Nutritional ecology and bioenergetics of muskrats (*Ondatra zibethicus*) in a southern Manitoba marsh.

by

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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DOCTOR OF PHILOSOPHY

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FACULTY OF GRADUATE STUDIES

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NUTRITIONAL ECOLOGY AND BIOENERGETICS OF MUSKRATS (*Ondatra zibethicus*) IN A SOUTHERN MANITOBA MARSH

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

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Abstract

Muskrats (*Ondatra zibethicus*) are versatile feeders capable of exploiting diverse food sources and habitats, yet little is known regarding their energy and nutritional requirements. The aim of this study was to assess the physiological mechanisms that enable muskrats to cope with seasonal changes in nutrient quality and availability. This investigation consisted of six interrelated projects. Project I entailed 30 complete digestibility-, energy-, and nitrogen-balance trials on 6 lab-acclimated muskrats fed 5 emergent plant diets. Dry matter (DM) digestibilities ranged from 61.2 to 70.6%. Microbial fermentation of fiber accounted for >40% of metabolizable energy intake (MEI), indicating that muskrats can digest fiber as well as many ruminants. To determine if digestive efficiency varied with seasonal changes in nutrient quality, project II involved testing 32 field-acclimatized muskrats maintained on mixed diets approximating those consumed in nature. From July to December, muskrats exhibited increases in DM intake and DM, energy and fiber digestibility ($P < 0.05$). Muskrats had difficulty maintaining nitrogen (N) balance on diets composed solely of aquatic vegetation in summer, but not during winter, when their daily N requirements were 26% lower.

Projects III and IV explored two potential mechanisms that could enable muskrats to meet their seasonal N requirements. The former project examined the potential nutritional benefits derived by muskrats that supplement their diet with animal tissue. Gain in body mass, intake of DM, MEI, and N digestibility all increased with rising levels of meat consumption ($P < 0.0001$). The main focus of project IV entailed measuring the

rate of ^{14}C-urea hydrolysis in 32 field-acclimatized muskrats during spring, summer, fall and winter to test whether muskrats conserve body N by recycling urea. Muskrats exhibited higher rates of urea hydrolysis (ca. 400%) and a lower serum urea N/creatinine ratio in fall and winter, compared to spring and summer ($P < 0.0001$). My findings suggest that urea recycling and the opportunistic consumption of animal tissue may both contribute significantly to the maintenance of N balance in wild muskrats.

The final phase of my thesis entailed a two-part study of the seasonal bioenergetics of captive and free-ranging muskrats to determine whether, and to what extent, wild populations are energetically and/or nutritionally stressed. The first phase examined seasonal adjustments in gut and organ morphology, MEI, basal metabolic rate (BMR), blood chemistry, and endogenous lipid and protein stores of 94 field-acclimatized muskrats. Mass-independent BMR ($kJ \cdot kg^{0.67} \cdot hr^{-1}$) varied significantly over the year ($P < 0.0001$), with February values >31% higher than those collected in July. Body lipid stores were lowest from May through September (<2% of body mass), increased to a peak value of 9.24±0.47% in February, and then were rapidly depleted in early spring ($P < 0.0001$). The second phase involved monitoring temporal changes in the body composition ($n = 129$) and forage intake ($n = 33$) of individually marked, free-ranging muskrats using the deuterated water technique. Results from these studies suggest that the daily intake of DM and MEI track the deposition/mobilization of body lipid stores, being significantly higher in winter (75.5 - 76.9 $g \cdot kg^{-0.75}$; 706.9 - 713.1 $kJ \cdot kg^{-0.75}$) than during mid-summer (54.9 - 59.7 $g \cdot kg^{-0.75}$; 406.0 - 438.6 $kJ \cdot kg^{-0.75}$). Annual adjustments in basal energy expenditure, diet selection, energy intake, blood chemistry parameters, gut

and organ masses, and body lipid stores appear to be closely linked to seasonal changes in forage quality and energy and nutrient availability. Such behavioral and physiological adjustments in response to seasonal shifts in diet quality appear to be important adaptations enabling this semi-aquatic rodent to cope with the rigors of season in a Manitoba prairie marsh.

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(and celebrating) my many antics during this period, and shall always cherish their friendship.

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Finally, I wish to dedicate this document to all the people who helped, in one way or another, this thesis come together (especially the above named). Without you I am nothing and never will be.

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Abbreviations used in the Text

ADF	acid detergent fiber
ADN	apparent digestible nitrogen
AE	assimilated energy
BM	body mass
BMR	basal metabolic rate
D_2O	deuterated water
DCP	dietary crude protein
DE	digestible energy
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
DNI	daily nitrogen intake
DPM	disintegrations per minute
GEI	gross energy intake
GIT	gastrointestinal tract
IFBM	ingesta-free body mass
kJ	kilojoule
ME	metabolizable energy
MEI	metabolizable energy intake
N	nitrogen

Abbreviations used in the text ..cont'd

NDF	neutral detergent fiber
NDS	neutral detergent solubles
orts	uneaten forage rations
T_3	triiodothyronine
T_4	thyroxine
TBW	total body water
TNB	tissue nitrogen balance
TND	true nitrogen digestibility
$\dot{V}O_2$	rate of oxygen consumption

I don't know if you'd call it words of wisdom, but what I learned from this race that maybe you can take home with you is: what it took for me to pull it together during those 23 miles, what it took for me to catch Thomas, was an absolute trust in the powers that be, and my ability to continue and keep on the pace and never once have an ounce of doubt about what I was doing out there. I hope that each of you can find that strength when it gets difficult.

- Mark Allen
6-time Ironman Champion

General Introduction

The semiaquatic muskrat (*Ondatra zibethicus*) inhabits a diverse range of wetland habitats extending from subtropical rivers and costal marshes in the south, to arctic tundras and deltas in the north (Errington 1963). The broad geographical distribution of this rodent has commonly been attributed to its versatile feeding habits, as muskrats often make extensive use of a wide variety of food sources (Takos 1947). Because they usually consume only the basal parts of emergent aquatic plants, muskrats incur a protracted and relatively heavy impact on forage species (Danell 1977, 1978). Large amounts of vegetation are also harvested for house building materials (Danell 1979). Consequently, these rodents are often considered to be the most important vertebrate species influencing marsh vegetation structure (Krummes 1946; McCabe 1982). In fact, an abnormally high muskrat population can reduce a productive marsh into a shallow, open-water lake ("eat out") in less than a year (Danell 1979; McCabe 1982).

Managed muskrat populations can, however, benefit marsh ecosystems (Danell 1979) and contribute to waterfowl productivity (Krummes 1940; Cartwright 1946). Muskrats create numerous and variable-sized patches of open-water in the vicinity of their lodges, and the extent of these openings increase when the same house sites are occupied over successive years (Danell 1977, 1979). Bird numbers and species diversity have been shown to peak when the ratio of dense cover to open-water approaches 50:50, creating a "hemi-marsh" condition (Weller and Spatcher in McCabe 1982). These openings created by muskrats are preferred by nesting waterfowl (Krummes 1940; Cartwright 1946)

for several reasons:

1) due to the irregular shape of the grazed areas, an extensive boundary is established between the open water and emergent hydrophytes, thus increasing the ability of ducklings to exploit invertebrate populations within the dense stands of emergent hydrophytes (Danell 1979).

2) open-water areas usually are rapidly colonized by submerged hydrophytes such as *Potamogeton* spp. and *Myriophyllum* spp. (Danell 1977). Submerged hydrophytes include several important food species for waterfowl, and are a necessary substrate for many invertebrate groups (Danell 1979; McCabe 1982).

3) emergent detritus from muskrat feeding activity increases invertebrate abundance.

4) muskrat mounds provide resting sites and concentrated food sources for wildlife, with more than 75% of mound plant species eaten by waterfowl (Krummes 1940; Kangas and Hannan 1985).

Muskrat foraging may also benefit a marsh's floral community. As pointed out by McCabe (1982), light grazing by muskrats may actually stimulate the growth and stem weight of emergent hydrophytes. Long term use of emergents by muskrats may also result in increased plant diversity, both in terms of community structure and number of species (Danell 1979). This is achieved through various means:

1) selective feeding by the muskrat on the dominant aquatic emergent reduces competition, allowing greater species richness (McCabe 1982).

2) transport of food to feeding platforms may aid in the dispersal of rhizomes of many aquatic plants, including cattail (*Typha* spp.) and *Phragmites* sp. (Danell 1977).

3) the developmental sequence of mound succession benefits many non-dominant plant species, with biomass levels for these species 35 times greater on mounds than in the surrounding marsh (Kangas and Hannan 1985).

Due in large part to the need for marsh managers to maintain wetlands at their maximum potential for perpetuating wildlife resources, substantial work has been completed on the natural history (Errington 1963), feeding habits (Stearns and Goodwin 1941; Takos 1947), and population and reproductive ecology of muskrats (Boutin and Birkenholz 1987; Messier, Virgl and Marinelli 1990; Clark and Kroeker 1993). However, considering the significant role this rodent plays in modifying marsh ecosystems, our knowledge of their nutritional requirements and seasonal bioenergetics is limited, at best.

The thermoregulatory tactics and aquatic energetics of this amphibious rodent have been well-documented (MacArthur 1979, 1984, 1986; MacArthur and Krause 1989). However, a foremost consideration in any study of mammalian energetics is the flow of energy through the species in question. Although assimilation efficiencies have been reported for several microtines fed a wide range of diets (Batzli and Cole 1979; Hammond and Wunder 1991), the number of species for which nutritional requirements are known with any precision is relatively few (Robbins 1993). Furthermore, little is known regarding seasonal variation in forage digestibility or, for that matter, any other

aspect of seasonal adaptation to nutritional stress in herbivorous rodents.

In response to these needs, I examined the physiological and behavioral strategies that enable muskrats to cope with seasonal fluctuations in forage quality and nutrient availability in freshwater marsh environments. A second objective of my study was to determine the intake and expenditure of energy by muskrats on a seasonal basis. Not only will this information provide much-needed insight into the forage requirements and aquatic energetics of muskrats, but it is a necessary step in quantifing the impact of muskrats on marsh vegetation. Such information will help wildlife managers form sound marsh management guidlines critical to maintaining wetlands in their most natural, diverse and productive state (McCabe 1982).

In brief, this study involves six interrelated projects. Projects I and II dealt with food intake, nutrient selection, and assimilation efficiencies of muskrats maintained on (1) single-genera aquatic vegetation diets and (2) mixed aquatic plant diets approximating those consumed in different seasons in nature. Expanding on this phase, projects III and IV explored the potential benefits of animal tissue consumption and urea recycling on the seasonal maintenance nitrogen requirements of muskrats. Projects V and VI entailed studies of the seasonal energy intake, basal energy expenditure, blood chemistry, gut and organ morphology as well as body mass and lipid stores of captive and free-living muskrats. In conjunction with these projects, I acquired data on seasonal changes in the energy content and nutrient profile of broadleaf cattail (*Typha latifolia*), the dominant food source of muskrats from my study area, to help determine whether, and to what extent, free-living muskrats are energetically and/or nutritionally stressed.

Knowledge of how herbivores utilize fiber and protein in their diet is essential to understanding the energy and nitrogen requirements for maintenance, growth and reproduction (Wunder 1992). Accordingly, my primary objective in Part I was to acquire baseline information on the nutritional physiology and bioenergetics of muskrats, specifically in terms of nutrient assimilation and nitrogen balance. This study, completed in the summer of 1990, used lab-acclimated animals fed single-genera hydrophyte diets, consisting of sedge (*Carex atherodes*), bulrush (*Scirpus validus*) or hybrid-cattail (*Typha* x *glauca*). One of the objectives of this study was to compare digestibilities of these principal emergent species, in order to clarify some of the controversy pertaining to the habitat selection of muskrats (Errington 1963; Welch 1980).

Building upon the foundation established in Part I, the second project involved seasonal investigations of acclimatized muskrats maintained on mixed diets approximating those consumed in nature. Small mammals inhabiting temperate and subarctic zones exhibit a variety of adaptations for coping with the seasonal energetic and nutritional constraints imposed by their environment. For example, in small microtine rodents, Gross, Wang and Wunder (1985) and Hammond and Wunder (1991) demonstrated that reduced energy availability (ie. increased food fiber) and reduced temperature were both factors that induced the development of a larger gut capacity, slowed passage rate, and increased forage digestibility. However, confirmation of these trends in natural populations is generally lacking (Korn 1992). As the quality and availability of principal food items are expected to change throughout the year (Batzli 1987), the objective of Part II was to test whether seasonal variability in diet quality is accompanied by compensatory

changes in diet selection, daily energy intake, and digestive capacity of wild muskrats.

Throughout the year, muskrats consume forages containing high levels of fiber and, at times, low protein content. As protein is often assumed to constitute a primary dietary constraint limiting the growth and reproduction of mammalian herbivores (Loeb, Schwab and Demment 1991; Robbins 1993), Projects III and IV explored two potential mechanisms whereby muskrats could meet their seasonal nitrogen requirements: carnivory and urea recycling.

The consumption of animal matter has often been reported in food preference studies conducted on muskrats (Errington 1941; Stearns and Goodwin 1941; Triplet 1983; Convey, Hanson and MacKay 1989; Neves and Odom 1989). This behavioral tactic has also been observed in the omnivorous white-tailed antelope squirrel (*Ammospermophilus leucurus*) which consumes small quantities of animal tissue (8% of intake) to alleviate seasonal deficiencies in dietary plant nitrogen (Karasov 1982). Consequently, carnivory may represent an important strategy muskrats employ to meet their daily nitrogen requirements. To assess the nutritional benefits derived by muskrats consuming animal matter, specifically in terms of nitrogen balance, Part III compared the assimilation efficiencies of muskrats maintained on a 100% cattail shoot diet with those of muskrats fed a cattail shoot diet supplemented with two levels (5 and 15%) of animal tissue.

Urea recycling involves the conservation of urea, a nitrogenous waste product normally voided in the urine. In animals demonstrating this phenomenon, urea is transferred from the bloodstream into the alimentary tract where it is hydrolysed by bacterial urease into ammonia and carbon dioxide (Richards 1972; Robbins. 1974; Nelson

et al. 1975). Ammonia produced at this point may be synthesized into bacterial protein, absorbed into the bloodstream, or voided in the feces (Mould and Robbins 1981). Upon reaching the liver, blood-borne ammonia joins the nitrogen pool available for the synthesis of nonessential amino acids (Richards 1972). Urea recycling has been shown to contribute significantly to the nitrogen economy of hibernating bears (*Ursus americanus*), two species of ground squirrels (Nelson et al. 1975; Steffen et al. 1980; Bintz and Torgerson 1981), as well as captive deer (*Odocoileus virginianus*) and elk (*Cervus canadensis*) maintained on low protein forage (Robbins et al. 1974; Mould and Robbins 1981). However, no previous study has addressed whether non-hibernating, simple-stomached herbivores also conserve body nitrogen by recycling urea. Hence, the primary objective of Part IV was to ascertain whether urea hydrolysis, a necessary step in urea recycling, occurs in muskrats and, if so, whether the intensity of this hydrolysis varies with the seasonal shifts in the protein content of broadleaf cattail.

Annual variation in the quality and availability of forage has been implicated as a major factor influencing dietary intake, body mass, body composition, digestive efficiency, serum chemistry, and basal metabolic rate (Wunder, Dobkin and Gettinger 1977; Morton and Lewis 1980; Merritt 1984, 1986; Virgl and Messier 1992*a*, 1992*b*; Nagy, Grower and Stetson 1995). While laboratory studies of acclimated animals provide important information regarding the plasticity and functional capacity of individual organ systems, field studies are necessary to evaluate how much of this functional range animals actually exploit in their natural environments (Tomasi and Horton, 1992).

Results I obtained in Part II suggested that muskrats have an increased appetite

in fall and winter. It is conceivable that this response was an artifact resulting from the presentation of rations *ad libitum*. However, an increased food intake during these periods could also be selected for to store fat and thus cope with potential shortages during late winter - early spring. To obtain reliable estimates of the daily intake of fresh vegetation and assimilated energy of individually marked muskrats, Part V of my project entailed monitoring temporal changes in the water influx of free-living muskrats using the well-established deuterated water (D_2O) technique (Costa 1987; Robbins 1993). This technique involves injecting a known quantity of isotope into the animal, allowing the tracer to equilibrate for several hours, and then withdrawing a blood sample for analysis. Because the turnover of D_2O in the body is a function of metabolism (Costa 1987), the rate of disappearance of the tracer, determined from sequential blood samples, can be used to estimate the water influx of free-ranging animals (Nagy, Shoemaker and Costa 1976). This study provided an essential step in quantifying the forage requirements and energy exchange of free-ranging muskrats.

The principal objective of Part VI was to test whether seasonal changes in the energy content and nutrient profile of broadleaf cattail is accompanied by parallel adjustments in organ morphology, basal metabolic rate, serum chemistry, and proximate composition of acclimatized muskrats. This study was conducted in concert with the seasonal digestibility trials (Part II), to assess the relationships among daily energy intake, basal metabolism, and organ and tissue masses within individual muskrats. The initial level of isotope dilution provided by the D_2O technique outlined in Part V also provided an estimate of the animals' total body water (TBW) content. Utilizing the known

proximate composition data from field-acclimatized muskrats, I found a strong inverse relationship between TBW and body fat content. Thus, a second objective of Part VI was to examine temporal adjustments in body size and endogenous energy reserves of tagged, free-living muskrats to resolve whether individuals undergo adaptive seasonal adjustments in mass or endogenous energy reserves. Such information is critical to defining the seasonal energetic and nutritional constraints that a prairie marsh environment imposes on these prominent North American rodents.

PART I

DIGESTIBILITY AND ASSIMILATION OF NATURAL FORAGES BY MUSKRATS

Abstract

Knowledge of the forage intake and digestive efficiencies of muskrats is essential for developing an understanding of the habitat requirements of these rodents and their impact on emergent vegetation. I performed 30 complete digestibility-, energy-, and nitrogen-balance trials on 6 adult male muskrats fed 5 diets: (1) sedge shoot, (2) softstem bulrush shoot, (3) hybrid cattail shoot, (4) cattail rhizome, and (5) a combination of cattail shoot and rhizome. Dry matter (DM) digestibilities ranged from 61.2 to 70.6%. Neutral detergent fiber (NDF) digestibilities varied from 40.0 to 59.6% for these emergent plant diets with NDF levels ranging from 44.6 to 62.1%. Microbial fermentation of fiber accounted for 39.4% of digestible energy intake. These findings suggest that muskrats can digest fiber as well as can many ruminants and pseudoruminants, but more efficiently than can other rodents. Apparent digestibility of dietary crude protein was highest ($P < 0.001$) on sedge (73.6%) and lowest ($P = 0.001$) on the cattail rhizome diet (7.2%). However, the daily nitrogen intake required by muskrats to maintain tissue balance on a cattail rhizome diet (0.599 g $N \cdot kg^{-0.75} \cdot day^{-1}$) was less than half the daily intake required for all other diets combined (1.266 g $N \cdot kg^{-0.75} \cdot day^{-1}$)($P < 0.001$). This implies the existence of a protein conservation mechanism by which muskrats could negate the effects of low dietary crude protein during winter.

Introduction

A goal in wetland management is to maintain marshes at, or near, their maximum potential for perpetuating wildlife resources. Achieving this objective requires an understanding of the muskrat's role in modifying marsh ecosystems, because this rodent is often the most prominent vertebrate consumer of marsh vegetation (Krummes 1940; McCabe 1982). Muskrats are versatile feeders, capable of exploiting diverse food sources (Errington 1941; Bellrose 1950; Danell 1977, 1978), yet little is known regarding their energy and nutritional requirements (Westworth 1974; Welch 1980; Jelinski 1989). Estimates of the rate of food intake, energy assimilation, and coefficients of digestibility are essential to developing an understanding of muskrat's nutritional needs. Such information should help managers assess the relative nutrient value of different forages and the potential role of these animals in providing biological control of aquatic emergents (Danell 1979; McCabe 1982). Our understanding of habitat selection and population dynamics of muskrats in wetland ecosystems also would be enhanced (Messier, Virgl and Marinelli 1990; Clark and Kroeker 1993).

My objective was to evaluate digestibilities of 5 common emergent forages consumed by muskrats in northern prairie marshes. I also estimated the minimum energy required for maintenance on each diet, as well as the efficiency with which muskrats convert dietary protein into body tissue.

Materials and Methods

I livetrapped 6 adult male muskrats at Oak Hammock Marsh, Manitoba (50°06'N, 97°07'W), in early May 1990. Upon capture, animals were transported to the University of Manitoba, where they were held in controlled environment chambers maintained at 14 ±1 C with a 12 hour light:12 hour dark photoperiod (MacArthur 1979). Relative humidity was kept at 77-94% for the study duration. Except during digestion trials, muskrats were maintained on a diet of Wayne Lab-Blox® rodent chow supplemented with apples and carrots. All animals were acclimated to the holding facilities for ≥3 weeks before digestion trials were initiated. I derived *in vivo* digestion estimates, using the total balance trial method in which all feed ingested and wastes produced were weighed and subsequently analyzed (Robbins 1993). This and all subsequent phases of this study were conducted with authorization of a University-approved animal research protocol (C91-50).

During digestion trials, animals were housed individually in digestion cages (106 x 52 x 46 cm), each furnished with a fiberglassed nest box (27 x 23 x 25 cm). I suspended each cage over a hardware cloth screen (0.3-cm mesh) for fecal collection; a plastic drape beneath the screen caught and directed all urine into a collection vial containing 0.7 ml concentrated HCl. Drinking water was provided *ad libitum*.

I performed 30 digestion trials between 23 May and 14 September 1990. Each trial consisted of a 5-day pretrial session when muskrats adjusted to the test ration and digestion cage, followed by a 5-day fecal and urine collection period. Successive trials with different diets were punctuated by rest periods, each ≥5 days, when muskrats were

fed the commercial laboratory diet.

I tested 5 emergent plant diets: (1) sedge shoot (*Carex atherodes*), (2) softstem bulrush (*Scirpus validus*) shoot, (3) hybrid cattail shoot (*Typha* x *glauca*), (4) cattail rhizome, and (5) a mixed cattail diet consisting of 67% shoot and 33% rhizome. I selected the mixed diet to test for possible associative digestion effects (Robbins 1993), and the predominance of shoots in this diet was based on analyses of early summer feeding platforms used by muskrats. Forage rations were collected at Delta Marsh, Manitoba (50°11'N, 98°23'W) and stored at 5°C to reduce plant respiration and deterioration. Vegetation was harvested on the day before each pretrial and trial session. Only the lower 25-40 cm of each plant was presented to animals. This preparatory step was based on space restrictions in the digestion cage and on the natural preference of muskrats for basal portions of emergent plants (Westworth 1974; Danell 1977; Welch 1980).

During digestion trials completed between 19 June and 31 August, I randomly assigned muskrats the cattail shoot, mixed cattail, and bulrush shoot diets. I tested all animals on the sedge diet between 23 May and 15 June, and on the cattail rhizome diet between 22 August and 14 September. I tested the sedge diet first, because other emergents were unavailable in the spring, and sedge often constitutes the principal forage consumed by muskrats during this season (Takos 1947; Sather 1958; Danell 1978). I tested the cattail rhizome diet last because roots and rhizomes of aquatic plants constitute a major portion of the autumn and winter diet of muskrats (Takos 1947; Bellrose 1950; Jelinski 1989). Each muskrat was assigned a given diet only once, thus providing 6

digestibility estimates for each of the 5 diets tested.

In each trial, muskrats were fed preweighed (*ad libitum*) rations 3 times daily. A sample of each test ration was also weighed and left on a tray outside the digestion cage. During each daily collection period, I weighed the ration sample and all feces and uneaten rations (orts) to the nearest 0.01 g, then froze them at -20°C for subsequent analyses. I recorded mass and total volume of urine produced daily, and pooled urine samples for each animal for the 5-day trial. Muskrats were weighed daily (0900 h) to determine mass changes during pretrial and trial periods.

Ration samples, orts, and feces were oven-dried to constant mass (48-72 hr) at 70°C, and then ground through a 1-mm-mesh screen in a Wiley mill. A portion of each ground sample was sent to a feed analysis laboratory (Department of Animal Science, University of Manitoba) for protein (Kjeldahl N x 6.25), acid detergent fiber (ADF) and neutral detergent fiber (NDF) determinations (Goering and Van Soest 1970, AOAC 1984). I calculated neutral detergent solubles (NDS) as 100% - NDF. I analyzed urine samples for nitrogen and energy content. Gross energy content of food, feces, and urine was obtained by duplicate measurements in an adiabatic oxygen bomb calorimeter (Parr 1241 Calorimeter, Parr Instrument Co., Moline, Il.). Urine samples were first lyophilized and then mixed with a known mass of mineral oil to ensure complete combustion. I determined total ash content of each sample by combustion of 2-g samples for 2 hours at 600°C. The proportion of energy obtained from consumed fiber (ration NDF - orts NDF) was calculated following Hammond and Wunder (1991). Animal and diet effects were evaluated using 2-way analysis of variance and mean values were compared with

t-tests for pairwise comparison of least-squares means (Procedure GLM, SAS Institute Inc. 1990).

Results

Ration Analyses

The gross energy content of the 5 emergent plant diets fed to muskrats varied inversely with ash content (Table 1-1). On an ash-free basis, the energy content of these diets varied over a narrow range, from 18.1 to 18.9 $kJ \cdot g^{-1}$. The protein content of sedge was 2.5-3.0 times higher than that of any other diet tested (Table 1-1). Fiber content generally was highest for bulrush and lowest for the cattail rhizome diet (Table 1-1). Dry matter content was low in all 5 emergent diets (8.2-13.2%).

Not all diets were tested concurrently, and some observed differences may reflect changes in plant phenology. However, results for bulrush, mixed cattail, and cattail shoots were from samples collected over 2 months, and ranged from newly emerged to mature shoots. Variance for these samples is small and generally consistent with that of the diets tested first (sedge) and last (cattail rhizomes) in this study (Table 1-1).

Intake and Digestibility of Nutrients

During the first 48-72 h of each pre-trial period, muskrats generally exhibited low levels of vegetation intake and consequently lost body mass. This trend presumably occurred in response to the abrupt shift in diet from lab chow to aquatic vegetation. However, intake of vegetation and body mass reached a plateau by the final 48 h of each

Table 1-1. Chemical composition of 5 emergent plant diets fed to adult male muskrats (n = 6) during 10-day digestibility trials, conducted in southern Manitoba, 1990.

Item	Sedge shoot		Bulrush shoot		Cattail shoot		Cattail rhizome		Cattail shoot and rhizome	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Ash (%)	10.0C[a]	0.0	12.6B	1.1	12.6B	0.6	17.0A	0.5	14.2B	0.6
Dry matter content (%)	13.2A	0.5	10.1B	0.7	8.7C	0.4	9.5BC	0.3	8.2C	0.0
Gross energy ($kJ \cdot g^{-1}$)	17.0A	0.0	16.3B	0.2	16.0BC	0.1	15.1D	0.2	15.5CD	0.1
Ash-free energy ($kJ \cdot g^{-1}$)	18.9A	0.0	18.7AB	0.1	18.3BC	0.1	18.2BC	0.4	18.1C	0.1
Crude protein (%)	16.8A	0.4	6.7B	0.5	6.0BC	0.3	5.5C	0.1	5.9BC	0.3
NDS[b] (%)	41.6BC	0.3	37.9C	1.8	42.0BC	1.3	55.4A	2.7	45.4B	1.7
NDF[c] (%)	58.4BC	0.3	62.1C	0 1.8	58.0BC	1.3	44.6A	2.7	54.6B	1.7
ADF[d] (%)	31.3C	0.0	38.6A	0.9	35.9B	0.6	25.9D	0.3	31.5C	1.8

[a] Within each row, means sharing the same letter are not significantly different ($P > 0.05$).
[b] NDS = neutral detergent solubles
[c] NDF = neutral detergent fiber
[d] ADF = acid detergent fiber

pre-trial period, with both variables remaining relatively stable throughout the trial period.

Analysis of variance of pooled data for all 5 diets revealed no differences ($P > 0.05$) among individual animals in any of the variables tested. Daily DM and gross energy intake by muskrats were highest on the cattail rhizome diet, but similar for the remaining diets (Table 1-2). Digestibility coefficients for DM, DE, and metabolizable energy (ME) also tended to be highest for the cattail rhizome diet (Table 1-3). Apparent DCP digestibility was highest (73.6%; $P < 0.001$) for the sedge and lowest (7.2%; $P = 0.001$) for the cattail rhizome diet (Table 1-3).

Fiber digestibility varied little among diets. Though the model was not significant ($P = 0.090 - 0.523$), NDF and ADF digestibilities tended to be lowest on cattail rhizome and highest on bulrush and sedge diets (Table 1-3). Dry matter digestibility varied inversely with dietary NDF (DM digestibility = 89.14 - 0.44 NDF, $r^2 = 0.26$, $n = 30$, $P = 0.004$), but showed no relationship with forage ADF content. Digestibilities of NDF and ADF varied with the percentages of these constituents in the diet: NDF digestibility = -6.47 + 1.06 NDF ($r^2 = 0.39$, $n = 30$, $P < 0.001$); ADF digestibility = 22.38 + 0.92 ADF ($r^2 = 0.14$, $n = 30$, $P = 0.042$). Digestibility of the NDS fraction was highest for muskrats fed the cattail rhizome diet ($P = 0.043$). Apparent digestible NDS correlated with the NDS content of the diet ($r^2 = 0.99$, $n = 30$, $P < 0.001$). The regression equation relating these variables (NDS digestibility = 17.979 + 0.923 NDS) yielded a true NDS digestibility of 92.3%.

Table 1-2. Dry matter (DMI), gross energy (GEI), digestible energy (DEI), and metabolizable energy (MEI) intake of 6 adult male muskrats fed 5 emergent plant diets, in southern Manitoba, 1990.

Item	Sedge shoot $\bar{x}$	Sedge shoot SE	Bulrush shoot $\bar{x}$	Bulrush shoot SE	Cattail shoot $\bar{x}$	Cattail shoot SE	Cattail rhizome $\bar{x}$	Cattail rhizome SE	Cattail shoot and rhizome $\bar{x}$	Cattail shoot and rhizome SE
$\bar{x}$ Body mass (g)	901.4B[a]	44.0	889.7B	40.6	896.0B	56.1	964.5A	52.4	912.0B	49.4
DMI (g)	32.5B	1.3	35.0B	2.4	28.6B	3.4	47.3A	5.3	31.2B	3.9
DMI ($g \cdot kg^{-0.75} \cdot day$)	35.4B	1.8	38.5B	2.7	31.4B	3.9	48.7A	5.0	33.3B	3.4
GEI/BM[b] ($kJ \cdot kg^{-0.75} \cdot day$)	602.7B	29.7	618.3B	45.6	483.8B	66.4	776.4A	73.6	521.0B	56.3
DEI/BM ($kJ \cdot kg^{-0.75} \cdot day$)	367.6B	21.9	375.5B	22.9	288.4B	49.4	534.8A	65.3	296.0B	36.8
MEI/BM ($kJ \cdot kg^{-0.75} \cdot day$)	327.5B	20.9	354.0B	23.2	264.5B	47.6	521.4A	64.6	277.3B	36.6

[a] Within each row, means sharing the same letter are not significantly different ($P > 0.05$).
[b] BM = body mass $kg^{0.75}$.

Table 1-3. Apparent digestibility (%) of nutrients in 5 emergent plant diets fed to 6 adult male muskrat in southern Manitoba, 1990.

Item	Sedge shoot $\bar{x}$	SE	Bulrush shoot $\bar{x}$	SE	Cattail shoot $\bar{x}$	SE	Cattail rhizome $\bar{x}$	SE	Cattail shoot and rhizome $\bar{x}$	SE
Dry matter	64.0B[a]	1.5	65.9AB	1.2	62.7B	2.0	70.6A	3.4	61.2B	2.8
Digestible energy	60.9B	1.5	61.1AB	1.1	57.7B	3.4	68.3A	3.3	56.9B	2.8
Metabolizable energy	54.2B	1.6	57.5B	1.0	52.3B	3.9	66.5A	3.3	53.1B	3.0
Dietary crude protein	73.6A	1.2	33.4C	4.6	47.1B	2.1	7.2D	6.6	27.5C	6.0
Neutral detergent solubles	68.5B	1.0	72.9B	0.9	69.2B	2.3	79.6A	3.7	69.3B	2.0
Neutral detergent fiber	59.6A	2.7	59.4A	1.6	53.5A	3.4	40.0B	7.6	50.0AB	4.1
Acid detergent fiber	55.5AB	3.3	63.1A	1.5	52.0B	3.9	44.7B	7.7	47.1B	3.4

[a] Within each row, means sharing the same letter are not significantly different ($P > 0.05$).

Partitioning of Dietary Nitrogen

For all diets except sedge, the mean daily nitrogen intake (DNI) of muskrats was similar (Table 1-4). The DNI of muskrat fed sedge was >2 times that recorded for any other diet ($P < 0.001$). The regression of apparent digestible nitrogen (ADN) on DNI yielded the equation ADN (g $N \cdot kg^{-0.75} \cdot day^{-1}$) = -0.293 + 0.965 DNI ($r^2 = 0.92$, $n = 30$, $P < 0.001$). This equation provides a true nitrogen digestibility (TND) estimate of 96.5%. Calculated TND estimates for individual diets were 98.2% for sedge, 95.1% for bulrush, 98.5% for cattail shoot, 99.5% for cattail rhizome, and 88.4% for mixed cattail.

Mean fecal nitrogen loss varied little with diet. However, mean urinary nitrogen loss was highly variable (Table 1-4; $P < 0.001$). Endogenous urinary nitrogen loss was only 0.041 g $N \cdot kg^{-0.75} \cdot day^{-1}$, following the regression total urinary nitrogen excreted (g $N \cdot kg^{-0.75} \cdot day$) = 0.041 + 0.710 DNI ($r^2 = 0.52$, $n = 30$, $P < 0.001$). On all diets, urinary energy loss was correlated with urinary nitrogen ($r^2 = 0.65$, $n = 30$, $P < 0.001$) and ration nitrogen levels ($r^2 = 0.63$, $n = 30$, $P < 0.001$).

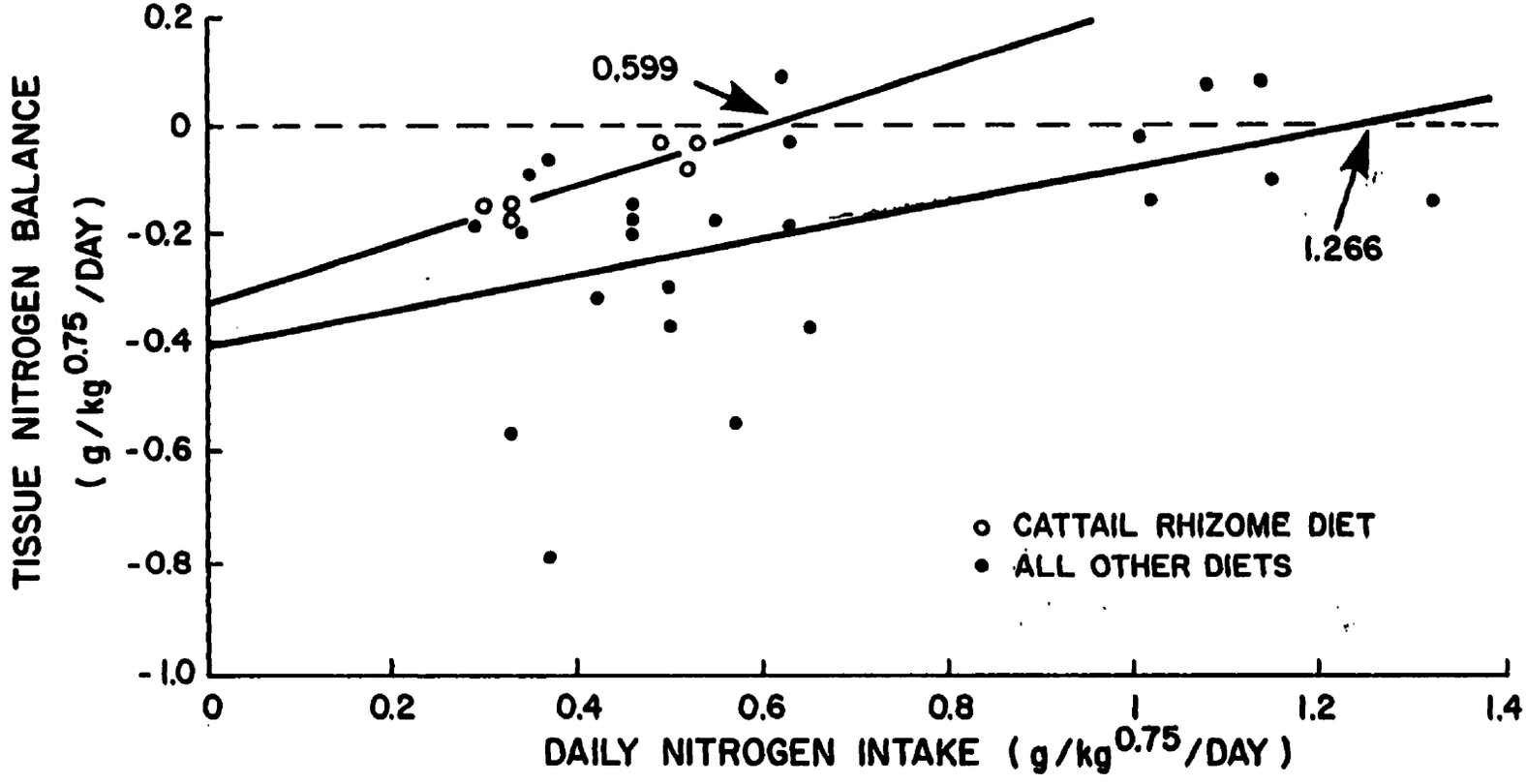
Regressing tissue nitrogen balance on DNI for all diets except cattail rhizome revealed that muskrats must consume 1.266 g $N \cdot kg^{-0.75} \cdot day^{-1}$ to maintain nitrogen balance (Fig. 1-1). Following the method of Holter, Hayes and Smith (1979), the level of dietary crude protein needed to meet this daily nitrogen requirement was 22.8% at a mean forage intake of 34.65 g $\cdot kg^{-0.75} \cdot day^{-1}$. However, the maintenance nitrogen requirements of muskrats fed the cattail rhizome diet were only 0.599 g $N \cdot kg^{-0.75} \cdot day^{-1}$. This is less than half the DNI required on the other diets (Fig. 1-1), and could be met with a crude protein level in cattail rhizomes of only 7.69%.

Table 1-4. Daily intake and partitioning of dietary nitrogen in 6 captive adult male muskrats fed 5 emergent plant diets, in southern Manitoba, 1990.

Item ($g \cdot kg^{-0.75} \cdot day^{-1}$)	Sedge shoot $\bar{x}$	Sedge shoot SE	Bulrush shoot $\bar{x}$	Bulrush shoot SE	Cattail shoot $\bar{x}$	Cattail shoot SE	Cattail rhizome $\bar{x}$	Cattail rhizome SE	Cattail shoot and rhizome $\bar{x}$	Cattail shoot and rhizome SE
Nitrogen intake	1.12A[a]	0.05	0.51B	0.06	0.47B	0.03	0.42B	0.04	0.44B	0.05
Fecal nitrogen loss	0.30BC	0.02	0.34AB	0.04	0.25C	0.02	0.38A	0.02	0.32ABC	0.04
Urinary nitrogen loss	0.87A	0.06	0.51B	0.10	0.47BC	0.07	0.15D	0.02	0.31CD	0.05
Tissue nitrogen balance	-0.04A	0.04	-0.33C	0.13	-0.25BC	0.07	-0.10AB	0.03	-0.19ABC	0.05
Apparent digestible nitrogen	0.83A	0.04	0.18BC	0.03	0.22B	0.01	0.04D	0.03	0.12C	0.03

[a] Within each row, means sharing the same letter are not significantly different ($P > 0.05$).

Fig. 1-1. The relationship between tissue nitrogen balance (TNB) and daily nitrogen intake (DNI) of 6 adult male muskrats fed aquatic emergent plant diets during summer and early autumn, 1990. The 5 rations tested were sedge shoot, bulrush shoot, cattail shoot, cattail rhizome, and a mixture of cattail shoot and rhizome. Regression lines were fitted by the method of least-squares (cattail rhizome diet: TNB = -0.334 + 0.557 DNI, $n = 6$, $r^2 = 0.83$, $P = 0.012$; other diets combined: TNB = -0.410 + 0.324 DNI, $n = 24$; $r^2 = 0.22$, $P = 0.022$).


TISSUE NITROGEN BALANCE
(g / kg 0.75 / DAY)
0.2
0
-0.2
-0.4
-0.6
-0.8
-1.0
0.599
1.266
o CATTAIL RHIZOME DIET
• ALL OTHER DIETS
0
0.2
0.4
0.6
0.8
1
1.2
1.4
DAILY NITROGEN INTAKE (g / kg 0.75 / DAY)

Discussion

Digestibility of Nutrients

Muskrat densities in marshes vary with the types of emergent vegetation present (Boutin and Birkenholz 1987; Messier, Virgl and Marinelli 1990). Clark and Kroeker (1993) found that vegetation succession can influence recruitment and survival of muskrats in prairie marshes. However, the extent to which demographic factors and habitat selection are influenced by diet remains unknown (Lacki et al. 1990). Although forage quality and palatability have been implicated as possible factors influencing diet choice of muskrats (Errington 1941; Takos 1947; Bellrose 1950), I found little variation in digestive efficiencies. Muskrats appeared to digest sedge, bulrush, and cattail shoots to similar extents during summer, implying that other variables such as forage availability, predation, water level, or suitability for lodge construction may have a greater bearing on habitat selection.

Although no associative digestion effect was observed on the mixed cattail diet, all 6 muskrats showed a preference for the rhizome component of this ration. The diet consisted of 67% shoot and 33% rhizome, yet the rhizome fraction constituted 52% of DM intake. Muskrats preference for the rhizome fraction was also demonstrated in laboratory feeding trials performed by Akkermann (1975), and likely reflects the superior nutritional value of this portion of the plant.

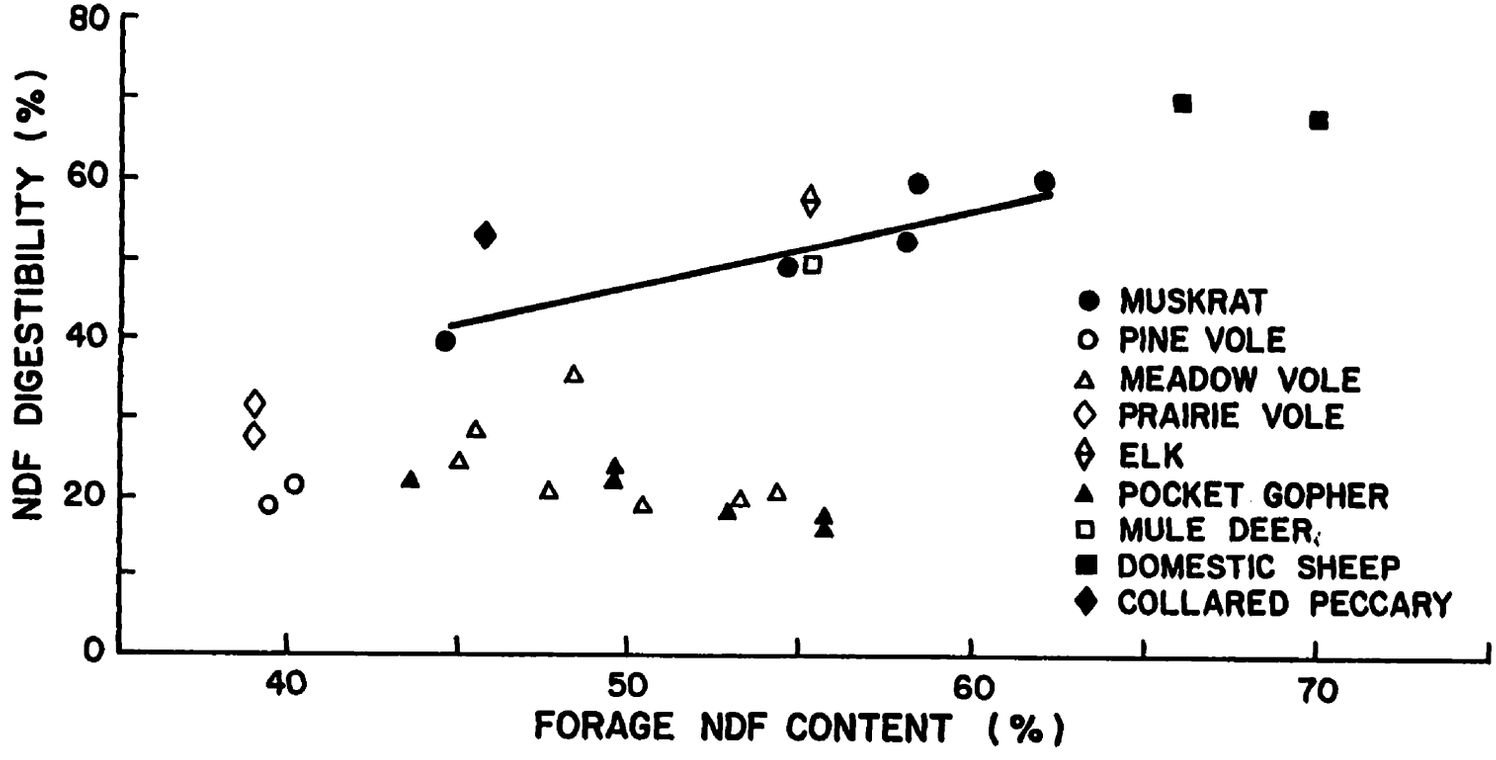
The DM digestibilities reported herein are 20-25% higher than estimates derived for other rodent species fed diets of similar fiber content. Results reported by Batzli and Cole (1979) suggest that brown lemmings (*Lemmus sibiricus*) digest sedge (*C. aquatilis*)

only about half as well as the muskrats maintained in this study on a similar sedge (*C. atherodes*) diet. The DM and gross energy digestibilities of lemmings fed *C. aquatilis* were only 33.2 and 34.0%, respectively, compared with 64.0 and 60.9% for muskrats maintained on *C. atherodes*.

Several recent studies have indicated that small herbivores can utilize plant NDF as a major source of dietary energy. The NDF fraction accounted for 19-32% of DE in rodents fed diets containing 39-51% NDF (Castle and Wunder 1994; Hammond and Wunder 1991; Justice and Smith 1992; Nagy and Negus 1993). By comparison, muskrats obtained an average of 39.4% of their DE intake from the microbial fermentation of fiber ($\bar{x}$ NDF consumed = 43.1%). Whereas meadow voles (*Microtus pennsylvanicus*) experience high mortality when dietary NDF exceeds 55% (Keys and Van Soest 1970; but see Castle and Wunder 1994), muskrats maintained mass and appeared healthy on diets containing up to 67% NDF. These findings suggest that the muskrat's capacity to digest plant fiber exceeds that of other small rodents and may rival digestion capabilities of several ruminants and pseudoruminants (Fig. 1-2). Muskrats apparently can digest fiber with an efficiency comparable with that of collared peccaries (*Tayassu tajacu*) (Carl and Brown 1986), mule deer (*Odocoileus hemionus*), and elk (*Cervus elaphus*)(Baker and Hansen 1985). It is doubtful these similarities arise from variability in plant digestion inhibitors, because the lignin content of cattail shoots (3.4%, Lacki et al. 1990) is close to that (4.7-5.4%) of diets tested in the latter studies.

Forage quality and gut morphology of mammals are strongly interdependent. Increased dietary fiber, for example, often stimulates caecal growth, thereby lengthening

Fig. 1-2. The relationship between neutral detergent fiber (NDF) digestibility and forage NDF content of rodent and ruminant herbivores. Data are included for muskrats (this study), pine voles, *Microtus pinetorum* (Servello et al. 1985), meadow voles, *Microtus pennsylvanicus* (Keys and Van Soest 1970; Servello et al. 1985), prairie voles, *Microtus ochrogaster* (Hammond and Wunder 1991), pocket gophers, *Thomomys bottae* (Loeb, Schwab and Demment 1991), collared peccaries, *Tayassu tajacu* (Carl and Brown 1985), mule deer, *Odocoileus hemionus*, and elk, *Cervus elaphus* (Baker and Hansen 1985), and domestic sheep (data of Keys and Van Soest 1970). The regression line was fitted to the muskrat data by the method of least squares (NDF digestibility = -6.47 + 1.06 forage NDF, $r^2 = 0.39$, $P < 0.001$).


NDF DIGESTIBILITY (%)
80
60
40
20
0
40
50
60
70
FORAGE NDF CONTENT (%)
MUSKRAT
PINE VOLE
MEADOW VOLE
PRAIRIE VOLE
ELK
POCKET GOPHER
MULE DEER
DOMESTIC SHEEP
COLLARED PECCARY

digesta retention time and facilitating digestion of cell wall constituents (Gross, Wang and Wunder 1985; Hammond and Wunder 1991; Loeb, Schwab and Demment 1991). Therefore, the muskrat's ability to handle large quantities of dietary fiber may reflect the species' large, well-developed caecum (Virgl and Messier 1992*a*; Part II), allowing for a high fermentation capacity and large absorptive surface area (see Fig. 4-6). The high thermoregulatory costs of aquatic foraging, especially in winter, also may select for large gut capacity and high fiber digestibility in the muskrat.

Like the muskrat, nutria (*Myocastor coypus*) also feed predominantly on basal shoots and rhizomes of aquatic plants, including cattail and bulrush. Nutrias reportedly have a long digesta retention time (45 hr) and high NDF digestibility (48%) on a diet composed of 32.5% NDF (Sakaguchi and Nabata 1992). As Sakaguchi and Nabata (1992) pointed out, these traits could reflect the occurrence of coprophagy in this South American rodent. Coprophagy was not prevented in my experiments and may have contributed to the high fiber digestibilities I observed.

Partitioning of Dietary Nitrogen

My estimate of TND in the muskrat (96.5%) is close to values (94-98.5%) reported for other similar-sized caecal fermentators (Nagy, Shoemaker and Costa 1976; Carl and Brown 1985; Meyer and Karasov 1989). High TND and relatively low true NDS digestibility (92.3%) observed in muskrats may, to some extent, reflect the presence of secondary plant compounds in the emergent diets (Robbins 1993).

Extrapolating the regression of ADN on DNI to zero nitrogen intake yielded a

metabolic fecal nitrogen loss of 0.293 g $N \cdot kg^{-0.75} \cdot day^{-1}$, or 0.78 g N per 100g DM intake. The latter estimate is near the upper limit of the range reported for caecal digestors (0.3-0.9 g N per 100 g DM intake). This is to be expected because high-fiber forages tend to yield large metabolic nitrogen losses in feces (Robbins 1993).

Muskrats voided 93-198 ml urine per day, a trend likely reflecting the high water content of ingested forage. However, the estimated endogenous urinary nitrogen loss of 0.04 g $N \cdot kg^{-0.75} \cdot day^{-1}$ is lower than in other nonruminant eutherians studied (range: 0.13-0.18 g $N \cdot kg^{-0.75} \cdot day^{-1}$; Robbins 1993).

The finding that muskrats tended to be in a slightly negative nitrogen balance on all 5 emergent plant diets (Table 1-4) suggests that animals consuming these diets alone would be unable to maintain body protein over extended periods. This observation, together with the high dietary crude protein requirement (22.8%) of muskrats fed emergent vegetation, suggests the need for a supplemental source of nitrogen in the summer diet. Such a need could explain the consumption of animal matter in at least some muskrat populations (Errington 1941; Stearns and Goodwin 1941). Although muskrats may feed opportunistically on clams, mussels, snails, and carrion throughout the year, their consumption of animal matter often appears highest in summer (Triplet 1983; Convey, Hanson and MacKay 1989; Neves and Odom 1989), when dietary nitrogen requirements presumably are greatest.

My estimate of the minimum level of dietary protein (7.69%) required for muskrats to maintain tissue nitrogen balance on cattail rhizomes is close to winter protein levels previously reported for this plant component (7.61-7.75%; Freudethal 1922; Stearns

and Goodwin 1941). This estimate is lower than the 15% crude protein that is generally recognized as a minimal requirement in rodent diets (Jelinski 1989). How muskrats reduce their nitrogen requirements on cattail rhizomes, the dominant forage consumed by these rodents during fall and winter is unknown. Urea recycling, coprophagy, urine drinking, and selective foraging are all potential physiological and behavioral tactics by which mammals can negate the effects of low dietary protein (Smith et al. 1975; Robbins 1993).

PART II

SEASONAL CHANGES IN GUT MASS, FORAGE DIGESTIBILITY AND NUTRIENT SELECTION OF WILD MUSKRATS

Abstract

The aim of this study was to determine if seasonal variability in diet quality and cold stress are accompanied by compensatory changes in nutrient selection, energy intake and digestive capacity of seasonally acclimatized muskrats. I hypothesized that in summer, muskrats meet their energy and nutrient requirements by selectively consuming high-protein, low-fiber aquatic plants. I also predicted that muskrats use fiber as an important energy source during those periods of the year when the nutritional value and diversity of forage species are lowest. At such times, muskrats should be most dependent on microbial fermentation and should exhibit maximal gut size and digestive efficiency. As predicted, muskrats offered natural forage increased the fraction of protein while reducing the proportion of fiber in their diet during summer, but not during spring or winter digestibility trials. From July to December, muskrats exhibited increases in dry matter intake, gut mass, and forage digestibility. The increase in hindgut mass was accompanied by an 18.5% rise in NDF digestibility, while the proportion of digestible energy derived from the fermentation of fiber increased from 38.4% in July, to 53.2% in December. During winter muskrats were able to reduce their dietary nitrogen requirements by 26.0%. My results suggest that changes in the absorptive surface area and volume of the gut are important adaptations for promoting nutrient assimilation during periods when muskrats are challenged both by high maintenance costs and a limited choice of diets.

Introduction

The high metabolic rate:gut capacity ratio of small herbivorous mammals dictates a high energy intake, especially during periods of reproductive activity or low environmental temperature (Demment and Van Soest 1985). In nature, the quality and availability of principal food items also change throughout the year (Hammond 1993). Small herbivorous mammals have several options for maximizing energy and nutrient assimilation when thermoregulatory costs are high and forage quality or availability is reduced. One is to optimize forage intake and retention time, allowing the gut to operate at, or near, maximum capacity (Sibly 1981). However, on poor quality forage, increasing the level of food intake or digesta retention time may not be sufficient to meet metabolic demands (Justice and Smith 1992; Hammond 1993). Selective consumption of high quality, low-fiber foods may also attenuate nutritional stress, although such foods must be available in adequate quantities (Justice and Smith 1992; Hammond 1993). A third option involves phenotypic adjustments to increase the surface area and volume of the absorptive region of the gut (Derting and Bogue 1993; Hammond 1993). These changes could involve villus hypertrophy or gut hyperplasy (Brugger 1991), responses which theoretically should offset the loss of assimilation efficiency resulting from increased rates of forage intake.

In recent years, numerous laboratory studies have correlated changes in intestinal morphology with differences in reproductive status, temperature, and food quality or quantity (see Miller, Xia and Norrie 1990; Hammond and Wunder 1991). However,

confirmation of these results for natural populations is generally lacking (Korn 1992).

In response to these needs, I initiated a study to determine if seasonal variability in diet quality and cold stress are accompanied by compensatory changes in diet selection, daily energy intake, and digestive capacity of wild muskrats. I hypothesized that the high thermoregulatory costs of aquatic foraging (MacArthur 1984) may have led to selection in this species for high energy and nutrient assimilation from aquatic vegetation. I expected muskrats to use fiber as an important energy source during those periods of the year when high-quality, low-fiber foods are unavailable. I also predicted that during summer, energy needs are largely met by selective consumption of low-fiber, high-protein forage, thus reducing the muskrat's dependence on microbial fermentation.

During late autumn, the translocation of nutrients from plant shoots to underground root structures, coupled with the establishment of persistent ice cover, physically limits the foraging range of this rodent while increasing the cost of feeding. I therefore hypothesized that muskrats enhance their forage assimilation capabilities in winter by increasing the size and absorptive capacity of the gut. My earlier study of laboratory-acclimated muskrats tested in early autumn revealed that these animals require reduced levels of dietary nitrogen on a 100% cattail rhizome diet (Fig. 1-1). As cattail rhizomes are the principal constituent of the muskrat's winter diet (Takos 1947; Jelinski 1989), I hypothesized that a similar decline in dietary nitrogen requirements may occur in winter-acclimatized muskrats.

Materials and Methods

Animals

Ninety-four muskrats were livetrapped at Oak Hammock Marsh, Manitoba (50°06'N, 97°07'W), between May 8, 1991 and April 17, 1992. Animals were immediately transported to the Animal Holding Facility, University of Manitoba, and housed individually at 14±1°C with a 12L:12D photoperiod (MacArthur 1979). These conditions (photoperiod and temperature) were chosen as "neutral", and selected to maintain consistancy among the various digestion trial periods. Experiments were performed during each of six test periods (n = number of animals tested): May 8-June 8 (n = 17), July 3-28 (n = 17), September 9-October 1 (n = 18), November 20-December 13 (n = 18), January 27-February 17 (n = 12), and April 8-17 (n = 12).

Digestibility Trials and Composition of Experimental Diets

A total of 32 digestibility and food intake trials were completed during the four test periods between May and December (n = 8 per period), and each animal was tested only once. However, one muskrat from the December digestibility trials was excluded from the analyses, since it exhibited low forage intake and lost >10% of body mass. Each digestibility trial was initiated on the day after animal capture, and consisted of a 5-d pretrial session when the muskrat adjusted to the test ration and digestion cage, followed by a 5-d fecal and urine collection period. Digestion trial protocol, sample analyses, and calculations of apparent digestibility coefficients are provided in Part I. Ration composition for each test period was based on field analyses of muskrat feeding

sites and estimates of forage availability in Oak Hammock Marsh (Takos 1947). Feeding sites were closely examined and the proportion of each plant species present was recorded. For convenience, ration mixtures were rounded to the nearest 5% (fresh mass) for each plant type. During the 5-d pretrial periods, uneaten portions were examined to confirm ration selection choices. The experimental ration in May consisted of 50% cattail shoot, 25% cattail rhizome, 20% bladderwort (*Utricularia vulgaris*), and 5% sedge shoot. In July, muskrats were fed a ration consisting of 70% cattail shoot, 10% cattail rhizome, and 5% each of softstem bulrush shoot, white-top (*Scolocholoa festucacea*) shoot, sedge shoot and duckweed (*Lemna minor*). The September ration consisted of cattail rhizomes (60%), cattail shoots (30%) and white-top shoots (10%). Vegetation for digestibility trials was harvested on the day preceding each pretrial and trial session, and stored at 5°C. In December, muskrats were presented with a 100% cattail rhizome ration collected in late fall, just prior to freeze-up. These rhizomes were allowed to air-dry, then packed in peat moss and stored at 3°C until used. Subsequent analyses of stored rhizomes showed minimal degradation of these samples between late-October and mid-December, and were comparable to rhizomes collected from two muskrat feeding lodges in December 1995 (see Table A1-1).

All ration, ort and fecal samples were analyzed for ash, protein (Kjeldahl N x 6.25) and energy. Due to the high starch content of some dietary components, acid detergent fiber, and neutral detergent fiber were determined using a modified Van Soest technique (Goering and Van Soest 1970) with Termamyl 120L (Åman and Hesselman 1984). Lyophilized urine samples were analyzed for nitrogen and energy content only.

Food Intake and Selectivity

To test for behavioral selection of specific dietary constituents, I subtracted the level of ash, energy, crude protein, NDF and ADF in the orts, from that in the ration offered to muskrats. Thus, intake for each dietary component was estimated as:

% component in ration x food offered (g) - % component in orts x orts (g).

The mass of each component consumed was divided by dry matter intake to calculate the fraction of that component in the diet, and this value was then compared against ration levels. The intake of daily digestible energy derived from the microbial fermentation of consumed ADF or NDF was calculated, following Hammond and Wunder (1991).

Gut Morphology

All food was removed at 0900 h on the day following completion of digestibility trials. Animals were killed at 1200 - 1300 h with an overdose of Halothane anesthetic (M.T.C. Pharmaceuticals), weighed, and their alimentary tracts removed, separated, and cleared of mesentery. Lengths of the stomach, small intestine, caecum and large intestine were recorded to the nearest 1 mm by suspending each organ vertically along a meter rule. Gut contents were removed by rinsing with physiological saline and isolated gut segments were stored at -20°C. Digestive organs were freeze-dried (72 h) and weighed to the nearest 0.1 mg on an analytical balance (Mettler model AJ100).

To determine if acclimation during digestibility trials affected gut morphology, 9-10 additional (control) animals were live-trapped during each test period, and all

measurements except digestibility were recorded from these muskrats within 2-d of capture.

Statistics

Proportions of dietary nutrients offered to, and selectively consumed by muskrats within each season were compared using paired *t*-tests. All gut morphology variables were compared for males vs. females, adults vs. subadults (see Part VI for aging criteria), and digestibility trial vs. control animals, using two-way ANCOVAs with ingesta-free body mass as the covariate (SAS Institute 1990). These comparisons enabled me to determine which variables could be pooled. I evaluated seasonal diet and animal effects on body mass, food intake, digestibility and energy and nitrogen partitioning using 2-way ANOVAs. All seasonal differences between means were tested using Tukey's studentized range test. Significance was set at the 5% level, and means are presented ±1 standard error (SE).

Results

There were no significant effects of sex, age class or acclimation on any morphological variable involving the total gut, stomach, small intestine or caecum ($P > 0.05$). For these variables, data for different sexes, age classes, and test groups (digestibility trials and controls) were therefore pooled. The dry mass of the large intestine was, however, greater in females than in males ($F_{[1,42]} = 5.13$, $P = 0.0295$), and

greater in subadults than in adults ($F_{[1,61]} = 4.19$, $P = 0.0456$).

Diet Selectivity

Muskrats selectively consumed specific components in the rations offered during each of the four test periods (Table 2-1). In May, the energy content of the food ingested was 3.8% (630 $J \cdot g^{-1}$ DMI) above that measured in the ration offered ($P = 0.0052$). During the same period, muskrats reduced ash intake 22.9% below the ration value (85.4 $mg \cdot g^{-1}$ DMI vs. 110.8 $mg \cdot g^{-1}$ DMI; $P = 0.0002$). Muskrats presented with a mixed ration containing 62.4% NDF in July, reduced NDF consumption 18.4% below that offered ($P < 0.0001$). However, muskrats fed a 100% cattail rhizome ration in December, increased NDF intake 15.4% over the ration value ($P = 0.029$). Muskrats presented with mixed rations selected for protein in July and September, when ration protein content was lowest ($P < 0.0001$).

Gut Morphology

Dry Mass. The dry mass of the total gut changed significantly over the year ($F_{[5,88]} = 10.64$, $P < 0.0001$). It was lowest in May and July, increased in fall, and peaked in December (Fig. 2-1; $P < 0.05$). Changes in the dry mass of the small intestine, caecum, and large intestine accounted for most of this increase in total gut mass.

Though not statistically significant, stomach dry mass was lowest in spring and summer, and greatest in fall and winter. The dry mass of the small intestine increased from May to September ($P < 0.05$), and continued to rise throughout the winter (Fig. 2-1).

Table 2-1. Comparison of the nutrient composition of experimental rations offered to, and selectively consumed by muskrats

	Ash (%)	Energy Content ($KJ \cdot g^{-1}$)	Crude Protein (%)	NDF (%)	ADF (%)
May					
Ration offered	11.08 (0.20)	16.62 (0.08)	11.39 (0.27)	59.47 (1.35)	31.81 (1.69)
Diet consumed	8.54 (0.48)	17.25 (0.17)	11.30 (0.29)	59.88 (1.90)	27.23 (1.76)
	***	*	(NS)	(NS)	(NS)
July					
Ration offered	10.54 (0.04)	16.59 (0.01)	6.58 (0.12)	62.44 (0.14)	36.62 (0.09)
Diet consumed	11.83 (0.33)	16.48 (0.09)	8.96 (0.28)	50.95 (1.13)	26.51 (0.85)
	**	(NS)	***	***	***
September					
Ration offered	6.68 (0.21)	17.02 (0.06)	5.92 (0.04)	52.25 (0.56)	27.29 (0.00)
Diet consumed	5.60 (0.51)	17.18 (0.19)	7.66 (0.32)	49.81 (3.35)	23.61 (1.38)
	(NS)	(NS)	***	(NS)	*
December					
Ration offered	7.75 (0.07)	16.74 (0.01)	7.84 (0.04)	43.33 (0.62)	22.96 (0.07)
Diet consumed	6.79 (0.38)	16.83 (0.05)	8.29 (0.35)	49.99 (2.66)	24.77 (1.03)
	*	(NS)	(NS)	*	(NS)

Note: Values are presented as mean (SE); $n = 8$ for all samples except September ADF ($n = 4$).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

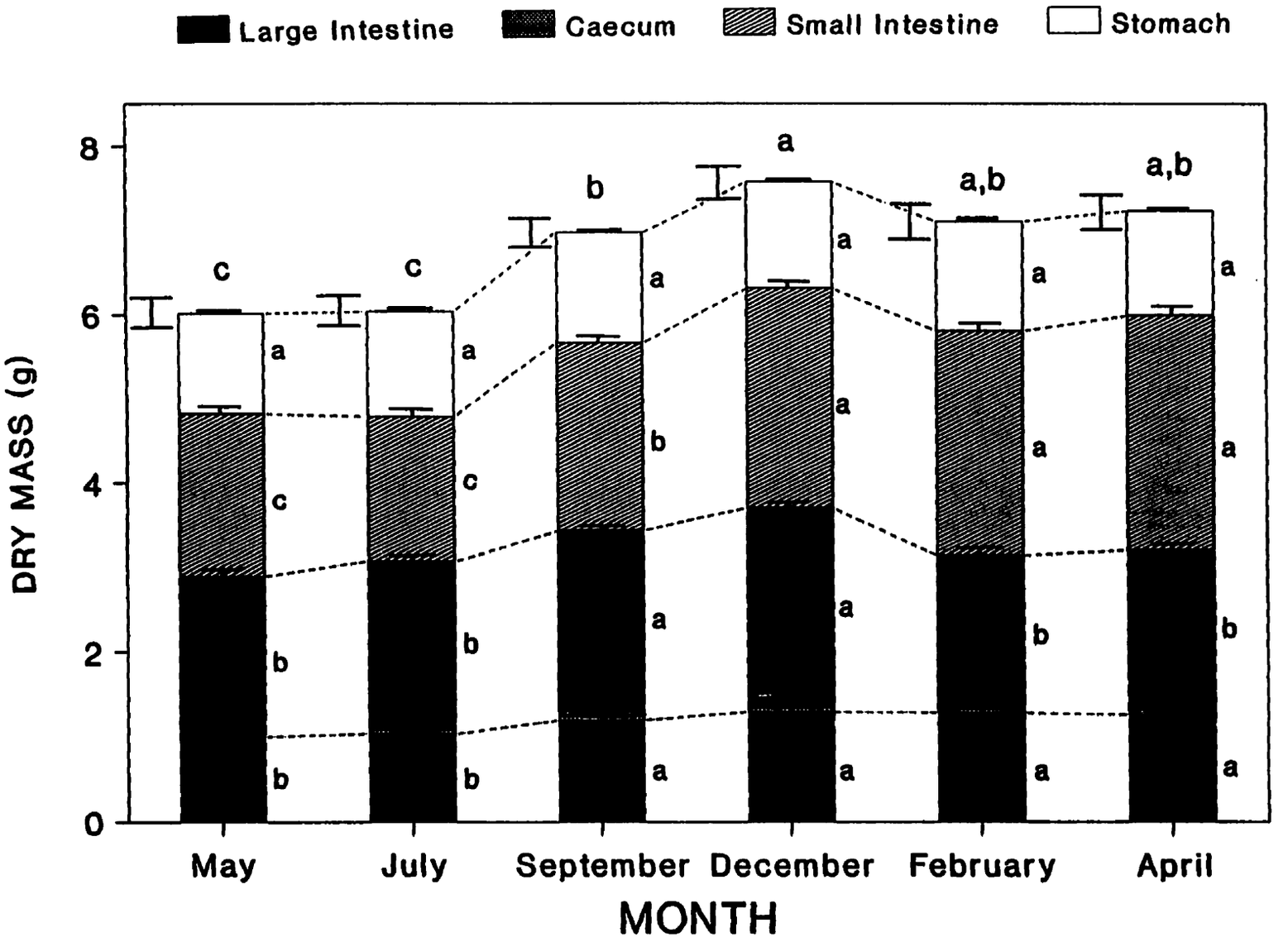
Although not significantly different from muskrats in December and February, April-caught animals exhibited the heaviest small intestines. Caecal dry mass increased from spring to fall, reaching peak values in September and December ($P < 0.05$), and then declined to spring levels (Fig. 2-1). The dry mass of the large intestine was lowest in May and July ($P < 0.05$), increased from July to December, and then remained elevated until spring.

Gut Lengths. Although there were no significant changes in the lengths of the small intestine, large intestine, or total gut ($F_{[5,88]} = 0.74\text{-}1.37$, $P > 0.05$), these parameters were consistently highest in the December-trapped muskrats (data not shown). There was seasonal variation in the lengths of the stomach ($F_{[5,88]} = 4.62$, $P = 0.0009$) and caecum ($F_{[5,88]} = 2.87$, $P = 0.0191$). Stomach length increased as winter progressed, and peaked in April. Caecum length generally increased from summer to mid-winter, but then declined from December to February ($P < 0.05$).

Digestibility Trials

Intake. During May and July, dry matter and gross energy intake were relatively constant among animals, averaging 60 $g \cdot kg^{-0.75} \cdot day^{-1}$ and 1011 $KJ \cdot kg^{-0.75} \cdot day^{-1}$, respectively (Table 2-2). However, muskrats increased DMI by 26.2% and gross energy intake by 27.3% in the September and December digestibility trials ($P < 0.01$). Fecal energy output (GE - DE) showed no definite trend, though it was highest in May and September (Table 2-2). Urinary energy loss (DE - ME) increased from May to July ($P < 0.05$), but then declined

Fig. 2-1. Seasonal changes in the dry gut mass of 94 field-acclimatized muskrats. The error bar to the left of each histogram represents ±1 SEM for the entire gut; error bars within histograms denote 1 SEM for individual gut compartments (n = 12-18 animals/month). Means sharing the same letters for individual gut segments and for the whole gut are not significantly different ($P > 0.05$). Note: all means presented are adjusted means with ingesta-free body mass ($\bar{x}$ = 772 g) as the covariate.


Large Intestine
Caecum
Small Intestine
Stomach
DRY MASS (g)
0
2
4
6
8
May
July
September
December
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MONTH
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Table 2-2. Effects of season and diet on food intake, apparent digestibility, and energy gain of field-acclimatized muskrats

	May (n = 8)	July (n = 8)	September (n = 8)	December (n = 7)
Body mass (g)	1000[a] (27)	901[a,b] (37)	1010[a] (50)	826[b] (44)
Dry matter intake ..... ($g \cdot kg^{-0.75} \cdot day^{-1}$)	59.72[b] (2.61)	60.30[b] (1.74)	75.87[a] (3.06)	75.55[a] (5.95)
Digestibility (%)				
Dry matter	44.39[c] (2.50)	54.82[a,b] (2.37)	49.23[b,c] (2.09)	59.84[a] (3.16)
Digestible energy	41.61[b] (2.41)	52.70[a] (2.29)	46.98[a,b] (1.86)	55.96[a] (3.32)
Metabolizable energy	39.08[b] (2.50)	48.86[a] (2.26)	46.53[a,b] (0.94)	54.89[a] (3.30)
Crude protein	25.81[a,b] (3.08)	38.37[a] (2.88)	15.35[b] (5.04)	21.66[b] (5.37)
NDS	48.92[b] (4.22)	70.02[a] (1.52)	55.73[a,b] (4.85)	59.05[a,b] (6.10)
NDF	40.65[b] (2.05)	40.15[b] (3.05)	38.76[b] (4.73)	58.60[a] (2.30)
ADF	34.10[b] (3.66)	29.07[b] (4.06)	41.03[b*] (6.05)	62.77[a] (2.07)
Energy gained ($KJ \cdot kg^{-0.75} \cdot day^{-1}$)				
Gross energy	1027.6[b] (37.6)	993.5[b] (27.9)	1303.9[a] (57.1)	1269.3[a] (98.0)
Metabolizable energy	406.0[c] (35.1)	482.9[b,c] (19.6)	599.7[a,b] (11.3)	706.9[a] (78.0)
Digestible energy	431.5[c] (34.8)	520.9[b,c] (19.6)	605.8[a,b] (11.0)	721.3[a] (86.2)
NDF	249.9[a,b] (17.5)	201.1[b] (14.1)	259.1[a,b] (46.6)	360.7[a] (25.3)
NDS	210.6[b] (28.7)	341.1[a,b] (13.5)	375.5[a,b] (49.8)	409.3[a] (76.9)
ADF	97.9[b] (14.4)	76.7[b] (11.1)	134.1[a,b*] (24.9)	199.3[a] (21.0)

Note: Values are presented as mean (SE). Within each row, means sharing the same letter are not significantly different ($P > 0.05$).
[*] n = 4.

sharply in Sep

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higher than fo
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soluble (NDS *ction*
December or *September*
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($F_{[3,27]}$ = 2.86 P < 0.05
(Table 2-3). *Mean* fec
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paralleled uri *y* cnerg
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revealed two *signi*ct se
0.69, df = 14; *Septembe*
These models *predict* tu
winter (0.811 $W_{kg}^{-0.75}$

sharply in September and December, when it was <25% of July values (Table 2-2).

Apparent Digestibility. The DE and ME coefficients of muskrats tested in December were higher than for muskrats tested in May, but not July and September (Table 2-2). NDF and ADF digestibilities were highest in December. Digestibility of the neutral detergent soluble (NDS) fraction was highest in July, though not significantly greater than in December or September. Crude protein digestibility was highly variable, ranging from a minimum of 15.35% in September to a maximum of 38.37% in July (Table 2-2).

Intake and Partitioning of Dietary Nitrogen

The mean daily nitrogen intake (DNI) of muskrats was similar throughout the year ($F_{[3,27]} = 2.86$, $P = 0.056$), ranging from 0.86 $g \cdot kg^{-0.75}$ in July to 1.08 $g \cdot kg^{-0.75}$ in May (Table 2-3). Mean fecal nitrogen loss was similar for May, September and December trials, but was reduced in July. In contrast, mean urinary nitrogen loss was 16.5 times greater in July than in September (Table 2-3). This component of nitrogen loss closely paralleled urinary energy loss in that it remained high in spring and summer before dramatically falling in autumn. Regressing tissue nitrogen balance (TNB) on DNI revealed two distinct seasonal models (May and July: TNB = -1.300 + 1.189 DNI, r^2 = 0.69, df = 14; September and December: TNB = -0.561 + 0.691 DNI, $r^2 = 0.62$, df = 14). These models predict that the minimum DNI required to meet TNB is lower in fall and winter (0.811 $g \cdot kg^{-0.75} \cdot day^{-1}$) than during spring and summer (1.091 $g \cdot kg^{-0.75} \cdot day^{-1}$).

Table 2-3. Seasonal intake and partitioning of dietary nitrogen in field-acclimatized muskrats

Variable ($g \cdot kg^{-0.75} \cdot day^{-1}$)	May (n = 8)	July (n = 8)	September (n = 8)	December (n = 7)
Nitrogen intake	1.08[a] (.04)	0.86[b] (.02)	0.93[a,b] (.06)	0.97[a,b] (.08)
Fecal nitrogen	0.79[a] (.02)	0.53[b] (.03)	0.77[a] (.03)	0.74[a] (.03)
Urinary nitrogen	0.25[b] (.02)	0.66[a] (.03)	0.04[c] (.01)	0.12[c] (.03)
Nitrogen balance	0.03[a] (.02)	-0.32[b] (.03)	0.12[a] (.06)	0.11[a] (.06)
Apparent digestible nitrogen ...	0.28[a] (.04)	0.33[a] (.03)	0.16[a] (.06)	0.23[a] (.07)

Note: Values are presented as mean (SE). Within each row, means sharing the same letter are not significantly different ($P > 0.05$).

Discussion

Seasonal Changes in Gut Morphology and Digestive Efficiency

Laboratory studies have established that small endotherms can dramatically alter the size and absorptive capacities of specific regions of the alimentary tract in response to changes in fiber content, reproductive status, and temperature. For instance, voles exposed to low temperatures increase forage intake and the size of the small intestine, resulting in greater energy absorption (Gross, Wang and Wunder 1985; Hammond and Wunder 1991). Similarly, small herbivores presented with low quality rations (increased fiber) increased forage intake as well as the volume and surface area of the caecum and large intestine (Hammond and Wunder 1991; Loeb, Schwab and Demment 1991). However, few studies (Miller, Xia and Norrie 1990; Hammond 1993) have demonstrated that similar modifications in gut size or absorptive capacity occur in wild populations. My findings clearly indicate that gut size and forage digestibility of free-ranging muskrats do not remain static, but fluctuate in response to temporal changes in energy demand and fiber intake. It is important to recognize that muskrats in this study were fed natural diets that varied with season. Therefore, I could not clearly distinguish dietary effects from other factors that might have contributed to the observed seasonal trends in gut size and digestive function. The observed changes in gut mass could not be attributed to changes in the composition of this tissue, since the wet:dry mass ratio of the alimentary tract showed little seasonal variation. Furthermore, I observed little correspondence between gut mass and changes in body composition (ie. fat content) during summer or late-winter (see Fig. 6-2).

Muskrats undergo a significant reduction in the mass of intestinal tissue and body fat reserves between April and May (this study; Virgl and Messier 1992*a*). During the breeding season, males experience a more rapid decline in body fat stores than females, a trend that may reflect the high cost of establishing male breeding territories (Virgl and Messier 1992*a*). As gut tissue is metabolically expensive to maintain (Brugger 1991), it may be adaptive for males to reduce intestinal mass and mobilize surplus fat reserves in early spring. Unfortunately, no females were collected during the breeding season in this study. Virgl and Messier (1992*a*) reported that female muskrats have larger digestive tracts than males from June through August. It is likely that the females in my study population also maintained larger guts to meet the high energy demands of pregnancy and lactation (see Part VI for further explanation).

Muskrats collected in July demonstrated no appreciable change in total gut mass, gut length, or DMI, compared to spring-caught animals. However, digestive efficiencies were consistently higher in July. The presence of secondary plant compounds and structural digestion inhibitors are known to reduce digestibility of forages consumed by small herbivores (Robbins 1993), and many mammals appear to select foods based on levels of digestive inhibitors (Miller, Xia and Norrie 1990). Seasonal variability in lignin concentration of the experimental rations was small (range: 3.22-4.97%), and it is unlikely these differences led to the changes in fiber utilization and digestibility that I observed. Although it is conceivable that associative digestion effects may have influenced forage digestibility estimates, results in Part I suggest that these factors were generally insignificant (Table 1-3).

Small herbivores are expected to prefer foods that can be rapidly absorbed and are low in fiber (Loeb, Schwab and Demment 1991), and the high digestibility values recorded during July are probably the result of forage selection. Muskrats presented with a mixed ration containing 62.4% NDF and 6.58% protein in July selectively consumed vegetation containing only 51.0% NDF (18.4% below ration level), but 8.96% protein (36.2% above ration level; Table 2-1). Evidently, muskrats in summer are able to meet their energy needs by selectively consuming lower levels of fiber, rather than by increasing gut mass. Selective feeding reduces the dependence of many small herbivores on microbial fermentation (Justice and Smith 1992). Indeed, the fraction of energy derived from this source declined from 59.5% of DE intake in May, to 38.4% in July (Table 2-2). It is noteworthy that the latter value is still well above previously reported estimates for other small herbivores maintained on high fiber rations (but see Castle and Wunder 1994). Prairie voles, wood rats (*Neotoma* spp.) and collared lemmings (*Dicrostonyx groenlandicus*) obtained only 19-36% of DE from rations containing 39-60% NDF (Castle and Wunder 1994; Hammond and Wunder 1991; Justice and Smith 1992; Nagy and Negus 1993).

My data suggest that the muskrat's ability to digest fiber actually exceeds predictions based on body mass. According to the computer simulations of Justice and Smith (1992), a 1-kg herbivore fed an alfalfa-based ration containing 60% NDF, should exhibit a NDF digestibility of 24% and obtain 33% of its DE through microbial fermentation. In May, muskrats consuming a mixed ration containing 59.9% NDF displayed a NDF digestibility of 40.7%, while obtaining 59.5% of their DE from fiber.

According to the model developed by Justice and Smith (1992), my fiber utilization values correspond to those predicted for a 20-kg animal. My values represent a conservative estimate of fiber digestibility in muskrats, since caecum mass was lowest in spring (Fig. 2-1). These findings are in accord with my earlier study indicating that muskrats readily adapt to high-fiber diets and utilize these structural carbohydrates as an important energy source (Fig. 1-2; Table 1-3).

An increased caecal capacity permits a larger volume of fermentable particles to be retained in the gut, and hence provides more time for microbial fermentation. The muskrat possesses a large haustrated caecum which empties into a complex (8-spiral) proximal colon (Luppa 1957; Fig. 4-6) similar to that described for the Scandinavian lemming, *Lemmus lemmus* (Vorontsov 1967). Sperber, Björnhag and Ridderstråle (1983) have shown that the convolutions of the lemming's proximal colon create a separation mechanism which traps and provides for a direct flow of bacteria and fine particles back towards the caecum. Selective retention of fluid and small particles in the caecum should permit more complete digestion of dry matter, since fine particles have an increased fermentable surface area and will support a higher concentration of bacteria.

In muskrats, the dry masses of the total alimentary tract, small intestine, caecum, and large intestine had all increased by September. Despite these gains, digestive efficiencies were slightly lower in September than in July (Table 2-2). Since NDF levels of the ingested diet were similar for both months, the small reduction in digestibilities of DM, DE and ME in September are likely a response to the 25.8% increase in daily DMI ($g \cdot kg^{-0.75}$). As pointed out by Robbins (1993), reduced digestibility need not be

maladaptive if it leads to an increase in total nutrient absorption. Indeed, results for the September digestibility trial indicated that muskrats increased their daily ME intake 24.2% over July values (Table 2-2). This gain in energy absorption is likely linked to the increase in small intestine mass, and perhaps digestive transporters (Hammond and Diamond 1992). The continued enlargement of this organ during fall and winter probably served to further extract easily digestible nutrients from the diet (eg. NDS and protein).

The notable increases in dry mass of the caecum and large intestine between July and September are presumably the consequence of increased DMI, since the NDF content of ingested forage remained close to 50% (Table 2-1). It appears paradoxical that the 11% gain in caecal dry mass from July to September was correlated with a 1.5% decrease in NDF digestibility. However, over the same period, the proportion of daily energy gained from the fermentation of NDF and ADF increased 4.2% and 7.5%, respectively. December-trapped animals fed a 100% cattail rhizome ration consumed portions of the rhizomes that were high in fiber (15% above ration offered). Consequently, December-caught muskrats consumed equivalent amounts of fiber as animals trapped in September (37.8 $g \cdot kg^{-0.75} \cdot day^{-1}$). However, the 8% gain in caecal mass of muskrats in December appeared to increase both their NDF digestibility (38.76 to 58.60%) and the proportion of DE derived from NDF (42.56% to 53.23%).

Muskrats begin accumulating body fat stores in September (Virgl and Messier 1992*a*; Fig. 6-2; Fig. 6.3), presumably to offset potential food shortages in late winter. Dry masses of the total gut, caecum, and large intestine peaked in December-trapped muskrats, and I interpret this as a mechanism to maximize energy absorption and the

accumulation of body lipid reserves. The observed gains in gut mass probably also contributed to the substantial increases in digestibilities (10-20%) for the DM, DE, ME, NDF and ADF components from September to December (Table 2-2).

It is possible that there has been strong selective pressure for muskrats to maximize energy derived from cattail rhizomes, which account for a large proportion of the muskrat's winter diet (Takos 1947). My results support this hypothesis, as muskrats in December were able to increase ME intake by 17.9% over September values, even though gross energy intake was 2.7% lower. I similarly reported a high DM digestibility (70.6%) and ME intake (521.4 $KJ \cdot kg^{-0.75} \cdot day^{-1}$) in my earlier study of laboratory-acclimated muskrats consuming a 100% cattail rhizome diet (Part I).

Seasonal Nitrogen Dynamics

Since nitrogen is often a limiting nutrient in the diet of herbivores, its digestibility and retention is crucial (Loeb, Schwab and Demment 1991). My findings suggest that the minimum nitrogen intake required to meet tissue nitrogen balance is 0.280 $g \cdot kg^{-0.75} \cdot day^{-1}$ less in fall and winter than during spring and summer. In my previous study (see Part I), I found a similar reduction in the nitrogen requirements of muskrats maintained on a 100% cattail rhizome ration in late summer and early fall. Furthermore, muskrats in the present study were able to maintain tissue nitrogen balance in September and December (Table 2-3) on diets containing only 8% protein. This value is only half that generally recognized as constituting a minimal requirement in rodent diets (Jelinski 1989).

It is possible that muskrats may, like some hibernators (Steffen et al. 1980; Bintz

and Torgerson 1981), conserve body nitrogen in winter by reducing urinary nitrogen output through urea recycling. The recycling of urea formed from tissue nitrogen may be important to the nitrogen economy of animals when nitrogen intake is inadequate to meet daily requirements (Robbins et al. 1974).

The large caecum, selective increase in dietary fiber intake, and possibly the recycling of nitrogen, may all contribute to maintaining intestinal bacteria and protozoan populations in winter. Furthermore, muskrats are known to practice coprophagy (K.L. Campbell, unpublished observations), but no steps were taken to either prevent or quantify this behavior in the present study. Thus, it is conceivable that coprophagy may have contributed not only to the high fiber digestibilities reported herein, but may, along with urea recycling, be an important mechanism utilized by muskrats to meet their seasonal nitrogen requirements.

PART III

DIGESTIBILITY OF ANIMAL TISSUE BY MUSKRATS

Abstract

I examined the potential nutritional benefits derived by muskrats that supplement their aquatic plant diet with animal tissue. Digestibility, energy and nitrogen balance trials were conducted with adult muskrats fed each of three diets: 100% cattail shoots; 95% cattail shoots, 2.5% fathead minnows (*Pimephales promelas*) and 2.5% muskrat flesh; and 85% cattail shoots, 10% fathead minnows and 5% muskrat flesh. Muskrats presented with diets containing meat selectively increased the proportion of animal tissue ingested above that offered in the ration ($P < 0.001$). Gain in body mass, dry-matter intake, and coefficients of dry matter, energy and protein digestibility all increased with rising levels of meat consumption. Muskrats can efficiently digest high levels of animal tissue (>50% dry matter intake) with no apparent loss of ability to digest fiber. Moreover, free-ranging muskrats consuming diets containing only 3.6-6.1% animal tissue can meet their maintenance daily nitrogen requirements (1.02 g $N \cdot kg^{-0.75}$ body mass) solely from meat. I conclude that meat consumption, even at low levels, can be nutritionally beneficial to muskrats in nature.

Introduction

Protein is often assumed to constitute a primary dietary constraint limiting the growth and reproduction of mammalian herbivores (Loeb, Schwab and Demment 1991; Robbins 1993). Seasonal deficiencies in dietary protein may be compounded by the high fiber content of mature plants which reduces the amount of readily digestible energy that small herbivores can extract from such diets (Hammond 1993).

The muskrat feeds predominantly on aquatic plants that are high in fiber and low in nitrogen (Table 1-1; Appendix I). My previous digestion trials (Parts I and II) suggested that muskrats consuming diets composed solely of aquatic vegetation have difficulty maintaining nitrogen balance in summer (Table 1-4; Table 2-3). It is currently unknown how free-ranging muskrats meet their daily nitrogen requirements. One possibility involves the opportunistic feeding on concentrated nitrogen sources such as animal matter, which may help to alleviate seasonal deficiencies in dietary crude protein (Karasov 1982).

The consumption of animal tissue, including muskrat flesh, has often been reported in food preference studies of *O. zibethicus* (Errington 1941; Stearns and Goodwin 1941; Triplet 1983; Convey, Hanson and MacKay 1989; Neves and Odom 1989). Ching and Chih-Tang (1965), for example, reported that animal tissue accounted for 6.6% of the daily food intake by muskrats. Unfortunately, the nutritional benefits of this dietary component are poorly understood because little is known regarding the digestibility and assimilation of animal flesh by mammalian herbivores.

This study evaluates the potential nutritional benefits of animal tissue ingestion by muskrats, specifically in terms of energy and nitrogen balance. I hypothesized that muskrats, when presented with a choice, would actively select a diet containing a higher level of animal tissue than that offered in a mixed ration containing aquatic vegetation and vertebrate animal matter. I also predicted that coefficients for dry matter digestibility, energy assimilation, and nitrogen retention would all increase with rising levels of meat consumption by muskrats.

Materials and Methods

Eight adult male muskrats were captured at Oak Hammock Marsh, Manitoba (50°06'N, 97°07'W) in mid-May 1993. Animals were housed, fed and acclimated following the methods presented in Part I.

A total of 24 digestibility and food-intake trials were completed over six measurement periods between June 9 and August 14, 1993. Muskrats were tested once on each of three rations and the order of ration presentation was randomized. Consecutive tests on the same individual were separated by a minimum of 14 days. Each digestion trial lasted 5 days and was preceded by a 5-day adjustment period during which muskrats were fed the test ration. Digestion trial protocol, sample analyses, and calculations of apparent digestibility coefficients are provided in Part I. Ash content was determined by combusting 2-g duplicate samples for 6h at 600°C (AOAC, 1984).

In all trials, muskrats were presented with a total daily ration (1000 g) that was

approximately twice the daily intake previously recorded for captive muskrats (see Part I). On a wet-weight basis, the test rations consisted of: (a) 100% cattail shoots (hereafter referred to as 0% meat ration), (b) 95% cattail shoots, 2.5% fathead minnows and 2.5% muskrat flesh (5% meat ration), and (c) 85% cattail shoots, 10% fathead minnows and 5% muskrat flesh (15% meat ration). On a dry-weight basis, rations offered to muskrats consisted of 16.5% animal tissue in the 5% meat ration and 38.9% in the 15% meat ration. Cattail is the dominant food source of muskrats in northern prairie marshes, and muskrat flesh and fathead minnows are forms of animal tissue known to be available to muskrat populations in Oak Hammock Marsh (A.P. Dyck, personal communication). These rations were also selected to provide ranges of protein and energy availability (Table 3-1) that might be encountered by muskrats in nature.

Cattail shoots were harvested at Oak Hammock Marsh on the day before each pretrial and trial session. Minnows were collected from a retention pond near the University of Manitoba, rinsed, and frozen whole in sealed plastic bags at -20°C. Skeletal muscle tissue was removed from frozen muskrat carcasses and was similarly stored at -20°C. All tissues were thawed immediately prior to presenting rations to muskrats.

Following the completion of all three digestibility trials, each muskrat was anestethized with Halothane and a blood sample withdrawn by heart puncture (see Appendix II; Table A2-2).

Ration and animal effects were evaluated using the general linear models procedure of SAS (Sas Institute, Inc. 1990), and treatment means were compared with *t*-tests for pairwise comparison of least-squares means, with significance set at 0.017

Table 3-1. Nutrient composition of the three food items fed to muskrats in feeding trials conducted at the University of Manitoba, June 9-August 14, 1993. Means ± SE are indicated.

Food item	*n*	Cattail shoots	Fathead minnows	Muskrat flesh
Dry Matter (%)	30	6.0 ± 0.2	19.4 ± 0.2	24.9 ± 0.4
Energy ($kJ \cdot g^{-1}$)	12	15.7 ± 0.7	20.7 ± 0.1	23.4 ± 0.2
Crude Protein (%)	12	13.2 ± 2.5	65.7 ± 0.9	82.7 ± 2.2
NDF[a] (%)	12	51.7 ± 0.9	---	---
Ash (%)	12	16.4 ± 0.5	14.6 ± 0.3	4.6 ± 0.1

[a] NDF = Neutral detergent fiber

(0.05/3 treatments). Proportions of food items in rations offered to, and in diets consumed by muskrats were compared using paired *t*-tests. Predictive equations were derived by least-squares regression analysis. All means are presented ± 1 standard error.

Results

In 15 of the 16 digestibility trials involving animal tissue, muskrats consumed a higher proportion of meat than was offered in the test rations (Table 3-2, $P < 0.001$). This selectivity resulted in muskrats ingesting diets that consisted of 30.3% (5% meat ration) and 59.3% (15% meat ration) animal tissue on a dry- weight basis (Table 3-2). One muskrat on the 15% meat ration consumed animal tissue at a level slightly below that offered in the diet. This individual was subsequently excluded from the analyses because its consumption of tissue was 18-28% lower than that of other animals on the 15% meat ration.

Body mass and energy intake of muskrats increased with rising levels of meat consumption (Table 3-3). Intake of metabolizable energy was >2 times greater on the 15% meat ration than on the 0% meat ration ($P < 0.001$). Similarly, dry matter intake (DMI) was 59% higher on the 15% meat ration than on the 0% meat ration (Table 3-3, $P < 0.001$). Despite the progressive gain in DMI, the digestibility coefficients for DM, digestible energy, metabolizable energy, protein, neutral detergent fiber, and neutral detergent solubles all increased with the level of meat consumption (Table 3-3).

As expected, daily nitrogen intake, nitrogen retention, and urinary nitrogen loss

Table 3-2. Proportions of food items (dry weight basis) offered to and consumed by muskrats in feeding trials conducted at the University of Manitoba, June 9-August 14, 1993. Means ± SE and number of trials (in parentheses) are indicated.

Food item	5% Meat ration (n = 8)	15% Meat ration (n = 7)
Cattail Shoots:		
% in ration offered	83.5 ± 0.6	61.1 ± 1.1
% in diet consumed	69.7 ± 2.0	40.7 ± 1.3
	P = 0.0001[a]	P = 0.0001
Whole Fish:		
% in ration offered	7.3 ± 0.2	26.3 ± 1.4
% in diet consumed	14.1 ± 1.3	38.6 ± 3.3
	P = 0.0001	P = 0.0049
Muskrat Tissue:		
% in ration offered	9.2 ± 0.4	12.6 ± 1.5
% in diet consumed	16.2 ± 0.9	20.7 ± 3.3
	P = 0.0001	P = 0.0439

[a] P-value for paired t-test comparing the proportion of a food item offered in ration, and the proportion of that same food item in the diet actually consumed by muskrats.

Table 3-3. Body mass, energy intake, and apparent digestibilities of adult male muskrats maintained on three diets differing in animal tissue content. Feeding trials were conducted at the University of Manitoba, June 9-August 14, 1993. Means ± SE and number of trials (in parentheses) are indicated.[a]

Variable	0% Meat ration ($n = 8$)	5% Meat ration ($n = 8$)	15% Meat ration ($n = 7$)
Body mass (g)[b]	911.5A ± 33.7	931.7A ± 33.0	986.7A ± 37.4
Mass change (g)	-37.3C ± 8.7	-4.9B ± 9.3	29.1A ± 8.1
Daily intake:			
Dry matter ($g \cdot kg^{-0.75}$)	31.2B ± 2.2	36.5B ± 3.2	46.7A ± 2.9
Gross energy ($kJ \cdot kg^{-0.75}$)	482.0B ± 35.9	618.4B ± 60.7	837.8A ± 65.1
DE ($kJ \cdot kg^{-0.75}$)	310.7C ± 26.9	477.1B ± 47.5	698.0A ± 51.1
ME ($kJ \cdot kg^{-0.75}$)	287.9C ± 25.2	434.3B ± 47.2	641.6A ± 53.0
Digestibility (%):			
Dry matter	67.4B ± 1.2	77.1A ± 2.0	80.7A ± 1.2
Digestible energy	64.2C ± 1.6	82.7B ± 1.5	89.6A ± 0.7
Metabolizable energy	59.4C ± 1.6	77.0B ± 1.4	85.3A ± 0.4
Dietary crude protein	61.9B ± 6.2	85.6A ± 1.5	91.2A ± 0.7
Neutral detergent solubles	73.1B ± 1.5	81.0A ± 1.3	82.9A ± 1.1
Neutral detergent fiber	57.7A ± 2.9	66.1A ± 4.5	67.4A ± 2.6

[a] Within each row, means sharing the same letter are not significantly different ($P > 0.017$).

[b] Average body mass during trial.

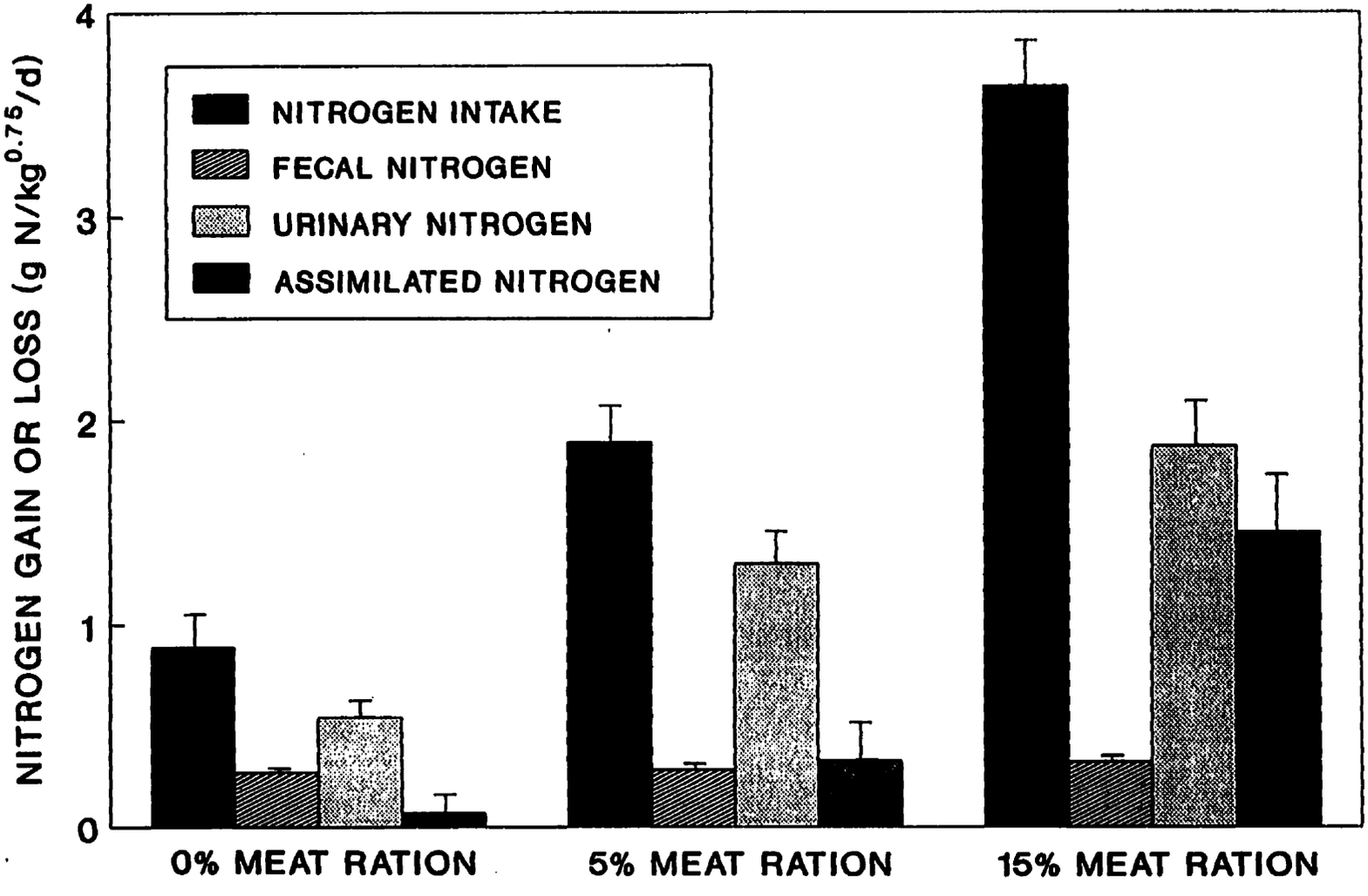
increased concurrently with meat consumption, while fecal nitrogen loss varied little among diets (Fig. 3-1). Regressing daily tissue nitrogen balance on DNI yielded the equation: daily tissue nitrogen balance = -0.560 + 0.548 DNI ($r^2 = 0.74$, df = 23). From this regression, the maintenance nitrogen requirement of muskrats was estimated to be 1.02 g $N \cdot kg^{-0.75} \cdot day^{-1}$.

Discussion

Numerous studies published on the food habits and nutrient requirements of microtine rodents indicate animal tissue contributes <10% of the total diet (Batzli 1985). Unfortunately, little or no attention has been paid to the nutritional significance of meat consumption by these small herbivores. My results clearly indicate that low levels of meat ingestion can lead to significant gains in the energy and protein absorption of muskrats. Associative effects arising from feeding herbivores mixed diets have been reported (Bjorndal 1991), and it is possible that this phenomenon may be responsible, at least in part, for the high digestibilities I observed.

Although muskrats possess a specialized digestive tract adapted to efficiently process aquatic vegetation, there is no evidence of morphological adaptations for handling a carnivorous diet (Luppa 1956). Nevertheless, it appears that muskrats do not suffer any loss in digestive efficiency resulting from the consumption of animal tissue, even at high levels of ingestion (>50% DMI; Table 3-3). Indeed, DM and ME digestibilities of muskrats fed the 15% meat ration were 10-47% higher than those recorded for muskrats

Fig. 3-1. Relationship of daily nitrogen intake to fecal nitrogen loss, urinary nitrogen loss and net nitrogen gain in muskrats fed three rations differing in animal tissue content. Values are presented as means + 1 SE.


NITROGEN GAIN OR LOSS (g N/kg$^{0.75}$/d)
4
3
2
1
0
NITROGEN INTAKE
FECAL NITROGEN
URINARY NITROGEN
ASSIMILATED NITROGEN
0% MEAT RATION
5% MEAT RATION
15% MEAT RATION

fed only emergent plant diets (Table 1-3; this study). The high DM and ME digestibilites recorded on the 15% meat ration cannot be ascribed to variation in DMI, since the range of DMI for the three diets tested in this study (31.2-46.7 $g \cdot kg^{-0.75} \cdot day^{-1}$) was similar to that (31.4-48.7 $g \cdot kg^{-0.75} \cdot day^{-1}$) reported in Table 1-3. There was no evidence that the ability of this rodent to digest plant fiber was compromised by the addition of animal tissue to the diet (Table 3-3). While NDF digestibility was highest on the 15% meat ration, the proportion of daily ME intake derived from the fermentation of fiber decreased from 51.1% (147.50 $kJ \cdot kg^{-0.75} \cdot day^{-1}$) on the 0% meat ration, to only 16.4% (106.72 $kJ \cdot kg^{-0.75} \cdot day^{-1}$) on the 15% meat ration. However, muskrats on the 0% meat ration ingested 39% more NDF than animals on the 15% meat ration. This factor would protentially allow for a longer fiber retention period and hence, enhanced fiber digestibility on the 15% meat ration.

My estimate of DE digestibility on the 15% meat ration (89.6±0.7%) is close to the values reported for carnivores consuming meat (95.5%), fish (95.3%), and whole bird or mammal diets (85.3%)(see Robbins 1993). Muskrats maintained on the 5% and 15% meat rations also exhibited apparent crude protein digestibilities (85.6 and 91.2%, respectively) similar to those of carnivores (78.9-96.8%) fed pure meat rations (Davidson et al. 1978; Harper, Travis and Glinsky 1978; Powers, Mautz and Pekins 1989). More importantly, assuming a true nitrogen digestibility of 94% (this study), my data suggest that non-reproducing adult muskrats can meet their daily maintenance nitrogen requirement (1.02 g $N \cdot kg^{-0.75}$ body mass) from the consumption of only 31.2 g of muskrat flesh or 53.1 g of fathead minnows on a wet-weight basis. These values represent only

3.6-6.1% of the estimated wet mass of aquatic vegetation required each day to sustain free-ranging muskrats (ca. 875 $g \cdot kg^{-1}$; see Table 5-2). A similar pattern has been described by Karasov (1982) for the white-tailed antelope squirrel (*Ammospermophilus leucurus*), which can meet its daily nitrogen requirements solely from the animal tissue component of an omnivorous diet (8% of total intake).

It is clear from this study that the consumption of animal matter has the potential to play a major role in the nitrogen economy of muskrats (Fig. 3-1). This may be especially true for juvenile cohorts, since meat consumption should enable young to maximize somatic growth, thereby achieving a larger body size at the onset of winter. Occasional meat consumption may also benefit muskrat populations existing in marginal habitats characterized by low quality, protein-deficient forage (Messier, Virgl and Marinelli 1990). The question remains, however, if muskrats are so efficient at utilizing animal flesh as an energy and nitrogen source, why is carnivory not more prevalent in nature? Perhaps the increased foraging time required, coupled with a greater risk of injury or predation, have selected against a stronger reliance on animal tissue in the diet of these rodents. It is possible that muskrats obtain most of their meat as scavengers, and that the amount of meat in their diet varies with the availability of prey. However, such opportunistic consumption of animal tissue is difficult to document in nature, and perhaps this habit is more widespread in wild muskrat populations than was previously thought.

PART IV

UREA RECYCLING IN MUSKRATS:

A POTENTIAL NITROGEN CONSERVING TACTIC?

Abstract

The rate of ^{14}C-urea hydrolysis was determined in 32 field-acclimatized muskrats maintained on natural diets during spring, summer, fall and winter. I hypothesized that urea recycling occurs in muskrats during all seasons, and that the conservation of tissue nitrogen via this mechanism is most prevalent in fall and winter, when forage protein levels are lowest. Muskrats exhibited higher rates of urea hydrolysis and a lower serum urea nitrogen/creatinine ratio in fall and winter, compared to spring and summer. Even after correcting for seasonal differences in blood urea pool size, the adjusted rate of urea hydrolysis was 67% higher in fall/winter than in spring/summer. There was no evidence that the maintenance nitrogen requirements of muskrats fed natural vegetation were affected by seasonal changes in the amino acid composition of the diet. I suggest that increased levels of urea recycling, coupled with adaptive mechanisms for reducing nitrogen excretion and possibly conserving carbon skeletons of essential amino acids, may allow muskrats to reduce their nitrogen requirements on fall and winter diets. My finding that ^{14}C-urea hydrolysis occurred during all four sampling periods suggests that nitrogen derived from this source may also be critical to supporting large hindgut microbe populations that enable this rodent to exploit the appreciable fiber content of its aquatic plant diet throughout the year.

Introduction

At temperate and northern latitudes, the protein content of vegetation can fluctuate dramatically throughout the year. During periods when dietary protein is limiting, the digestibility and retention of nitrogen can be critical factors influencing growth, reproduction and survival of free-ranging animals (Loeb, Schwab and Demment 1991). Consequently, considerable research has been done on seasonal changes in nitrogen metabolism of herbivores, including the role of urea recycling (Robbins et al. 1974; Mould and Robbins 1981). In animals demonstrating this phenomenon, urea is transferred from the bloodstream into the gastrointestinal tract where it is hydrolysed by bacterial urease into ammonia and carbon dioxide (Richards 1972; Nelson et al. 1975). Ammonia produced at this point can either be incorporated into bacterial protein or diffuse into the bloodstream. Upon reaching the liver, blood-borne ammonia joins the nitrogen pool available for the synthesis of non-essential amino acids (Richards 1972). In simple-stomached animals, the incorporation of nitrogen from inorganic compounds into amino acids is greater when the dietary intake of protein is inadequate to meet daily requirements (Richards 1972), a condition prevalent during hibernation or long-term fasts (Nelson et al. 1975; Harlow and Buskirk 1991).

The potential benefits of urea recycling to non-hibernating, simple-stomached herbivores during periods of low protein availability are unknown. An excellent model for investigating this question is provided by the muskrat. I previously found that muskrats consuming natural vegetation exhibited urinary nitrogen excretion rates that were

nearly 6 times lower in fall and winter, compared to spring and summer (Table 2-3). Consequently, the maintenance nitrogen requirement calculated for this rodent was >25% lower during fall and winter, when dietary protein levels of aquatic vegetation appeared to be lowest (Table 2-1; Appendix I). The quality of dietary protein is known to influence the daily requirements for this nutrient in mammals (Robbins 1993). It is possible that, despite their low nitrogen content, the fall and winter diets of muskrats contain a higher proportion of essential amino acids than in other seasons. Alternatively, muskrats may reduce nitrogen excretion in fall and winter by recycling urea. Throughout the year, muskrats consume forages containing high levels of neutral detergent fiber (43-62%) and rely strongly upon microbial fermentation to meet their daily energy requirements. However, in hindgut fermenters, an appreciable proportion of the daily protein intake is likely absorbed before it reaches the hindgut. Because urea hydrolysis provides a nitrogen source for the synthesis of microbial proteins in the cecum (Chilcott and Hume 1984), this reaction may be essential for maintaining high microbial populations year-round, thus permitting muskrats to exploit the appreciable fiber content of their aquatic plant diets.

The principal objective of this study was to determine if urea hydrolysis, a necessary step in urea recycling, occurs in muskrats and, if so, whether the intensity of this hydrolysis varies with seasonal shifts in the protein content of cattail, the dominant food source of muskrats in northern prairie marshes. I hypothesized that urea recycling occurs in muskrats throughout the year, and that the conservation of tissue nitrogen via this mechanism is most prevalent during fall and winter, when maintenance nitrogen

requirements and forage protein levels are lowest. Additionally, I examined seasonal changes in the amino acid composition of the muskrat's natural forage to determine if diet quality might also contribute to the low maintenance nitrogen requirement previously observed during fall and winter.

Materials and Methods

Eight adult muskrats were livetrapped at Oak Hammock Marsh, Manitoba (50°06'N, 97°07'W), during each of four test periods: 27 July-10 August 1994 (3 males, 5 females), 11-20 October 1994 (8 males), 4-24 January 1995 (5 males, 3 females), and 16-26 May 1995 (6 males, 2 females). Animals were housed individually at 14±1°C with a 12L:12D photoperiod (MacArthur 1979) for 1-4 d prior to testing. Each muskrat was tested only once (n = 32 muskrats in total). To minimize disturbances to digestive physiology, including microbial function, muskrats were fed vegetation approximating their natural diet in each test period. Accordingly, during May and July, muskrats were fed fresh aquatic vegetation, consisting predominately of cattail shoots and leaves *ad libitum*. Muskrats in the October trials were maintained exclusively on fresh cattail rhizomes *ad libitum*. In January, muskrats were fed cattail rhizomes collected just prior to freeze-up and stored at 5°C. Muskrats were not fasted prior to metabolic testing.

To test for the occurrence and evaluate the intensity of urea hydrolysis, each muskrat was lightly anesthetized with Halothane (M.T.C. Pharmaceuticals) and injected intraperitoneally with 5 μCi of ^{14}C-urea (8.92 X 10^{-5} mmol·L^{-1}) diluted in 0.5 mL sterile

physiological saline (Harlow and Buskirk 1991). The dosage was measured to the nearest 0.1 mg by weighing the sample syringe (1cc Tuberculin) on an analytical balance (Mettler model AJ100) before and after injection. The animal was then transferred to a darkened, 11.5-L glass box fitted with a heavy plexiglass lid and a removable wire screen floor, where it remained for a period of 8 h. The metabolic chamber was installed in a controlled-temperature cabinet held at 22±1°C and dry, CO_2-free air was pumped through the chamber at a rate of 2 $L \cdot min^{-1}$ (Fig. 4-1). Flow rate was monitored with a Matheson rotameter calibrated against a model 1057 Brooks Vol-U-Meter. A small fraction (5%) of the exhaust gas from the chamber was routed to a Beckman F-3 paramagnetic O_2 analyzer connected to a strip-chart recorder (SE-120, BBC Goerz Metrawatt). The remaining exhaust gas was passed sequentially through two organic CO_2 traps, each containing 250 mL of ethylene glycol monomethylether and ethanolamine in a 2:1 volume ratio (Jaffay and Alvarez 1961). As a precautionary measure, exhaust gas from the chamber was routed through a final CO_2 trap of soda lime before exiting to the atmosphere.

Upon completion of a trial, two 0.6-mL aliquots of fluid were removed from each organic CO_2 trap and placed into separate scintillation vials, each containing 15 mL of Beckman Ready Safe scintillation cocktail. Each vial was assessed for ^{14}C activity using a Beckman LS 6000TA liquid scintillation counter, and the values were summed to give total activity of expired ^{14}C. To correct for between-animal variation in metabolic rate, the production of $^{14}CO_2$ was expressed as disintegrations per minute (dpm) of $^{14}CO_2$ expired$\cdot mL^{-1}$ O_2 consumed. The total oxygen consumed during the 8-h experiment was

Fig. 4-1. Diagram of the apparatus used to monitor rates of oxygen consumption and $^{14}CO_2$ production by muskrats, July 27, 1994 - May 26, 1995.

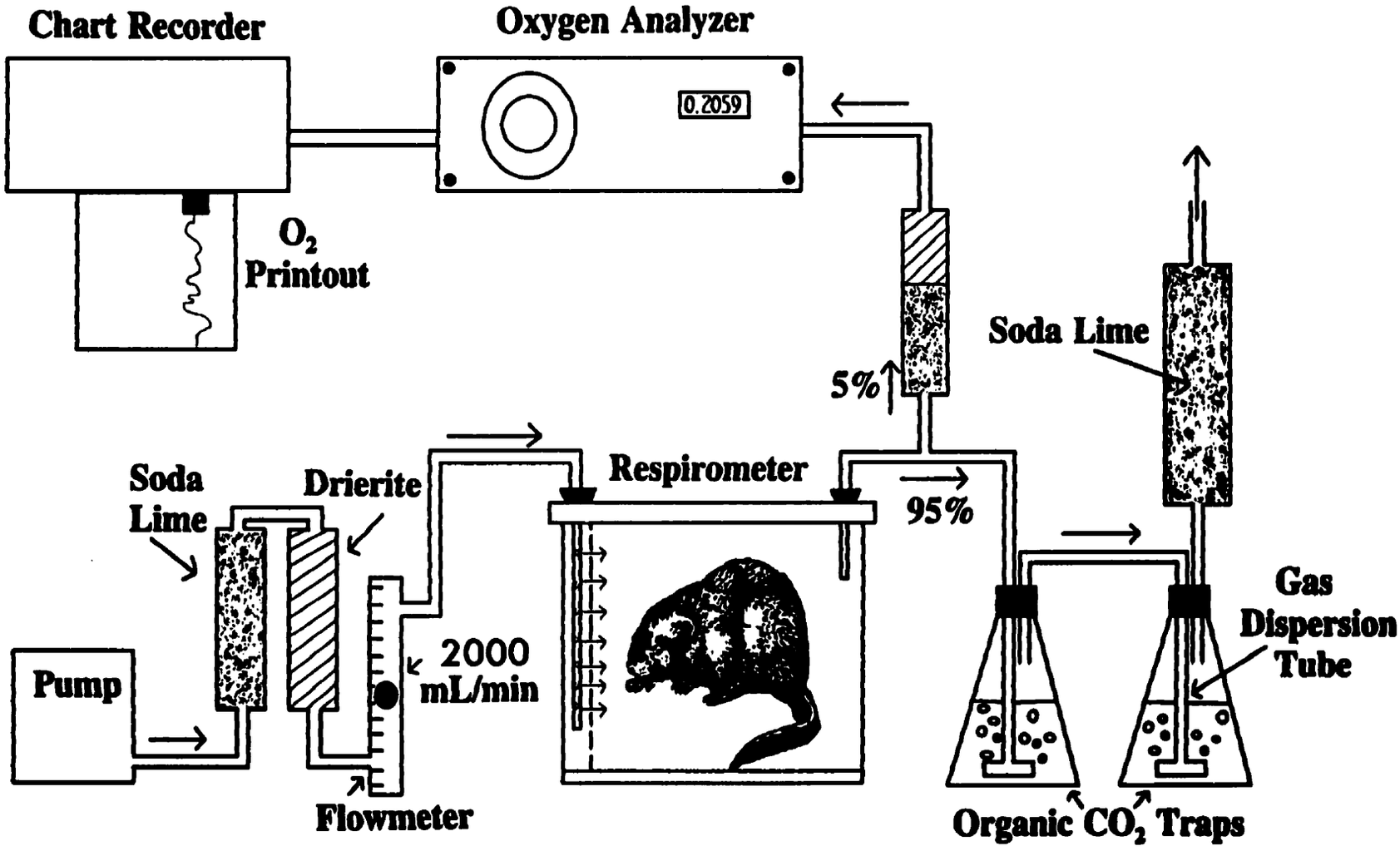


Chart Recorder
Oxygen Analyzer
0.2059
O_2 Printout
5%
Soda Lime
Soda Lime
Drierite
Respirometer
95%
Pump
2000 mL/min
Gas Dispersion Tube
Flowmeter
Organic CO_2 Traps

determined using a Jandel Digitizing Tablet and Sigma-Scan software to integrate area beneath the O_2 tracing (Dyck and MacArthur 1993).

To test for seasonal changes in the ratio of serum urea to serum creatinine, blood samples were collected from a separate group of 59 muskrats livetrapped in Oak Hammock Marsh from May 1991 to April 1992 (see Part II; Appendix II). Serum urea and creatinine concentrations were determined with a Coulter Discrete Chemical Analyzer (Manitoba Agriculture Veterinary Services, Winnipeg, Manitoba).

To document seasonal changes in the crude protein content of cattail in Oak Hammock Marsh, I periodically collected random plant samples in 1991. Cattail rhizome samples were also obtained from two separate food caches found inside winter feeding lodges in early-December 1995. Plant samples were separated into stem, leaf and rhizome components, oven-dried at 70°C, and ground through a 1-mm mesh screen in a Wiley mill. A portion of each ground sample was sent to a feed analysis laboratory (Department of Animal Science, University of Manitoba) for determination of crude protein content (Kjeldahl N x 6.25).

To test for possible seasonal changes in protein quality of the diet, samples of the natural mixed forages fed to muskrats in a previous study (see Part II) were evaluated for their amino acid composition. The spring diet tested consisted of 50% cattail shoot, 25% cattail rhizome, 20% bladderwort, and 5% sedge shoot. The summer diet consisted of 70% cattail shoot, 10% cattail rhizome, and 5% each of sedge shoot, soft-stem bulrush shoot, whitetop shoot, and duckweed. The fall diet consisted of cattail rhizomes (60%), cattail shoots (30%) and white-top shoots (10%). The winter diet was comprised entirely

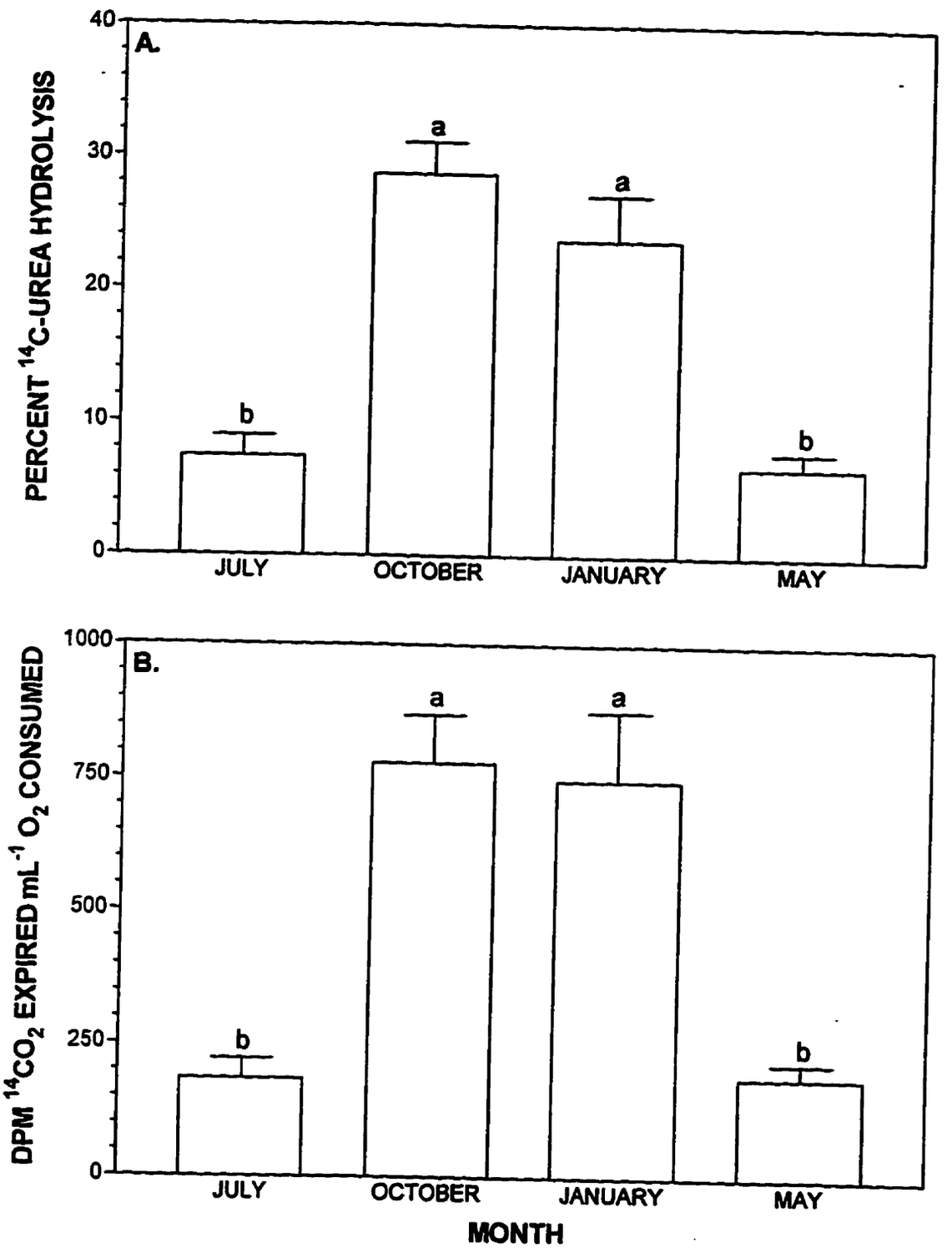
of cattail rhizomes. Following a modified procedure of Mills, Rotter and Marquardt (1989), a 100-mg portion of each forage sample was combined with 4 mL of 6 N HCl and 0.1 mL 2-Octanol, evacuated for 1 min, then hydrolysed at 110°C for 24 h. The hydrolysate was neutralized with 4.1 mL of 25% (wt/vol) NaOH, and made up to 50 mL with a sodium citrate buffer (pH 2.2). The amino acid content of each sample was determined using ion exchange chromatography with ninhydrin detection on a LKB 4151 *Alpha Plus*® Amino Acid Analyzer (Department of Animal Science, University of Manitoba).

Treatment effects were initially evaluated for differences due to season, sex, and sex x season, using a 2-way ANOVA. Since no sex-related differences were apparent ($P > 0.05$), data for both sexes were pooled in all subsequent analyses. Mean seasonal values for pooled data were compared with 1-way ANOVA and Tukey's studentized range test (SAS Institute 1990). Significance was set at the 5% level, and means are presented with 1 standard error (SE). Upon completion of testing, muskrats were held for a minimum of 48 h before being released at their site of capture.

Results

The occurrence of ^{14}C-urea hydrolysis was documented in all muskrats tested in this study, with peak levels recorded in October and January ($F_{[3,28]} = 23.58$, $P < 0.0001$; Fig. 4-2*a*). The percentage of injected ^{14}C-urea that was hydrolyzed during the 8-h trial increased from an average of 6.7-7.4% in May and July, to 23.7-28.7% in October and

Fig. 4-2. Seasonal changes in the proportion of urea hydrolysed by 32 field-acclimatized muskrats (n = 8 per month). (A) Percentage of injected ^{14}C-urea collected as $^{14}CO_2$ in expired air. (B) Disintegrations per minute (dpm) of $^{14}CO_2$ expired·mL^{-1} O_2 consumed. Vertical lines denote 1 SEM. Means sharing the same letter are not significantly different ($P > 0.05$).


A.
PERCENT ^{14}C-UREA HYDROLYSIS
40
30
20
10
0
a
a
b
b
JULY
OCTOBER
JANUARY
MAY
B.
DPM $^{14}CO_2$ EXPIRED mL^{-1} O_2 CONSUMED
1000
750
500
250
0
a
a
b
b
JULY
OCTOBER
JANUARY
MAY
MONTH

January ($P < 0.05$). The $^{14}CO_2$ production (dpm of $^{14}CO_2$ expired·mL^{-1} O_2 consumed) of fall- and winter-acclimatized muskrats was 4 times higher than values recorded from animals caught in spring and summer ($F_{[3,28]} = 16.13$, $P < 0.0001$; Fig. 4-2*b*).

The serum urea/creatinine ratio of muskrats demonstrated strong seasonal variation, and was significantly lower from September to April than during May and July ($F_{[5,53]} = 11.36$, $P < 0.0001$; Fig. 4-3). Though measured in a different group of muskrats collected in a different year, serum urea/creatinine ratios (Fig. 4-3) appeared to vary inversely with the rate of ^{14}C-urea hydrolysis (Fig. 4-2). Muskrats sampled in both years exhibited similar seasonal patterns in body composition (Fig. 6-2; Fig. 6-3), and measurements of diet quality and vegetation structure were consistent within the two collection periods (K.L. Campbell, unpublished data; see also Table 5-1 and Table A1-1).

The crude protein content of cattail shoots and leaves underwent dramatic seasonal changes, with shoot levels declining from 14.1% in June, to only 2.2% by late-September ($F_{[2,15]} = 55.91$, $P < 0.0001$; Fig. 4-4). Over this same period, the protein content of cattail rhizomes exhibited the opposite trend ($F_{[5,22]} = 3.89$, $P = 0.0112$; Fig. 4-4), with the highest recorded value (9.1%) observed in the rhizome samples recovered from two food caches in December. Nonetheless, this peak value for rhizomes is generally lower than the protein content of cattail shoots and leaves during the summer months (Fig. 4-4).

No discernable seasonal changes were apparent in the protein quality of the mixed diets approximating those consumed by free-ranging muskrats (Table 4-1). While the levels of individual amino acids varied seasonally, the total fraction of essential amino acids in each diet remained close to 42%.

Fig. 4-3. Seasonal changes in the ratio of serum urea nitrogen to serum creatinine in 59 field-acclimatized muskrats tested between May 1991 and April 1992 (see Appendix A2-1 for individual serum values). Vertical lines denote 1 SEM. Means sharing the same letter are not significantly different ($P > 0.05$). Values in parentheses indicate number of muskrats sampled in each month.

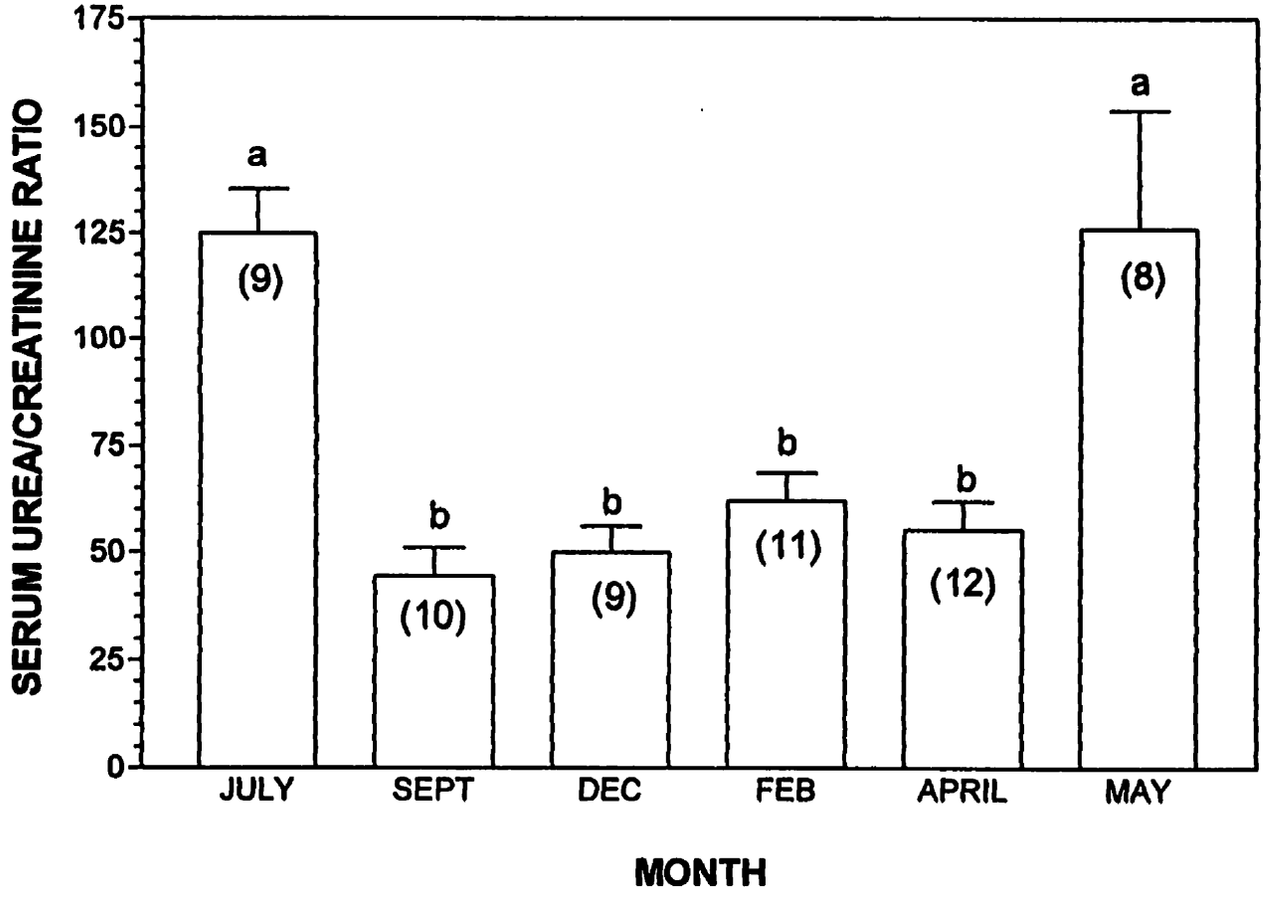

SERUM UREA/CREATININE RATIO
175
150
125
100
75
50
25
0
a
(9)
b
(10)
b
(9)
b
(11)
b
(12)
a
(8)
JULY
SEPT
DEC
FEB
APRIL
MAY
MONTH

Fig. 4-4. Seasonal changes in the crude protein content (dry mass basis) of the rhizomes, shoots and leaves of cattail collected at Oak Hammock Marsh, Manitoba, from May to October 1991. The December value is based on samples collected from two muskrat food caches on December 2 and December 13, 1995, respectively. Each monthly mean is based on plant samples collected from six randomly selected sites, except for October, when only 2 sites were sampled. Vertical lines denote 1 SEM.

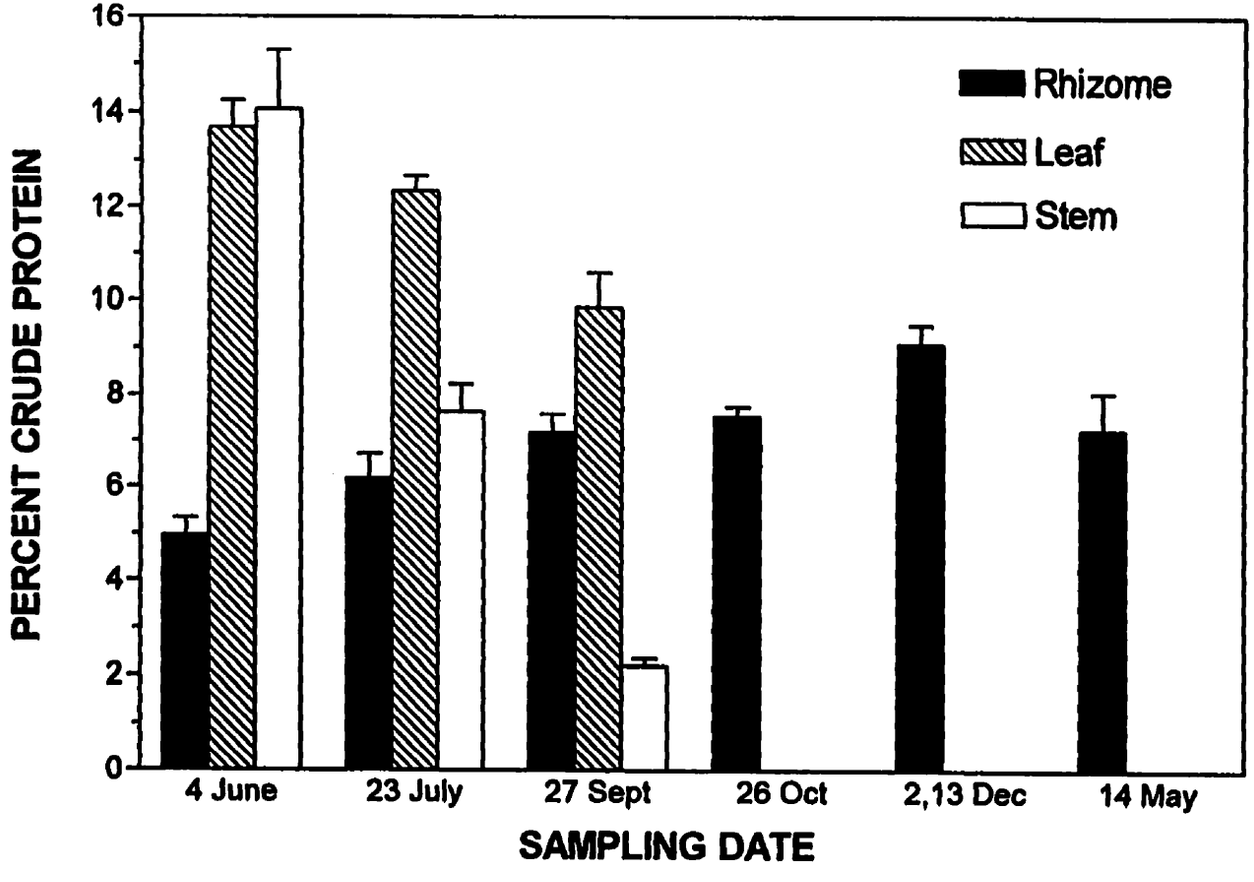

PERCENT CRUDE PROTEIN
16
14
12
10
8
6
4
2
0
Rhizome
Leaf
Stem
4 June
23 July
27 Sept
26 Oct
2,13 Dec
14 May
SAMPLING DATE

Table 4-1. Seasonal changes in the crude protein, amino acid and ammonia content (dry mass basis) of natural mixed forages approximating those consumed by free-ranging muskrats.

Component	May	July	September	December
% Crude Protein:	11.3	9.0	7.7	8.3
% Amino Acid:				
Essential:				
Arginine	4.28	8.24	9.24	8.76
Histidine	2.37	2.20	2.73	2.50
Isoleucine	3.04	1.11	0.15	2.45
Leucine	7.35	6.09	5.43	6.34
Threonine	4.38	5.58	4.35	4.42
Lysine	5.19	5.26	6.08	4.12
Methionine	2.88	2.53	1.37	2.66
Phenylalanine	5.41	4.60	4.48	4.67
Valine	6.56	6.73	8.76	6.92
Total:	**41.46**	**42.34**	**42.59**	**42.84**
Non-Essential:				
Aspartate	16.09	14.89	19.10	19.61
Glutamate	12.98	12.99	12.81	11.02
Serine	6.85	7.39	7.06	7.02
Proline	4.94	7.33	3.45	4.24
Glycine	5.78	4.84	5.26	5.17
Alanine	7.09	6.28	5.59	4.98
Tyrosine	1.96	1.14	0.65	1.43
Total:	**55.69**	**54.86**	**53.92**	**53.47**
% Ammonia	2.85	2.72	3.40	3.60

Note: In May, the diet consisted of 50% cattail shoot, 25% cattail rhizome, 20% bladderwort (*Utricularia vulgaris*), and 5% sedge (*Carex atherodes*) shoot. In July, the diet consisted of 70% cattail shoot, 10% cattail rhizome, and 5% each of sedge shoot, soft-stem bulrush (*Scirpus validus*) shoot, whitetop (*Scolocholoa festucacea*) shoot, and duckweed (*Lemna minor*). The September diet consisted of cattail rhizomes (60%), cattail shoots (30%) and whitetop shoots (10%). The December diet consisted exclusively of cattail rhizomes.

Discussion

These results strongly suggest that urea recycling occurs throughout the year in muskrats, and that this nitrogen-conserving tactic is most prevalent in fall and winter (Fig. 4-2). Animals on low protein diets typically exhibit reduced plasma urea concentrations (Robbins 1993) and, in humans at least, the rate of urea hydrolysis is proportional to the concentration of urea in body fluids (Richards 1972). These observations have led to speculation (Harlow 1987) that protein-deficient diets are associated with a reduced rate of urea hydrolysis in non-ruminant herbivores. However, muskrats demonstrated increased rates of urea hydrolysis in fall and winter (Fig. 4-2) when dietary protein (Table 4-1) and the serum urea/creatinine ratio (Fig. 4-3) were lowest. Low serum urea/creatinine values have been associated with the recycling of nitrogenous wastes and the conservation of lean body mass in polar bears (*Ursus maritimus*) (Ramsay, Nelson and Stirling 1991). The low urea/creatinine values observed in muskrats from late-fall to early spring are consistent with the relatively high level of urea hydrolysis observed during fall and winter, and may be indicative of a reduced rate of total protein catabolism (Harlow and Buskirk 1991).

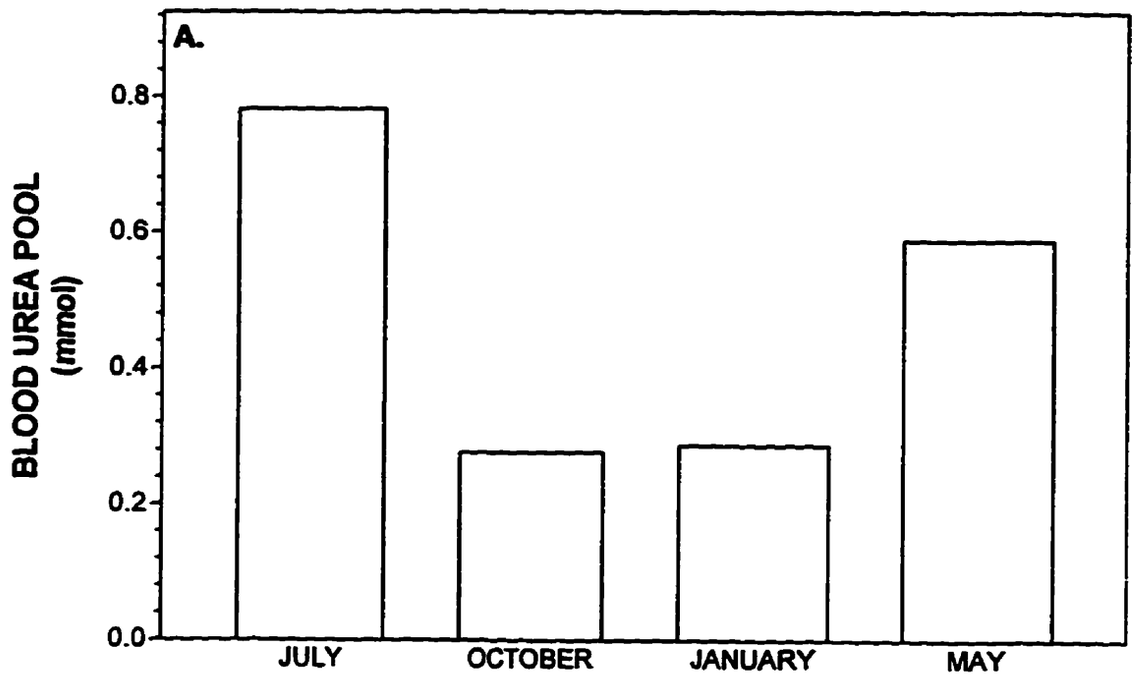
The observed seasonal trend in the rate of urea hydrolysis might be due, at least in part, to differences in ^{14}C-urea dilution arising from monthly variation in blood urea pool size. To address this possibility, I estimated the total blood urea content of muskrats in each season, using serum urea concentrations and previous measurements of blood volume in field-acclimatized muskrats (MacArthur 1990). Serum urea values collected

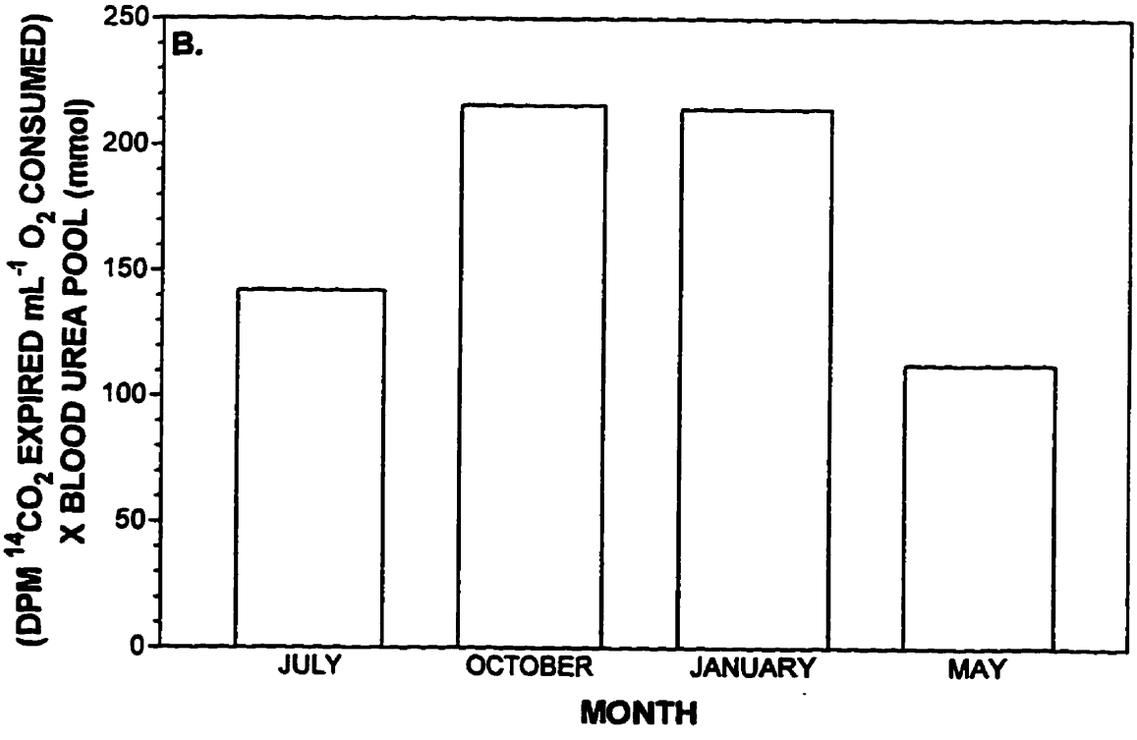
on dates closest to those of the urea hydrolysis trials were used in calculations. My estimates of blood urea pool size followed a similar seasonal pattern to serum urea concentration, reaching minimal values in fall and winter (Fig. 4-5*a*). However, when the total blood urea pool was multiplied by the activity of expired $^{14}CO_2$ (dpm of $^{14}CO_2$ expired·mL^{-1} O_2 consumed) to compensate for variation in initial ^{14}C-urea dilution, a strong seasonal trend in the rate of urea hydrolysis was still apparent (Fig. 4-5*b*). The adjusted rate of urea hydrolysis was 67% greater in October-January than in May-July. Thus, the seasonal patterns reported herein cannot be attributed to monthly variation in blood urea pool size or metabolic rate, but appear to reflect adaptive responses to temporal changes in diet quality. It is important to note that muskrats in this study were fed natural diets that varied seasonally, in order to minimize disturbances in digestive function. Therefore, at this stage I cannot conclusively separate the effects of diet quality from other factors that might account for the observed seasonal trends in the rate of urea hydrolysis.

The digestive tract of muskrats undergoes pronounced seasonal changes, attaining maximal size in fall and winter (Virgl and Messier 1992*a*; Fig. 2-1). Animals with a larger gut capacity should theoretically possess higher levels of bacterial urease, and thus should demonstrate higher rates of urea hydrolysis. My results support this hypothesis, since seasonal changes in the adjusted rate of urea hydrolysis (Fig. 4-5) followed a similar trend to cecum size in muskrats (Fig. 2-1).

Free-ranging muskrats rely on diets that undergo significant reductions in crude protein content between spring and fall (Table 4-1; Fig 4-4). During this period, muskrats

Fig. 4-5. Seasonal changes in (A) the estimated blood urea pool size (mmol urea), and (B) the rate of ^{14}C-urea hydrolysis in muskrats adjusted for initial ^{14}C-urea dilution (see text for details).


A.
BLOOD UREA POOL (mmol)
0.0
0.2
0.4
0.6
0.8
JULY
OCTOBER
JANUARY
MAY


B.
(DPM $^{14}CO_2$ EXPIRED mL^{-1} O_2 CONSUMED) X BLOOD UREA POOL (mmol)
0
50
100
150
200
250
JULY
OCTOBER
JANUARY
MAY
MONTH

show a pronounced decline in nitrogen excretion (Table 2-4). Two factors could contribute to this decline. First, the average energy content of the fall and winter diet (17.01 $kJ \cdot g^{-1}$) was, relative to the crude protein content (7.98%), higher than in spring and summer (16.87 $kJ \cdot g^{-1}$ and 10.13% crude protein). This difference in the proportion of energy to protein in the diet may have resulted in more efficient utilization of dietary protein in fall and winter, and hence less nitrogen excretion in the urine (Robbins et al. 1974). Second, since reabsorption of urea by the kidneys is passive, and closely linked to the reabsorption of water, the amount of urea excreted increases with urine output (West 1985). Thus, the fall decline in nitrogen excretion could also reflect the marked seasonal changes in the water content of aquatic vegetation, and hence urine production, between spring/summer ($\bar{x}$ = 252 $mL \cdot day^{-1}$) and fall/winter ($\bar{x}$ = 39 $mL \cdot day^{-1}$; K.L. Campbell, unpublished data). Furthermore, urea resorption from the bladder is known to increase during periods of low urine output in several mammalian species (Nelson et al. 1975; Harlow 1987), and it is possible that during fall and winter muskrats also reduce urea excretion in this manner.

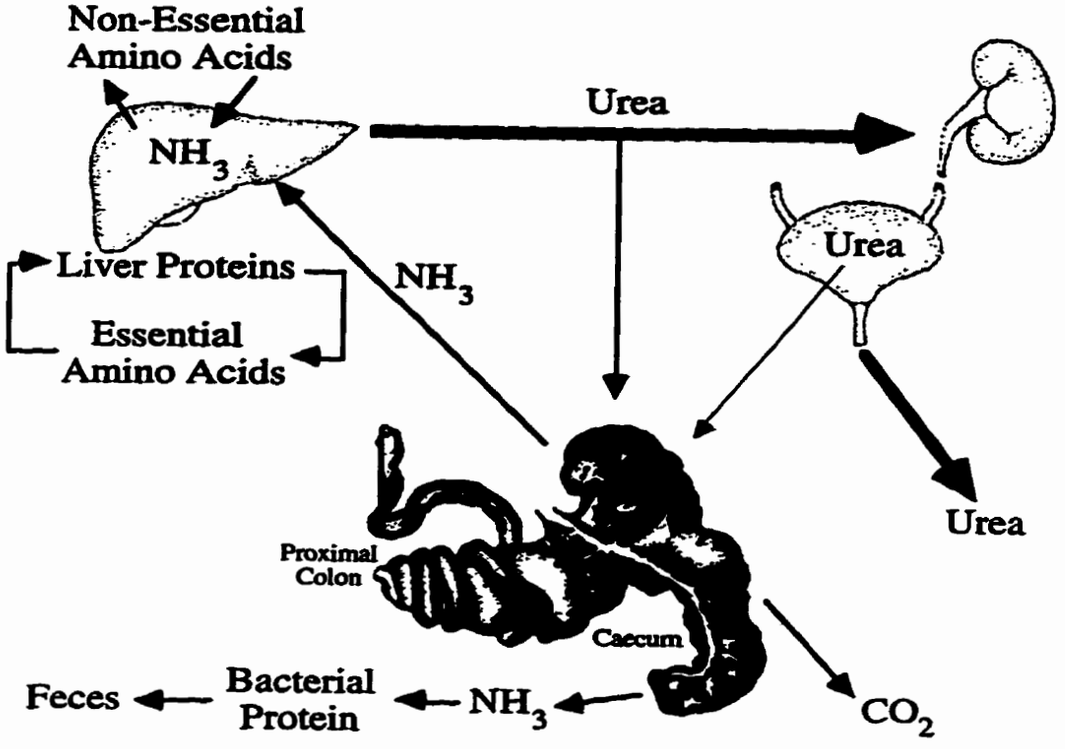
Ammonium ions derived from urea in the digestive tract have two potential fates. They can be utilized by cecal and colonic bacteria for protein synthesis, or transported to the liver where they are incorporated into proteins or reconverted into urea. Nitrogen is thus free to circulate from the gut to the liver as ammonium ions, and from the liver to the gut as urea, until its eventual loss in urine, feces, sweat, or sloughed cells (Robbins 1993). The observed increase in the rate of hydrolysis from May-July to October-January (Figs. 4-2 and 4-5*b*), combined with the decrease in urea excretion, suggests that

ammonium ions have a greater potential to re-enter this cycle in fall and winter, compared to spring and summer (Fig. 4-6). Thus, assuming adequate energy is available to the hindgut microflora, muskrats should be capable of substantially increasing the total amount of urea recycled during fall and winter. These are periods when forage diversity and dietary protein levels are lowest, and when the thermoregulatory costs of aquatic foraging are greatest. A similar finding has been observed in ruminants, in which a greater proportion of the urea synthesized in the liver is recycled when dietary protein and plasma urea concentrations are lowest (Robbins et al. 1974; Mould and Robbins 1981). Although the nitrogen recovered by urea recycling in simple-stomached animals can be incorporated into at least three essential amino acids in the liver if their α-keto acid analogs are present, the extent of this conversion is believed to be nutritionally insignificant (Richards 1972).

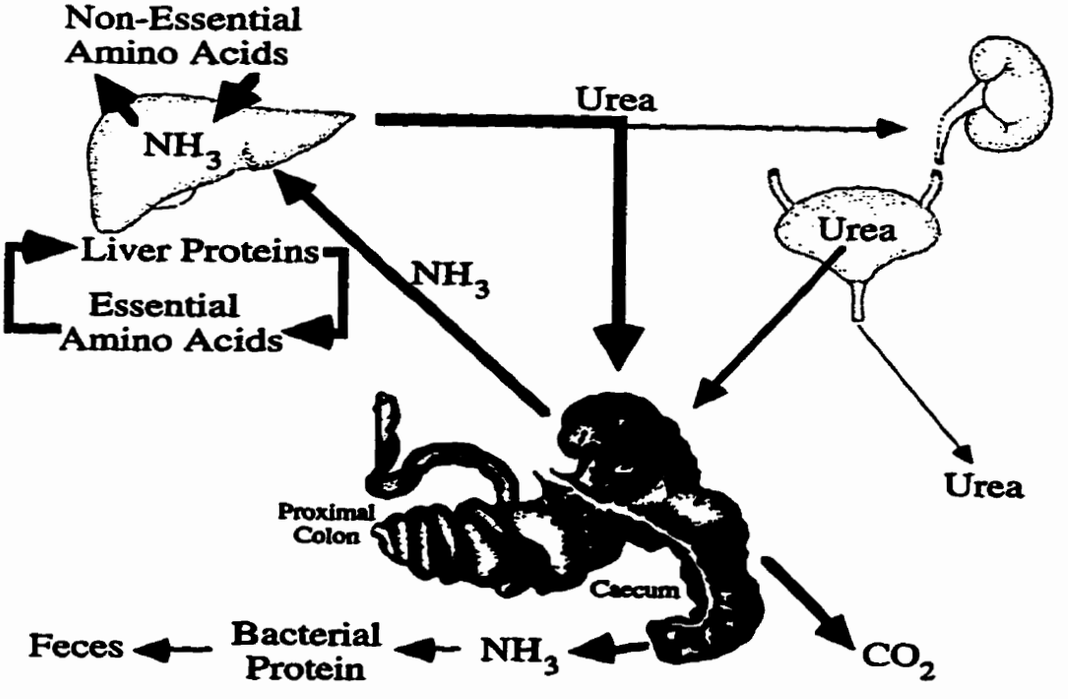
Muskrats maintained exclusively on aquatic plant diets demonstrate a true protein digestibility of 96.5% (see Part I). Thus, almost all ingested protein is absorbed prior to reaching the cecum. The proportion of recycled urea which is utilized by microbes in the gut of non-ruminants ranges from 44% in domestic rabbits (Regoeczi et al. 1965), to 53-59% in rock hyrax, *Procavia habessinica* (Hume, Rübasmen and Engelhardt 1980). My finding that ^{14}C-urea hydrolysis occurred during all four sampling periods suggests that nitrogen derived from this source may be critical to supporting large hindgut microbe populations that enable this rodent to exploit the appreciable fiber content of its aquatic plant diet (43-62%; Table 1-1; Table 2-1) throughout the year. A similar advantage has been suggested for the ringtail possum, *Pseudocheirus peregrinus*, which consumes high-

Fig. 4-6. Possible pathways involved in urea recycling of seasonally acclimatized muskrats. Thick bars represent preferential pathways in different seasons.

Spring-Summer



Fall-Winter



fiber, low-nitrogen forage and relies strongly upon microbial fermentation to meet its daily energy requirements (Chilcott and Hume 1984).

Ruminants and other foregut fermenters can absorb amino acids of bacterial origin directly from the small intestine (Robbins 1993). However, no active transport mechanism for amino acids has been demonstrated in the lower digestive tract of mammals (Chilcott and Hume 1984). Thus, in hindgut fermenters, bacterial proteins produced in the lower gut are presumably lost to the host, unless consumed and digested in the stomach and small intestine (Alexander 1993). Coprophagy is recognized as an important nitrogen-conserving tactic in animals that exhibit this behavior (Alexander 1993; Robbins 1993). Muskrats in this study were observed to practice coprophagy before metabolic trials in both summer and winter. However, no attempt was made to quantify seasonal changes in the frequency of this behavior.

Muskrats from Oak Hammock Marsh do not appear to lose lean tissue mass during fall and winter (see Part VI). Furthermore, during these seasons, muskrats can meet their maintenance nitrogen requirements on diets containing only 10.7 mg nitrogen·g^{-1} dry matter (assuming a dry matter intake of 75.7 g·$kg^{-0.75}$·d^{-1}). This value is close to the minimum nitrogen content of plant diets required for ruminants to remain in nitrogen balance (8 mg nitrogen·g^{-1} dry matter; Robbins 1993), and is >18% below the minimum requirement predicted for white-tailed antelope squirrels, *Ammospermophilus leucurus* (Karasov 1982).

The question remains, then, how do muskrats meet their essential amino acid requirements in fall and winter on diets containing so little protein? One possibility is

approach of Robbins et al. (1974), and assuming a true nitrogen digestibility of 96.5% and an endogenous urinary nitrogen loss of 0.041 $g \cdot kg^{-0.75} \cdot d^{-1}$ (Table 1-4), I estimated the mean (±SE) biological value of the aquatic plant diets consumed by muskrats. This value increased from 52.7±7.2% (n = 16) in spring and summer to 96.5±1.9% (n = 15) in fall and winter ($F_{[1,29]} = 34.75$, $P < 0.0001$). The gain in biological value cannot be attributed to seasonal changes in either dietary protein quality (Table 4-1) or daily nitrogen intake (0.97 $g \cdot kg^{-0.75}$ in spring and summer; 0.95 $g \cdot kg^{-0.75}$ in fall and winter; Table 2-4). This difference could reflect increased catabolism of dietary protein for energy in summer, with increased production and excretion of urea (Robbins et al. 1974). However, my earlier studies indicated that muskrats can meet their daily maintenance energy requirements from non-protein sources in all seasons. I suggest that the increased levels of urea recycling in fall- and winter-acclimatized muskrats, together with adaptive mechanisms for reducing nitrogen excretion and possibly conserving carbon skeletons of essential amino acids, may permit muskrats to increase the biological value of aquatic vegetation. Such responses would enable these animals to lower both their daily maintenance nitrogen and essential amino acid requirements during those periods of the year when they are nutritionally challenged.

PART V

SEASONAL CHANGES IN WATER FLUX, FORAGE INTAKE AND ASSIMILATED ENERGY OF FREE-RANGING MUSKRATS

Abstract

Knowledge of the seasonal energy and forage requirements of free-ranging muskrats is essential for evaluating the habitat requirements and potential impact of this species on aquatic vegetation. I obtained 33 seasonal estimates of the daily intake of water, fresh vegetation, dry matter and assimilated energy of 27 free-ranging muskrats. Water influx and consumption of fresh vegetation were highest from spring through fall. However, owing to the lower water content and higher digestibility of the winter diet, daily intake of DM and AE were significantly higher ($P < 0.05$) in winter (76.9 $g \cdot kg^{-0.75}$; 713.1 $kJ \cdot kg^{-0.75}$) than during mid-summer (54.9 $g \cdot kg^{-0.75}$; 438.6 $kJ \cdot kg^{-0.75}$). If corrections are made for wastage and use of vegetation for house construction, these food consumption estimates can be utilized to assess the potential impact of muskrats on the primary productivity of prairie marsh ecosystems.

Introduction

It is well established that the foraging and lodge construction activities of muskrats can substantially alter the vegetation structure of marsh ecosystems (Pelikán, Svoboda and Květ 1970; Danell 1978, 1979). Consequently, several researchers have attempted to estimate the daily food consumption (Butler 1940; Ching and Chih-Tang 1965; Akkermann 1975) and resulting impact of muskrats on marsh vegetation (Pelikán, Svoboda and Květ 1970; Clark, personal communication). However, predictions based on studies of captive muskrats may not accurately reflect the forage requirements of wild populations, especially when these studies fail to allow for annual variation in plant phenology, diet composition, and the energy costs of free existence. In an earlier study of acclimatized muskrats fed natural mixed diets (Part II), I observed that the DMI of these rodents was >26% higher in fall and winter than during spring and summer (Table 2-2). However, this was a laboratory study of penned animals, and it is not known if similar patterns in food consumption occur in wild populations of muskrats.

A promising technique for estimating food intake of unrestrained animals involves the use of the biological water tracers deuterium and tritium (Robbins 1993). Deuterated water is particularly suited to field studies because it is inexpensive, non-radioactive, and relatively easy to analyze (Costa 1987; Robbins 1993). In principle, the rate of disappearance of this tracer from the body water pool can be used to estimate the water influx of animals (Lifson and McClintock 1966; Shoemaker, Nagy and Costa 1976; Nagy and Costa 1980). If the metabolic water production of the animal and the moisture and

energy content of consumed vegetation are known, and remain constant during the measurement period, then the intake of DM and AE can be estimated from measurements of water influx (Shoemaker, Nagy and Costa 1976; Peppard et al. 1993). This technique is most accurate when applied to species that meet all of their water requirements from the consumption of diets with high moisture contents (Shoemaker, Nagy and Costa 1976; Costa 1987). The semi-aquatic muskrat is ideally suited for application of the D_2O technique. Given the water-resistant qualities of the muskrat's pelage and the high water content (>80%) of ingested forage, any error associated with water exchange across the skin and lungs should be relatively minor.

The objective of this study was to obtain reliable measures of water influx from which I could estimate the daily intake of vegetation and AE of free-ranging muskrats in different seasons. Ultimately, such data should enable researchers to refine estimates of the seasonal forage and energy requirements of muskrats, which is essential to developing a better understanding of the role of these animals in modifying marsh ecosystems (McCabe 1982; Clark, personal communication). This information is also vital to the interpretation of seasonal dynamics in protein and lipid reserves of wild populations (Virgl and Messier 1992*a*).

Materials and Methods

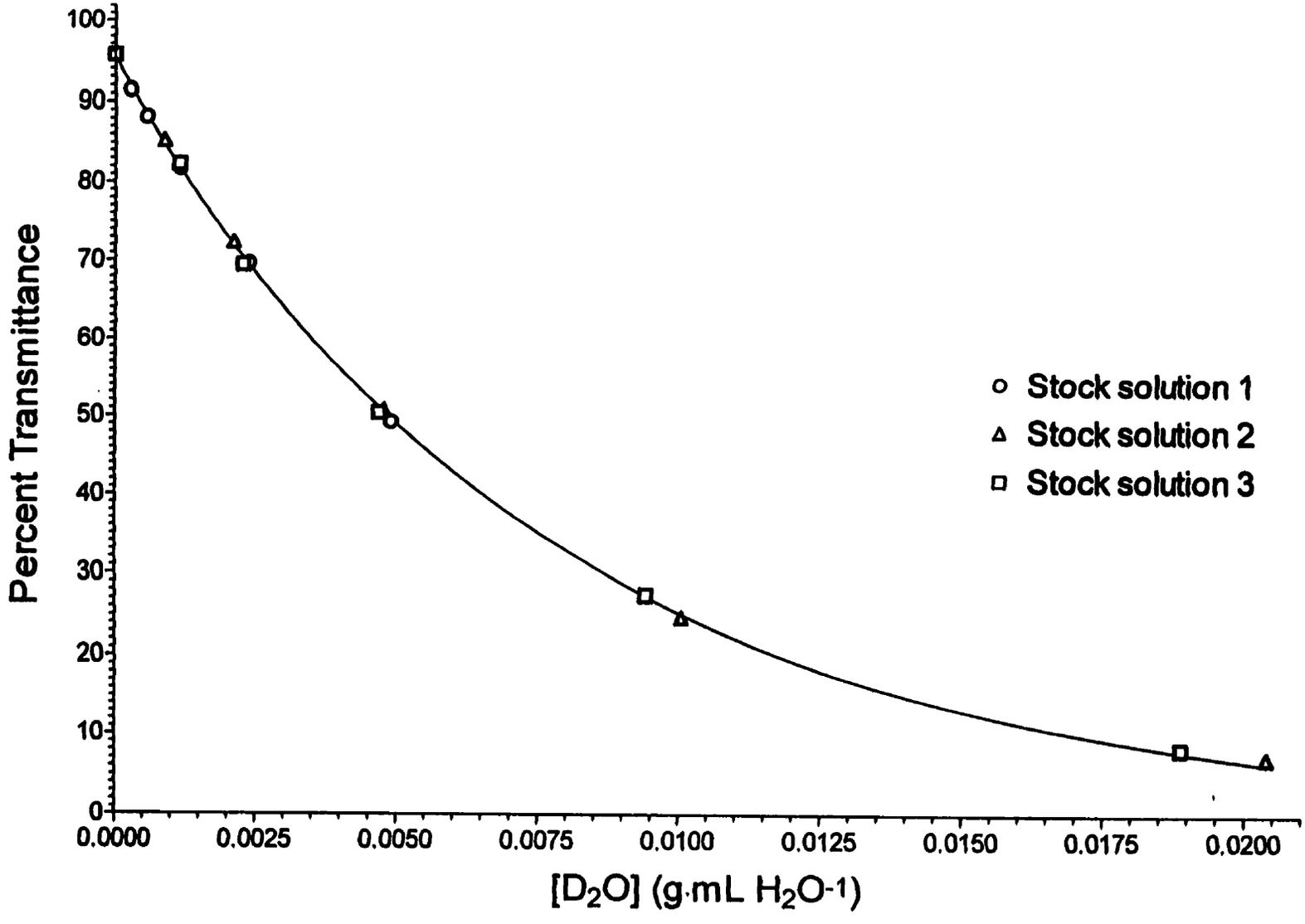
A total of 58 muskrats were live-trapped at Oak Hammock Marsh, injected with D_2O, and released. Recapture success was 45%, providing 33 estimates of water flux

from 27 animals during five sampling periods: 12-15 July 1994 (5 males, 4 females), 13-28 September 1994 (5 males, 2 females), 6-15 December 1994 (6 males, 5 females), 20-24 February 1995 (2 males, 3 females), and 9-12 May 1995 (1 male).

During each sampling period, live traps were set twice daily, and captured muskrats were transported by canoe or covered sled to a nearby laboratory at the Institute for Wetland and Waterfowl Research, Oak Hammock Marsh Conservation Center. Here, muskrats were weighed, lightly anesthetized with Halothane (M.T.C. Pharmaceuticals), and administered a preweighed, intraperitoneal injection of D_2O (99.9% purity, ICN Biochemicals) at a dosage of 3 $g \cdot kg^{-1}$ body mass. The dosage was measured to the nearest 0.1 mg by weighing the D_2O injection syringe before and after ejection. Following injection, each animal was marked with #1 monel ear tags and a subcutaneous microchip transponder (Avid Marketing Inc.) inserted near the base of the tail. After a 3-h equilibration period, the animal was again anesthetized, weighed, and a 3-mL blood sample obtained by cardiac puncture. This step was performed to estimate the animal's TBW content and to obtain the initial serum concentration of D_2O. Tagged muskrats were released at their sites of capture and an attempt was made to re-trap them 48-72 hours later. Recaptured muskrats were weighed, anesthetized, and a second blood sample taken.

Blood samples were allowed to clot at 4°C, centrifuged, and the serum removed and stored at -20°C. Serum samples were distilled by vacuum sublimation using an apparatus modeled after that of Stansell and Mojica (1968). The percent transmittance of the purified water distillate was measured at 2510.0 cm^{-1} (wavelength = 3.8 μm)

Fig. 5-1. Calibration curve constructed from three deuterium stock solutions illustrating the relationship between deuterium concentration [D_2O] and the percent transmittance of the spectrophotometer. The regression performed yielded the equation: [D_2O] = ln (read/95.315)/-132.846 (r^2 = 0.9998, df = 15).


Percent Transmittance
100
90
80
70
60
50
40
30
20
10
0
0.0000
0.0025
0.0050
0.0075
0.0100
0.0125
0.0150
0.0175
0.0200
[D2O] (g·mL H2O-1)
Stock solution 1
Stock solution 2
Stock solution 3

using a Model 881 Perkin-Elmer dual-beam infrared spectrophotometer and standard calcium fluoride cells. The D_2O concentration of each sample was determined from a calibration curve which accounted for background levels of D_2O in body fluids (Fig. 5-1).

The composition, dry matter, and energy content were determined following the procedures outlined in Part I, and digestibility coefficients of natural diets were taken from Part II.

Calculations

To evaluate the accuracy of the D_2O technique for estimating TBW, 15 additional animals were trapped and sacrificed after blood sampling at 3-h post-injection. Carcasses were skinned, eviscerated, and freeze-dried to constant mass to determine their true TBW content (mL). The D_2O technique overestimated the TBW content of muskrats by an average of 7.6±0.9%. Therefore, all estimates of TBW derived by this method were multiplied by a correction factor of 0.929.

Because my calibration curve corrected for background deuterium levels, I calculated TBW from the initial dilution of D_2O, using a modified equation of Peppard et al. (1993):

$$[1] \quad \text{TBW (mL)} = \frac{D_2O \text{ injected (g)}}{\text{Serum } D_2O \text{ concentration (g } D_2O \cdot g^{-1} H_2O)} \times 0.929$$

Equations provided in Nagy and Costa (1980) were used to calculate water efflux and influx based on the progressive dilution of D_2O in body fluids. Because animal mass varied during every trial, the equations used were those derived for calculating water

efflux [eqn. 4] and influx [eqn. 5] when TBW content increases or decreases linearly with time (Nagy and Costa 1980, pg. R455). Final TBW estimates were determined from the equation:

[2] $$TBW_{final} = [(Mass_{final} - Mass_{initial}) \times \%TBW_{initial}] + TBW_{initial}$$

Dry matter intake was calculated from the equation of Shoemaker, Nagy and Costa (1976) for a non-drinking animal in steady state:

[3] intake of dry matter ($g \cdot kg^{-1}$ body mass$\cdot day^{-1}$) =

$$\frac{\text{mL } H_2O \text{ influx} \cdot kg^{-1} \text{ body mass} \cdot day^{-1}}{\text{mL } H_2O \cdot g^{-1} \text{ DM} + (\text{ME coeff.} \times kJ \cdot g^{-1} \text{ DM} \times 0.030 \text{ mL } H_2O \cdot kJ^{-1} \text{ ME})}$$

where ME = metabolizable energy. The coefficients of ME and DM and the energy content of vegetation consumed by muskrats are presented in Table 5-1. Metabolic water production was estimated by assuming a conversion factor of 0.030 mL $H_2O \cdot kJ^{-1}$ ME (Schmidt-Nielsen 1983). The amount of fresh vegetation consumed by muskrats was determined by dividing DMI by the DM content of the diet in each test period. Seasonal estimates of the daily intake of AE ($kJ \cdot kg^{-0.75}$) were derived from equation [5] of Shoemaker, Nagy and Costa (1976, pg. 365).

Statistics

Treatment effects were evaluated initially for differences due to season, sex, and sex x season, using a 2-way ANOVA (SAS Institute 1990). Since no sex-related differences were apparent ($P > 0.05$), data for both sexes were pooled. Significance was

Table 5-1. Values used to estimate the mean daily water influx and intake of vegetation, dry matter, and assimilated energy of captive and free-ranging muskrats, Oak Hammock Marsh, Manitoba, 1994-1995.

Month	Dry matter (%)	Metabolizable energy digestibility[a] (%)	Energy content of forage ($kJ \cdot g^{-1}$ dry matter)
July	6.43	48.86	16.36
Sept	10.61	46.53	17.24
Dec-Feb	16.97	54.89	16.88
May	7.83	39.08	16.94

[a] Values from Table 2-2.

set at the 5% level and means are presented ±1 standard error (SE).

Results

In all but 3 cases, muskrats lost body mass over the 48 - 72 h measurement interval (Table 5-2). However, mass loss was not significantly different among months ($F_{[3,28]} = 1.51$, $P = 0.233$). Water influx varied seasonally ($F_{[3,28]} = 17.81$, $P < 0.0001$), ranging from 423 to 915 mL $H_2O \cdot kg^{-1} \cdot day^{-1}$ (Table 5-2). The calculated intake of fresh vegetation also varied seasonally ($F_{[3,28]} = 14.19$, $P < 0.0001$), with the highest values recorded in spring and summer (Table 5-2). Dry matter intake ($g \cdot kg^{-1} \cdot day^{-1}$ and $g \cdot kg^{-0.75} \cdot day^{-1}$) peaked in December and February when water influx and intake (wet mass) of fresh vegetation were lowest (Table 5-2). Similarly, the daily intake of AE ($kJ \cdot kg^{-0.75} \cdot day^{-1}$) was highest in winter ($P < 0.05$), when it was 62.6% and 25.9% greater than in July and September, respectively. These trends are consistent with the reduced water content and higher ME digestibility of winter forage (Table 5-1). The DM content of forage (Table 5-1) was a reasonable predictor of both daily water influx and intake of fresh vegetation: water influx (mL $H_2O \cdot kg^{-1} \cdot day^{-1}$) = 1086.9 - [38.5 x %DM] ($r^2 = 0.662$, df = 32, $P < 0.0001$); vegetation intake (g wet mass$\cdot kg^{-1} \cdot day^{-1}$) = 1123.8 - [37.0 x %DM] ($r^2 = 0.612$, df = 32, $P < 0.0001$). Although no significant differences were detected between sexes for any of the variables measured, females tended to exhibit the highest intake of vegetation and AE within each sampling period.

Table 5-2. Seasonal changes in estimated mean (±SE) daily water influx and intake of vegetation, dry matter and assimilated energy of free-ranging muskrats, Oak Hammock Marsh, Manitoba, 1994-1995.

	July (n = 9)		Sept (n = 7)		Dec (n = 11)		Feb (n = 5)		May (n = 1)
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$
Body mass (g)	881A[a]	33	817AB	86	695B	43	686AB	48	825
Change in BM[b] (g)	-69	21	-42	13	-70	8	-34	15	-6
Water Influx ($mL \cdot kg^{-1}$ BM$\cdot day^{-1}$)	841.0A	68.0	648.0B	63.7	444.0C	17.0	423.4C	45.3	914.6
Vegetation Intake (g wet mass$\cdot kg^{-1}$ BM$\cdot day^{-1}$)	884.2A	71.5	704.8AB	69.3	506.0C	19.4	482.5BC	51.6	975.8
Dry Matter Intake ($g \cdot kg^{-1}$ BM$\cdot day^{-1}$)	56.9B	4.6	74.8A	7.4	85.9A	3.3	81.9A	8.8	76.4
Dry Matter Intake ($g \cdot kg$ BM$^{-0.75} \cdot day^{-1}$)	54.9B	2.8	70.6AB	7.4	78.1A	3.1	74.4AB	7.9	72.8
Assimilated Energy ($kJ \cdot kg$ BM$^{-0.75} \cdot day^{-1}$)	438.6C	33.8	566.2BC	59.6	724.0A	28.6	689.1AB	73.3	482.0

[a] Within each row, means sharing the same letter are not significantly different ($P > 0.05$).
[b] BM = Body mass.

Discussion

Free-living rodents have been shown to lose mass with repeated captures (Kaufman and Kaufman 1994). On average, muskrats lost 7.4% of body mass between the first and second captures, which is equivalent to approximately 50% of a typical gut fill (K.L. Campbell, unpublished data). Nonetheless, monthly estimates of DMI and AE obtained by the D_2O method (54.9 - 78.1 $g \cdot kg^{-0.75} \cdot day^{-1}$ and 438.6 - 724.0 $kJ \cdot kg^{-0.75} \cdot day^{-1}$) were nearly identical (two-sample *t*-tests, df = 13 - 16, t = 0.241 - 1.589, P = 0.133 - 0.813) to those derived from total balance digestion trials (59.7 - 75.9 $g \cdot kg^{-0.75} \cdot day^{-1}$ and 406.0 - 706.9 $kJ \cdot kg^{-0.75} \cdot day^{-1}$) involving field-acclimatized muskrats (Table 2-2). Results of both studies indicate that muskrats increase their intake of DM and AE during fall and winter, and are consistent with the finding that muskrats maintain the largest amount of gut tissue during these periods (Virgl and Messier 1992*a*; Fig. 2-1). An increased intake of forage combined with enhanced energy and nutrient digestibility during these seasons may also account for the substantial fat accumulation beginning in fall and continuing throughout winter (Virgl and Messier 1992*a*; Fig. 6-2).

In winter, cattail rhizomes have a high DM content (Table 5-1), are rich in energy and soluble carbohydrates, and are highly digestible (Table 1-3; Table 2-2). Consumption of a diet consisting primarily of cattail rhizomes apparently allows muskrats to increase their intake of DM and AE while actually reducing their gross intake of wet vegetation. Because individual tubers can attain a mass of several hundred grams (K.L. Campbell, personal observation), it is possible that muskrats in winter can meet their maintenance

energy requirements with fewer daily excursions than in summer, when they feed predominately on the basal stems of emergent plants. This winter diet may be adaptive, given that the low water temperature and translocation of nutrients from plant shoots to underground root structures should, in theory, increase the energetic cost of aquatic foraging in this species.

Muskrats should preferentially select habitats that enhance survival and reproduction (Messier, Virgl and Marinelli 1990; Messier and Virgl 1992). It is well known that cattail is a preferred food of muskrats (Pelikán, Svoboda and Květ 1970; Lacki et al. 1990) and Clark (1994) recently demonstrated that cattail stands are favored locations for construction of winter lodges. Moreover, muskrats overwintering in stands of cattail exhibited the highest survival rates and greatest gains in body mass (Clark and Kroeker 1993; Clark 1994). These observations are consistent with my previous findings that coefficients of DM and ME digestibility are highest on diets of cattail rhizomes (Table 1-3; Table 2-2).

Management Implications

My field trials indicate that muskrats consume 750-1000 g fresh vegetation·kg^{-1} body mass·day^{-1} from spring until late fall. This estimate is slightly above the daily spring and fall food requirement (734 g·kg^{-1}) reported by Ching and Chih-Tang (1965) for muskrats held in outdoor enclosures, but is lower than the average daily intake of 1250 g·kg^{-1} (range = 875-1800 g·kg^{-1}) reported by Akkermann (1975) for captive muskrats in summer. However, the range I estimated for free-living muskrats is similar to that (894

$g \cdot kg^{-1}$ body mass) calculated from the mean daily food consumption of lab-acclimated muskrats (577 ± 26 $g \cdot kg^{-1}$ body mass; Part I) assuming a daily energy expenditure in the field equivalent to 1.55 times basal metabolic rate (see Part VI). Pelikán, Svoboda and Květ (1970) estimated that the quantity of vegetation that was harvested by muskrats, accounting for wastage of plants used for lodge construction, was at least 2-3 times the amount actually consumed by these rodents. Assuming muskrats harvest twice as much vegetation as they consume, I estimate that these rodents would remove 2.3-3.0 kg fresh vegetation$\cdot kg^{-1}$ body mass$\cdot day^{-1}$ during the growing season.

My field estimate of winter food consumption (550-600 g vegetation$\cdot kg^{-1}$ body mass$\cdot day^{-1}$) is close to the mean intake (513 $g \cdot kg^{-1} \cdot day^{-1}$) I measured for captive, winter-acclimatized muskrats fed cattail rhizomes (unpublished data). However, it is well below the 1200 $g \cdot kg^{-1} \cdot day^{-1}$ reported by Akkermann (1975) for captive muskrats fed a cattail rhizome diet in winter. Although wastage of vegetation during winter has never been quantified, observations from laboratory feeding trials (K.L. Campbell, unpublished data) suggest that it may be equivalent to 50-100% of the animal's intake. At these levels of wastage, muskrats would remove 775-1200 g vegetation$\cdot kg^{-1} \cdot day^{-1}$ in winter. Assuming a 5 month period of ice cover, I estimate that each adult muskrat would harvest between 597 and 821 kg fresh vegetation$\cdot kg^{-1} \cdot year^{-1}$, or 59-83 kg dry matter$\cdot kg^{-1} \cdot year^{-1}$. These predictions are based upon crude estimates of forage removal, and may vary according to habitat type. To refine these estimates and more accurately predict the impact of muskrats on wetland habitat, further research is required to quantify the wastage of vegetation by foraging muskrats.

This study provides a first, essential step in quantifying the forage requirements and energy exchange of free-ranging muskrats. Since muskrats can digest several emergent plant species equally well, my seasonal values for intake of DM and AE (Table 5-2) can be used to estimate the consumption of vegetation by muskrats occupying a wide range of emergent plant communities. When combined with reliable estimates of the amount of vegetation wasted or used in house construction, these data can be utilized to assess the impact of muskrats on the primary productivity of a variety of wetland habitats.

PART VI

NUTRITION AND THE ENERGETIC TACTICS OF MUSKRATS: MORPHOLOGICAL AND METABOLIC ADJUSTMENTS TO SEASONAL SHIFTS IN DIET QUALITY

Abstract

Basal metabolic rate, serum T_4 level, lean organ mass and body composition were measured in 94 captive, seasonally-acclimatized muskrats between May 1991 and April 1992. Seasonal measurements of oxygen consumption, body water content and mass were obtained from an additional 124 captive or free-ranging animals in 1994-95. Mass-independent basal metabolic rate ($kJ \cdot kg^{-0.67} \cdot h^{-1}$) and serum T_4 level ($nmol \cdot L^{-1}$) varied significantly over the year ($P < 0.0001$), with mean values in February that exceeded July values by 31.1% and 77.2%, respectively. These variables tracked seasonal changes in the neutral detergent soluble content of broadleaf cattail, the dominant food source of muskrats in the study population. From July through February, masses of the alimentary tract, liver, spleen, and heart increased, while kidney mass declined. Body fat stores varied significantly over both years, with peak values measured in February. However, lean body and pelt mass exhibited little seasonal variation ($P > 0.05$). Stepwise multiple regression and principal component analyses suggested that variation in BMR was associated most closely with changes in the mass of the heart and alimentary tract. Annual variation in basal energy expenditure, serum T_4 level and organ masses of wild muskrats appear to be linked to seasonal shifts in forage NDS content and energy intake, and may be important factors relating to the annual pattern of fat accretion and mobilization in this semiaquatic rodent.

Introduction

Muskrats are the largest members of the subfamily Arvicolidae, an attribute possibly linked to the species' long semiaquatic history (Zakrzewski 1974; MacArthur 1989). Exploitation of wetland environments by muskrats has selected for a number of physiological adaptations to mitigate thermoregulatory stresses, especially in winter (Fish 1979; MacArthur 1979, 1984). During this season, ice cover and low water temperatures restrict daily movements, increase thermoregulatory costs (Macarthur 1979, 1984, 1986), and reduce the diversity of aquatic vegetation upon which the animals feed. Unlike most non-hibernating rodents, muskrats accrue substantial lipid stores during late fall and winter (Jelinski 1988; Virgl and Messier 1992*a*, 1992*b*). It has been suggested that fat storage in this rodent may be facilitated by winter reductions in thyroid activity and lean body mass, and hence reduced basal metabolic costs (Aleksiuk and Frohlinger 1971; Virgl and Messier 1992*a*, 1995). However, I found that the intake of assimilated energy was substantially higher (>60%) in winter than in summer (Table 5-2). Consequently, it is still not clear whether lipid deposition in muskrats during winter results from an increase in energy intake, a reduction in metabolic activity, or perhaps some combination of both these factors (Salsbury and Armitage 1994).

Annual variation in the availability of energy and other nutrients has also been implicated as a major factor influencing body mass (Merritt 1986; Nagy, Grower and Stetson 1995), gut and organ morphology (Aleksiuk and Frohlinger 1971; Virgl and Messier 1992*a*, 1992*b*), blood chemistry (Morton and Lewis 1980; DelGiudice, Mech and

Seal 1990), and basal metabolic rate, BMR (Wunder, Dobkin and Gettinger 1977; Wunder 1978; Merritt 1984, 1986) of free-ranging animals. However, to date, few studies have adequately integrated seasonal changes in nutrient availability with physiological adjustments in energy expenditure and allocation. Recent studies (Konarzewski and Diamond 1994; Speakman and McQueenie 1996) have suggested that the masses of organs associated with the absorption, metabolism, transport and excretion of ingested nutrients may be regulated by current energetic demands. These organs exhibit high mass-specific rates of metabolism, and their relative masses may account for significant variation in BMR (Gross, Wang and Wunder 1985; Daan, Masman and Groenewold 1990) and daily energy expenditure (Hammond and Diamond 1992, 1994; Hammond et al. 1994; Konarzewski and Diamond 1994). Although a causal link between BMR, energy intake, and organ morphology has been documented in laboratory mice (Konarzewski and Diamond 1994; Speakman and McQueenie 1996), it is not known if a similar relationship applies also to wild populations (Daan, Masman and Groenewold 1990).

To address these issues, I initiated a study to first determine if seasonal changes in the energy and nutrient profile of broadleaf cattail, the dominant food source of muskrats in prairie marshes, is accompanied by modifications in BMR, thyroid activity, organ morphology, lean body mass and proximate composition of muskrats. I also applied regression analyses to measurements of daily energy intake, BMR and organ and tissue masses of muskrats, to assess the interrelationships among these traits within individuals. Although seasonal adjustments in body size and endogenous energy reserves have been demonstrated in several species (Iverson and Turner 1974; Nagy, Grower and

Stetson 1995), few longitudinal data exist for individuals in wild populations (Merritt 1984, 1986). Therefore, my final objective was to monitor temporal changes in the mass and lipid content of individually marked, free-living muskrats using the deuterium oxide (D_2O) dilution technique. Knowledge of the seasonal dynamics of fat and protein reserves is necessary to establish whether somatic growth continues throughout winter in this species. Such information is vital to defining the seasonal energetic and nutritional constraints that a northern wetland environment imposes on these amphibious rodents.

Materials and Methods

Vegetation analyses

To evaluate seasonal changes in the energy content and nutrient composition of cattail at Oak Hammock Marsh, Manitoba (50°06'N, 97°07'W), I periodically collected plant samples at randomly selected sites from May through October 1991. Samples of cattail rhizome were also obtained from two separate food caches found inside winter feeding dens in early December 1995. Plant samples were separated into stem, leaf and rhizome components, oven-dried at 70°C, and ground through a 1-mm mesh screen in a Wiley mill. The gross energy content of each sample was obtained by duplicate measurements in an adiabatic oxygen bomb calorimeter (Parr 1241 Calorimeter, Parr Instrument Co., Moline, Ill.). Ash content was determined following combusion of duplicate 2-g samples at 600°C for 2 h. A portion of each ground sample was also sent to a feed analysis laboratory (Department of Animal Science, University of Manitoba) for neutral detergent fiber determinations using a modified Van Soest technique (Goering and

Van Soest 1970) that employed Termamyl 120L (Åman and Hesselman 1984). For the purposes of this study, forage quality is defined by the level of neutral detergent solubles, and is equivalent to 100% - NDF.

Metabolic trials

Resting rates of oxygen consumption ($\dot{V}O_2$) were measured on a total of 125 seasonally acclimatized adult and subadult muskrats live-trapped at Oak Hammock Marsh in 1991-92 ($n = 92$) and 1994-95 ($n = 33$). Immediately following capture, muskrats were transported to the Animal Holding Facility, University of Manitoba, and were housed individually at 14±1°C with a 12L:12D photoperiod (MacArthur 1979).

In 1991-92, experiments were performed during each of six test periods (n = 11-18 animals per period): May 10-June 2, July 4-21, September 13-25, November 22-December 6, January 29-February 16, and April 10-16. Within 48 h of capture, metabolic measurements were obtained following a 16- to 24-h fast to ensure muskrats were post-absorptive. During metabolic tests, animals were held at 15±0.5°C in a darkened, 11.5-L glass chamber fitted with a heavy plexiglass lid and a removable wire screen floor. A positive-pressure, open-circuit respirometry system (MacArthur 1984) was used, in which the inlet flow rate of dry, CO_2-free air was maintained at 3.5 $L \cdot min^{-1}$ with a Matheson rotameter calibrated against a model 1057 Brooks Vol-U-Meter. Exhaust gas from the chamber was split into two streams. One stream was routed through drierite followed by soda lime/drierite, and then through either a Beckman F-3 paramagnetic or an Applied Electrochemistry S3-A oxygen analyzer connected to a two-channel chart recorder (SE-

120, BBC Goerz Metrawatt). The second stream was routed through drierite and then through an Applied Electrochemistry CD-3A carbon dioxide analyzer connected to the second channel of the recorder. Muskrats were allowed 1 h to adjust to the metabolic chamber, followed by an additional 2-3 h for data collection. Minimum steady-state rates of oxygen consumption and CO_2 production were calculated for three periods, each of at least 5-min duration (Wang and Peter 1975). The respiratory quotient derived from these measurements was used to convert $\dot{V}O_2$ to units of heat production (Stanier, Mount and Bligh 1984).

As part of another study (see Part IV), additional measurements of $\dot{V}O_2$ were obtained from 33 muskrats that were collected over four test periods in 1994-95 (n = 8-9 animals per period): July 27-August 10, October 11-20, January 4-24 and May 16-26. These animals were not fasted prior to testing and metabolic trials were completed within 1-5 days of capture. In each case, the animal was lightly anaesthetized and injected with a small dose of ^{14}C-urea 15 minutes before the start of an 8-h metabolic trial. In the 1994-95 trials, chamber temperature was kept at 22°±1°C, inlet flow rate was held at 2 $L \cdot min^{-1}$, and only $\dot{V}O_2$ was recorded. In most of these metabolic trials, minimum $\dot{V}O_2$ was determined during the final 2-3 h of each run. In both studies, muskrats were weighed before and after each trial and the average mass for each trial calculated. Each animal was tested only once.

Proximate analyses of carcasses

On the day following metabolic testing, 62 of the muskrats studied in 1991-92

were euthanized with an overdose of Halothane anesthetic (M.T.C. Pharmaceuticals). The remaining 32 animals were used in 10-d digestibility trials and then sacrificed in a like manner (see Part II). Blood samples were obtained from 89 of these muskrats and allowed to clot (see Appendix II). Following centrifugation, serum was extracted and stored on ice. Serum thyroxine (T_4) levels were determined with a fluorescence polarization immunoassay technique utilizing competitive antigen binding methodology (Levinson, Goldman and Burch 1992).

Standard body measurements were obtained from freshly killed animals, and the heart, kidneys, liver, adrenals, spleen and gastrointestinal tract excised. Following the method of Elder and Shanks (1962), the baculum was removed from each male, frozen, and subsequently used to distinguish breeding adults (≥10 months) from pre-breeding subadults (<10 months). Beer and Meyer (1951) and Aleksiuk and Frohlinger (1971) reported a strong relationship between age and adrenal mass of muskrats. I found that age estimated from adrenal mass was identical to that predicted from baculum morphology (r = 1.0, n = 72, $P < 0.0001$), and therefore used adrenal mass to age females. Organ-free carcasses were skinned and passed repeatedly through a Krefft meat grinder until the homogenate was a consistent color and texture. The homogenate was re-weighed and stored at -20°C. The pelt, internal organs and a representative sample (20-30%) of each carcass homogenate were freeze-dried to constant mass (minimum 72 h) to determine their respective water contents. Each of these components was then finely ground, separately, in a Black and Decker Model CBM100 coffee bean grinder. Ground organs were subsequently added back to the ground carcass samples in proportion to their

contribution to carcass dry matter. Carcass and pelt samples were sent to a commercial laboratory (Norwest Labs, Winnipeg, MB) and analyzed separately for lipid and protein content. Neutral lipid content was determined by refluxing each sample with petroleum ether in a Soxhlet apparatus (Method 954.02, AOAC 1990), and the crude protein content of each sample was estimated as Kjeldahl nitrogen x 6.25 (Method 979.09, AOAC 1990). The ash content of each carcass and pelt was determined by combusting duplicate 2-g samples at 600°C for 2 h. For each animal, fat-free pelt mass was calculated by subtracting pelt lipid content from dry pelt mass after lyophilization. Mass of skeletal muscles was estimated by subtracting the dry-masses of the pelt, total lipids, ash and all internal organs from the ingesta-free body mass (IFBM) of each carcass. The mean (±SE) muscle mass derived by this method (47.73±0.50% of IFBM) was consistent with that reported for other mammals (44.4 - 49.5%; Calder 1984, p20).

Deuterium oxide studies

A total of 129 estimates of total body water (TBW) were obtained from 91 muskrats over five sampling periods: July 12-15, 1994 (n = 19), September 13-28, 1994 (n = 34), December 6-16, 1994 (n = 34), February 14-24, 1995 (n = 19), and May 9-12, 1995 (n = 23). Only animals >400 g were tested, as the water content of muskrats <400 g may not provide an accurate estimate of body fat content (Virgl and Messier 1993). Live-traps were set twice daily and captured muskrats were transported by canoe or covered toboggan to the Institute for Wetland and Waterfowl Research at Oak Hammock Marsh. Here, animals were lightly anaesthetized, weighed, sexed, and administered a

Oxygen consumption and thyroid activity

Owing to the considerable variation in body mass (range = 461-1241 g), it was essential to correct for the effects of size variation before attempting to interpret seasonal differences in $\dot{V}O_2$ (Wunder, Dobkin and Gettinger 1977). Unfortunately, the intraspecific scaling of $\dot{V}O_2$ to body mass has been reported in few wild species, and no published data are available for muskrats (McNab 1988). Therefore, I pooled all of my basal $\dot{V}O_2$ data for the two years of study, and regressed log basal $\dot{V}O_2$ (mL $O_2 \cdot hr^{-1}$) on log body mass (kg). This procedure yielded the allometric equation: $\dot{V}O_2 = 700\ mass^{0.676}$ ($r^2 = 0.431$, df = 124, $P = 0.0001$). I therefore calculated mass-independent $\dot{V}O_2$ and BMR using body $mass^{0.67}$, as recommended by Heusner (1982). Basal $\dot{V}O_2$ data for 1991-92 and 1994-95 animals were analyzed separately. Mean basal $\dot{V}O_2$ for each month was compared by sex by age class and their interaction terms, using two-way ANOVA (SAS Institute 1990). A similar test was used to compare monthly serum T_4 concentrations. To avoid any possible bias associated with expressing metabolic rate as a ratio of body mass (Packard and Boardman 1988), I also evaluated seasonal variation in $\dot{V}O_2$ (mL $O_2 \cdot hr^{-1}$) and BMR ($kJ \cdot kg^{-1} \cdot hr^{-1}$) with a 2-way ANCOVA, using average body mass as the covariate.

Proximate analyses of carcasses

Preliminary tests indicated that IFBM accounted for more of the variation in body composition than did a principal component analysis using estimates of structural size. I therefore compared body composition variables (water, ash, protein and total lipids) and organ masses for males versus females, adults versus subadults, and post-digestibility trial

(11 d in captivity) versus recent capture (<3 d in captivity), using two-way ANCOVAs with IFBM as the covariate.

Deuterium oxide studies

Body fat estimates determined from the D_2O method were analyzed using (a) only the initial fat estimate for each muskrat ($n = 91$) and (b) the initial and recapture estimates combined ($n = 129$), with predicted IFBM as the covariate. As both seasonal models were highly significant ($P < 0.0001$), only the results for all 129 measurements combined are presented.

Changes in mass and TBW space were assessed from measurements of individuals captured over successive trapping periods.

Individual variation in energy intake, BMR and organ masses

One of my primary objectives was to examine individual and seasonal variability in BMR in relation to changes in organ and tissue morphology. I entered these variables into a stepwise multiple regression model using BMR as the dependent variable and the masses of each organ and tissue as independent predictor variables (McDevitt and Speakman 1994). However, given the potential for autocorrelation among organ and tissue masses, I also conducted a principal component factor extraction analysis on the masses of these organs and tissues ($n = 10$ per animal) to derive uncorrelated orthogonal axes. As independent predictors of BMR, scores for all morphological axes were then entered into a stepwise multiple regression analysis for each individual (Speakman and

McQueenie 1996). Relationships between energy intake and BMR, and between energy intake and organ morphology were assessed using simple correlation analysis.

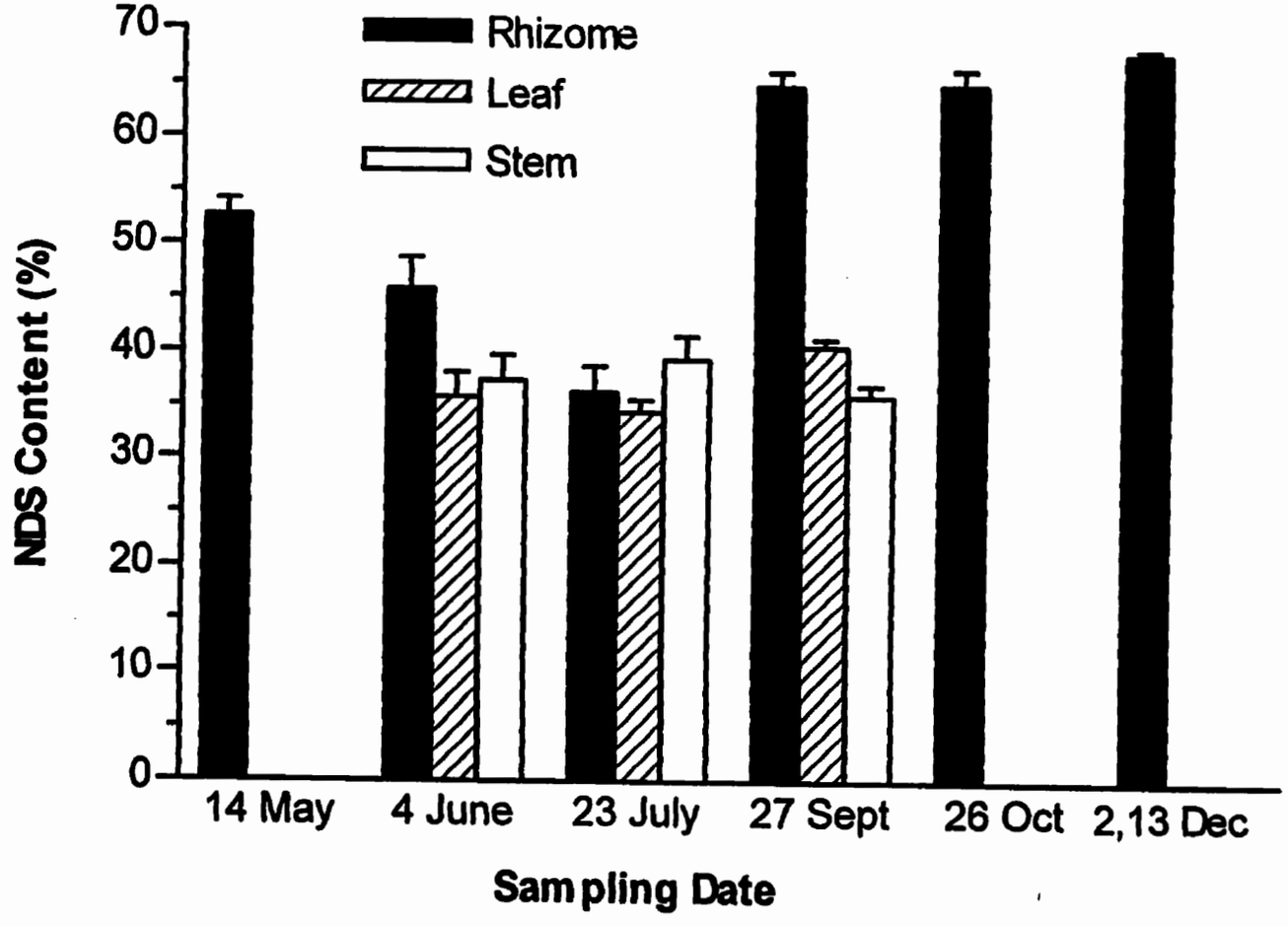
In all comparisons, significance was set at the 5% level and means are presented with ±1 standard error.

Results

Forage quality

Monthly variation in NDS content of cattail shoots and leaves ranged from 36.0 to 40.5% (Fig. 6-1; Table A1-1), and was significant only for leaves ($F_{[2,15]} = 4.09$, $P = 0.0382$). The mean energy content of cattail shoots was highest in June (17.59±0.19 kJ·g^{-1}) and September (17.64±0.05 kJ·g^{-1}) and lowest in July (16.32±0.11 kJ·g^{-1}). This trend reflected changes in the ash content of cattail shoots, which was lowest in September (6.15±0.24%) and highest in July (11.98±0.58%; Table A1-1). The leaf component of cattail consistently had the highest energy content, ranging from 17.88±0.15 kJ·g^{-1} in June to 18.81±0.08 kJ·g^{-1} in September ($F_{[2,15]} = 9.62$, $P = 0.0021$). The mean NDS content of cattail rhizomes also varied seasonally ($F_{[5,22]} = 27.12$, $P < 0.0001$); it was lowest in July (36.3±2.4%), increased to 64.8±1.5% by September, and remained near this value through early winter (Fig. 6-1). Over this same period, the gross energy content of cattail rhizomes varied only from 16.26 kJ·g^{-1} to 16.86 kJ·g^{-1} ($F_{[5,22]} = 1.54$, $P = 0.2198$). Seasonally, the gross energy content of cattail leaves and rhizomes varied inversely with their respective ash contents (5.45±0.22% - 13.17±0.45%; Table A1-1).

Fig. 6-1. Seasonal changes in the neutral detergent soluble (NDS) content of the rhizomes, shoots and leaves of cattail (*Typha latifolia*) collected at Oak Hammock Marsh, Manitoba, from May through October 1991. The December value is based on samples collected from two muskrat food caches in December 1995. Each monthly mean is based on plant samples collected from six randomly selected sites, except for October, when only two sites were sampled. Vertical lines denote 1 SEM.


Rhizome
Leaf
Stem
NDS Content (%)
70
60
50
40
30
20
10
0
14 May
4 June
23 July
27 Sept
26 Oct
2,13 Dec
Sampling Date

Metabolic trials

On a mass-specific (per gram) basis, subadults generally exhibited higher rates of oxygen consumption than adults in both 1991-92 and 1994-95 (Table 6-1). However, as winter progressed, differences between these age groups diminished. When metabolic data were corrected for intraspecific size variation using mass$^{0.67}$ or ANCOVA, I observed no effect of age class, sex, or their interaction terms ($P > 0.05$; range = 0.0709-0.9314). Consequently, adult and subadult muskrats of both sexes were pooled in all subsequent analyses. In both years, I observed significant seasonal changes in basal $\dot{V}O_2$ (1991-92: $F_{[5,86]} = 6.93$, $P < 0.0001$; 1994-95: $F_{[3,29]} = 3.03$, $P = 0.0450$), with lowest rates observed from April through September, and highest rates from October through February. Mass-independent BMR ($kJ \cdot kg^{-0.67} \cdot hr^{-1}$) varied significantly from May 1991 through April 1992 ($F_{[5,83]} = 8.34$, $P < 0.0001$); mean values obtained in February 1992 were >31% higher than those recorded in July 1991 (Fig. 6-2).

The concentration of serum T_4 also varied seasonally ($F_{[5,83]} = 15.18$, $P < 0.0001$), ranging from 25.25±1.63 $nmol \cdot L^{-1}$ in July to 44.73±2.29 $nmol \cdot L^{-1}$ in February (Fig. 6-2). Serum T_4 level generally tracked seasonal changes in forage quality, organ masses, percent body fat and BMR (Fig. 6-1; Fig. 6-2).

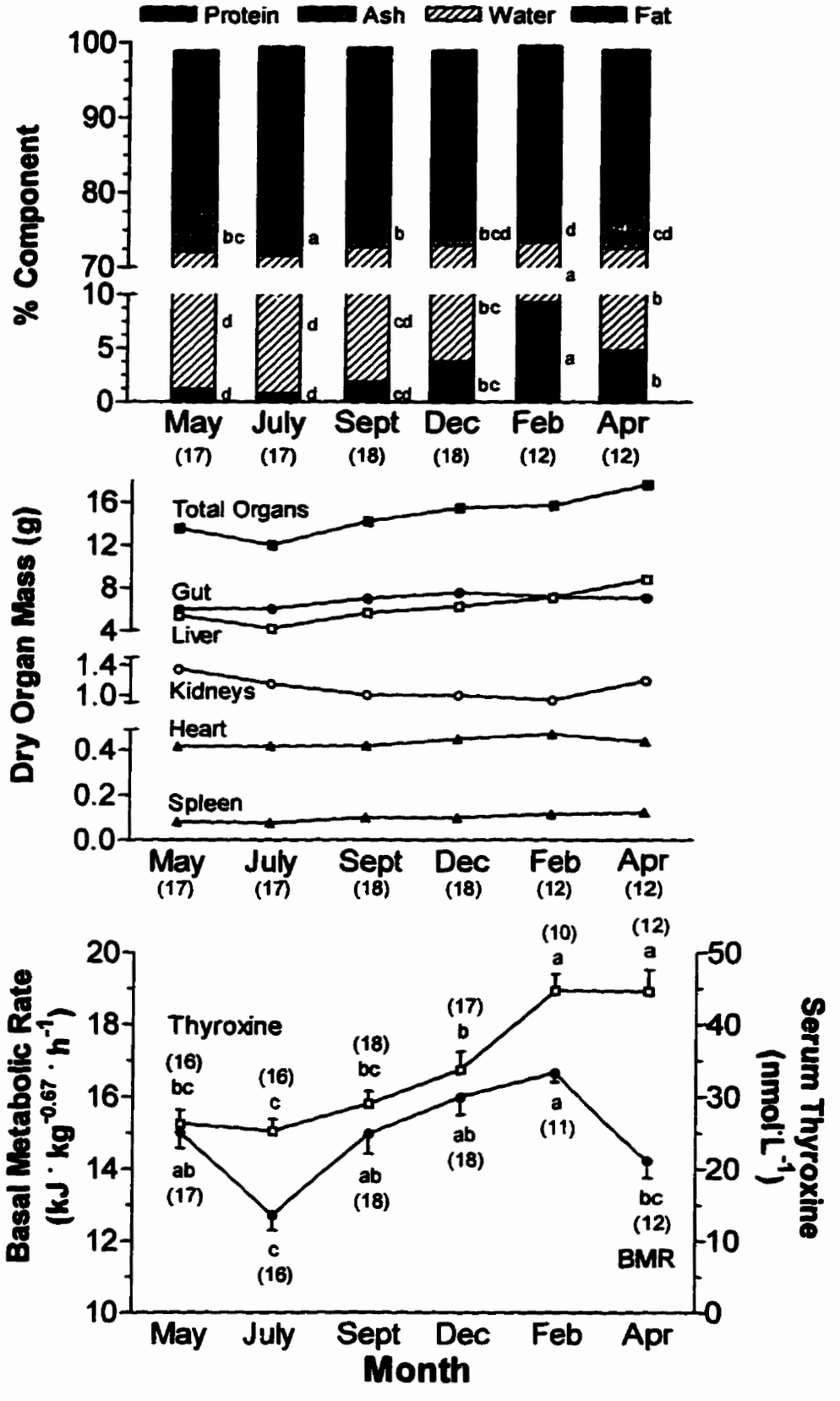
Proximate analyses of carcasses

Total body protein content was relatively constant, accounting for about 21% of IFBM in all sampling periods ($F_{[5,88]} = 1.098$, $P = 0.368$; Fig. 6-2). Consequently, body protein content (g) was strongly predicted by IFBM (g): protein = -9.71 + 0.22 x IFBM

Table 6-1. Seasonal variation in mean (±SE) body mass and basal rate of oxygen consumption ($\dot{V}O_2$) of 125 acclimatized muskrats live-trapped at Oak Hammock Marsh, Manitoba, in 1991-92 and 1994-95.

	Adult			Subadult		
	n	Mass (g)	$\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)	*n*	Mass (g)	$\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
1991-92						
May	17	933±28	0.761±0.023	-	-----	-----
July	16	916±26	0.627±0.025	-	-----	-----
Sept	10	1065±36	0.731±0.038	8	714±25	0.800±0.054
Dec	4	872±69	0.726±0.025	14	617±24	0.924±0.039
Feb	2	1114±84	0.805±0.002	9	855±18	0.845±0.017
April	5	929±21	0.739±0.023	7	795±40	0.711±0.034
1994-95						
August	8	922±32	0.667±0.029	-	-----	-----
October	2	1053±3	0.715±0.037	6	666±30	0.844±0.029
January	2	841±41	0.710±0.030	7	569±31	0.790±0.021
May	8	804±27	0.681±0.032	-	-----	-----

Fig. 6-2. Seasonal changes in (a) body composition, (b) dry organ masses, and (c) serum thyroxine levels and basal metabolic rate (BMR) of acclimatized muskrats. Means sharing the same letters are not significantly different ($P > 0.05$). Vertical lines denote 1 SEM; sample sizes are given in parentheses. All organ values are adjusted means with ingesta-free body mass ($\bar{x} = 772$ g) as the covariate.


Protein
Ash
Water
Fat
% Component
100
90
80
70
10
5
0
bc
a
b
bcd
d
a
cd
d
d
d
d
cd
cd
bc
bc
a
b
b
May
July
Sept
Dec
Feb
Apr
(17)
(17)
(18)
(18)
(12)
(12)
Dry Organ Mass (g)
16
12
8
4
1.4
1.0
0.4
0.2
0.0
Total Organs
Gut
Liver
Kidneys
Heart
Spleen
May
July
Sept
Dec
Feb
Apr
(17)
(17)
(18)
(18)
(12)
(12)
Basal Metabolic Rate
(kJ · kg-0.67 · h-1)
20
18
16
14
12
10
Thyroxine
(16)
bc
(16)
c
(18)
bc
(17)
b
(10)
a
(12)
a
ab
(17)
c
(16)
ab
(18)
ab
(18)
a
(11)
bc
(12)
BMR
50
40
30
20
10
0
Serum Thyroxine
(nmol·L-1)
May
July
Sept
Dec
Feb
Apr
Month

(r^2 = 0.957, df = 93, P < 0.0001) and TBW (g): -0.145 + 0.305 x TBW (r^2 = 0.900, df = 93, P < 0.0001). The ash content of muskrats varied with sampling period ($F_{[5,88]}$ = 14.739, P < 0.0001), and was highest in July and lowest from December to April. Body lipid stores were lowest (<2% of IFBM) from May to September. Lipid reserves increased in winter, reaching a peak value of 9.24±0.47% in February, and were rapidly depleted in early spring ($F_{[5,88]}$ = 48.894, P < 0.0001; Fig. 6-2). As expected, body water content also exhibited strong seasonal variation ($F_{[5,88]}$ = 35.350, P < 0.0001), varying inversely with body fat content: %fat = 77.08 - 1.06 x ingesta-free %TBW (r^2 = 0.8649, df = 93, P < 0.0001). Subadults had lower water content but higher lipid reserves than adults (P < 0.05).

Deuterium oxide studies

Total body water (%) estimated from D_2O dilution studies of live muskrats in 1994-95 followed a similar seasonal trend to the 1991-92 results based on carcass analyses. The average lipid content of free-ranging muskrats (Fig. 6-3) ranged from a low of 0.8±0.4% in July, to a maximum of 7.5±0.9% in February ($F_{[4,124]}$ = 35.534, P < 0.0001).

A total of 50 recaptures were obtained from 34 tagged muskrats that had been previously caught and sampled. Recapture data indicated that all muskrats gained mass during the July-September trapping intervals (n = 12). From September through May, muskrats >800 g lost mass in 86% of all cases (n = 7), while 87% of animals <800 g (n = 31) maintained or increased mass ($\bar{x}$ increase = 10.6±2.5%). For the latter cohort, TBW

Fig. 6-3. Seasonal changes in the body fat content of wild muskrats measured via the deuterium oxide dilution technique (see text for details). A total of 124 measurements were obtained from 91 animals captured at Oak Hammock Marsh, Manitoba, between July 12, 1994 and May 12, 1995. Means sharing the same letters are not significantly different ($P > 0.05$). Vertical lines denote 1 SEM; numbers within bars indicate sample sizes for each month.

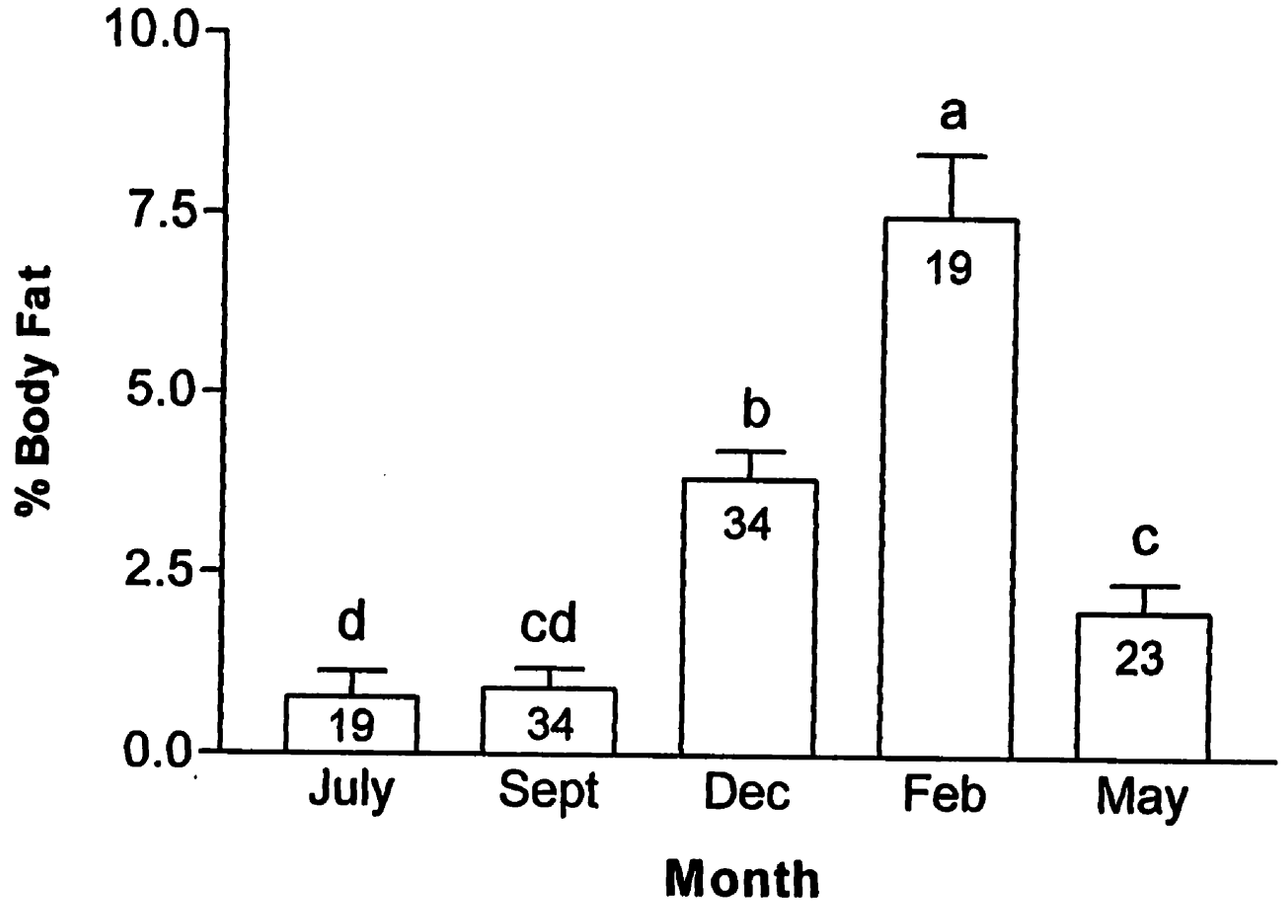
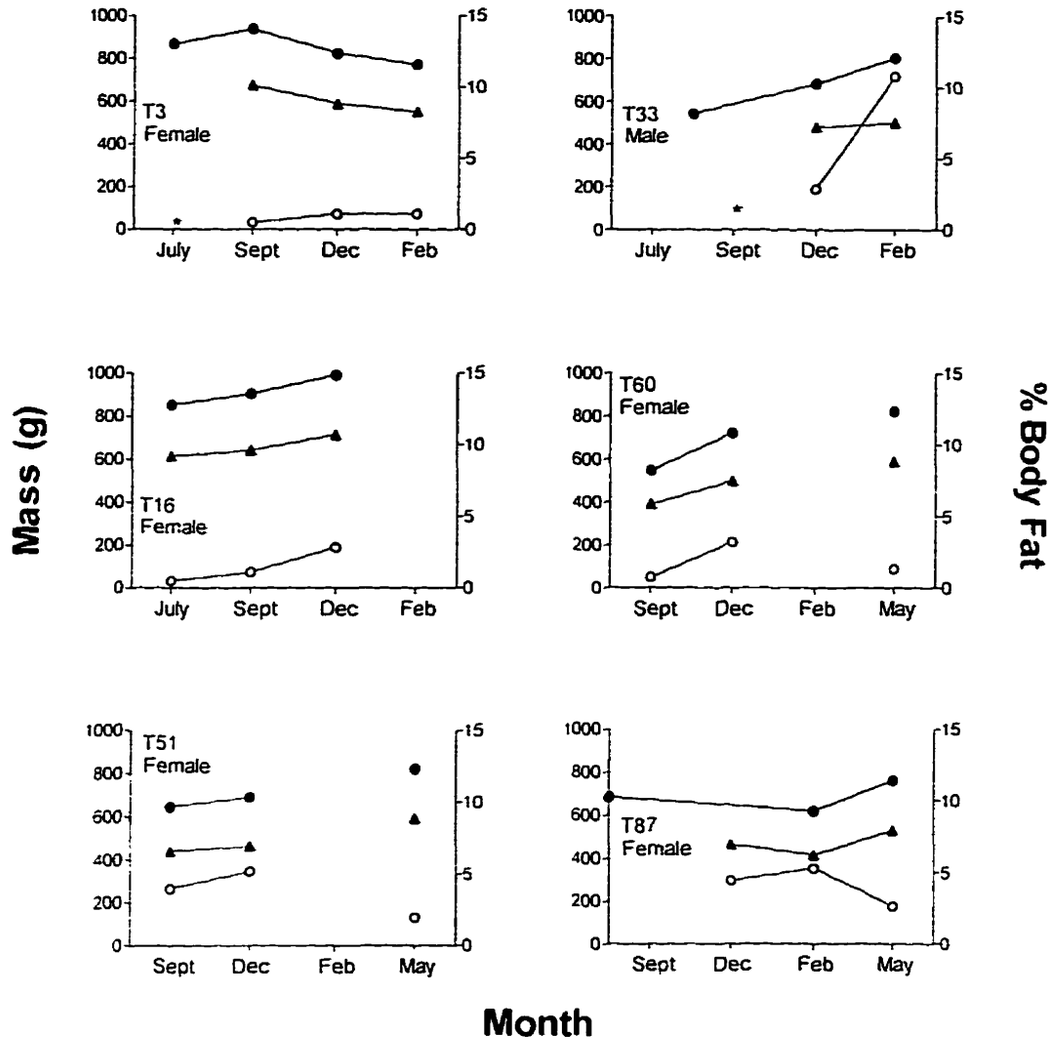

10.0
7.5
5.0
2.5
0.0
% Body Fat
a
19
b
34
c
23
d
19
cd
34
July
Sept
Dec
Feb
May
Month

Fig. 6-4. Temporal changes in body mass (•), total body water (▲) and fat content (o) of representative muskrats that were recaptured over consecutive trapping periods. Total body water (g) and percentage body fat content of each animal was estimated using the deuterium oxide dilution technique (see text for details). Asterisks denote cases when body fat calculations yielded negative values.


Mass (g)
% Body Fat
T3
Female
T33
Male
T16
Female
T60
Female
T51
Female
T87
Female
July
Sept
Dec
Feb
May
Month

increased by an average of 6.6±2.5%, with the greatest increases occurring during the September-December (+8.5%) and February-May (+16.9%) trapping interval (Fig. 6-4).

Organ masses and their relationship to BMR

The masses of most individual organs increased from summer to late-winter, a pattern clearly reflected in total organ mass (Fig. 6-2; $F_{[5,88]} = 20.20$, $P < 0.0001$). Indeed, muskrats exhibited hypertrophy of the alimentary tract (+17.9%; Fig. 2-1), heart (+12.7%), liver (+68.2%) and spleen (+51.0%) from July through February (Fig. 6-2; $F_{[5,88]} = 3.79$ - 32.37, $P < 0.005$). However, over this same period, kidney mass declined by 19.1%. Although values were generally highest in February, I observed no significant seasonal variation in the fat-free mass of either skeletal muscle ($F_{[5,88]} = 1.41$, $P = 0.230$) or pelt ($F_{[5,88]} = 1.74$, $P = 0.134$).

For the 89 muskrats for which I had measurements of both organ masses and BMR, I performed a stepwise multiple regression (with both forward selection and backward elimination) using BMR ($kJ \cdot d^{-1}$) as the dependent variable, and the masses of selected organs and tissues as independent predictor variables (McDevitt and Speakman 1994). In a stepwise multiple regression with forward selection, heart, alimentary tract and fat mass entered as significant predictors, explaining 33.0% of the variation in BMR (Table 6-3). However, with backward elimination, four variables (heart, alimentary tract, pelt and ash) entered as significant predictors, explaining 33.1% of the variation in BMR (Table 6-3).

Given the potential for autocorrelation among these variables, I also employed a

Table 6-2. Principal components analysis of seasonal variation in the dry masses of 10 organs and tissues measured in 89 acclimatized muskrats caught at Oak Hammock Marsh, Manitoba, May 8, 1991 through April 17, 1992.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	5.383	1.800	0.806	0.599	0.492	0.316
Proportion of variation ...	53.8	18.0	8.1	6.0	4.9	3.2
Cumulative variation	53.8	71.8	79.8	85.8	90.8	93.9
Eigenvectors:						
Alimentary tract	0.716	0.401	0.333	-0.150	-0.110	-0.346
Liver	0.688	0.501	-0.145	-0.128	-0.406	0.125
Heart	0.842	0.069	-0.200	-0.055	0.209	0.292
Kidneys	0.744	-0.389	-0.319	0.070	-0.344	-0.011
Adrenals	0.656	-0.194	0.583	-0.327	0.020	0.224
Spleen	0.546	0.439	0.298	0.639	0.010	0.091
Skeletal muscle	0.947	-0.203	-0.106	0.044	0.018	-0.054
Pelt	0.899	-0.164	-0.083	0.002	0.201	-0.159
Ash	0.787	-0.515	-0.026	0.077	0.181	-0.087
Fat	0.285	0.815	-0.291	-0.171	0.281	-0.032

Table 6-3. Output of stepwise multiple regression analysis with BMR set as the dependent variable and either (a) dry masses of all 10 organs and tissues or (b) scores on all 10 principal components describing variability in organ dry masses entered as the independent variables. Measurements are based on 89 seasonally acclimatized muskrats live-trapped at Oak Hammock Marsh, Manitoba, between May 8, 1991 and April 17, 1992.

Predictor	Coefficient	SE	r^2	*P* Value
Heart[a]	192.371	75.553	0.2242	0.0127
Fat	0.472	0.212	0.0686	0.0288
Alimentary tract ...	10.280	4.788	0.0368	0.0347
Heart[b]	242.089	94.452	0.2241	0.0122
Ash	-2.218	0.9506	0.0617	0.0220
Alimentary tract ...	10.180	4.915	0.0150	0.0414
Pelt	1.924	1.037	0.0302	0.0669
PC1[c]	22.726	4.629	0.2049	0.0000
PC2	14.239	4.767	0.0766	0.0037
PC6	-7.740	4.677	0.0227	0.1017

[a]Forward selection procedure: BMR ($kJ \cdot d^{-1}$) = 172.32 + 192.37 heart (g) + 0.472 fat (g) + 10.28 alimentary tract (g); $r^2 = 0.3296$, df = 3, $F = 13.77$, $P < 0.0001$.

[b]Backward elimination procedure: BMR ($kJ \cdot d^{-1}$) = 159.53 + 242.09 heart (g) - 2.218 ash (g) + 10.18 alimentary tract (g) + 1.924 pelt (g); $r^2 = 0.3311$, df = 4, $F = 10.516$, $P < 0.0001$.

[c]Backward elimination procedure: BMR ($kJ \cdot d^{-1}$) = 337.54 + 22.73 (PC1) + 14.24 (PC2) - 7.74 (PC6); $r^2 = 0.3042$, df = 3, $F = 12.24$, $P < 0.0001$.

principal component analysis to extract orthogonal axes of variability in organ and tissue masses of muskrats (Table 6-2). From this analysis, two dominant principal components (PC1 and PC2) emerged (eigenvalues > 1.0). The first principal component was influenced primarily by the masses of skeletal muscle, pelt, heart, ash content, kidneys, and alimentary tract. The second principal component was strongly dominated by the mass of body lipid stores. The scores of all 10 principal components were entered as independent predictor variables into a stepwise multiple regression with BMR treated as the dependent variable (Speakman and McQueenie 1996). With the forward selection procedure, two principal components (PC1 and PC2) proved to be significant predictors of BMR, explaining 28.2% of the variation in BMR. However, with backward elimination, the sixth principal component also entered as a significant predictor of BMR (Table 6-3). The regression equation was dominated by the first principal component which, alone, explained 20.5% of the variation in BMR. The second (body fat content) and sixth (alimentary tract, adrenals, and heart) principal components explained a further 7.7 and 2.3% of the variance, respectively.

Relationship of daily energy intake to BMR and organ masses

I obtained estimates of both BMR (this study) and daily gross energy intake, GEI (Table 2-2) for 32 seasonally-acclimatized muskrats (n = 8 in each of May, July, September and December). These variables were significantly correlated (r = 0.38; df = 31, P = 0.031; Table 6-4), indicating that animals with higher basal metabolic rates tended also to have higher rates of energy intake. For these same 32 animals, daily GEI varied

Table 6-4. Correlation coefficients relating the gross energy intake ($kJ \cdot day^{-1}$) to organ mass (g) in seasonally acclimatized muskrats.

Variable	r^a	P
Stomach	0.52	0.002
Small Intestine	-0.11	0.549
Caecum	0.38	0.032
Large Intestine	0.42	0.017
Alimentary Tract	0.30	0.094
Heart	0.50	0.004
Liver	0.32	0.075
Adrenals	0.34	0.054
Spleen	0.20	0.284
Kidneys	0.14	0.463

[a]df = 31 in all cases.

positively with the dry masses of the stomach, caecum and large intestine, but not the small intestine (Table 6-4). Furthermore, GEI correlated significantly with heart mass, marginally with adrenal and liver mass, and not at all with either splenic or kidney mass.

Discussion

Nutrition has been described as the "mechanistic thread" linking wildlife populations to their environment (DelGiudice, Mech and Seal 1990; Robbins 1993). Variation in the energy and nutrient content of forage has been associated with changes in body mass and composition (Batzli and Esseks 1992), serum levels of thyroid hormone (Eales 1988) and BMR (Veloso and Bozinovic 1993). Not surprisingly, I found that annual changes in forage quality (Fig. 6-1) and energy intake (Table 2-2; Table 5-2) were accompanied by changes in basal energy expenditure, serum T_4 concentration, body lipid stores and organ sizes of seasonally acclimatized muskrats (Fig. 6-2). Since I was studying seasonally acclimatized animals, I cannot conclusively isolate the effects of diet quality and energy intake from temperature, photoperiod and other factors that may have contributed to the observed seasonal trends. Additionally, as no females were collected during the breeding season, these results exclude the energetic costs associated with pregnancy and lactation.

Recently, considerable research effort has focused on the association between the energy requirements of rodents and morphological changes in the organs principally involved with energy uptake, metabolism, transport and excretion (Konarzewski and

Diamond 1994; McDevitt and Speakman 1994; Speakman and McQueenie 1996). These studies suggest that the sizes of the alimentary tract, heart, liver and kidneys increase or decrease in concert with energetic demands of the animal. As these organs have intrinsically high rates of metabolism (Konarzewski and Diamond 1994; Speakman and McQueenie 1996), their relative masses should strongly influence BMR (Daan, Masman and Groenewold 1990). Consequently, to accommodate a higher rate of food intake without compromising digestive efficiency, organs involved with nutrient uptake and metabolism should exhibit hypertrophy, resulting in an elevated BMR (Konarzewski and Diamond 1994; Speakman and McQueenie 1996). These same organs should exhibit atrophy when food availability is low, reducing basal energetic costs (Weiner 1992). As will be seen below, my results generally support these predictions. Moreover, the timing of these physiological adjustments appears to have an important bearing on the seasonal patterns of lipid deposition and utilization of muskrats.

Relationships between organ masses, BMR and daily energy intake

My finding that seasonal changes in GEI tracked changes in stomach, caecum, large intestine, heart and liver mass (Fig. 6-2) is consistent with the hypothesis that enlargement of these organs is associated with the need to absorb, metabolize and transport additional nutrients ingested (Hammond and Diamond 1994; Konarzewski and Diamond 1994). The best predictors of BMR of muskrats were the sizes of the heart and alimentary tract, as evidenced by their dominant effect in multiple regression models. Masses of these organs, as well as those of the kidneys, skeletal muscle, pelt and body

ash, also dominated the first principal component. These findings support the view (Konarzewski and Diamond 1994) that the alimentary tract and heart, while constituting a small percentage of IFBM (3.25±0.06%), also incur high maintenance costs. Body fat was also a significant predictor of BMR in both multiple regression and principal component (PC2) analyses (Table 6-2; Table 6-3). While brown adipose tissue was not measured in this study, seasonal changes in the mass of this tissue reported by Aleksiuk and Frolinger (1971) followed a similar pattern to the observed body fat and BMR measurements (Fig. 6-2). It is conceivable that the relationship I observed between BMR and total body fat is linked to seasonal changes in brown adipose tissue and, therefore, thermogenic capacity (McDevitt and Speakman 1994) of muskrats. Thus, seasonal adjustments in the masses of these highly metabolically active tissues may have contributed to the substantial variation in BMR (31%) observed from July through February (Fig. 6-2).

The observation that kidney mass of muskrats declined in winter was reported also by Aleksiuk and Frohlinger (1971). Kidney mass has been shown to increase in cold-stressed laboratory mice, presumably to dispose of the additional metabolic wastes associated with an increased rate of metabolism (Hammond et al. 1994; Konarzewski and Diamond 1994). However, this finding suggests that energy intake may not be the primary factor regulating kidney mass of wild muskrats. Rather, the seasonal trend in kidney mass is consistent with the observation that muskrats exhibit substantially higher rates of urine output and water flux in summer than in winter (Part IV; Table 5-2).

If the masses of organs associated with nutrient assimilation are important

determinants of both BMR and rate of food intake, then these latter variables should be strongly correlated. In fact, several studies have suggested a link between BMR and field metabolic rate of free-ranging animals (Peterson, Nagy and Diamond 1990; Weiner 1992). However, few attempts have been made to collect both BMR and daily energy intake from the same individuals, or to test for this relationship on a seasonal basis in either captive or free-ranging animals (Weiner 1992; Salsbury and Armitage 1994). My results clearly indicate that seasonal changes in MEI of both captive and free-living muskrats (Table 2-2; Table 5-2) closely follow seasonal adjustments in BMR (Fig. 6-5). Furthermore, in the 32 animals for which I had measurements of both BMR and GEI, I found that these variables were closely correlated ($P = 0.031$).

I previously found that the daily intake of dry matter by captive (Table 2-2) and free-ranging (Table 5-2) muskrats is substantially higher in winter (74.4 - 78.1 $g \cdot kg^{-0.75}$) than in summer (54.9 - 59.7 $g \cdot kg^{-0.75}$). Results of the present study indicate that forage NDS content is also highest in winter (Fig. 6-1). This factor, together with the high digestibility of cattail rhizomes (Table 1-3; Table 2-2), may at least partially explain why the total metabolizable energy intake (MEI) of free-living muskrats is 64% greater in winter than in summer (Table 5-2). The alimentary tract, presumably responding to the additional energy intake, undergoes substantial enlargement over this same period, enabling muskrats to maintain and even increase digestive efficiency (Table 2-2). The masses of the stomach, caecum, large intestine, but not small intestine, were positively correlated with the level of energy intake. The correspondence between size of the lower digestive tract and daily GEI is not surprising, given that muskrats obtain up to 62% of

their daily MEI from the fermentation of fiber (Part I; Table 2-2).

Food quality and energy intake may also be directly involved in the regulation of BMR via T_4, the primary hormone of the thyroid gland. Energy-induced increases in the availability of circulating T_4 and its conversion to triiodothyronine (T_3) would be expected to elevate BMR (Eales 1988; McNabb 1992). Furthermore, chronically elevated T_4 and T_3 levels may induce hypertrophy of metabolically active organs (Konarzewski and Diamond 1994). My findings are consistent with these arguments and, in fact, variation in serum T_4 levels of muskrats (Fig. 6-2) followed a similar trend to seasonal changes in forage quality (Fig. 6-1), energy intake (Table 2-2; Table 5-2), organ masses and BMR (Fig. 6-2).

Seasonal adjustments in body mass and BMR

For most arvicolid rodents, winter is characterized by low ambient temperatures coupled with reduced availability and quality of forage (Wunder 1984). In response to the high thermoregulatory demands imposed by winter foraging, many arvicolid species are thought to undergo adaptive reductions in body mass (Iverson and Turner 1974; Wunder, Dobkin and Gettinger 1977). This tactic confers two potential advantages: (1) it reduces absolute metabolic costs in winter when energy availability is assumed to be lowest (Wunder 1984), and (2) it permits a greater mass-specific thermogenic response to acute cold exposure (Wunder, Dobkin and Gettinger 1977).

Muskrats do not appear to conform to this model since they often increased body mass (Fig. 6-4), yet exhibited higher mass-specific $\dot{V}O_2$ and mass-independent BMR,

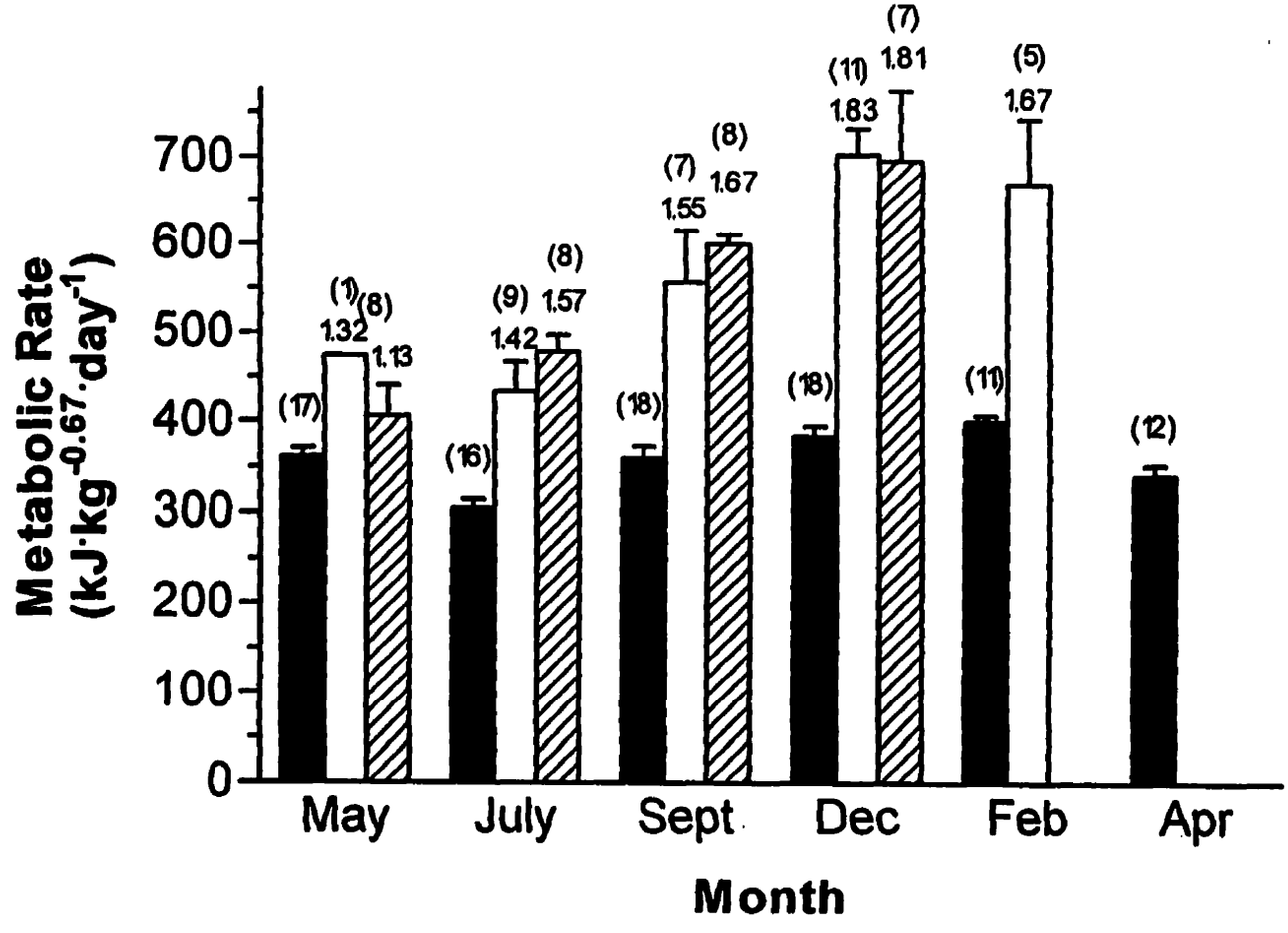
beginning in late fall and continuing throughout winter (Table 6-1; Fig. 6-2). While a reduction in mass should theoretically lower basal energy requirements, it may also facilitate heat loss, owing to an increased body surface area:mass ratio (Merritt 1986). Given the low tolerance of muskrats to immersion hypothermia (Fish 1979; MacArthur 1984), there may be little, if any, energetic advantage to reducing lean body mass in winter.

A high BMR has been linked to an up-regulation in maximal metabolic rate (Wunder, Dobkin and Gettinger 1977; Wunder 1984). While maximal $\dot{V}O_2$ was not examined in this study, the BMR of muskrats was highest in winter, a period when muskrats are foraging in near-freezing water and when daily energy expenditure appears to be greatest (Fig. 6-5). An earlier study (MacArthur 1979) established that free-living muskrats elevate body temperature by up to 1.2°C prior to initiating major foraging bouts in winter. Conceivably, an elevated BMR could facilitate this predive storage of body heat and thus contribute to delaying the onset of immersion hypothermia (MacArthur 1984). An elevated BMR might also expedite rewarming between foraging bouts (MacArthur 1984, 1986) and, in conjunction with communal nesting, could also contribute to maintaining a buffered microenvironment in winter dwelling lodges (MacArthur and Aleksiuk 1979).

Seasonal patterns of fat accretion and mobilization

My findings confirm earlier studies indicating that muskrats accrue substantial lipid stores during winter (Aleksiuk and Frohlinger 1971; Jelinski 1988; Virgl and Messier

Fig. 6-5. Seasonal changes in basal metabolic rate (BMR) of acclimatized muskrats (solid bars; this study) and the metabolizable energy intake (MEI) of captive (hatched bars; Table 2-2) and free-ranging (open bars; Table 5-2) muskrats. Numbers indicate the mean MEI:BMR ratio and the sample sizes (in parenthesis) for each month. Vertical lines denote 1 SEM.


Metabolic Rate
(kJ·kg-0.67·day-1)
700
600
500
400
300
200
100
0
(17)
(1) 1.32
(8) 1.13
(16)
(9) 1.42
(8) 1.57
(18)
(7) 1.55
(8) 1.67
(18)
(11) 1.83
(7) 1.81
(11)
(5) 1.67
(12)
May
July
Sept
Dec
Feb
Apr
Month

1992*a*, 1992*b*). This is somewhat surprising, given that low temperatures and ice-cover restrict daily movements, impose high thermoregulatory costs during swimming, and reduce the diversity of aquatic plants available to foraging muskrats. It has been suggested that the positive energy balance achieved by muskrats in winter is facilitated by reductions in motor activity, lean body mass and BMR (Aleksiuk and Frohlinger 1971; Virgl and Messier 1992*a*, 1995). My findings are not entirely consistent with this hypothesis. I found, for instance, that muskrats maintained lean muscle mass (Fig. 6-2) while increasing BMR and MEI from mid-summer through late-winter (Fig. 6-5). Moreover, most subadults for which I obtained recapture data in winter gained mass and exhibited increases in TBW from September through May. As the absolute protein mass of muskrats is closely tied to water space (this study; Virgil and Messier 1993), these data strongly suggest that somatic growth continues during the winter months in my study population. Nagy and associates (1995) and Merritt (1986) reported similar patterns in seasonal body mass of collared lemmings (*Dicrostonyx groenlandicus*) and short-tailed shrews (*Blarina brevicauda*), respectively. Like the muskrat, these species also have been shown to have access to abundant, high-quality foodsources and buffered subnivean microclimates during winter.

Recently, interest has been expressed in the energy turnover rates of free-living animals, measured as the ratio of daily energy expenditure (field metabolic rate or MEI) to BMR (Daan, Masman and Groenewold 1990; Peterson, Nagy and Diamond 1990; Weiner 1992). In terrestrial mammals, this ratio typically varies from 1.30 to 5.25 (Karasov 1992). The MEI:BMR ratios derived for penned, seasonally-acclimatized and

this study) suggests that tissue reserves may contribute to the reproductive success of free-living muskrats. However, males and females appear to exploit different energetic tactics during this period. Measurements collected during the breeding season (this study; Part V), suggest that males adopt a "frugal strategy" (Koteja 1996) characterized by reductions in food intake, BMR and masses of organs that are metabolically expensive to maintain. Adoption of these energy-conserving tactics could enable males to increase their reliance on body fat reserves and thereby minimize foraging effort, while maximizing time allotted to reproductive activities. Virgl and Messier (1992*a*) reported that the alimentary tract of females enlarges from June through August, likely in response to the high energetic demands of gestation and lactation (Hammond 1992, 1994; Speakman and McQueenie 1996). Thus, during the breeding season, females may adopt an energetically "wasteful strategy" (Koteja 1996) characterized by a higher daily intake of energy, a higher BMR and larger organ masses than is the case for males. Clearly, further research is required to delineate the metabolic costs of reproduction in muskrats, especially of females. These data are vital to developing a fuller understanding of the seasonal allocation of energy and nutrients in this prominent North American rodent.

Summary and Conclusions

Winter is associated with a short photoperiod, cold temperatures, restricted food availability and low forage quality (Wunder 1984). Consequently, for most small mammals existing at temperate latitudes, logic dictates that winter should be both energetically and nutritionally stressful (Hammond 1993). Prior to the onset of this study, it was assumed this tenet also applied to the semiaquatic muskrat. For instance, the quality and availability of the principal forage items consumed by muskrats was long thought to subside from summer to winter (Aleksiuk and Frolinger 1971). During the spring and summer, it was reasonably assumed that emergent hydrophytes were relatively high in protein and low in fiber, providing muskrats with a reliable and easily digestible source of nutrients. By fall however, plant senescence and the translocation of nutrients from plant shoots to underground root structures were expected to reduce the diversity and availability of such high-quality, low-fiber foods. Contributing to this constraint is the long-term establishment ($\geq$ six months) of persistent ice-cover, an element expected to limit both the feeding range and foraging efficiency of this species. Additionally, as cold water is among the most thermally challenging environments facing mammals anywhere on earth (MacArthur 1989), the attainment of adequate energy and nutrients by muskrats must more than offset the heightened metabolic costs of foraging in near freezing water.

While it is generally accepted that aquatic foraging by muskrats is energetically expensive (Fish 1979; MacArthur 1979, 1984, 1986), my results suggest that from a

nutritional standpoint, winter is not particularly stressful to these animals. This and other recent studies (see Jelinski 1989; Virgl and Messier 1992*a*, 1992*b*) have suitably demonstrated that in spite of the high energetic costs of aquatic foraging in winter, muskrats accrue substantial fat deposits during this season. Furthermore, my findings suggest that these rodents are able to accomplish this feat while increasing both their basal metabolism and lean tissue mass. This then invites the question, how are muskrats able to compensate for the presumed nutritional shortfalls and energetic demands imposed by winter? The answer, it seems, lies in the nature of the aquatic plants upon which muskrats feed, and in the exceptional ability of these rodents to exploit this diet.

A primary finding of Part I, that was also corroborated in Parts II and III, was that muskrats can digest plant fiber with an efficiency surpassing predictions based on allometry (Justice and Smith 1992), and well above previously reported values for other small mammals maintained on high fiber diets. Whereas other rodents experience high mortality when dietary NDF exceeds 55%, muskrats maintained mass and appeared healthy on diets containing up to 67% NDF. In fact, when maintained on a natural diet containing 62% NDF (see Part II), muskrats obtained up to 60% of their digestible energy intake from the fermentation of fiber. Indeed, the ability of muskrats to digest fiber is comparable to that of many ruminant and pseudoruminant species (Fig. 1-2). This finding is quite remarkable, considering the ruminant stomach has long been regarded to be the most efficient in digesting fiber.

Muskrats are the largest North American representatives of the subfamily Microtinae, an attribute considered to be thermally adaptive for an aquatic lifestyle

(MacArthur 1989). However, considering the large, well-developed caecum of muskrats (see Fig. 4-6), an equally tenable selective pressure for large size in this species may be the high fiber content of aquatic vegetation (see Appendix 1). Large body size is known to be an important adaptation for herbivores, especially those species which rely heavily upon nutrients obtained from the microbial fermentation of fiber (like muskrats). Fiber digestion is positively associated with retention time which, in turn, is a direct function of body size (Demment and Van Soest 1985; Justice and Smith 1992).

Muskrats from my study site forage principally upon cattail, an emergent hydrophyte characterized by high-fiber, high-protein shoots and leaves in summer and relatively low-fiber, low-protein rhizomes in winter (Fig. 4-4; Fig. 6-1). Logically, one would expect that muskrats feeding on a predominantly cattail-based diet should have little trouble meeting their maintenance nitrogen requirements in summer. However, a second major finding of Part I, and one also corroborated in Part II, was that muskrats had difficulty maintaining nitrogen balance in spring and summer on diets consisting solely of aquatic plants. While this apparent nitrogen shortfall may result from both a low energy-to-nitrogen ratio of aquatic vegetation and a high water influx in spring and summer (see Part IV), of more immediate concern for muskrats during these seasons is meeting their nitrogen requirements for maintenance, growth and reproduction.

The opportunistic consumption of concentrated protein (and low-fiber) sources, such as animal tissue, is one option by which free-living muskrats can meet their daily nitrogen requirements during the summer months. In fact, carnivory has often been reported in studies of food preference of muskrats (Errington 1941; Sterns and Goodwin

1941; Triplet 1983; Convey, Hanson and MacKay 1989; Neves and Odem 1989). The central findings of Part III were that the apparent digestibilities of dry matter, metabolizable energy and protein all increased with the level of animal tissue presented in the diet. More significantly, a strong correlation existed between nitrogen (protein) intake and nitrogen balance. These results suggest that carnivory in muskrats may serve an important role in meeting nitrogen balance, particularly during the summer months when the availability of animal tissue may be greatest. Secondly, as will be considered in more detail below, selective consumption of low-fiber foods may be a tactic muskrats employ to increase their metabolizable energy intake in summer, rather than relying on the more energetically expensive option of maintaining increased gut and accessory organ masses.

Findings of Parts I and II suggested that muskrats were generally unable to meet their nitrogen requirements during spring and summer, when the protein levels of their aquatic plant diets are highest. Paradoxically, however, results of these same experiments suggested that during winter, muskrats maintained on a relatively low-protein diet of cattail rhizomes were able to satisfy their maintenance nitrogen requirements. This led to a test of the hypothesis that muskrats, like some ruminants and hibernating rodents (Robbins et al. 1974; Mould and Robbins 1980; Steffen et al. 1980; Harlow and Buskirk 1991), conserve body nitrogen by reducing urinary nitrogen output through urea recycling. My results indicated that the rate of urea hydrolysis was 67% higher in fall and winter than in spring and summer. This finding suggests that increased levels of urea recycling, coupled with adaptive mechanisms for reducing nitrogen excretion and perhaps conserving

carbon skeletons of essential amino acids, may allow muskrats to lower their maintenance nitrogen requirements on fall and winter aquatic plant diets. Additionally, because a considerable proportion of ingested protein may be absorbed before it reaches the hindgut, nitrogen liberated from urea recycling may be an important nitrogen source for hindgut microbes. Consequently, the recycling of urea may aid in maintaining a vigorous hindgut microbe population and may explain, at least in part, the remarkable fiber digesting capabilities of this amphibious rodent.

Laboratory studies have shown that small endotherms exposed to cold temperatures or high-fiber diets increase food intake (Gross, Wang and Wunder 1985; Hammond and Wunder 1991; Derting and Bogue 1993). However, reduced digestive efficiency often results, since digestibility tends to vary inversely with feeding rate (Sibly 1981). To compensate, many small mammals increase the size of the alimentary tract, especially the small intestine and caecum (Hammond and Wunder 1991; Derting and Bogue 1993; Hammond 1993). These changes presumably increase the surface area and volume of the absorptive region of the gut, allowing for longer digesta retention time and greater absorptive efficiency (Loeb, Schwab and Demment 1991). However, due to the high turnover of gut tissue, the alimentary tract is one of the most metabolically expensive tissues to maintain (Gross, Wang and Wunder 1985; Brugger 1991; Hammond and Diamond 1992, 1994). Consequently, during the summer months when a wide array of food is available, it appears to be more advantageous for muskrats to selectively forage on high-quality, low-fiber plant sources, rather than by maintaining an enlarged alimentary tract. Indeed, digestibility trials conducted during the summer of 1991 (see Part II)

demonstrated that muskrats presented with a mixed diet containing 62.4% NDF and 6.58% protein, selectively consumed vegetation containing only 51.0% NDF (18.4% below ration level), but 8.96% protein (36.2% above ration level). Moreover, as noted above, muskrats that supplement their aquatic plant diet with animal tissue (Part III), even at low levels, may reap substantial nutritional benefits. Utilization of such tactics, together with the mobilization of surplus fat reserves during spring, may reduce the foraging effort required by these rodents and hence provide more time for dispersal and reproductive activities. In fact, the dependence on fat reserves was greatest in early spring when metabolizable energy intake levels were lowest (Table 5-2). However, the maintenance of minimal fat stores in the summer months does not necessarily mean that muskrats are nutritionally stressed during this period. It may simply indicate that energy intake is precisely balancing energy expenditure.

Beginning in late-summer, the translocation of nutrients from cattail shoots to underground rhizomes converts these root structures from a high-fiber forage to a diet rich in energy and soluble carbohydrates, and one with a relatively low water content (Appendix 1; Table 5-1). This phenolic change in the characteristics of cattail rhizomes has several important implications for muskrats in winter. For example, findings of Part V suggest that the low water content of cattail rhizomes allows muskrats to increase their intake of dry matter and assimilated energy while actually reducing their gross energy intake of wet vegetation during the fall and winter months. Because individual tubers can attain a mass of several hundred grams in winter, these findings suggest that in the face of declining water temperatures, muskrats are able to minimize the time and energy

expended in food gathering while maximizing metabolizable energy intake by selecting a diet of cattail rhizomes. Furthermore, because urea excretion increases with urine output (West 1985), utilization of this low-water content diet may partially account for the low nitrogen excretion and enhanced urea recycling observed in acclimatized muskrats during fall and winter.

Results of Parts II, V and VI suggest that a causal relationship exists between seasonal changes in the energy intake, organ morphology and basal metabolic rate of acclimatized muskrats (Fig. 6-2; Fig. 6-5). Indeed, beginning in late fall, muskrats exhibited parallel increases in metabolizable energy intake, basal metabolic rate, and masses of the alimentary tract, heart, spleen and liver. These adaptations appeared to be facilitated by a phenolic shift in the quality and nutrient profile of cattail, from a relatively high-fiber forage in the summer to a low-fiber, highly digestible food source in winter. Faced with potential late-winter freeze-outs and no option to breed during the ice-bound season, it may be advantageous for muskrats to increase forage intake and thus accrue lipid reserves beginning in late fall. To accommodate the additional intake of dry matter without compromising digestive and nutrient processing efficiencies, muskrats respond by increasing the masses of the alimentary tract and liver (Fig. 2-1). The observed enlargement of the heart in winter may be beneficial for transporting additional nutrients to various parts of the body during periods of high energy demand, such as rewarming following foraging bouts. Irrespective of need, such systems are metabolically costly to maintain, a direct consequence of which should be an increase in basal metabolic rate (Daan, Masman and Groenewold 1990; Konarzewski and Diamond 1994). Indeed,

the basal rate of metabolism of muskrats increased by >31% from mid-summer to late-winter. However, over this same period, muskrats were able to increase their intake of metabolizable energy by an average of 64%. Consequently, as demonstrated in Figure 6-5, it appears that the adoption of this metabolically "wasteful" strategy, perhaps in conjunction with other adaptations (eg. communal nesting, reduced motor activity), allows muskrats to realize a surfeit of energy for growth and lipid deposition during the winter months. Adoption of such tactics are undoubtedly critical to meeting the seasonal nutritional and energetic requirements of muskrats and may at least partially explain the broad geographical distribution and successful exploitation of a wide range of wetland habitats by this prominent North American rodent.

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Appendix I. Proximate composition of plant samples collected at Oak Hammock Marsh, Manitoba, 1991 and 1995

Table A1-1. Proximate composition (±SE) of plant samples collected at Oak Hammock Marsh, Manitoba, 1991 and 1995

	Ash (%)	Energy Content (kJ/g)	Crude Protein (%)	NDF (%)	ADF (%)
1991					
May 14					
Cattail rhizome[a]	8.07±0.98	16.68±0.15	7.23±0.82	47.28±1.51	20.86±0.88
Bladderwort[b]	16.17±1.60	16.71±0.31	19.81±2.43	37.02±1.67	42.52±3.68
Sedge[b]	6.26±0.53	18.21±0.07	16.97±0.82	62.92±1.00	32.16±0.09
June 4					
Cattail shoots[a]	10.49±0.40	17.59±0.19	14.05±1.22	62.62±2.34	39.12±2.03
Cattail leaves[a]	10.33±0.53	17.88±0.15	13.69±0.55	64.22±2.36	39.65±1.39
Cattail rhizome[a]	7.16±0.74	16.56±0.12	4.96±0.38	53.24±2.91	26.41±1.93
Bladderwort[c]	26.39	14.25	21.17	41.94	40.36
Sedge[b]	10.00±0.41	17.87±0.11	18.16±0.51	63.52±1.35	33.85±1.65
July 23					
Cattail shoots[a]	11.98±0.53	16.32±0.11	7.63±0.61	60.64±2.17	36.35±1.88
Cattail leaves[a]	7.64±0.46	18.61±0.22	12.34±0.32	65.64±1.20	40.41±0.80
Cattail rhizome[a]	13.17±0.48	16.26±0.07	6.18±0.54	63.70±2.39	40.02±2.28
Sedge[b]	9.46±0.75	17.65±0.18	12.39±1.23	63.57±1.04	35.31±0.01
White-top[b]	3.74±0.37	18.39±0.06	6.20±0.43	60.42±0.71	34.29±1.53
Duckweed[b]	19.66±1.28	14.15±0.13	9.05±0.25	43.31±0.53	22.38±0.55
September 27					
Cattail shoots[a]	6.15±0.22	17.64±0.05	2.20±0.16	64.06±1.09	42.22±1.11
Cattail leaves[a]	5.45±0.20	18.81±0.08	9.85±0.75	59.49±0.76	38.43±0.73
Cattail rhizome[a]	8.02±0.79	16.60±0.19	7.18±0.40	35.22±1.33	19.34±1.13
White-top[b]	4.44±0.03	18.25±0.05	5.49±0.56	58.54±3.66	34.84±2.45
October 26					
Cattail rhizome[b]	7.56±0.02	16.67±2.95	7.53±0.22	35.13±2.57	18.80±0.99
1995					
December 2, 13					
Cattail rhizome[a]	6.44	16.86	10.14	31.82	16.69
Cattail rhizome[a]	5.99	16.86	8.05	32.60	16.34

[a] $n = 6$
[b] $n = 2$
[c] $n = 1$

Statistics

Seasonal diet, sex and age effects were evaluated initially on blood and urine variables using 1-way ANOVAs. Data were pooled when differences related to diet, sex and age were nonsignificant ($P > 0.05$). All seasonal differences between means were tested using Duncan's New Multiple Range Test. Significance was set at the 5% level, and means are presented ±1 standard error (SE).

Serum samples from two sick animals collected in 1991-1992 were omitted from the analysis. Another animal (93-6; 15% diet) exhibited low food consumption values and appeared lethargic during its only feeding trial. Following blood sampling, this animal was subsequently removed from the experimental protocol and euthanized (data are presented for comparative purposes; Table A2-2).

In the 1991-1992 study, serum characteristics for magnesium ($\bar{x}$ = 2.93±1.68 mmol·L^{-1}; n = 72), glutamic oxaloacetic transaminase (GOT; $\bar{x}$ = 151.8±12.8 U·L^{-1}; n = 92) and gamma glutamyl transpeptidase (GGT; $\bar{x}$ = 10.8±2.3 U·L^{-1}; n = 72) did not vary ($P > 0.05$) by season, diet, sex or age and are not included in Table A2-2.

Table A2-1. Seasonal changes in mean (±SE) serum and urine chemistry variables of 90 acclimatized muskrats collected at Oak Hammock Marsh, Manitoba, 1991 - 1992.

Variable	May		July		Sept		Dec		Feb		April	
	n	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$
Hematocrit, %												
Adult	16	$42.17^{c}\pm0.82$	16	$40.80^{c,d}\pm0.95$	10	$38.11^{d}\pm1.14$	4	$49.13^{a}\pm4.00$	2	$48.82^{a,b}\pm1.19$	7	$42.93^{b,c}\pm0.90$
Subadult	-	-	-	-	8	$42.48^{b}\pm0.85$	13	$49.35^{a}\pm0.46$	9	$50.04^{a}\pm1.78$	5	$43.08^{b}\pm2.31$
$F_{[1,56]}$=10.45, P=0.0021												
Hemoglobin, g·dL^{-1}												
Adult	16	$15.68^{b}\pm0.48$	16	$14.94^{b,c}\pm0.40$	10	$14.06^{c}\pm0.46$	4	$18.11^{a}\pm1.08$	2	$18.58^{a}\pm0.16$	7	$16.04^{a,b}\pm0.43$
Subadult	-	-	-	-	8	$15.25^{b}\pm0.35$	13	$19.38^{a}\pm0.37$	9	$19.34^{a}\pm0.64$	5	$16.23^{b}\pm0.72$
$F_{[1,56]}$=13.02, P=0.0007												
Male	16	$15.68^{b,c}\pm0.48$	15	$15.01^{b,c}\pm0.42$	18	$14.59^{c}\pm0.32$	9	$19.80^{a}\pm0.60$	6	$20.10^{a}\pm0.74$	6	$16.31^{b}\pm0.54$
Female	-		1	13.90^{b}	-		8	$18.27^{a}\pm0.33$	5	$18.11^{a}\pm0.41$	6	$15.93^{b}\pm0.55$
$F_{[1,38]}$=5.11, P=0.0297												
Cholesterol, mmol·L^{-1}												
$F_{[5,84]}$=6.35, P<0.0001	16	$0.58^{b}\pm0.05$	16	$0.74^{b}\pm0.07$	18	$0.72^{b}\pm0.08$	17	$0.79^{b}\pm0.04$	11	$1.17^{a}\pm0.12$	12	$0.70^{b}\pm0.09$
Glucose, mmol·L^{-1}												
Adult	16	$6.3^{b}\pm0.5$	16	$4.5^{c}\pm0.5$	10	$7.2^{a,b}\pm0.4$	4	$6.9^{a,b}\pm0.3$	2	$9.2^{a}\pm0.8$	7	$8.0^{a}\pm0.2$
Subadult	-	-	-	-	8	$8.7^{b}\pm0.4$	13	$7.7^{b}\pm0.2$	9	$9.7^{a}\pm0.5$	5	$8.0^{b}\pm0.4$
$F_{[1,56]}$=7.73, P=0.0074												

Phosphorous, mmol·L^{-1}												
$F_{[5,84]}$=6.34, P<0.0001	16	2.3^{a}±0.2	16	2.3^{a}±0.1	18	2.5^{a}±0.1	17	1.7^{b}±0.1	11	2.5^{a}±0.1	12	2.16^{a}±0.11
Calcium, mmol·L^{-1}												
$F_{[5,84]}$=8.53, P<0.0001	16	2.5^{c,d}±0.03	16	2.4^{d}±0.03	18	2.7^{a}±0.04	17	2.6^{b,c}±0.02	11	2.6^{a,b}±0.02	12	2.6^{a,b}±0.04
Chloride, mmol·L^{-1}												
$F_{[5,84]}$=5.39, P=0.0002	16	97.4^{b,c}±0.6	16	97.7^{b,c}±0.8	18	97.4^{b,c}±0.7	17	100.9^{a}±0.7	11	98.6^{a,b}±1.0	12	95.25^{c}±1.18
Triglycerides, mg·dL^{-1}												
Adult	7	17.5^{c}±5.6	3	15.1^{b,c}±4.9	4	62.4^{a}±19.3	4	89.2^{a}±16.8	2	69.1^{a,b}±28.2	7	67.9^{a}±8.9
Subadult	-	-	-	-	8	50.3±5.3	13	38.6±3.7	9	38.4±5.0	5	45.6±6.9
$F_{[1,50]}$=22.92, P<0.0000												
Sodium, mmol·L^{-1}												
$F_{[5,84]}$=15.95, P<0.0001	16	140.1^{c}±0.7	16	140.7^{c}±0.3	18	142.6^{b}±0.4	17	141.0^{c}±0.6	11	145.6^{a}±0.6	12	145.1^{a}±0.3
Potassium, mmol·L^{-1}												
Digestibility	8	4.3^{a}±0.2	8	3.9^{a,b}±0.2	8	4.1^{a}±0.1	8	3.6^{b}±0.1	-	-	-	-
Control	8	5.2^{a}±0.5	8	4.1^{b}±0.2	10	4.4^{b}±0.1	9	4.2^{b}±0.2	11	3.9^{b}±0.2	12	4.4^{b}±0.2
$F_{[1,65]}$=10.53, P=0.0019												
Na:K ratio												
Digestibility	8	33.7^{b}±1.6	8	36.8^{a,b}±1.1	8	35.3^{b}±1.1	8	39.8^{a}±1.1	-	-	-	-
Control	8	28.2^{c}±2.2	8	34.5^{a,b}±1.3	10	32.2^{b,c}±0.7	9	33.9^{a,b}±1.3	11	37.6^{a}±1.6	12	33.7^{b}±1.2
$F_{[1,65]}$=14.92, P=0.0003												
Serum urea nitrogen, mmol·L^{-1}												
Digestibility	8	5.1^{a}±1.6	8	5.4^{a}±0.9	8	2.0^{b}±0.2	8	2.7^{a,b}±0.4	-	-	-	-
Control	8	8.2^{a}±1.4	8	8.8^{a}±0.5	10	3.3^{c}±0.5	9	2.9^{c}±0.2	11	5.3^{b}±0.6	12	3.9^{b,c}±0.6
$F_{[1,65]}$=5.07, P=0.0277												

Alkaline phosphatase, $U \cdot L^{-1}$												
Adult	16	586±48	16	465±33	10	475±52	4	445±45	2	524±244	7	534±91
Subadult	-	-	-	-	8	720±63	13	601±55	9	694±48	5	525±102
$F_{[1,56]}$=8.66, P=0.0047												
Male	16	586^{b}±48	15	462^{b}±35	18	584^{b}±49	9	$592^{a,b}$±80	6	780^{a}±47	6	$572^{a,b}$±60
Female	-		1	509	-	-	8	533±43	5	522±65	6	488±119
$F_{[1,39]}$=4.10, P=0.0499												
Creatine phosphokinase, $U \cdot L^{-1}$												
Digestibility	8	521^{b}±90	8	1125^{a}±192	8	652^{b}±69	8	374^{b}±52	-	-	-	-
Control	8	2094^{a}±892	8	$1617^{a,b}$±316	10	$1081^{a,b}$±335	9	441^{b}±83	11	527^{b}±65	12	395^{b}±49
$F_{[1,65]}$=4.99, P=0.0290												
Albumin, $G \cdot L^{-1}$												
Adult	16	$30.9^{a,b}$±0.7	16	29.0^{b}±1.3	10	32.5^{a}±1.0	4	$31.5^{a,b}$±0.5	2	$33.6^{a,b}$±0.2	7	$32.0^{a,b}$±1.6
Subadult	-	-	-	-	8	$35.8^{a,b}$±1.0	13	$33.8^{a,b}$±0.9	9	36.4^{b}±1.0	5	32.4^{b}±1.7
$F_{[1,56]}$=7.84, P=0.0070												
Globulin, $G \cdot L^{-1}$												
Adult	16	$24.3^{a,b}$±1.0	16	27.4^{a}±1.1	10	$24.7^{a,b}$±1.3	4	20.8^{b}±1.1	2	$25.9^{a,b}$±0.7	7	$23.3^{a,b}$±1.5
Subadult	-	-	-	-	8	18.8^{b}±0.8	13	17.9^{b}±0.9	9	18.7^{b}±0.7	5	22.8^{a}±1.4
$F_{[1,56]}$=26.73, P<0.0001												
Male	16	24.3^{b}±1.0	15	27.2^{a}±1.2	18	$22.0^{b,c}$±1.1	9	17.5^{d}±0.6	6	$19.4^{c,d}$±1.6	6	$20.5^{b,c,d}$±0.3
Female	-	-	1	$29.1^{a,b}$	-	-	8	19.8^{c}±1.4	5	$20.7^{b,c}$±1.5	6	25.7^{a}±1.4
$F_{[1,39]}$=6.89, P=0.0124												

AG ratio												
Adult	16	$1.30^{a}\pm0.05$	16	$1.08^{b}\pm0.06$	10	$1.35^{a}\pm0.09$	4	$1.53^{a}\pm0.06$	2	$1.30^{a,b}\pm0.04$	7	$1.41^{a}\pm0.12$
Subadult	-	-	-	-	8	$1.93^{a}\pm0.09$	13	$1.95^{a}\pm0.11$	9	$1.98^{a}\pm0.11$	5	$1.45^{b}\pm0.14$
$F_{[1,56]}=26.73$, $P<0.0001$												
Male	16	$1.30^{c}\pm0.05$	15	$1.08^{d}\pm0.06$	18	$1.61^{b}\pm0.09$	9	$1.95^{a}\pm0.12$	6	$1.97^{a,b}\pm0.16$	6	$1.62^{b}\pm0.04$
Female	-	-	1	$1.10^{a,b}$	-	-	8	$1.74^{a}\pm0.15$	5	$1.72^{a,b}\pm0.17$	6	$1.24^{b}\pm0.13$
$F_{[1,39]}=6.89$, $P=0.0124$												
Total bilirubin, $\mu mol \cdot L^{-1}$												
$F_{[4,67]}=11.95$, $P<0.0001$	-	-	16	$8.5^{a}\pm0.9$	18	$5.0^{b}\pm0.3$	15	$7.5^{a}\pm0.7$	11	$8.9^{a}\pm0.7$	12	$3.6^{b}\pm0.4$
Urinary urea nitrogen, $mg \cdot dL^{-1}$												
Adult	14	$443^{b}\pm149$	15	$272^{b}\pm55$	6	$39^{c}\pm17$	3	$520^{a,b,c}\pm434$	2	$974^{a,b}\pm30$	5	$920^{a}\pm292$
Subadult	-	-	-	-	6	$90^{b}\pm20$	11	$109^{b}\pm32$	5	$619^{a}\pm196$	3	$239^{b}\pm203$
$F_{[1,39]}=4.47$, $P=0.0408$												
Digestibility	7	224 ± 119	8	154 ± 41	5	23 ± 6	5	79 ± 14	-	-	-	-
Control	7	$643^{a}\pm259$	7	$407^{a,b}\pm85$	7	$94^{b}\pm17$	9	$263^{a,b}\pm146$	7	$720^{a}\pm150$	8	$665^{a}\pm224$
$F_{[1,53]}=4.95$, $P=0.0304$												
Urinary creatinine, $mg \cdot dL^{-1}$												
$F_{[5,65]}=11.05$, $P<0.0001$	14	$13.1^{b,c}\pm2.7$	15	$11.2^{c}\pm2.1$	12	$17.0^{b,c}\pm3.3$	14	$15.8^{b,c}\pm6.0$	8	$51.7^{a}\pm31.6$	8	$26.4^{b}\pm13.9$
Urinary U:C ratio												
$F_{[5,64]}=2.51$, $P=0.0388$	14	$25.8^{a}\pm4.8$	15	$30.7^{a}\pm9.1$	12	$5.0^{b}\pm1.7$	14	$11.2^{b}\pm4.1$	7	$18.2^{a,b}\pm5.1$	8	$27.9^{a,b}\pm11.2$

Note: Within each row, means sharing the same letter are not significantly different ($P > 0.05$).

Table A2-2. Serum chemistry of nine adult male muskrats maintained on three diets differing in animal tissue content (0, 5, 15%). Feeding trials were conducted at the University of Manitoba, June 9-August 14, 1993.

Variable	93-5 (0%)	93-9 (0%)	93-3 (5%)	93-4 (5%)	93-7 (5%)	93-8 (5%)	93-1 (15%)	93-2 (15%)	93-6 (15%)
Hematocrit, %	44.75	44.00	46.25	43.50	43.75	40.25	46.50	46.00	-
Magnesium, $mmol \cdot L^{-1}$	1.01	0.93	1.20	0.92	1.28	1.04	1.28	1.05	1.07
Sodium, $mmol \cdot L^{-1}$	145	143	140	143	144	143	143	144	142
Potassium, $mmol \cdot L^{-1}$	4.9	4.7	5.1	4.2	5.8	4.5	4.8	4.1	5.2
Na/K ratio	30	30	27	34	25	32	30	35	27
Chloride, $mmol \cdot L^{-1}$	101	101	96	93	98	96	99	96	96
Urea nitrogen, $mmol \cdot L^{-1}$	9.1	8.6	7.7	11.6	7.1	8.2	19.3	15.5	15.5
Creatinine, $mmol \cdot L^{-1}$	0.06	0.08	0.06	0.06	0.07	0.09	0.06	0.07	0.05
Serum U:C ratio	151.7	107.5	128.3	193.3	101.4	91.1	321.7	221.4	310.0
Glucose, $mmol \cdot L^{-1}$	5.0	5.4	6.9	7.0	8.8	7.1	6.4	8.3	6.7
Creatine phosphokinase, $U \cdot L^{-1}$	332	219	504	82	190	183	129	175	292
Glutamic oxaloacetic transaminase, $U \cdot L^{-1}$	40	46	58	16	25	48	74	32	36
Alanine aminotransferase, $U \cdot L^{-1}$	74	125	120	27	56	63	116	80	59
Gamma glutamyl transpeptidase, $U \cdot L^{-1}$	21	32	<1	0	2	0	21	1	0
Alkaline phosphatase, $U \cdot L^{-1}$	430	515	216	236	237	292	474	314	189