 1951; Hayward, 1965; Sawicka-Kapusta, 1968; Fedyk, 1977), but there is little information on whether increases are due to changes in either brown or white adipose tissue, or in both.

It has been suggested by Sealander (1972) that white fat is of less importance than brown fat in the overall seasonal adjustment of C. rutilus. This suggestion is based on the finding that white fat represents a more constant fraction of the body weight when compared with brown fat. Such was not the case in C. gapperi and M. pennsylvanicus, as both types of adipose tissue showed wide variations in mass throughout the year, but they differed with regards to the seasonal pattern of change. Although both tissues reached maximum mass in winter, increases in brown fat mass of both species started in late summer, whereas increases in white fat started

in early winter. This suggests that during the early stages of cold-acclimatization in the fall an increase in thermogenic capacity, through an increase in brown fat mass relative to body weight, is favored over an increase in lipid reserves *per se*, but increased lipid storage in the form of white fat may play a significant role under winter conditions, when there is a deep snow cover and the amount of supranivean activity is reduced (Pruitt, 1957) with a possible reduction in foraging (Stebbins, 1972).

Other studies on seasonal changes in brown fat of small nonhibernators have shown a similar pattern of tissue mass increases over the fall and early winter, but results differ slightly from present results with regards to the time of the year in which maximum brown fat mass is attained. The relative mass of the left axillary brown fat depot in *T. hudsonicus* (Aleksiuk, 1971), and of the interscapular depot in *O. zybethica* (Aleksiuk and Frohlinger, 1972), in *C. rutilus* (Sealander, 1972), and in *P. leucopus* (Lynch, 1973) have been shown to reach a maximum in January. In a study of seasonal changes in the total brown fat mass relative to body weight in *M. pennsylvanicus* in Alberta, Didow and Hayward (1969) have found that mass of this tissue in immature meadow voles reached a maximum in January, whereas in mature animals it reached a maximum in November, with a subsequent decline over the winter months. Since the snow cover had already reached a depth of 25 cm by the end of November, in the year encompassed by the study of Didow and Hayward (1969), the

authors suggested that the increase in the total brown fat mass in mature meadow voles to a maximum prior to winter, and its subsequent decrease during the winter months, were indications that brown fat thermogenesis becomes less important once the microclimate is made more stable and warmer by the presence of an adequate snow cover. The further increase in relative brown fat mass of immature meadow voles to a maximum in January was interpreted as an indication that young animals, which have not yet fully developed their thermoregulatory mechanisms, place a greater reliance on brown fat thermogenesis than mature animals (Didow and Hayward, 1969). An effect of age on the mass of brown adipose tissue relative to body weight has also been reported in other species (Dawkins and Hull, 1964; Tarkkonen and Julka, 1968).

The suggestion that brown fat thermogenesis is of particular value during the early stages of acclimatization in the fall, when temperatures are very low and the animals do not yet have the protection of a subnivean microclimate (Didow and Hayward, 1969; Aleksiuk, 1971; Sealander, 1972) is supported by the results obtained for C. gapperi and M. pennsylvanicus. The lower-than-average snowfall in the autumn and early winter of 1976-1977 in Manitoba resulted in the hiemal threshold not being reached until late January. The lack of a protected microclimate in November, December, and January, when mean minimum temperatures were at least 10, and sometimes as low as 25 degrees below 0^{o}C, must have imposed unusually severe requirements for heat production in

the vole population. Accordingly, increases in brown fat mass of both species continued throughout the fall and early winter until February, when the snow cover had reached a depth of 25-28 cm, sufficient to provide enough insulation for a warmer microclimate. At that time the mean absolute mass of brown fat ceased to rise, and the mean body weight started to increase, leading to a decline in the relative mass of the tissue during the following months.

The decline in relative and absolute mass of brown fat seen in C. gapperi in December was mostly due to a decrease in the percentage of lipids in the tissue. This decrease in lipid reserves is also probably related to the late arrival of snow. The lack of a protected microclimate, coupled with colder than usual nights in December, may have imposed so great a requirement for heat production in red-backed voles that it resulted in net mobilization of their lipid reserves. This view is further supported by the fact that white fat also showed a decline in mass due to a decrease in the percentage of lipids in December. Since no M. pennsylvanicus were captured in December, it is not known whether these voles showed a similar decline in the mass and percentage of lipids of their adipose tissues at that time.

It is possible that part of the increase in the relative and absolute mass of brown fat in both species in January and February was due to the fact that samples for those months consisted of animals captured at the Delta Field Station. There were no significant differences, however, in

brown fat mass relative to body weight of red-backed voles and meadow voles captured at the two locations from March to May, 1977, and significant differences in absolute mass of brown fat were found only between samples of M. pennsylvanicus for May, 1977.

Maximum relative brown fat mass of C. gapperi and M. pennsylvanicus (7% and 8%, respectively) was much higher than in the mature and immature meadow voles (1.8% and 2.2%, respectively) studied by Didow and Hayward (1969) in Alberta. Part of this discrepancy is no doubt due to the fact that total relative brown fat mass of the Albertan voles was calculated as a percentage of the total body weight, without taking into account the weight of the gastrointestinal contents, as was done in the present study. However, this and other differences in procedure, such as the use of frozen voles by Didow and Hayward (1969), as opposed to freshly-killed animals in the present study, are not likely to account completely for the much higher values which were found in the voles in Manitoba. Present results for C. gapperi and M. pennsylvanicus, although also somewhat higher, are in closer agreement with results obtained by Sealander (1972) in C. rutilus. The relative mass of the interscapular brown fat depot in C. gapperi and M. pennsylvanicus, at its maximum, was 1.41 and 1.10% of body weight, respectively. The maximum mass of the same depot in C. rutilus was 0.95% of the body weight (Sealander, 1972). The greater brown fat mass of voles in Manitoba, when compared to voles in Alberta (Didow and Hayward,

1969), and Alaska (Sealander, 1972) may be related to a greater need for brown fat thermogenesis of the Manitoban voles, due to the unusually harsh conditions in the fall and early winter of 1976-1977.

It should further be noted that studies by Hissa and Tarkkonen (1969) on M. agrestis and C. glareolus in Finland provide another example of conflicting results. These investigators found that although the relative mass of the interscapular brown fat depot increased slightly between summer and winter (0.38 to 0.41% in C. glareolus, and 0.21 to 0.25% in M. agrestis), differences between the two seasons were not statistically significant. It is possible, however, that more pronounced changes in the total mass of the tissue may have occurred which were not paralleled by changes in the interscapular depot. This suggestion is based on the observation that in C. gapperi and M. pennsylvanicus the ratio - interscapular brown fat : total brown fat mass - declined slightly during the period of large increases in total brown fat mass in the fall. If growth of this tissue in response to changes in the environment occurs to the same extent in all depots, then one would expect that ratio to remain relatively constant throughout the year.

The discrepancies in relative mass of brown fat which have been found in these various studies may indicate that, although the presence of brown fat and its growth in response to increased thermogenic needs may be a characteristic of small nonhibernators, the actual mass of the tissue in a

given population depends also on the prevailing regional environmental conditions, such as length of the fall critical period.

Seasonal changes in brown fat mass of C. gapperi and M. pennsylvanicus were not accompanied by similarly pronounced changes in the proportions of lipid, water, and protein in the tissue. There was no obvious seasonal pattern of increase in the percentage of lipid in brown fat over the autumn and winter months, as seen in T. hudsonicus (Aleksiuk, 1971) and O. zibethica (Aleksiuk and Frohlinger, 1971). The slight increase in monthly mean percentage of lipids in brown fat of both species in January and February may have been due to the fact that voles captured at the Delta Field Station had a higher percentage of this component in their brown fat than voles captured in Winnipeg, although differences were significant only in M. pennsylvanicus in March. Variations in the lipid component were always accompanied by reverse changes in the water component, a situation also observed by other investigators (Aleksiuk, 1971; Aleksiuk and Frohlinger, 1971). A lack of marked seasonal changes in the percentages of lipid and water in brown adipose tissue has been reported also for M. pennsylvanicus in Alberta (Didow and Hayward, 1969).

The percentage of protein in brown fat is often used as an indicator of the thermogenic capacity of the tissue. Presumably, increases in the proportion of metabolically-active nitrogen-containing compounds in the tissue corre-

spond to an increase in its heat-producing capacity. The stable percentage of protein in brown fat of C. gapperi and M. pennsylvanicus throughout the year indicates that the thermogenic capacity of the tissue does not change on a seasonal basis, as does its mass. A similar lack of seasonal effect on the percentage of protein in brown fat has also been reported for other populations of small nonhibernators (Didow and Hayward, 1969; Aleksiuk, 1971; Aleksiuk and Frohlinger, 1971). In addition, no statistically significant differences were found on the incorporation of labeled amino acids into protein by brown adipose tissue between summer- and winter-acclimatized M. pennsylvanicus, in response to exposure temperatures of 15 and 25°C (Narayansingh and Aleksiuk, 1972).

Results for free-ranging populations are in contrast with results obtained from studies on cold-induced changes in brown fat in the laboratory. This tissue in cold-acclimated rats has been shown to have both a higher mass and a higher proportion of protein than brown fat in control rats (Babineau and Page, 1950; Smith and Roberts, 1964; Roberts and Smith, 1967; Thomson et al., 1969; Portet et al., 1976). This suggests that the increase in thermogenic capacity of the animal due to cold-acclimation in the laboratory involves both an increase in mass, as well as an increase in the thermogenic capacity of brown fat tissue itself. Seasonal acclimatization, however, involves changes in brown fat mass, but apparently no changes in the metabolic capacity of the

tissue itself, as suggested by its unchanged protein content throughout the year. Thus, in nature the thermogenic capacity of brown fat may be kept at a relatively constant level, and changes in the total thermogenic capacity due to brown fat may be mainly a consequence of changes in the mass of this tissue.

Maintenance of a presumably high thermogenic capacity of brown fat at all times of the year would be advantageous to C. gapperi and M. pennsylvanicus, since it has been suggested that extra heat production for thermoregulation in small nonhibernators may be necessary even in summer (Didow and Hayward, 1969). These investigators found that in Alberta the microhabitat temperature of M. pennsylvanicus did not rise above 15°C even in the hottest times of the year encompassed by their study. This temperature is well below the lower critical temperature (25°C) of C. gapperi (McManus, 1974), and M. pennsylvanicus (Weigert, 1961). Even assuming that the microhabitat temperature of voles in Manitoba did rise to 25°C, thus eliminating the need for extra heat production for thermoregulation while the animals were in the nest, nocturnal foraging would, at least at times, expose these voles to temperatures considerably below their lower critical level, and they might have to resort to brown fat thermogenesis to maintain body temperature.

Present results for C. gapperi and M. pennsylvanicus do not support the suggestion that brown fat mass may be influenced by the reproductive physiology of the animal (Didow

and Hayward, 1969), nor that this tissue may act as an important energy source during pregnancy and lactation (Sealander, 1972), as there were no significant differences in mass and composition of brown fat between the two sexes in both species during the months of May, June, and July, 1976, when most of the females in the monthly samples were pregnant or lactating. The few significant differences which were found between male and female red-backed voles at other times of the year do not appear to be related to reproduction. Although the absolute brown fat mass of female C. gapperi was significantly lower than that of males in October, none of the females in the sample for that month showed signs of pregnancy or lactation. Since there were no differences in the percentage of brown fat between the sexes, the higher absolute mass of this tissue in males in the October sample is probably a reflection of their higher body weight. The only other difference between the two sexes of red-backed voles was found in the percentage of lipids in brown fat in March and April, 1977, with that for males being significantly higher. Again, this does not appear to result from lipid depletion due to lactation or embryonic development because none of the females in the sample for March showed signs of pregnancy or lactation, although two of the four females in the sample for April appeared to be in the early stages of gestation.

There was suggestive evidence that death in some of the snap-trapped C. gapperi did not occur instantly, which might have resulted in cold exposure for an undetermined period

prior to death. This would very likely lead to rapid utilization of brown fat for thermoregulation, which might be the reason for the significantly lower mass and lipid percentage in brown fat of snap-trapped when compared to live-trapped voles.

White fat in C. gapperi and M. pennsylvanicus differed from brown fat in that pronounced differences existed between the two sexes, and these were due to reproduction. Females of both species had a greater white fat mass than males during the breeding season, but variability among individual females was also greater than among males. These differences between males and females in the summer resulted in a slightly different seasonal pattern of change in white fat mass for the two sexes. Both males and females had a greater white fat mass in winter than in fall, but whereas males also had more white fat in winter than in the previous summer, females had similar amounts of white fat during these two seasons.

The rise in white fat mass of C. gapperi and M. pennsylvanicus in January and February could be partly due to the fact that samples for these months consisted of voles captured at the Delta Field Station. Animals from that location had a larger white fat mass than voles captured in Winnipeg in March, April, and May, 1977, although differences were statistically significant only in a few instances. A seasonal effect was also present, however, since voles captured in Winnipeg in March, April, and May, 1977 also had a greater white fat mass than voles captured at the same location dur-

ing the previous fall.

The percentage composition of white fat was also influenced by reproduction. Pregnant and/or lactating females had a much lower percentage of lipids, and higher percentages of water and protein in white fat than non-pregnant, non-lactating females. This lower lipid content in white fat of reproducing females is probably related to the increase in energy requirements due to gestation and lactation (Kaczmarski, 1966; Migula, 1969).

There were no significant differences in the percentage composition of white fat between male and female C. gapperi and M. pennsylvanicus throughout the year, when reproducing females were excluded from monthly samples. As in brown fat, compositional variations in this tissue were mostly due to changes in the lipid and water components, with changes in the percentage of lipids being inversely related to changes in the percentage of water. The percentage of protein in white fat remained unchanged throughout the year. This, too, might indicate that the metabolic capacity of white adipose tissue remains relatively constant throughout the year.

Since adipose tissues are the main sites of lipid storage in mammals, the lipid content in brown and white fat of C. gapperi and M. pennsylvanicus may be used as an indicator of the total lipid reserves in these animals. The increase in mass of brown and white adipose tissues in winter, coupled with a less pronounced increase in the percentage of lipids, led to a large increase in the lipid reserves of C. gapperi

and M. pennsylvanicus relative to body weight, with approximately half of the increase being due to an increase in brown fat lipids. C. gapperi had significantly more lipids stored in brown fat than in white fat from August to January, whereas M. pennsylvanicus had significantly more white fat lipids than brown fat lipids in April and May, 1977.

The significance of increased lipid reserves in winter for the survival of small nonhibernators has been the subject of controversy. Most authors suggest that the lipid stored represents a source of fuel which the animal may utilize during periods of low temperatures and low food availability (Sealander, 1951; Hayward, 1965; Sawicka-Kapusta, 1968; Fleharty et al., 1973). Others maintain that the actual amount of lipids stored is relatively too small to constitute the only source of metabolizable fuel for any considerable length of time (Schreiber and Johnson, 1975). In fact, it has been shown by Kolodziej-Banach (1976) that even when the lipid content of the common vole, Microtus arvalis, is at its maximum in late winter, the amount of lipids stored (an average of 2 g per individual) would be sufficient to cover maintenance costs for less than two days and could not, therefore, represent the sole energy supply for thermoregulation. The author suggested that subcutaneous fat might serve to provide thermal insulation.

Conclusions as to the particular role of increased lipid reserves in winter, however, may be arrived at only if the sites of increased deposition are also taken into consi-

deration. Present results for C. gapperi and M. pennsylvanicus show that approximately half of the increase in lipid reserves of these voles in winter can be attributed to an increase in lipids stored in brown fat, and, therefore, must be related to their need for a greater supply of substrate to support the heat-producing process in brown adipose tissue. Although the lipids stored in brown and white fat of red-backed and meadow voles are not likely to constitute their sole energy supply during winter, they do, nevertheless, represent a source of utilizable substrate which could be drawn upon during brief periods of stress and must contribute to survival in winter.

C. gapperi and M. pennsylvanicus must be able to secure enough food in winter to satisfy their energy requirements for thermoregulation. This could be achieved by changing to a food item readily available in winter, as is the case of C. gapperi in Manitoba, which feeds mainly on the bark of young trees during the cold seasons of the year (Criddle, 1932). Storage of food in the fall would also provide a steady supply of energy in winter, and although a hoarding behavior has not yet been demonstrated in C. gapperi and M. pennsylvanicus in nature, there is evidence that Clethrionomys in the Russian taiga store sufficient food to last all winter (Koshkina, 1957), and a pronounced hoarding behavior has been demonstrated in M. pennsylvanicus in the laboratory (Lanier, et al., 1974).

It is noteworthy that in C. gapperi and M. pennsylvanicus neither the mass nor the amount of lipids stored in brown and white fat in May, 1977 fell to levels as low as those seen in May, 1976. Year-to-year variations in patterns of lipid deposition have also been shown by Pucek (1973) in A. flavicolis and C. glareolus, in which greater differences were found when comparing the same season in different years than when comparing different seasons in the same year. Differences between two successive years have also been shown for M. arvalis, in which the pattern of change was similar in both years, increasing in fall and winter and decreasing in spring, but the amounts of lipid deposited were greater in the second year (Kolodziej-Banach, 1976). The possibility that lipid cycles of longer periodicity may be superimposed on annual cycles has also been suggested by Iverson and Turner (1975) for M. pennsylvanicus. It appears that the characteristics of seasonal variation in the total lipid content of small nonhibernators in different years may be linked to population dynamics such as variation in numbers, and the actual reproduction and mortality taking place (Kolodziej-Banach, 1976). Since present results show that a great part of the increase in the lipid reserves of C. gapperi and M. pennsylvanicus may be attributed to an increase in the amount of lipids stored in brown fat, differences in the seasonal pattern of change in the total lipid content from year to year may also be closely related to environmental conditions such as duration of the fall and spring critical periods.

Besides the pronounced seasonal changes in mass and in the amount of lipids stored in brown and white adipose tissues, C. gapperi and M. pennsylvanicus also showed considerably lower mean body weight, total length, and skeletal muscle mass in the fall. Lower body weight in fall and winter appear to be characteristic of northern populations of small nonhibernators (Sealander, 1951, 1966; Mezhzherin, 1964; Fuller et al., 1969; Brown, 1973; Iverson and Turner, 1974; Stebbins, 1976; Whitney, 1976). The two main causes for this smaller size are believed to be a change in the age structure of the population, which results in a predominance of younger, smaller animals in the fall-winter population (Sealander, 1966, 1972; Stebbins, 1976), and a weight loss by individuals in late summer and fall (Boikova and Boikov, 1972; Brown, 1973; Iverson and Turner, 1973; Whitney, 1978).

While present results for C. gapperi and M. pennsylvanicus offer no information with regard to seasonal changes in weight of individual animals, the data indicate that the apparent decline in size of both species in fall was due to an increased proportion of younger animals in the monthly samples. Moreover, total length and skeletal muscle mass remained virtually unchanged from September to February, but increased rapidly in March and April, which indicates that growth is reduced during fall and winter, but resumes again in spring.

A fall-winter decrease in growth in other populations of small nonhibernators has been previously reported by other

investigators (Aleksiuk and Frohlinger, 1971; Brown, 1973; Iverson and Turner, 1974; Stebbins, 1976; Fuller, 1977). Furthermore, laboratory studies have shown that muscle growth is inhibited by low temperatures, as evidenced by reduced DNA synthesis in skeletal muscle of warm-acclimated rats exposed to 7°C, when compared to warm-acclimated rats exposed to 24°C (Nusetti and Aleksiuk, 1975).

The advantage of reduced growth in overwintering populations of small nonhibernators are believed to be twofold: Firstly, maintenance of a smaller size in winter would lead to a reduction in the food requirements in direct proportion to size (Iverson and Turner, 1975). This has been confirmed by Stebbins (1978) in P. maniculatus, in which the daily consumption of energy (i.e., Kilocalories consumed/mean weight of animal/day) did not increase over the winter when energy requirements were greater. This could be achieved, at least partially, through a reduction in growth. Secondly, a reduction in the energy expended for cell proliferation and growth would make more energy available for thermoregulation (Sealander, 1966; Fuller, 1969; Stebbins, 1976, 1978).

It should be noted that the reduction in growth of skeletal muscle was not accompanied by changes in the percentage of protein in the tissue, but that the percentage of water decreased slightly and the percentage of lipids increased twofold. However, since the percentage of lipids in skeletal muscle is comparatively small (2 to 5%), it is doubtful that the higher lipid content of this tissue in

winter would contribute greatly to the total lipid reserves of the animal.

It can be concluded from these results that winter acclimatization in C. gapperi and M. pennsylvanicus involves an increase in mass of both types of adipose tissue. Whereas increases in brown fat start in late summer, in white fat they start in early winter. This indicates that during the early stages of cold-acclimatization in the fall an increase in thermogenic capacity is favored over an increase in lipid reserves per se. The amounts of brown fat deposited by C. gapperi and M. pennsylvanicus were higher than those reported for other species of voles, and this could be at least partly due to greater thermogenic requirements imposed upon the animals by the very severe conditions in fall and early winter of 1976-1977 in Manitoba.

Gestation and lactation led to a decline in the amount of lipids stored in white fat of female voles during the breeding season, but brown fat did not appear to serve as a source of fuel during reproductive processes.

Both species of voles had greater lipid reserves in winter than in summer. High lipid reserves in winter are apparently related to the maintenance of fuel supply for the thermogenic processes which take place in brown fat, since approximately half of the increase in lipids was due to greater amounts of this substrate stored in that tissue. This was particularly true for C. gapperi, which had more lipids stored in brown fat than in white fat for most of the

year.

Voles of both species captured in fall and winter were smaller than voles captured at other times of the year due to a predominance of younger, smaller animals in the samples. These young voles were animals born in the previous spring and summer, but which had not yet reached sexual maturity. Growth in the young voles was arrested in fall and winter, but resumed again in spring.

Increases in the thermogenic capacity and in the lipid reserves, as well as a probable decrease in the energy expended for cell proliferation and growth, may be considered as important physiological adjustments which together with such other changes as increased fur insulation, are held to contribute to the survival of C. gapperi and M. pennsylvanicus in winter.

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APPENDICES

Appendix 1A. Determination of total muscle mass.

Carcasses which had been selected for muscle mass determination were placed in glass jars filled with an enzyme bath, which was prepared by dissolving 1.2% powdered, commercial laundry detergent (Sunlight), 1.2% commercial laundry presoak powder (Bio-Ad), and 0.03% commercial papain-containing meat tenderizer in tap water.

The jars with contents were then transferred to an oven at 45-48^{o}C, and the digestion was allowed to proceed only until the muscle tissue could be easily detached from bones, to avoid loss of bone substance during digestion. The carcasses were then rinsed in tap water, and the muscle was separated from bones manually, with the aid of a dissecting microscope. The clean skeleton was rinsed in tap water over a finely-meshed metal screen, drained of excess water on paper towels, and weighed. Total muscle mass was recorded as the difference between carcass and skeleton weights.

The simple linear regression equation of muscle mass (Y) on carcass weight (X) was calculated for each of the two species. As the correlation coefficient between these two measurements was high in both species ($r > 0.99$), the muscle mass of individual voles for which muscle digestion was not performed was calculated by substituting their carcass weight in the regression equation for the species.

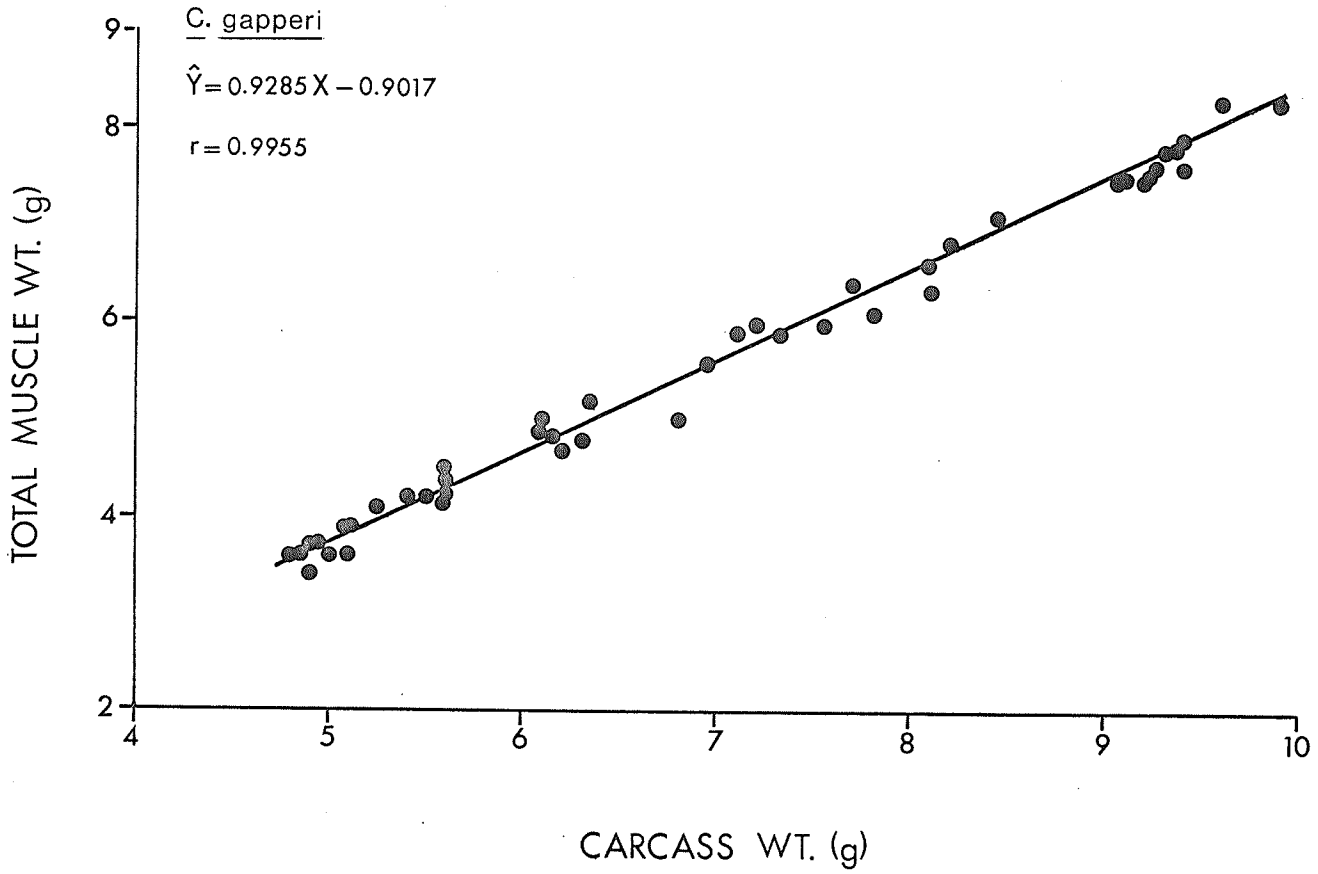
Appendix 1B. Weights of the carcass, skeleton, and total muscle tissue of individual C. gapperi submitted to enzyme bath digestion.

Animal No.	Weight (g)		
	Carcass	Skeleton	Muscle
14	7.14	1.22	5.92
16	7.30	1.42	5.89
22	7.69	1.25	6.44
23	9.37	1.78	7.59
33	9.20	1.67	7.53
42	6.32	1.48	4.83
45	6.17	1.45	4.73
49	5.62	1.37	4.25
51	4.76	1.21	3.55
52	5.63	1.24	4.39
54	5.03	1.45	3.58
55	4.87	1.20	3.67
57	5.46	1.31	4.15
62	4.92	1.50	3.42
63	5.07	1.46	3.61
67	5.24	1.16	4.07
72	4.93	1.24	3.69
75	6.77	1.77	5.00
79	5.60	1.38	4.21
80	5.08	1.24	3.85
88	5.10	1.19	3.91
89	4.82	1.22	3.60

Appendix 1B, Continued:

Animal No.	Weight (g)		
	Carcass	Skeleton	Muscle
94	5.42	1.22	4.20
101	6.12	1.09	5.03
104	9.25	1.63	7.62
108	7.21	1.24	5.97
111	5.57	1.09	4.47
113	6.13	1.21	4.93
115	6.35	1.18	5.17
122	6.95	1.35	5.60
126	8.22	1.47	6.76
130	9.92	1.65	8.27
131	6.14	1.29	4.85
133	9.62	1.34	8.28
134	8.43	1.36	7.07
135	9.07	1.57	7.50
136	9.38	1.48	7.90
137	9.04	1.51	7.53
138	9.35	1.56	7.78
139	8.09	1.54	6.55
144	8.11	1.77	6.34
149	7.82	1.70	6.12
150	9.30	1.50	7.80
153	7.54	1.54	6.01

Appendix 1C. Simple linear regression of total skeletal muscle weight (Y) on carcass weight (X) of C. gapperi. Each point represents data from one animal.


C. gapperi
$\hat{Y} = 0.9285X - 0.9017$
r = 0.9955
TOTAL MUSCLE WT. (g)
CARCASS WT. (g)
2
4
6
8
9
4
5
6
7
8
9
10

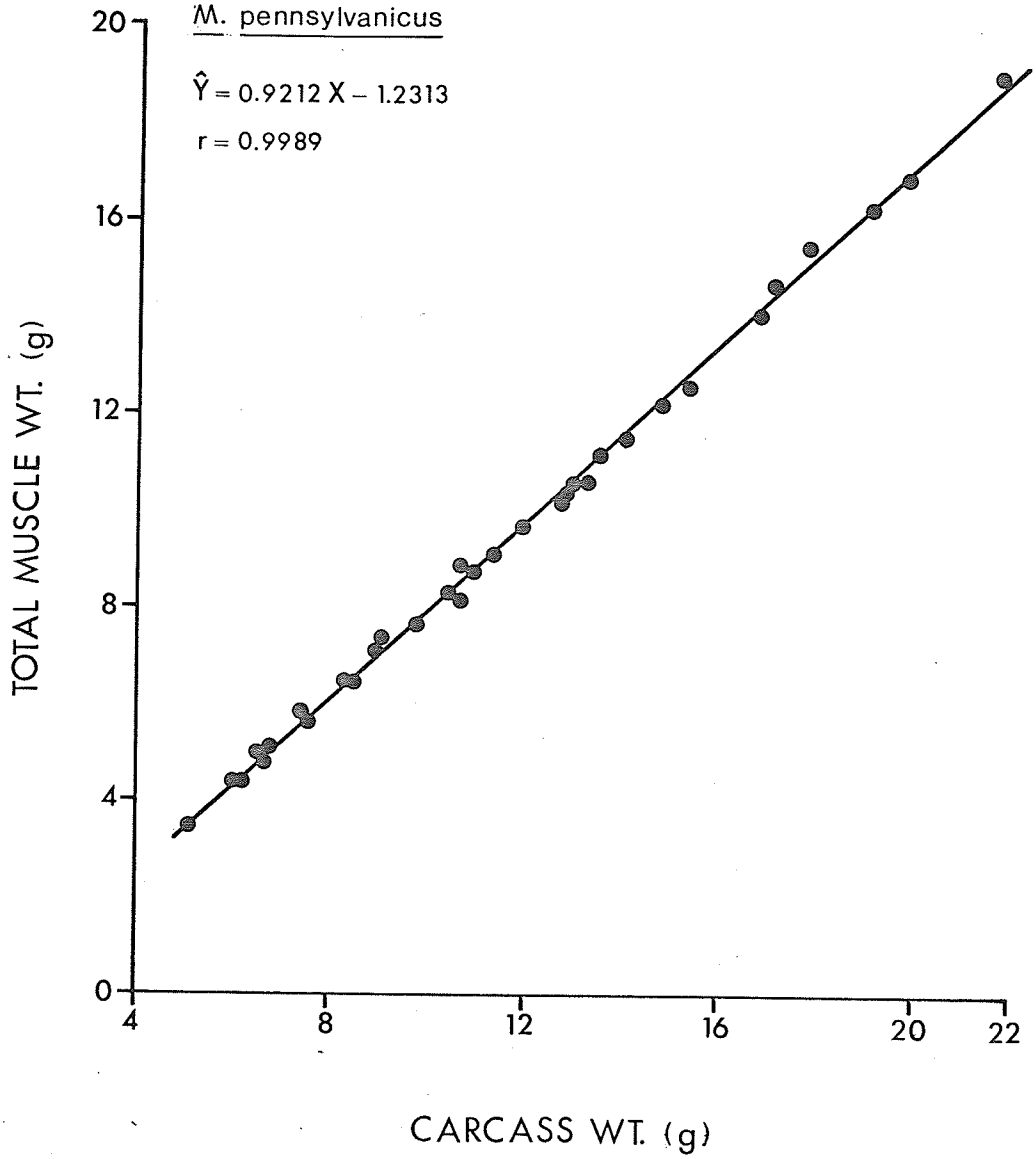
Appendix 1D. Weights of the carcass, skeleton, and total muscle mass of individual M. pennsylvanicus submitted to enzyme bath digestion.

Animal No.	Weight (g)		
	Carcass	Skeleton	Muscle
15	10.59	1.69	8.90
16	14.72	2.52	12.20
20	8.90	1.55	7.35
22	13.48	2.28	11.20
23	21.74	2.75	18.99
27	6.63	1.84	4.79
30	16.72	2.60	14.12
31	8.30	1.82	6.48
34	9.64	1.97	7.67
37	12.72	2.51	10.21
41	7.37	1.62	5.75
42	5.10	1.62	3.48
44	13.15	2.53	10.62
45	7.47	1.86	5.61
46	10.92	2.15	8.77
51	8.43	1.94	6.49
55	6.16	1.74	4.42
59	6.46	1.61	4.85
62	6.71	1.77	4.94
65	6.14	1.77	4.37
66	8.93	1.84	7.09
72	12.85	2.22	10.63

Appendix 1D, Continued:

Animal No.	Weight (g)		
	Carcass	Skeleton	Muscle
74	13.95	2.41	11.54
81	11.91	2.21	9.70
84	11.25	2.19	9.06
85	10.59	2.39	8.20
86	12.76	2.40	10.36
89	19.01	2.73	16.28
90	10.39	2.08	8.31
91	17.00	2.35	14.65
92	17.71	2.17	15.54
93	19.82	2.95	16.87
94	15.34	2.68	12.60

Appendix 1E. Simple linear regression of total skeletal muscle weight (Y) on carcass weight (X) of M. pennsylvanicus. Each point represents data from one animal.


M. pennsylvanicus
$\hat{Y} = 0.9212\,X - 1.2313$
r = 0.9989
TOTAL MUSCLE WT. (g)
CARCASS WT. (g)
0
4
8
12
16
20
4
8
12
16
20
22

Appendix 2. Preliminary standard determination of the percentages of lipid, water, and protein in liver tissue of a laboratory mouse.

Sample No.	Wet Tissue Wt. (mg)	% Lipid	% Water	% Protein
1	64.6	4.33	68.73	21.97
2	36.6	4.37	68.58	21.86
3	66.7	4.35	68.52	22.34
4	96.4	4.67	67.95	22.38
5	166.5	4.37	68.29	21.88
6	150.7	4.11	68.35	20.63
$\bar{X}$		4.37	68.40	21.84
SD		0.18	0.27	0.64

$\bar{X}$: Mean of six determinations.

SD: Standard deviation.

Appendix 3A. Micro-Kjeldahl procedure.

i. Digestion

Dry, lipid-free tissue samples were placed in 30 ml Kjeldahl digestion flasks. To each flask were added the following reagents:

2 ml of 36N H_2SO_4

1.6 g of K_2SO_4

0.5 ml of catalyst solution (10 g HgO/100 ml 4N H_2SO_4)

Two glass beads were added to the digestion mixture, and the sides of the flasks were washed with 1-2 ml of distilled water. Flasks were placed on a gas-heated digestion rack, and the contents were boiled vigorously until the mixture was clear. Digestion was continued for 45 minutes after clearing, giving a total digestion time of approximately 50 minutes.

ii. Distillation

Two to 3 ml of distilled water were added to the flasks to dissolve salts which formed upon cooling. The contents were then transferred to a micro-Kjeldahl distillation apparatus, with 5 washings of 1-2 ml of distilled water each. The digest was neutralized by adding 10 ml of alkali (200 g NaOH + 12.5 g $Na_2S_2O_5 \cdot 5H_2O$/500 ml H O), and was then steam distilled. The distillate was collected in 15 ml of a saturated, aqueous, boric acid solution, to which had been added 5 drops of an indicator mixture (2 parts 0.2% methyl red in 95% ethanol : 1 part 0.2% methylene blue in 95% ethanol). The distillation was stopped when 15 ml of the distillate had been

Appendix 3A, Continued:

collected, and the tip of the condenser was rinsed with 2-3 ml of distilled water directly into the flask containing the boric acid-distillate complex.

iii. Titration

The boric acid-distillate complex was titrated with 0.01N H_2SO_4 to the violet endpoint (i.e., until the mixture acquired a permanent violet color). The percentage of nitrogen in the sample was calculated by the equation:

$$\% N = \frac{\text{ml } H_2SO_4 \times 0.01 \times 14}{\text{mg of tissue}} \times 100, \text{ where:}$$

ml H_2SO_4 = volume of acid used to titrate the sample minus the volume used to titrate the blank;

0.01 = normality of the H_2SO_4 used for titration;

14 = equivalent weight of N; and

mg tissue = fresh weight of tissue sample.

The percentage of protein in the sample was calculated by multiplying the percentage of nitrogen by 6.25.

Appendix 3B. Percentage recovery of nitrogen by the micro-Kjeldahl method. Test standards consisted of an aqueous solution of urea[1], which contained 2 mg N/ml.

Trial No.	Standard (ml)	mg of N in Standard	mg of N Recovered	% Recovery
1	1.0	2.0	1.93	96.5
2	1.0	2.0	1.95	97.5
3	1.0	2.0	1.98	99.0
4	1.0	2.0	1.99	99.5
5	0.5	1.0	0.95	95.0
6	0.5	1.0	0.97	97.0
7	0.5	1.0	0.98	98.0
8	0.5	1.0	0.98	98.0
X ± SD				97.6 ± 1.4

[1]Prepared by making up 4.2858 g of urea to 1 liter with distilled water (Munro and Fleck, 1969).

Appendix 4. Comparison between live-trapped and snap-trapped C. gapperi.

	Live-trapped (n=3)		Snap-trapped (n=7)	
	$\bar{X}$	(SE)	$\bar{X}$	(SE)
Absolute mass of brown fat (g)	0.52	(0.07)	0.35	(0.01)**
Relative mass[1] of brown fat	4.97	(0.51)	3.32	(0.13)**
Brown fat composition: % Lipid	54.18	(5.08)	28.67	(1.59)**
% Water	32.62	(3.71)	51.43	(1.24)**
% Protein	10.68	(1.85)	16.00	(0.54)**
Absolute mass of white fat (g)	0.25	(0.06)	0.20	(0.02)
Relative mass of white fat	2.30	(0.53)	1.70	(0.24)
White fat composition: % Lipid	70.60	(4.23)	61.44	(3.98)
% Water	25.06	(3.73)	32.25	(3.48)
% Protein	3.23	(0.46)	2.90	(0.34)
Body weight (g)	11.61	(0.34)	11.26	(0.32)
Total length (mm)	113.83	(1.17)	112.43	(0.92)
Skeletal muscle mass (g)	4.12	(0.21)	4.12	(0.21)
Muscle composition: % Lipid	3.06	(0.42)	2.71	(0.22)
% Water	72.89	(1.07)	74.22	(0.93)
% Protein	20.98	(0.64)	20.04	(0.20)

[1]Total tissue mass as a percentage of the body weight.

**Indicates significant differences ($P < 0.01$) between the two groups of voles.

Appendix 5A. Monthly mean mass of brown fat as a percentage of body weight (% BF) in C. gapperi, from May, 1976 to May, 1977.

Month	% BF of Males and Females			% BF of Females			% BF of Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	1.46	(0.28)	4	1.29	(0.33)	3	1.68	(0.55)
Jun	15	1.81	(0.17)	7	1.77	(0.27)	8	1.85	(0.22)
Jul	12	1.82	(0.31)	5	1.85	(0.78)	7	1.85	(0.25)
Aug	12	2.79	(0.22)	6	2.48	(0.31)	6	3.11	(0.27)
Sep	11	4.03	(0.41)	4	3.61	(0.88)	7	4.27	(0.45)
Oct	13	4.75	(0.34)	6	4.37	(0.36)	7	5.52	(0.52)
Nov	9	5.76	(0.34)	6	5.99	(0.43)	3	5.31	(0.56)
Dec	3	4.50	(0.46)	1	5.24	———	2	4.13	(0.47)
Jan	9	6.50	(0.23)	5	6.63	(0.20)	4	6.34	(0.47)
Feb	11	7.35	(0.35)	3	6.58	(0.49)	8	7.63	(0.45)
Mar	18	5.93	(0.24)	8	5.72	(0.34)	10	6.10	(0.34)
Apr	15	5.25	(0.21)	4	5.15	(0.26)	11	5.28	(0.28)
May	13	4.07	(0.32)	4	4.41	(0.42)	9	3.92	(0.43)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for August means which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 5B. Monthly mean absolute mass of brown fat in C. gapperi, from May, 1976 to May, 1977.

	ABSOLUTE MASS OF BROWN FAT (g)								
	Males and Females			Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	0.27	(0.06)	4	0.24	(0.08)	3	0.31	(0.09)
Jun	15	0.32	(0.03)	7	0.31	(0.04)	8	0.32	(0.05)
Jul	12	0.25	(0.04)	5	0.27	(0.08)	7	0.24	(0.03)
Aug	12	0.40	(0.03)	6	0.35	(0.04)	6	0.44	(0.04)
Sep	11	0.50	(0.05)	4	0.44	(0.11)	7	0.53	(0.05)
Oct	13	0.61	(0.04)	6	0.52	(0.05)	7	0.69	(0.04)*
Nov	9	0.71	(0.03)	6	0.74	(0.03)	3	0.63	(0.06)
Dec	3	0.52	(0.07)	1	0.63	——	2	0.47	(0.07)
Jan	9	0.90	(0.07)	5	0.97	(0.09)	4	0.80	(0.09)
Feb	11	1.06	(0.06)	3	1.01	(0.10)	8	1.08	(0.07)
Mar	18	0.95	(0.05)	8	0.87	(0.07)	10	1.01	(0.06)
Apr	15	1.01	(0.05)	4	0.88	(0.06)	11	1.05	(0.06)
May	13	0.78	(0.07)	4	0.77	(0.07)	9	0.79	(0.10)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for July means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.05$).

Appendix 5C. Monthly mean percentage of lipid in brown fat in C. gapperi, from June, 1976 to May, 1977.

Month	% LIPID IN BROWN FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	15	52.39	(3.26)	7	53.38	(3.54)	8	51.53	(5.49)
Jul	11	51.62	(4.56)	5	53.70	(8.85)	6	49.88	(4.77)
Aug	12	58.87	(2.80)	6	62.19	(2.37)	6	55.54	(4.94)
Sep	11	62.06	(3.51)	4	59.05	(9.26)	7	63.77	(2.52)
Oct	12	60.05	(2.44)	6	59.29	(3.31)	6	60.87	(3.82)
Nov	9	63.30	(2.04)	6	64.61	(2.68)	3	60.67	(2.96)
Dec	3	54.18	(5.08)	1	62.63	———	2	49.96	(4.89)
Jan	9	67.61	(2.38)	5	70.88	(0.95)	4	63.52	(4.76)
Feb	11	66.59	(1.82)	3	66.41	(1.58)	8	66.66	(2.49)
Mar	18	63.00	(2.32)	8	57.88	(4.15)	10	67.09	(1.86)*
Apr	15	66.90	(1.51)	4	61.45	(2.66)	11	68.88	(1.45)*
May	13	62.19	(2.44)	4	62.69	(2.67)	9	62.26	(3.58)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for September and January means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.05$).

Appendix 5D. Monthly mean percentage of water in brown fat in C. gapperi, from June, 1976 to May, 1977.

Month	% WATER IN BROWN FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	15	34.40	(2.64)	7	33.02	(2.85)	8	35.61	(4.43)
Jul	12	36.93	(3.59)	5	34.96	(7.35)	7	38.34	(3.72)
Aug	12	31.11	(2.17)	6	28.03	(1.64)	6	34.19	(3.78)
Sep	11	27.31	(2.76)	4	30.11	(6.81)	7	25.71	(2.36)
Oct	12	29.26	(1.69)	6	29.43	(2.46)	6	29.09	(2.55)
Nov	9	26.16	(1.42)	6	25.46	(1.89)	3	27.57	(2.26)
Dec	3	32.62	(3.71)	1	26.31	——	2	35.77	(3.37)
Jan	9	23.60	(1.54)	5	21.73	(0.92)	4	25.94	(3.08)
Feb	11	24.31	(1.31)	3	24.87	(1.35)	8	24.10	(1.77)
Mar	18	26.15	(1.63)	8	29.23	(3.09)	10	23.68	(1.25)
Apr	15	23.78	(1.00)	4	26.78	(1.95)	11	22.69	(1.03)
May	13	27.14	(1.75)	4	26.85	(1.70)	9	26.16	(2.17)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for March means which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 5E. Monthly mean percentage of protein in brown fat in C. gapperi, from June, 1976 to May, 1977.

Month	% PROTEIN IN BROWN FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	14	8.35	(0.82)	6	7.74	(0.83)	8	8.80	(1.33)
Jul	11	9.89	(0.93)	5	9.10	(1.48)	6	10.55	(1.22)
Aug	11	8.38	(0.72)	5	8.31	(0.55)	6	8.44	(1.30)
Sep	11	8.08	(0.83)	4	9.22	(2.15)	7	7.43	(0.53)
Oct	12	8.07	(0.54)	6	9.02	(0.90)	6	7.13	(0.31)
Nov	9	8.06	(0.47)	6	7.53	(0.43)	3	9.13	(0.93)
Dec	3	10.68	(1.84)	1	8.23	———	2	11.90	(2.23)
Jan	9	6.87	(0.43)	5	6.24	(0.22)	4	7.66	(0.82)
Feb	11	6.40	(0.35)	3	6.42	(0.69)	8	6.40	(0.44)
Mar	18	7.52	(0.56)	8	8.41	(1.11)	10	6.81	(0.41)
Apr	15	6.63	(0.40)	4	7.24	(0.84)	11	6.41	(0.46)
May	13	7.23	(0.44)	4	7.53	(0.68)	9	7.10	(0.58)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for September, October, January, and March means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 6A. Monthly mean mass of brown fat as a percentage of body weight (% BF) in M. pennsylvanicus, from May, 1976 to May, 1977.

Month	% BF of Males and Females			% BF of Females			% BF of Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	0.79	(0.21)	5	0.66	(0.29)	2	1.09	(0.06)
Jun	14	1.53	(0.15)	5	1.28	(0.17)	9	1.67	(0.20)
Jul	11	2.05	(0.37)	5	2.14	(0.65)	6	1.97	(0.48)
Aug	8	1.92	(0.25)	6	1.74	(0.29)	2	2.48	(0.03)
Sep	10	2.83	(0.24)	5	2.66	(0.35)	5	3.00	(0.36)
Oct	6	3.07	(0.08)	4	3.10	(0.11)	2	3.01	(0.09)
Nov	5	4.01	(0.51)	2	4.77	(0.64)	3	3.50	(0.63)
Jan	3	5.08	(0.05)	2	5.12	(0.04)	1	5.00	——
Feb	4	6.11	(1.04)	2	5.36	(0.11)	2	6.86	(2.32)
Mar	9	4.81	(0.50)	6	5.12	(0.72)	3	4.21	(0.43)
Apr	12	3.85	(0.26)	5	4.16	(0.45)	7	3.63	(0.31)
May	12	2.81	(0.25)	5	3.28	(0.30)	7	2.47	(0.32)

Differences between means for males and females were tested by the Student's t-test for unpaired means. There were no significant differences.

Appendix 6B. Monthly mean absolute mass of brown fat in M. pennsylvanicus, from May, 1976 to May, 1977.

	ABSOLUTE MASS OF BROWN FAT (g)								
	Males and Females			Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	0.21	(0.06)	5	0.15	(0.06)	2	0.38	(0.03)
Jun	14	0.41	(0.04)	5	0.38	(0.06)	9	0.43	(0.05)
Jul	11	0.42	(0.06)	5	0.42	(0.09)	6	0.41	(0.09)
Aug	8	0.44	(0.06)	6	0.44	(0.08)	2	0.41	(0.05)
Sep	10	0.51	(0.05)	5	0.44	(0.05)	5	0.59	(0.09)
Oct	6	0.60	(0.05)	4	0.61	(0.08)	2	0.57	(0.04)
Nov	5	0.63	(0.06)	2	0.79	(0.09)	3	0.59	(0.09)
Jan	3	0.90	(0.05)	2	0.85	(0.03)	1	0.98	——
Feb	4	1.13	(0.16)	2	0.98	(0.07)	2	1.27	(0.32)
Mar	9	1.07	(0.15)	6	1.10	(0.23)	3	1.02	(0.12)
Apr	12	0.92	(0.06)	5	0.90	(0.12)	7	0.93	(0.06)
May	12	0.87	(0.08)	5	0.93	(0.14)	7	0.84	(0.11)

Differences between means for males and females were tested by the Student's t-test for unpaired means. There were no significant differences.

Appendix 6C. Monthly mean percentage of lipid in brown fat in M. pennsylvanicus, from June, 1976 to May, 1977.

Month	% LIPID IN BROWN FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	14	59.09	(3.21)	5	62.93	(3.66)	9	56.96	(4.55)
Jul	11	56.00	(4.63)	5	59.66	(7.18)	6	52.95	(6.33)
Aug	8	62.12	(5.85)	6	62.14	(7.83)	2	62.08	(6.36)
Sep	10	61.87	(2.75)	5	63.48	(2.94)	5	60.27	(4.92)
Oct	6	59.59	(3.25)	4	61.79	(4.64)	2	55.18	(0.47)
Nov	5	60.85	(4.29)	2	65.48	(7.19)	3	57.76	(5.68)
Jan	3	68.13	(0.63)	2	68.16	(1.09)	1	68.06	———
Feb	4	72.37	(4.23)	2	74.75	(2.39)	2	69.99	(7.98)
Mar	9	67.34	(3.72)	6	64.03	(5.14)	3	73.95	(0.73)
Apr	12	64.94	(2.71)	5	64.61	(5.56)	7	65.18	(2.89)
May	12	64.32	(3.13)	5	65.77	(2.88)	7	64.72	(4.53)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for March means, which were tested by the Behrens-Fisher test Cochran's modification). There were no significant differences.

Appendix 6D. Monthly mean percentage of water in brown fat in M. pennsylvanicus, from June, 1976 to May, 1977.

Month	% WATER IN BROWN FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	14	29.11	(2.57)	5	28.16	(2.93)	9	29.63	(3.80)
Jul	11	34.39	(4.69)	5	30.26	(5.63)	6	37.83	(7.36)
Aug	8	28.51	(5.06)	6	28.79	(6.79)	2	27.67	(4.82)
Sep	10	26.96	(2.10)	5	25.08	(2.35)	5	28.85	(3.55)
Oct	6	29.56	(2.20)	4	28.54	(3.31)	2	31.59	(0.94)
Nov	5	27.96	(3.01)	2	24.30	(4.94)	3	31.41	(3.82)
Jan	3	24.12	(0.21)	2	23.92	(0.06)	1	24.53	———
Feb	4	19.55	(2.33)	2	17.98	(1.56)	2	21.13	(5.00)
Mar	9	23.51	(2.65)	6	25.93	(3.65)	3	18.67	(0.32)
Apr	12	23.35	(2.20)	5	22.88	(4.61)	7	23.69	(2.25)
May	12	22.62	(2.29)	5	23.89	(3.26)	7	21.71	(3.32)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for March means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 6E. Monthly mean percentage of protein in brown fat in M. pennsylvanicus, from June, 1976 to May, 1977.

	% PROTEIN IN BROWN FAT								
	Males and Females			Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	12	8.08	(0.54)	4	7.01	(0.62)	8	8.62	(0.70)
Jul	10	8.71	(1.31)	4	6.81	(0.59)	6	9.98	(2.05)
Aug	8	6.22	(0.78)	6	6.41	(0.99)	2	6.80	(0.16)
Sep	9	7.44	(0.68)	5	6.98	(0.88)	4	8.01	(1.15)
Oct	6	8.32	(0.61)	4	7.82	(0.78)	2	9.31	(0.70)
Nov	5	7.60	(0.47)	2	7.31	(1.05)	3	7.80	(0.55)
Jan	3	6.04	(0.37)	2	6.29	(0.47)	1	5.55	———
Feb	4	6.13	(0.84)	2	5.46	(0.09)	2	6.81	(1.83)
Mar	8	6.22	(0.41)	5	6.27	(0.68)	3	6.15	(0.20)
Apr	12	6.34	(0.43)	5	6.43	(0.88)	7	6.27	(0.45)
May	12	6.40	(0.72)	5	6.20	(0.79)	7	6.55	(1.16)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for July and March means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 7A. Monthly mean mass of white fat as a percentage of body weight (% WF) in C. gapperi, from May, 1976 to May, 1977.

Month	% WF of Males and Females			% WF of Females			% WF of Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	6	2.73	(0.88)	3	3.92	(1.47)	3	1.54	(0.57)
Jun	15	3.09	(0.92)	7	5.24	(1.65)	8	1.21	(0.25)
Jul	12	2.97	(1.04)	5	5.96	(1.81)	7	0.84	(0.14)
Aug	12	1.66	(0.15)	6	1.63	(0.18)	6	1.69	(0.26)
Sep	11	1.73	(0.25)	4	1.33	(0.38)	7	1.96	(0.32)
Oct	13	2.31	(0.28)	6	2.08	(0.46)	7	2.50	(0.37)
Nov	9	2.81	(0.37)	6	3.17	(0.47)	3	2.10	(0.44)
Dec	10	1.77	(0.22)	4	2.21	(0.44)	6	1.48	(0.15)
Jan	9	3.72	(0.47)	5	4.03	(0.54)	4	3.32	(0.85)
Feb	11	5.26	(0.43)	3	4.54	(1.05)	8	5.53	(0.45)
Mar	18	4.62	(0.58)	8	4.17	(0.92)	10	4.98	(0.77)
Apr	15	4.66	(0.60)	4	3.63	(0.70)	11	5.04	(0.76)
May	13	3.72	(0.58)	4	4.45	(0.86)	9	3.39	(0.74)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for June and July means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 7B. Monthly mean absolute mass of white fat in C. gapperi, from May, 1976 to May, 1977.

	ABSOLUTE MASS OF WHITE FAT (g)								
	Males and Females			Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	6	0.50	(0.17)	3	0.72	(0.29)	3	0.28	(0.10)
Jun	15	0.57	(0.18)	7	0.97	(0.32)	8	0.22	(0.06)
Jul	12	0.53	(0.22)	5	1.11	(0.43)	7	0.11	(0.02)
Aug	12	0.24	(0.02)	6	0.23	(0.03)	6	0.24	(0.04)
Sep	11	0.22	(0.03)	4	0.16	(0.05)	7	0.25	(0.04)
Oct	13	0.28	(0.03)	6	0.25	(0.06)	7	0.31	(0.04)
Nov	9	0.34	(0.04)	6	0.39	(0.05)	3	0.25	(0.05)
Dec	10	0.20	(0.03)	4	0.25	(0.05)	6	0.17	(0.02)
Jan	9	0.53	(0.09)	5	0.61	(0.11)	4	0.43	(0.13)
Feb	11	0.78	(0.08)	3	0.73	(0.24)	8	0.79	(0.08)
Mar	18	0.75	(0.10)	8	0.65	(0.16)	10	0.83	(0.13)
Apr	15	0.92	(0.14)	4	0.64	(0.14)	11	1.03	(0.18)
May	13	0.71	(0.12)	4	0.79	(0.17)	9	0.68	(0.16)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for June and July means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 7C. Monthly mean percentage of lipid in white fat in C. gapperi, from June, 1976 to May, 1977.

Month	% LIPID IN WHITE FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	—	—	—	—	—	—	8	66.06	(5.46)
Jul	9	64.77	(4.59)	2	65.46	(12.83)	7	64.58	(5.32)
Aug	11	69.24	(2.69)	5	70.69	(1.36)	6	68.03	(4.97)
Sep	10	70.06	(2.64)	3	71.68	(2.69)	7	69.37	(3.69)
Oct	13	71.56	(3.03)	6	66.97	(5.23)	7	75.50	(3.01)
Nov	9	72.83	(2.70)	6	75.05	(2.70)	3	68.40	(6.02)
Dec	10	64.19	(3.25)	4	66.83	(7.06)	6	62.43	(3.18)
Jan	8	76.98	(2.23)	4	78.65	(3.19)	4	75.32	(3.35)
Feb	11	82.65	(1.16)	3	81.97	(2.62)	8	82.90	(1.37)
Mar	18	76.26	(1.76)	8	73.16	(2.97)	10	74.73	(3.55)
Apr	14	76.44	(2.23)	4	71.01	(6.78)	10	78.61	(1.46)
May	13	69.46	(3.17)	4	74.01	(2.61)	9	67.43	(4.35)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for August and April means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Monthly means do not include pregnant or lactating females.

Appendix 7D. Monthly mean percentage of water in white fat in C. gapperi, from June, 1976 to May, 1977.

Month	% WATER IN WHITE FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	—	—	—	—	—	—	8	28.40	(5.32)
Jul	9	29.45	(3.98)	2	27.92	(10.58)	7	29.88	(4.66)
Aug	11	25.46	(2.20)	5	24.70	(1.32)	6	26.10	(4.05)
Sep	10	23.53	(1.84)	3	24.11	(2.71)	7	23.27	(2.49)
Oct	13	22.75	(2.62)	6	26.17	(4.72)	7	19.82	(2.51)
Nov	9	22.04	(2.74)	6	19.57	(2.84)	3	26.98	(5.62)
Dec	10	30.09	(2.79)	4	27.84	(5.92)	6	31.60	(2.86)
Jan	9	18.51	(1.82)	5	16.93	(2.53)	4	20.48	(2.64)
Feb	11	13.84	(0.97)	3	13.89	(2.13)	8	13.82	(1.16)
Mar	18	17.20	(1.42)	8	18.15	(2.72)	10	16.63	(1.47)
Apr	15	18.21	(2.10)	4	23.73	(5.73)	11	16.20	(1.82)
May	13	25.74	(2.73)	4	21.42	(2.22)	9	27.66	(3.72)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for August means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Monthly means do not include pregnant or lactating females.

Appendix 7E. Monthly mean percentage of protein in white fat in C. gapperi, from June, 1976 to May, 1977.

Month	% PROTEIN IN WHITE FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	—	———	———	—	———	———	8	3.86	(0.41)
Jul	9	4.58	(0.64)	2	4.47	(1.22)	7	4.61	(0.80)
Aug	11	3.76	(0.51)	5	3.69	(0.38)	6	3.83	(0.92)
Sep	10	3.40	(0.34)	3	3.62	(0.09)	7	3.31	(0.49)
Oct	12	3.62	(0.44)	6	4.13	(0.71)	6	3.12	(0.48)
Nov	9	2.91	(0.21)	6	2.71	(0.26)	3	3.30	(0.24)
Dec	10	3.00	(0.27)	4	2.85	(0.52)	6	3.10	(0.32)
Jan	8	2.92	(0.27)	4	2.89	(0.12)	4	2.95	(0.57)
Feb	11	2.36	(0.19)	3	2.49	(0.30)	8	2.31	(0.25)
Mar	17	3.09	(0.32)	7	3.57	(0.67)	10	2.76	(0.26)
Apr	13	3.18	(0.32)	3	3.55	(0.83)	10	3.06	(0.35)
May	13	3.79	(0.29)	4	3.78	(0.46)	9	3.79	(0.39)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for January and March means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Monthly means do not include pregnant or lactating females.

Appendix 8A. Monthly mean mass of white fat as a percentage of body weight (% WF) in M. pennsylvanicus, from May, 1976 to May, 1977.

Month	% WF of Males and Females			% WF of Females			% WF of Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	4.85	(1.14)	5	6.54	(0.47)	2	0.62	(0.12)**
Jun	14	3.27	(0.61)	5	5.27	(1.05)	9	2.16	(0.45)*
Jul	11	2.89	(0.88)	5	4.34	(1.74)	6	1.68	(0.41)
Aug	8	2.25	(0.59)	6	2.40	(0.85)	2	1.80	(0.74)
Sep	10	3.02	(0.56)	5	3.75	(0.95)	5	2.28	(0.47)
Oct	6	1.62	(0.29)	4	1.63	(0.43)	2	1.61	(0.46)
Nov	5	2.83	(0.83)	2	3.94	(1.68)	3	2.10	(0.83)
Jan	3	7.25	(1.56)	2	8.14	(0.29)	1	5.48	——
Feb	4	5.13	(0.61)	2	6.03	(0.66)	2	4.25	(0.48)
Mar	9	5.90	(1.05)	6	4.83	(1.21)	3	8.03	(1.53)
Apr	12	5.37	(0.55)	5	6.11	(1.06)	7	4.84	(0.55)
May	12	4.08	(0.62)	5	4.00	(0.86)	7	4.14	(0.93)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for August means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences were indicated by * ($P < 0.01$), and by ** ($P < 0.001$).

Appendix 8B. Monthly mean absolute mass of white fat in M. pennsylvanicus, from May, 1976 to May, 1977.

	ABSOLUTE MASS OF WHITE FAT (g)								
	Males and Females			Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	1.17	(0.27)	5	1.55	(0.16)	2	0.22	(0.05)**
Jun	14	0.92	(0.18)	5	1.57	(0.31)	9	0.56	(0.11)**
Jul	11	0.65	(0.20)	5	0.92	(0.37)	6	0.43	(0.18)
Aug	8	0.53	(0.15)	6	0.61	(0.19)	2	0.31	(0.16)
Sep	10	0.57	(0.13)	5	0.68	(0.24)	5	0.46	(0.11)
Oct	6	0.32	(0.07)	4	0.33	(0.10)	2	0.30	(0.08)
Nov	5	0.44	(0.12)	2	0.58	(0.25)	3	0.35	(0.13)
Jan	3	1.26	(0.10)	2	1.36	(0.01)	1	1.07	——
Feb	4	0.95	(0.09)	2	1.10	(0.06)	2	0.81	(0.01)*
Mar	9	1.37	(0.29)	6	1.09	(0.36)	3	1.94	(0.38)
Apr	12	1.29	(0.13)	5	1.34	(0.25)	7	1.25	(0.15)
May	12	1.31	(0.21)	5	1.16	(0.30)	7	1.41	(0.30)

Differences between means for males and females were tested by the Student's t-test for unpaired means.

Significant differences are indicated by * ($P < 0.05$), and by ** ($P < 0.005$).

Appendix 8C. Monthly mean percentage of lipid in white fat in M. pennsylvanicus, from June, 1976 to May, 1977.

Month	% LIPID IN WHITE FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	—	—	—	—	—	—	9	80.64	(1.11)
Jul	8	74.71	(3.10)	2	84.48	(4.46)	6	71.45	(2.86)
Aug	6	76.78	(4.22)	4	79.65	(4.50)	2	71.05	(9.80)
Sep	9	70.91	(1.47)	4	71.67	(2.04)	5	70.30	(2.26)
Oct	6	66.93	(3.56)	4	70.19	(3.69)	2	60.40	(6.72)
Nov	5	73.12	(4.57)	2	79.56	(0.64)	3	68.82	(6.81)
Jan	3	81.48	(2.01)	2	81.77	(3.44)	1	80.91	—
Feb	4	81.46	(1.91)	2	82.47	(1.00)	2	80.46	(4.33)
Mar	8	82.06	(1.27)	5	81.99	(1.66)	3	82.16	(2.40)
Apr	12	78.50	(2.16)	5	80.82	(2.88)	7	76.84	(3.10)
May	12	77.11	(1.21)	5	75.83	(2.80)	7	78.02	(0.74)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for May means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Monthly means do not include pregnant or lactating females.

Appendix 8D. Monthly mean percentage of water in white fat in M. pennsylvanicus, from June, 1976 to May, 1977.

	% WATER IN WHITE FAT								
	Males and Females			Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	10	15.32	(0.96)	1	18.08	——	9	15.02	(1.02)
Jul	8	20.33	(2.60)	2	12.38	(3.73)	6	22.98	(2.46)
Aug	6	19.25	(3.68)	4	16.93	(3.85)	2	23.91	(9.02)
Sep	9	22.07	(2.17)	4	20.27	(4.10)	5	23.38	(2.43)
Oct	6	27.79	(3.06)	4	24.93	(2.93)	2	33.52	(6.35)
Nov	5	21.22	(4.14)	2	14.53	(2.42)	3	25.68	(5.51)
Jan	3	14.74	(1.33)	2	14.73	(2.31)	1	14.75	——
Feb	4	13.97	(0.67)	2	13.96	(0.02)	2	13.99	(1.63)
Mar	8	13.46	(1.31)	5	14.54	(1.43)	3	11.65	(2.55)
Apr	12	15.68	(1.20)	5	15.73	(2.53)	7	15.65	(1.23)
May	12	17.02	(1.61)	5	19.97	(3.14)	7	14.91	(1.28)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for February means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Monthly means do not include pregnant or lactating females.

Appendix 8E. Monthly mean percentage of protein in white fat in M. pennsylvanicus, from June, 1976 to May, 1977.

Month	% PROTEIN IN WHITE FAT								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	—	———	———	—	———	———	9	2.50	(0.22)
Jul	8	3.01	(0.31)	2	2.07	(0.76)	6	3.33	(0.25)
Aug	6	2.91	(0.51)	4	2.79	(0.63)	2	3.16	(1.18)
Sep	9	3.34	(0.39)	4	2.90	(0.87)	5	3.69	(0.19)
Oct	6	3.65	(0.42)	4	3.65	(0.60)	2	3.67	(0.67)
Nov	5	3.38	(0.51)	2	2.75	(0.57)	3	3.81	(0.72)
Jan	3	2.40	(0.27)	2	2.36	(0.45)	1	2.49	———
Feb	4	2.02	(0.08)	2	1.98	(0.06)	2	2.07	(0.16)
Mar	8	2.25	(0.19)	5	2.54	(0.19)	3	2.10	(0.27)
Apr	12	2.31	(0.12)	5	2.21	(0.24)	7	2.38	(0.12)
May	12	2.51	(0.19)	5	2.49	(0.24)	7	2.52	(0.28)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for September means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Monthly means do not include pregnant or lactating females.

Appendix 9A. Monthly mean lipid content (mg of tissue lipid/g of BW) in brown fat of C. gapperi, from June, 1976 to May, 1977.

	LIPID CONTENT OF BROWN FAT (mg/b BW)					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	7	9.85	(2.10)	8	10.31	(2.12)
Jul	5	11.50	(5.37)	6	10.09	(2.09)
Aug	6	15.96	(2.76)	6	17.90	(2.76)
Sep	4	23.70	(7.53)	7	27.57	(3.66)
Oct	6	26.28	(3.08)	6	31.71	(3.94)
Nov	6	39.13	(4.16)	3	32.32	(4.15)
Dec	1	32.84	———	2	20.84	(4.38)
Jan	5	46.98	(1.64)	4	40.93	(5.89)
Feb	3	43.81	(4.17)	8	50.95	(3.32)
Mar	8	33.99	(4.28)	10	41.07	(2.82)
Apr	4	31.47	(0.66)	11	36.55	(2.20)
May	4	27.56	(2.77)	9	25.46	(4.23)

Differences between means for males and females were tested by the Student's t-test for unpaired means, except for January and April means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 9B. Monthly mean lipid content (mg of tissue lipid/g of BW) in white fat of *C. gapperi*, from June, 1976 to May, 1977.

	LIPID CONTENT OF WHITE FAT (mg/g BW)					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	7	7.27	(2.00)	8	9.13	(2.05)
Jul	5	12.87	(2.22)	7	5.78	(1.20)*
Aug	6	11.25	(1.45)	6	12.12	(2.41)
Sep	3	12.25	(1.25)	7	14.05	(2.82)
Oct	6	15.05	(3.97)	7	19.41	(3.42)
Nov	6	24.21	(4.15)	3	14.81	(3.85)
Dec	4	15.96	(3.77)	6	9.34	(1.23)
Jan	4	28.61	(4.24)	4	25.79	(7.51)
Feb	3	37.61	(9.28)	8	46.17	(4.15)
Mar	8	31.22	(7.31)	10	40.13	(6.88)
Apr	4	25.83	(6.43)	10	35.13	(4.34)
May	4	33.29	(6.78)	9	24.95	(6.83)

Differences between means for males and females were tested by the Student's t-test for unpaired means.

Significant differences are indicated by * ($P < 0.02$).

Appendix 9C. Comparison between lipid content of brown and white fat in C. gapperi, from June, 1976 to May, 1977.

	TISSUE LIPID CONTENT (mg/b BW)					
	Brown Fat			White Fat		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	15	10.10	(1.44)	15	8.26	(1.41)
Jul	11	10.73	(2.55)	11	8.65	(1.66)
Aug	12	16.93	(1.88)	12	11.68	(1.35)*
Sep	11	26.28	(3.42)	10	13.51	(1.98)**
Oct	12	29.00	(2.52)	13	17.40	(2.56)**
Nov	9	36.86	(3.15)	9	21.07	(3.30)**
Dec	3	24.84	(4.73)	10	12.00	(1.89)*
Jan	9	44.29	(2.77)	8	27.20	(4.03)**
Feb	11	49.00	(2.75)	11	43.83	(3.86)
Mar	18	37.94	(2.53)	18	36.17	(4.99)
Apr	15	35.20	(1.71)	14	32.47	(3.66)
May	13	26.10	(2.98)	13	27.52	(5.13)

Differences between means for the two tissues were tested by the Student's t-test for unpaired means, except for March and April means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.05$), and by ** ($P < 0.01$).

Appendix 10A. Monthly mean lipid content (mg of tissue lipid/g of BW) in brown fat of M. pennsylvanicus, from June, 1976 to May, 1977.

	LIPID CONTENT OF BROWN FAT (mg/g BW)					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	5	8.15	(1.43)	9	9.94	(1.69)
Jul	5	14.37	(5.42)	6	11.31	(3.20)
Aug	6	11.77	(2.85)	2	15.36	(1.72)
Sep	5	16.79	(2.11)	5	18.07	(2.33)
Oct	4	19.31	(2.03)	2	16.56	(0.34)
Nov	2	31.68	(7.60)	3	20.94	(5.88)
Jan	2	34.90	(0.80)	1	34.04	———
Feb	2	40.04	(0.41)	2	49.76	(21.64)
Mar	6	34.46	(7.56)	3	31.14	(3.23)
Apr	5	27.80	(4.55)	7	23.67	(2.17)
May	5	21.65	(2.42)	7	16.50	(2.55)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for February means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 10B. Monthly mean lipid content (mg of tissue lipid/g of BW) in white fat of *M. pennsylvanicus*, from June, 1976 to May, 1977.

	LIPID CONTENT OF WHITE FAT (mg/g BW)					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	4	10.52	(2.30)	9	17.55	(3.73)
Jul	5	23.49	(15.04)	6	12.30	(3.11)
Aug	6	18.75	(7.47)	2	13.47	(7.00)
Sep	5	19.42	(4.42)	5	16.24	(3.54)
Oct	4	11.71	(3.34)	2	10.05	(3.88)
Nov	2	31.48	(13.62)	3	15.54	(7.41)
Jan	2	66.68	(5.18)	1	44.33	———
Feb	2	50.00	(5.82)	2	34.37	(5.65)
Mar	5	44.78	(11.56)	3	65.25	(10.89)
Apr	5	49.69	(8.34)	7	37.61	(5.22)
May	5	30.70	(6.94)	7	32.35	(7.24)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for July means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 10C. Comparison between lipid content of brown and white fat in M. pennsylvanicus, from June, 1976 to May, 1977.

	TISSUE LIPID CONTENT (mg/g BW)					
	Brown Fat			White Fat		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	15	9.30	(1.19)	13	15.39	(2.78)
Jul	11	12.70	(2.89)	11	17.45	(6.85)
Aug	8	12.67	(2.19)	8	17.43	(5.69)
Sep	10	17.43	(1.50)	10	17.83	(2.72)
Oct	6	18.39	(1.41)	6	11.15	(2.36)*
Nov	5	25.23	(4.80)	5	21.92	(7.09)
Jan	3	34.61	(0.54)	3	59.23	(8.03)
Feb	4	44.90	(9.27)	4	42.18	(5.60)
Mar	9	33.36	(5.00)	8	52.46	(8.63)
Apr	12	25.39	(2.24)	12	42.62	(4.74)**
May	12	18.64	(1.88)	12	31.66	(4.90)*

Means for the months of September, October, November, February, and March were tested for significant differences between the two tissues with the Student's t-test for unpaired means. All other monthly means were tested with the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.05$), and by ** ($P < 0.01$).

Appendix 11A. Monthly mean body weight of sexually mature and immature C. gapperi, from May, 1976 to May, 1977.

	BODY WEIGHT (g)					
	Mature			Immature		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	18.12	(0.83)	0	—	—
Jun	13	18.00	(0.59)	2	13.32	(0.29)
Jul	7	16.92	(1.25)	5	12.41	(0.14)
Aug	3	15.07	(0.73)	9	13.98	(0.26)
Sep	1	12.38	—	10	12.43	(0.31)
Oct	1	15.53	—	12	12.13	(0.23)
Nov	1	16.03	—	8	11.92	(0.18)
Dec	0	—	—	10	11.37	(0.24)
Jan	2	17.40	(2.23)	7	12.65	(0.46)
Feb	1	20.45	—	10	13.92	(0.37)
Mar	1	17.07	—	17	15.91	(0.42)
Apr	15	19.24	(0.56)	0	—	—
May	13	19.09	(0.53)	0	—	—

Appendix 11B. Monthly mean body weight of C. gapperi, from May, 1976 to May, 1977.

Month	MEAN BODY WEIGHT (g)								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	18.12	(0.83)	4	17.76	(1.41)	3	18.59	(0.81)
Jun	15	17.38	(0.67)	7	17.53	(0.58)	8	17.25	(1.18)
Jul	12	15.05	(0.97)	5	17.24	(1.87)	7	13.48	(0.55)
Aug	12	14.25	(0.25)	6	14.33	(0.46)	6	14.17	(0.27)
Sep	11	12.43	(0.28)	4	12.15	(0.34)	7	12.59	(0.40)
Oct	13	12.39	(0.34)	6	11.85	(0.29)	7	12.86	(0.53)
Nov	9	12.38	(0.48)	6	12.61	(0.70)	3	11.91	(0.40)
Dec	10	11.37	(0.24)	4	11.66	(0.53)	6	11.17	(0.21)
Jan	9	13.70	(0.87)	5	14.71	(1.38)	4	12.44	(0.60)
Feb	11	14.51	(0.68)	3	15.60	(2.44)	8	14.11	(0.44)
Mar	18	15.98	(0.40)	8	15.19	(0.58)	10	16.60	(0.49)
Apr	15	19.24	(0.56)	4	17.27	(1.44)	11	19.96	(0.41)*
May	13	19.09	(0.53)	4	17.55	(0.47)	9	19.77	(0.62)*

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for July and February means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.05$).

Appendix 11C. Monthly mean total length of C. gapperi, from May, 1976 to May, 1977.

Month	MEAN TOTAL LENGTH (mm)								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	129.64	(0.71)	4	129.75	(1.20)	3	129.50	(0.76)
Jun	15	127.13	(1.68)	7	130.14	(1.98)	8	124.50	(2.36)
Jul	12	122.29	(2.45)	5	126.80	(3.93)	7	118.21	(1.82)
Aug	12	117.25	(1.02)	6	117.92	(1.22)	6	116.58	(1.69)
Sep	11	114.86	(1.60)	4	113.25	(3.69)	7	115.79	(1.57)
Oct	13	113.38	(1.50)	6	111.50	(2.29)	7	115.00	(1.90)
Nov	9	113.33	(2.31)	6	114.67	(3.36)	3	110.67	(1.45)
Dec	10	112.85	(0.73)	4	112.63	(1.05)	6	113.00	(1.07)
Jan	9	117.83	(3.69)	5	123.80	(5.27)	4	110.38	(1.48)
Feb	11	116.50	(2.02)	3	120.83	(6.77)	8	114.88	(1.32)
Mar	17	120.59	(1.28)	8	117.94	(1.89)	9	122.89	(1.44)
Apr	15	128.13	(1.15)	4	127.13	(3.15)	11	128.50	(1.18)
May	13	128.65	(1.23)	4	129.00	(1.58)	9	128.50	(1.69)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means. There were no significant differences.

Appendix 11D. Monthly mean total muscle mass of C. gapperi, from May, 1976 to May, 1977.

Month	MEAN TOTAL MUSCLE MASS (g)								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	6.77	(0.34)	4	6.28	(0.42)	3	7.43	(0.26)
Jun	15	6.61	(0.38)	7	6.30	(0.10)	8	6.88	(0.72)
Jul	12	5.48	(0.34)	5	6.18	(0.66)	7	4.98	(0.23)
Aug	12	5.33	(0.10)	6	5.33	(0.14)	6	5.33	(0.16)
Sep	11	4.43	(0.16)	4	4.26	(0.28)	7	4.53	(0.19)
Oct	13	4.14	(0.15)	6	3.98	(0.17)	7	4.28	(0.24)
Nov	9	4.18	(0.17)	6	4.13	(0.20)	3	4.30	(0.36)
Dec	10	4.23	(0.15)	4	4.23	(0.36)	6	4.22	(0.11)
Jan	9	4.54	(0.31)	5	4.92	(0.50)	4	4.07	(0.12)
Feb	11	4.91	(0.30)	3	5.52	(1.82)	8	4.68	(0.17)
Mar	18	5.39	(0.17)	8	5.12	(0.14)	10	5.60	(0.27)
Apr	15	7.16	(0.25)	4	6.26	(0.56)	11	7.49	(0.20)*
May	13	7.18	(0.32)	4	6.16	(0.11)	9	7.64	(0.37)**

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for June, July, January, February, and May, 1977 means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.05$), and by ** ($P < 0.01$).

Appendix 12A. Monthly mean body weight of sexually mature and immature M. pennsylvanicus, from May, 1976 to May, 1977.

	BODY WEIGHT (g)					
	Mature			Immature		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	26.72	(2.26)	0	—	—
Jun	14	27.59	(1.07)	0	—	—
Jul	8	25.86	(3.23)	3	15.99	(1.10)
Aug	6	25.19	(1.40)	2	16.72	(1.74)
Sep	3	24.68	(2.87)	7	15.14	(0.67)
Oct	3	21.10	(0.95)	3	17.42	(1.56)
Nov	0	—	—	5	16.02	(0.73)
Jan	1	19.58	—	2	16.69	(0.72)
Feb	1	21.11	—	3	18.00	(0.61)
Mar	8	22.90	(0.92)	1	13.95	—
Apr	12	24.20	(1.39)	0	—	—
May	12	31.68	(1.62)	0	—	—

Appendix 12B. Monthly mean body weight of *M. pennsylvanicus*, from May, 1976 to May, 1977.

Month	MEAN BODY WEIGHT (g)								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	26.72	(2.27)	5	23.57	(1.41)	2	34.59	(1.08)**
Jun	14	27.59	(1.07)	5	29.68	(1.09)	9	26.43	(1.44)
Jul	11	23.17	(2.70)	5	22.03	(3.17)	6	24.12	(4.44)
Aug	8	23.07	(1.75)	6	25.19	(1.40)	2	16.72	(1.74)*
Sep	10	18.37	(1.61)	5	16.80	(1.63)	5	19.94	(2.79)
Oct	6	19.26	(1.16)	4	19.42	(1.81)	2	18.94	(0.62)
Nov	5	16.02	(0.73)	2	14.65	(0.02)	3	16.93	(0.85)
Jan	3	17.66	(1.05)	2	16.69	(0.72)	1	19.58	———
Feb	4	18.78	(0.89)	2	18.31	(0.91)	2	19.25	(1.87)
Mar	9	21.90	(1.28)	6	20.81	(1.79)	3	24.09	(0.27)
Apr	12	24.20	(1.39)	5	21.39	(1.03)	7	26.21	(1.99)
May	12	31.68	(1.62)	5	28.92	(2.59)	7	34.49	(1.91)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for November and March means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.02$), and by ** ($P < 0.01$).

Appendix 12C. Monthly mean total length of M. pennsylvanicus, from May, 1976 to May, 1977.

Month	MEAN TOTAL LENGTH (mm)								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	147.14	(3.52)	5	144.10	(4.07)	2	154.75	(3.75)
Jun	14	145.93	(1.97)	5	147.90	(2.29)	9	143.83	(2.62)
Jul	11	137.27	(4.33)	5	134.00	(6.94)	6	140.00	(5.76)
Aug	8	140.25	(4.77)	6	146.08	(3.69)	2	122.50	(3.50)*
Sep	10	128.15	(4.43)	5	124.10	(5.34)	5	132.20	(7.19)
Oct	6	130.10	(2.16)	4	130.63	(3.28)	2	129.00	(2.00)
Nov	5	123.00	(1.67)	2	122.00	(——)[1]	3	123.67	(2.96)
Jan	3	124.50	(4.54)	2	120.00	(1.00)	1	133.50	(——)
Feb	4	125.00	(3.76)	2	120.50	(1.50)	2	129.50	(6.50)
Mar	9	135.50	(2.02)	6	134.58	(3.05)	3	137.33	(0.33)
Apr	12	138.88	(2.80)	5	134.20	(2.67)	7	142.21	(4.10)
May	12	153.29	(2.42)	5	148.60	(3.26)	7	156.64	(2.96)

[1]Both females in the November sample measured 122.00 mm.

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for March means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.05$).

Appendix 12D. Monthly mean total muscle mass of M. Pennsylvanicus, from May, 1976 to May, 1977.

Month	MEAN TOTAL MUSCLE MASS (g)								
	Males and Females			Females			Males		
	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
May	7	10.38	(1.22)	5	8.60	(0.58)	2	14.83	(0.38)**
Jun	14	10.80	(0.47)	5	11.00	(0.26)	9	10.69	(0.72)
Jul	11	9.00	(1.31)	5	8.10	(1.62)	6	9.74	(2.08)
Aug	8	8.38	(0.64)	6	9.21	(0.38)	2	5.90	(0.99)**
Sep	10	6.40	(0.67)	5	5.48	(0.73)	5	7.32	(1.02)
Oct	6	6.58	(0.43)	4	6.59	(0.68)	2	6.57	(0.08)
Nov	5	5.35	(0.37)	2	4.57	(0.16)	3	5.86	(0.34)
Jan	3	5.41	(0.41)	2	5.02	(0.17)	1	6.20	———
Feb	4	5.86	(0.55)	2	5.52	(0.59)	2	6.19	(1.12)
Mar	9	6.74	(0.31)	6	6.52	(0.45)	3	7.19	(0.10)
Apr	12	8.49	(0.70)	5	6.89	(0.21)	7	9.64	(1.01)*
May	12	11.85	(0.99)	5	8.97	(0.55)	7	13.90	(1.11)**

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means.

Significant differences are indicated by * ($P < 0.05$), and by ** ($P < 0.01$).

Appendix 13A. Monthly mean percentage of lipid in skeletal muscle of male and female C. gapperi, from June, 1976 to May, 1977.

	% LIPID IN SKELETAL MUSCLE					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	6	1.33	(0.38)	8	1.58	(0.27)
Jul	5	1.84	(0.38)	7	1.23	(0.19)
Aug	5	1.83	(0.19)	6	1.61	(0.17)
Sep	4	2.42	(0.96)	7	2.14	(0.34)
Oct	6	2.35	(0.44)	7	2.42	(0.52)
Nov	6	3.34	(0.61)	3	2.46	(0.87)
Dec	4	3.22	(0.31)	6	2.54	(0.19)
Jan	5	4.03	(0.83)	4	3.31	(0.59)
Feb	3	3.42	(0.32)	8	4.35	(0.27)
Mar	8	2.77	(0.45)	10	3.55	(0.43)
Apr	4	2.13	(0.39)	11	2.65	(0.18)
May	4	3.05	(0.43)	9	2.2	(0.49)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means. There were no significant differences.

Appendix 13B. Monthly mean percentage of water in skeletal muscle of male and female C. gapperi, from June, 1976 to May, 1977.

	% WATER IN SKELETAL MUSCLE					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	6	74.44	(0.32)	8	74.53	(0.38)
Jul	5	75.02	(0.72)	7	74.40	(0.46)
Aug	5	73.88	(0.28)	6	74.14	(0.37)
Sep	4	74.16	(0.85)	7	74.70	(0.40)
Oct	6	73.84	(0.54)	7	73.82	(0.76)
Nov	6	73.17	(0.41)	3	72.98	(1.27)
Dec	4	72.25	(1.42)	6	74.87	(0.46)
Jan	5	72.96	(0.85)	4	72.98	(0.38)
Feb	3	72.15	(0.67)	8	71.86	(0.28)
Mar	8	73.27	(0.34)	10	73.11	(0.55)
Apr	4	75.14	(0.23)	11	73.74	(0.23)*
May	4	73.35	(0.72)	9	73.58	(0.42)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for February means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.005$).

Appendix 13C. Monthly mean percentage of protein in skeletal muscle of male and female C. gapperi, from June, 1976 to May, 1977.

	% PROTEIN IN SKELETAL MUSCLE					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	6	21.37	(0.62)	8	21.59	(0.69)
Jul	5	20.70	(0.72)	7	21.76	(0.32)
Aug	5	21.88	(0.31)	6	21.58	(0.22)
Sep	4	21.39	(0.45)	7	21.14	(0.18)
Oct	6	21.02	(0.31)	7	21.27	(0.46)
Nov	6	20.80	(0.30)	3	21.54	(0.44)
Dec	4	20.50	(0.62)	6	20.21	(0.20)
Jan	5	21.26	(0.50)	4	21.15	(0.22)
Feb	3	21.78	(0.47)	8	21.01	(0.17)
Mar	8	21.26	(0.24)	9	21.21	(0.11)
Apr	4	20.37	(0.40)	11	20.99	(0.22)
May	4	20.80	(0.17)	9	21.07	(0.44)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for February and May means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.

Appendix 14A. Monthly mean percentage of lipid in skeletal muscle of male and female M. pennsylvanicus, from June, 1976 to May, 1977.

	% LIPID IN SKELETAL MUSCLE					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	5	1.91	(0.35)	9	1.21	(0.31)
Jul	5	2.16	(0.92)	6	1.58	(0.28)
Aug	6	1.33	(0.29)	2	1.13	(0.76)
Sep	5	2.20	(0.36)	4	2.05	(0.48)
Oct	3	1.55	(0.42)	2	2.06	(0.83)
Nov	2	2.68	(0.64)	3	2.31	(0.58)
Jan	2	4.07	(0.90)	1	2.88	———
Feb	2	5.86	(0.35)	2	5.25	(1.46)
Mar	6	4.08	(1.16)	3	5.31	(0.50)
Apr	5	4.60	(0.88)	7	2.41	(0.43)*
May	5	2.54	(0.42)	7	2.56	(0.22)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for July means, which were tested by the Behrens-Fisher test (Cochran's modification).

Significant differences are indicated by * ($P < 0.02$).

Appendix 14B. Monthly mean percentage of water in skeletal muscle of male and female M. pennsylvanicus, from June, 1976 to May, 1977.

	% WATER IN SKELETAL MUSCLE					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	5	74.57	(0.17)	9	74.70	(0.34)
Jul	5	73.60	(0.69)	6	76.39	(1.56)
Aug	6	75.09	(0.36)	2	74.91	(0.77)
Sep	5	74.27	(0.54)	4	74.95	(0.28)
Oct	3	73.92	(0.50)	2	74.48	(0.52)
Nov	2	73.58	(0.13)	3	73.93	(0.61)
Jan	2	72.64	(0.12)	1	73.83	———
Feb	2	71.37	(0.08)	2	71.58	(0.39)
Mar	6	71.96	(0.98)	3	71.76	(0.69)
Apr	5	72.25	(0.88)	7	73.72	(0.45)
May	5	74.52	(0.34)	7	73.68	(0.27)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means. There were no significant differences.

Appendix 14C. Monthly mean percentage of protein in skeletal muscle of male and female M. pennsylvanicus, from June, 1976 to May, 1977.

	% PROTEIN IN SKELETAL MUSCLE					
	Females			Males		
Month	n	$\bar{X}$	(SE)	n	$\bar{X}$	(SE)
Jun	4	20.47	(0.34)	9	21.83	(0.39)
Jul	5	20.56	(0.25)	6	19.51	(1.41)
Aug	6	20.84	(0.45)	2	21.09	(0.47)
Sep	5	21.01	(0.43)	4	20.75	(0.18)
Oct	3	21.39	(0.33)	2	20.80	(0.13)
Nov	2	20.29	(0.57)	3	20.74	(0.20)
Jan	2	20.10	(0.90)	1	22.14	———
Feb	2	20.20	(0.09)	2	20.03	(1.53)
Mar	5	20.40	(0.54)	3	20.61	(0.17)
Apr	5	20.66	(0.54)	7	20.94	(0.26)
May	5	20.47	(0.68)	7	21.23	(0.31)

Differences between monthly means for males and females were tested by the Student's t-test for unpaired means, except for July means, which were tested by the Behrens-Fisher test (Cochran's modification). There were no significant differences.