Nutrition and the energetic tactics of muskrats (*Ondatra zibethicus*): morphological and metabolic adjustments to seasonal shifts in diet quality

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Abstract: Basal metabolic rate (BMR), serum thyroxine (T_4) concentration, lean organ mass, and body composition were measured in 94 captive, seasonally acclimatized muskrats (*Ondatra zibethicus*) 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 1994–1995. Mass-independent BMRs ($kJ \cdot kg^{-0.67} \cdot h^{-1}$) and serum T_4 concentrations ($nmol \cdot L^{-1}$) varied significantly over the year ($P < 0.0001$), with mean values in February exceeding July values by 31.1 and 77.2%, respectively. These variables tracked seasonal changes in the neutral detergent soluble (NDS) content of broadleaf cattails (*Typha latifolia*), the dominant food of muskrats in the study population. From July through February, alimentary tract, liver, spleen, and heart masses increased, while kidney mass declined. Body fat stores varied significantly over both years, with peak values measured in February. However, lean body and pelt masses 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 heart and alimentary tract masses. Annual variations in basal energy expenditure, serum T_4 concentration, and organ masses of wild muskrats appear to be linked to seasonal changes 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.

Résumé : Le taux de métabolisme de base (BMR), la concentration de thyroxine (T_4) sérique, la masse des organes sans les graisses et la composition corporelle ont été mesurés chez 94 Rats-musqués communs (*Ondatra zibethicus*) acclimatés aux conditions saisonnières, entre mai 1991 et avril 1992. Des mesures saisonnières de la consommation d'oxygène, du contenu hydrique total et de la masse ont été obtenues chez 124 animaux additionnels, libres ou en captivité, en 1994–1995. Le taux de métabolisme de base, indépendamment de la masse ($kJ \cdot kg^{-0,67} \cdot h$), et la concentration de T_4 sérique ($nmol \cdot L$) variaient significativement au cours d'une année ($P < 0,0001$) et les valeurs moyennes de ces variables en février excédaient celles de juillet de 31,1% dans le cas du taux de métabolisme et de 77,2% dans le cas de la concentration de T_4. Ces variables reflétaient les changements saisonniers de la fraction soluble dans les détergents neutres (NDS) des quenouilles (*Typha latifolia*), la principale source de nourriture de la population étudiée. De juillet à février, la masse du tube digestif, du foie, de la rate et du coeur ont augmenté, alors que la masse des reins a diminué. Les réserves de graisses ont varié significativement au cours des 2 années et les valeurs maximales ont été enregistrées en février. Cependant, la masse du corps sans les graisses et la masse de la fourrure variaient peu d'une saison à l'autre ($P > 0,05$). Une procédure de régression multiple pas à pas et des analyses des composantes principales ont montré que la variation du taux de métabolisme de base semble associée surtout aux changements de masse du coeur et du tube digestif. Les variations annuelles de la dépense énergétique de base, de la concentration de T_4 sérique et de la masse des organes chez les rats-musqués en nature semblent reliées aux changements saisonniers de la fraction NDS de la végétation consommée et aux changements dans la consommation d'énergie et peuvent représenter des facteurs à influence importante sur les patterns annuels d'accumulation et d'utilisation des graisses chez ce rongeur semi-aquatique.
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Introduction

The muskrat (*Ondatra zibethicus*) is the largest member of the subfamily Arvicolidae, the species' large size being an attribute possibly linked to its 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 that 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. In muskrats, unlike most nonhibernating rodents, substantial lipid stores accrue during late fall and winter (Jelinski 1989; Virgl and Messier 1992*a*, 1992*b*). It has been suggested that fat storage in this rodent may be facilitated by a winter reduction in thyroid activity and lean body mass, and hence a reduction in basal metabolic costs

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(Aleksiuk and Frohlinger 1971; Virgl and Messier 1992*a*, 1995). In a recent study of free-ranging muskrats (Campbell et al. 1998), however, we found that the intake of assimilated energy was substantially higher (>60%) in winter than in summer. 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 a combination of 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 the body mass (Merritt 1986; Nagy et al. 1995), organ morphology (Aleksiuk and Frohlinger 1971; Virgl and Messier 1992*a*, 1992*b*), blood chemistry (Morton and Lewis 1980; DelGiudice et al. 1990), and basal metabolic rate (BMR) (Wunder et al. 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 energy demands. These organs exhibit high mass-specific rates of metabolism, and their relative masses may account for significant variation in BMR (Daan et al. 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 there is a similar relationship in wild populations (Daan et al. 1990).

To address these shortcomings, we initiated a study to first determine if seasonal changes in the energy and nutrient profile of broadleaf cattails (*Typha latifolia*), the dominant food of muskrats in prairie marshes, are accompanied by modifications in BMR, thyroid activity, organ morphology, lean body mass, and proximate composition of muskrats. Although seasonal adjustments in body size and endogenous energy reserves have been demonstrated in several species (Iverson and Turner 1974; Nagy et al. 1995), few longitudinal data exist for individuals in wild populations (Merritt 1984, 1986). Therefore, our final objective was to monitor temporal changes in the mass and lipid content of individually marked free-living muskrats by means of the deuterium oxide (D_2O) dilution technique. Such information is vital to defining the seasonal energy and nutritional constraints imposed by a northern wetland environment on these amphibious rodents.

Materials and methods

Vegetation analyses

To evaluate seasonal changes in the energy content and nutrient composition of cattails at Oak Hammock Marsh, Manitoba (50°06′N, 97°07′W), we 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 from duplicate measurements made in an adiabatic oxygen bomb calorimeter (Parr 1241 Calorimeter, Parr Instrument Co., Moline, Ill.). Ash content was determined following combustion 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 (NDF) 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 as the neutral detergent soluble (NDS) content 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 livetrapped at Oak Hammock Marsh in 1991–1992 ($n = 92$) and 1994–1995 ($n = 33$). Immediately following capture, muskrats were transported to the Animal Holding Facility, University of Manitoba, and were housed individually at 14 ± 1°C with a 12 h light : 12 h dark photoperiod (MacArthur 1979). All muskrats were maintained on natural vegetation throughout their stay in captivity and were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

In 1991–1992, experiments were performed during 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 that 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 Plexiglas 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 · 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 CO_2 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 et al. 1984).

As part of another study (Campbell and MacArthur 1997), additional $\dot{V}_{O_2}$ measurements were obtained from 33 muskrats that were collected over four test periods in 1994–1995 ($n = 8$–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 d of capture. In each case, the animal was lightly anaesthetized and injected with a small dose of ^{14}C-urea 15 min before the start of an 8-h metabolic trial (see Campbell and MacArthur 1997). In the 1994–1995 trials, the chamber temperature was kept at 22° ± 1°C, the inlet flow rate was held at 2 L · min^{-1}, and only $\dot{V}_{O_2}$ was measured. 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, the muskrats were weighed before and after each trial, and each animal was tested only once.

Proximate analyses of carcasses

On the day following metabolic testing, 62 of the muskrats studied in 1991–1992 were euthanized with an overdose of halothane anaesthetic (M.T.C. Pharmaceuticals). The remaining 32 animals were used in 10-d digestibility trials and then sacrificed in a similar manner

(see Campbell and MacArthur 1996). Blood samples were obtained from 89 of these muskrats and allowed to clot. Following centrifugation, serum was extracted and stored on ice. Serum thyroxine (T_4) concentrations were determined with a fluorescence polarization immunoassay technique utilizing competitive antigen binding methodology (Levinson et al. 1992).

Standard body measurements were obtained from freshly killed animals, and the heart, kidneys, liver, adrenal glands, spleen, and gastrointestinal tract excised. Dry masses of the alimentary tracts of muskrats collected in 1991–1992 are reported in Campbell and MacArthur (1996). 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 old) from prebreeding subadults (<10 months old). Beer and Meyer (1951) and Aleksiuk and Frohlinger (1971) reported a strong relationship between age and adrenal mass in muskrats. We found that age estimated from adrenal mass was identical with that predicted from baculum morphology ($r = 1.0$, $n = 72$, $P < 0.0001$), and we 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 reweighed 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 (for a minimum of 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 the carcass dry matter. Carcass and pelt samples were sent to a commercial laboratory (Norwest Labs, Winnipeg, Man.) and analyzed separately for lipid and protein contents. Neutral lipid content was determined by refluxing each sample with petroleum ether in a Soxhlet apparatus (Method 954.02, Association of Official Analytical Chemists (AOAC) 1990), and the crude protein content of each sample was estimated as Kjeldahl nitrogen × 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 the 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 means of this method (47.73 ± 0.50% of IFBM) was consistent with that reported for other mammals (44.4–49.5%; Calder 1984, p. 20).

Deuterium oxide studies

In total, 129 estimates of total body water (TBW) content 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 TBW 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, and sexed, and a preweighed intraperitoneal injection of D_2O (99.9% purity, ICN Biochemicals) was administered. The dose (1–3 g $D_2O \cdot kg^{-1}$ body mass) was measured to the nearest 0.1 mg by weighing the sample syringe on an analytical balance (Mettler Model AJ100) before and after injection. Following injection, each animal was tagged with two Size 1 Monel ear tags and a subcutaneous microchip transponder (Avid Marketing Inc., Norco, Calif.) inserted near the base of the tail. After a 3-h equilibration period, muskrats were again anaesthetized and weighed, and a 3- to 4-mL blood sample was obtained by cardiac puncture. Following their recovery from anaesthesia (ca. 0.5 h), muskrats were released at their sites of capture.

Serum samples were distilled by vacuum sublimation (Stansell and Mojica 1968) and the deuterium concentration was measured at 2510.0 cm^{-1} (wavelength 3.8 μm) using a Model 881 Perkin–Elmer dual-beam infrared spectrophotometer and standard calcium fluoride cells. As the D_2O technique can overestimate the TBW content of mammals by up to 12.0% (Nagy and Costa 1980), a correction factor of 0.929 obtained from 15 muskrats with known water contents (Campbell et al. 1998) was applied to all TBW content estimates. To further correct for preformed water in the gut, and thus calculate the ingesta-free TBW content, we then multiplied the adjusted estimate of TBW content by a second correction factor (0.978) obtained by subtracting the average water content of the ingesta from the known TBW content of the above 15 animals.

Data analyses

Forage quality

Seasonal variation in the NDS and caloric contents of the stems, leaves, and rhizomes of cattails was assessed using one-way ANOVA followed by Tukey's Studentized range test for comparisons of means when significant model effects were detected.

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 et al. 1977). Unfortunately, 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, we pooled all of our basal $\dot{V}_{O_2}$ data for the two years of the study, and regressed log basal $\dot{V}_{O_2}$ (mL $O_2 \cdot h^{-1}$) on log body mass (kg). This procedure yielded the following allometric equation: $\dot{V}_{O_2} = 700\ \text{mass}^{0.676}$ ($r^2 = 0.431$, df = 124, $P = 0.0001$). We therefore calculated mass-independent $\dot{V}_{O_2}$ and BMR using body mass$^{0.67}$, as recommended by Heusner (1982). Basal $\dot{V}_{O_2}$ values for 1991–1992 and 1994–1995 animals were analyzed separately. Mean basal $\dot{V}_{O_2}$ values for each month were compared between males and females and between adults and subadults, including their interaction terms, using two-way ANOVA (SAS Institute Inc. 1990). A similar test was utilized to compare monthly serum T_4 concentrations. To avoid any bias associated with expressing metabolic rate as a ratio of body mass (Packard and Boardman 1988), we also evaluated seasonal variation in $\dot{V}_{O_2}$ (mL $O_2 \cdot h^{-1}$) and BMR (kJ $\cdot$ $kg^{-1} \cdot h^{-1}$) with a two-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 utilizing estimates of structural size. We therefore compared body composition variables (water, ash, protein, and total lipids) and organ masses for males versus females, adults versus subadults, and postdigestibility trial (11 d in captivity) versus recent capture (<3 d in captivity), using two-way ANCOVAs with IFBM as the covariate.

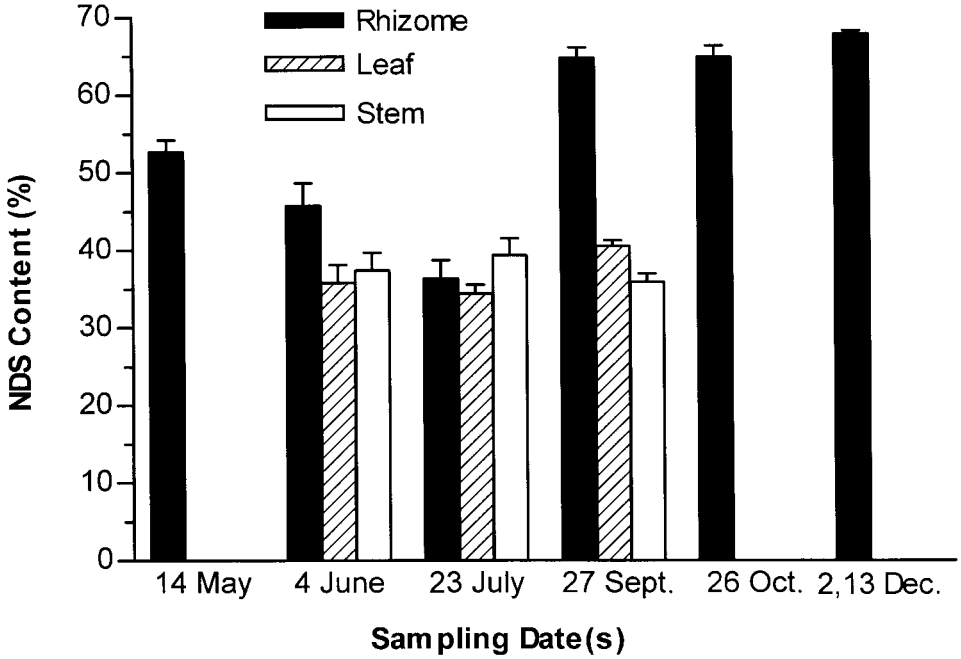
Deuterium oxide studies

Body fat content estimates determined by means of the D_2O method were analyzed using (*i*) only the initial fat estimate for each muskrat ($n = 91$) and (*ii*) 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 our primary objectives was to examine individual and seasonal variability in BMR in relation to changes in organ and tissue morphology.

Fig. 1. Seasonal changes in the neutral detergent soluble (NDS) content of rhizomes, shoots, and leaves of cattail (*Typha latifolia*) collected at Oak Hammock Marsh, Manitoba, from May through October 1991. The value for December 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.



We entered these variables into a stepwise multiple regression model using BMR as the dependent variable and the masses of the different organs and tissues as independent predictor variables (McDevitt and Speakman 1994). However, given the potential for autocorrelation among organ and tissue masses, we also conducted a principal component factor extraction analysis on them ($n = 10$ 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 ± 1 standard error.

Results

Forage quality

Monthly variation in NDS content of cattail shoots and leaves ranged from 36.0 to 40.5% (Fig. 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%). The leaf component of cattails 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. 1). Over the same period, the gross energy content of cattail rhizomes varied only from 16.26 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 (from 5.45 ± 0.22 to 13.17 ± 0.45%).

Metabolic trials

On a mass-specific (per gram) basis, subadults generally exhibited higher rates of oxygen consumption than adults in both 1991–1992 and 1994–1995 (Table 1). However, as winter progressed, the differences between these age groups diminished. When metabolic data were corrected for intraspecific size variation using mass$^{0.67}$ or ANCOVA, we observed no effect of age-class, sex, or their interaction terms ($P > 0.05$ in all cases). Consequently, data for adult and subadult muskrats of both sexes were pooled in subsequent analyses. In both years, we observed significant seasonal changes in basal $\dot{V}O_2$ (1991–1992: $F_{[5,86]} = 6.93$, $P < 0.0001$; 1994–1995: $F_{[3,29]} = 3.03$, $P = 0.0450$), with the lowest rates recorded from April through September and the highest rates from October through February. Mass-independent BMR (kJ · kg$^{-0.67}$ · h^{-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. 2).

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

Proximate analyses of carcasses

Total body protein content was relatively constant, accounting

Table 1. Seasonal variation in body mass and basal oxygen consumption rate ($\dot{V}_{O_2}$) of 125 acclimatized muskrats livetrapped at Oak Hammock Marsh, Manitoba, in 1991–1992 and 1994–1995.

	Adults			Subadults		
	n	Mass (g)	$\dot{V}_{O_2}$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)	*n*	Mass (g)	$\dot{V}_{O_2}$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
1991–1992						
May	17	933±28	0.761±0.023	—	—	—
July	16	916±26	0.627±0.025	—	—	—
September	10	1065±36	0.731±0.038	8	714±25	0.800±0.054
December	4	872±69	0.726±0.025	14	617±24	0.924±0.039
February	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
1994–1995						
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	—	—	—

for about 21% of IFBM during all sampling periods ($F_{[5,88]} = 1.10$, $P = 0.368$; Fig. 2). Body protein content (g) was strongly predicted by IFBM (g) and TBW (g): protein = –9.71 + 0.22 × IFBM ($r^2 = 0.96$, df = 93, $P < 0.0001$) and –0.145 + 0.305 × TBW ($r^2 = 0.90$, df = 93, $P < 0.0001$), respectively. The ash content of muskrats varied with the sampling period ($F_{[5,88]} = 14.74$, $P < 0.0001$), being 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 ($F_{[5,88]} = 48.89$, $P < 0.0001$; Fig. 2). As expected, body water content also exhibited strong seasonal variation ($F_{[5,88]} = 35.35$, $P < 0.0001$), varying inversely with body fat content: %fat = 77.08 – 1.06 × ingesta-free %TBW ($r^2 = 0.86$, df = 93, $P < 0.0001$). Subadults had a 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 1994–1995, followed a similar seasonal trend to the 1991–1992 results, based on carcass analyses. The average lipid content of free-ranging muskrats (Fig. 3) ranged from a minimum of 0.8 ± 0.4% in July to a maximum of 7.5 ± 0.9% in February ($F_{[4,124]} = 35.53$, $P < 0.0001$).

In total, 50 recaptures were made of 34 tagged muskrats that had been previously caught and sampled (Fig. 4). Recapture data indicated that all muskrats gained mass during the July–September trapping intervals ($n = 12$). From September through May, 86% of muskrats >800 g ($n = 7$) lost mass, while 87% of animals <800 g ($n = 31$) maintained or increased mass (mean increase = 10.6 ± 2.5%). For the latter cohort, TBW content increased by an average of 6.6 ± 2.5%, the greatest increases occurring during the September–December (+8.5%) and February–May (+16.9%) trapping intervals (Fig. 4).

Organ masses and their relationship to BMR

The masses of most individual organs increased between summer and late winter, a pattern clearly reflected in total organ mass (Fig. 2; $F_{[5,88]} = 20.20$, $P < 0.0001$). Muskrats exhibited hypertrophy of the alimentary tract (+17.9%), heart (+12.7%), liver (+68.2%), and spleen (+51.0%) from July through February ($F_{[5,88]} = 3.79–32.37$, $P < 0.005$). However, over the same period, kidney mass decreased by 19.1% (Fig. 2). Although values were generally highest in February, we observed no significant seasonal variation in 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 we had measurements of both organ masses and BMR, we 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 2). 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 2).

Given the potential for autocorrelation among these variables, we also employed a principal component analysis to extract orthogonal axes of variability in organ and tissue masses of muskrats (Table 3). 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 2). Not surprisingly, the regression equation was dominated by the first principal component, which alone explained 20.5% of the variation in BMR. The second (fat content) and sixth (alimentary tract, adrenals, and heart)

Fig. 2. Seasonal changes in body composition (*a*), dry organ masses (*b*), and serum thyroxine levels and basal metabolic rates (BMR) (*c*) of acclimatized muskrats. Means with the same letters are not significantly different ($P > 0.05$). Vertical lines denote 1 SEM; numbers in parentheses are sample sizes. Values for all organs are adjusted means with ingesta-free body mass (mean = 772 g) as the covariate.

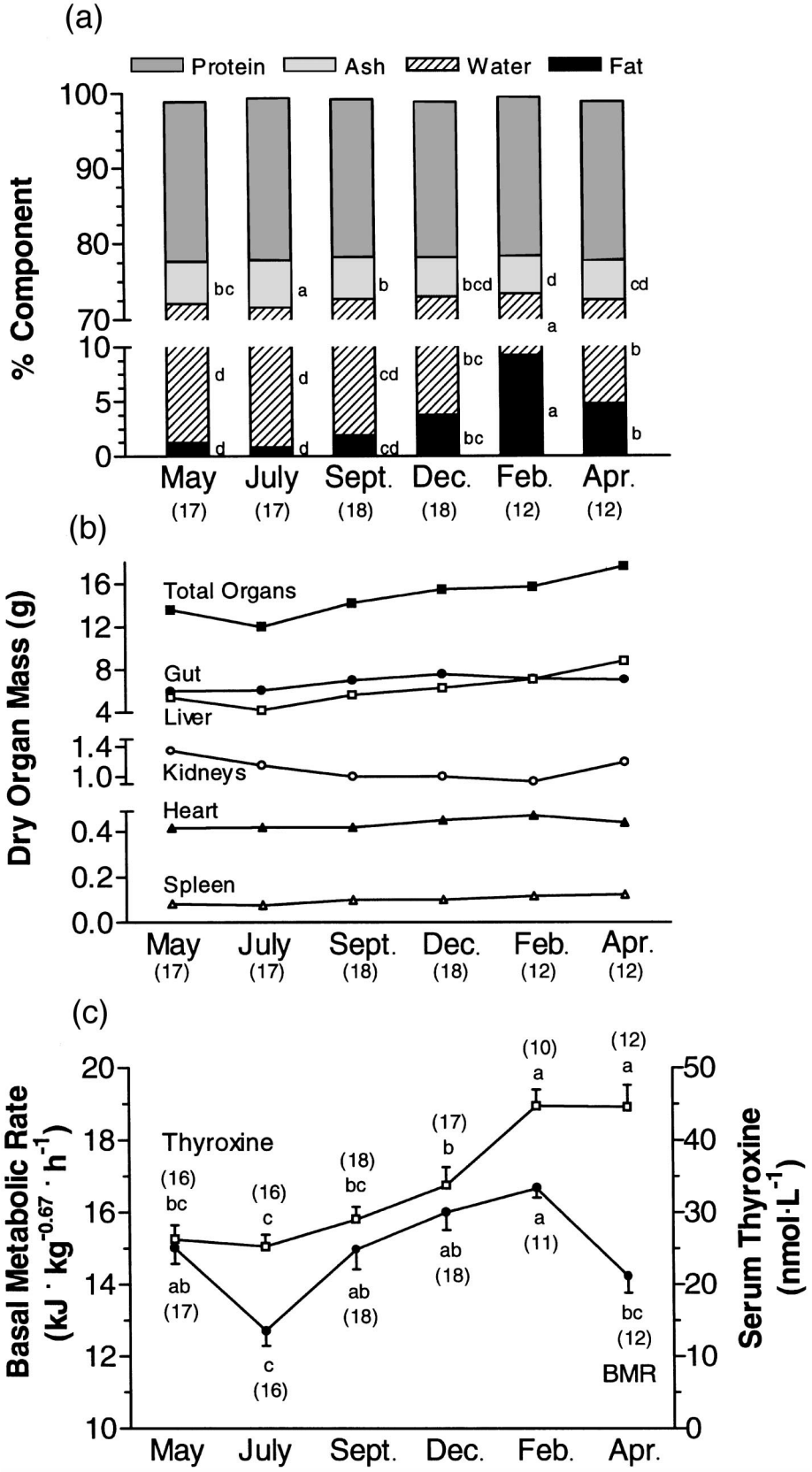


Fig. 3. Seasonal changes in the body fat content of wild muskrats measured via the deuterium oxide dilution technique (see the text for details). In total, 124 measurements were obtained from 91 animals captured at Oak Hammock Marsh, Manitoba, between July 12, 1994, and May 12, 1995. Means with the same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

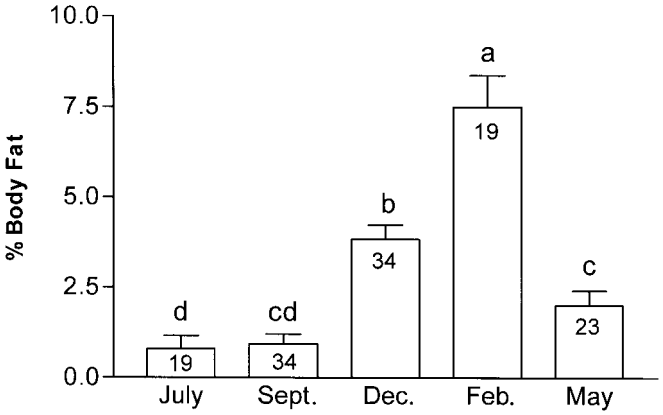


Table 2. Output of a stepwise multiple regression analysis with BMR set as the dependent variable and either dry masses of all 10 organs and tissues or scores on all 10 principal components describing variability in organ dry masses entered as the independent variables. Measurements are based on 89 seasonally acclimatized muskrats livetrapped at Oak Hammock Marsh, Manitoba, between May 8, 1991, and April 17, 1992.

Predictor	Coefficient	SE	r^2	P
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 components explained a further 7.7 and 2.3% of the variance, respectively.

Relationship of daily energy intake to BMR and organ masses

We obtained estimates of both BMR (this study) and daily gross energy intake, GEI (see Campbell and MacArthur 1996), 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, $P = 0.031$), indicating that animals with higher BMRs tended also to have higher rates of

Table 3. Principal component 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 4. Correlation coefficients relating gross energy intake ($kJ \cdot d^{-1}$) 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

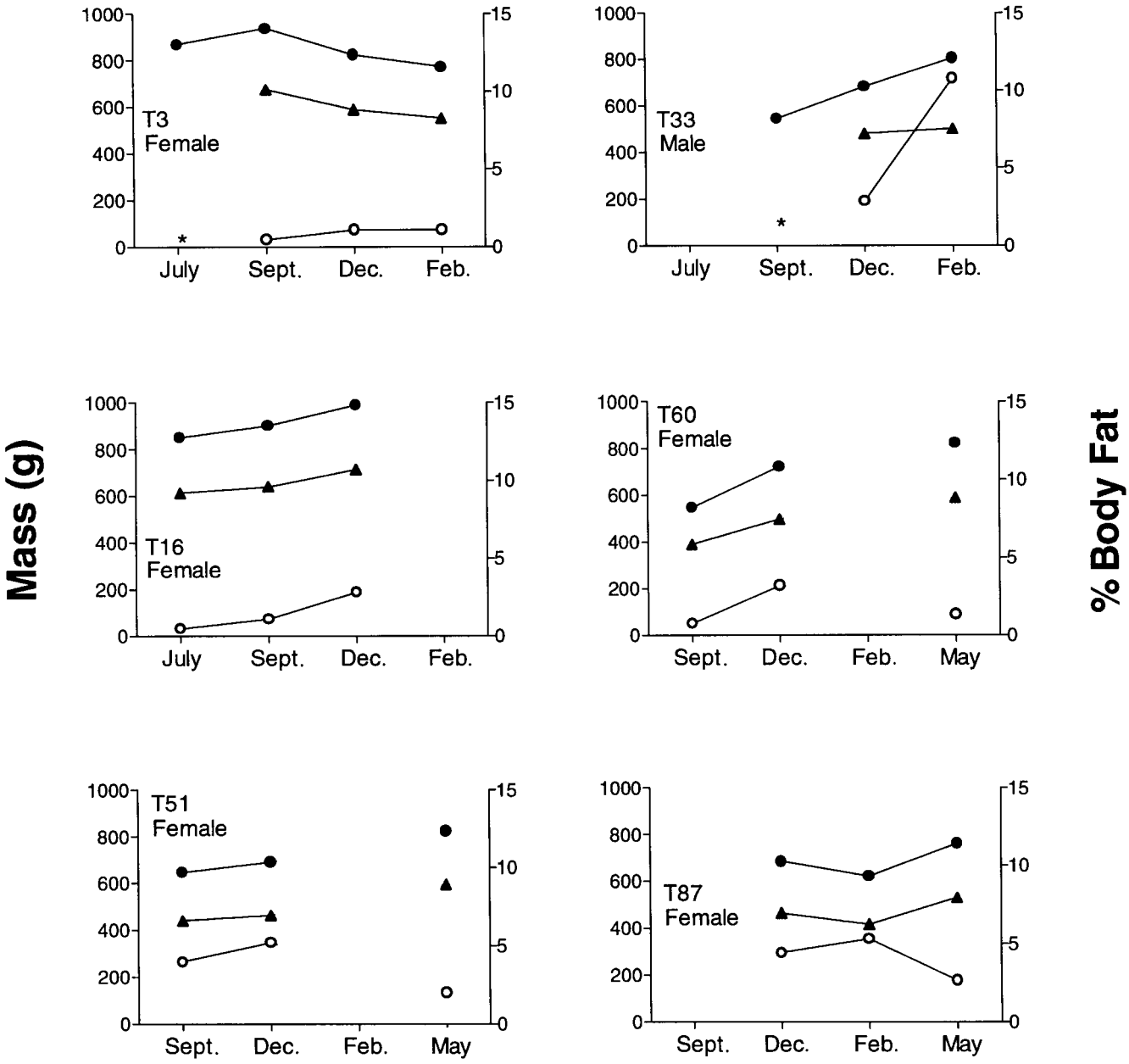
[a]df = 31 in all cases.

energy intake. For the same 32 animals, daily GEI varied positively with the dry masses of the stomach, caecum, and large intestine but not the small intestine (Table 4). Furthermore, daily GEI correlated significantly with heart mass and marginally with adrenal and liver mass, but not at all with either spleen or kidney mass.

Discussion

Nutrition has been described as the mechanistic thread linking wildlife populations to their environment (DelGiudice et al. 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, we found that annual changes in forage quality (Fig. 1) and energy intake (Campbell and MacArthur 1996; Campbell et al. 1998) were accompanied by changes in basal energy expenditure, serum T_4 concentration, body lipid stores, and organ sizes of seasonally acclimatized muskrats

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

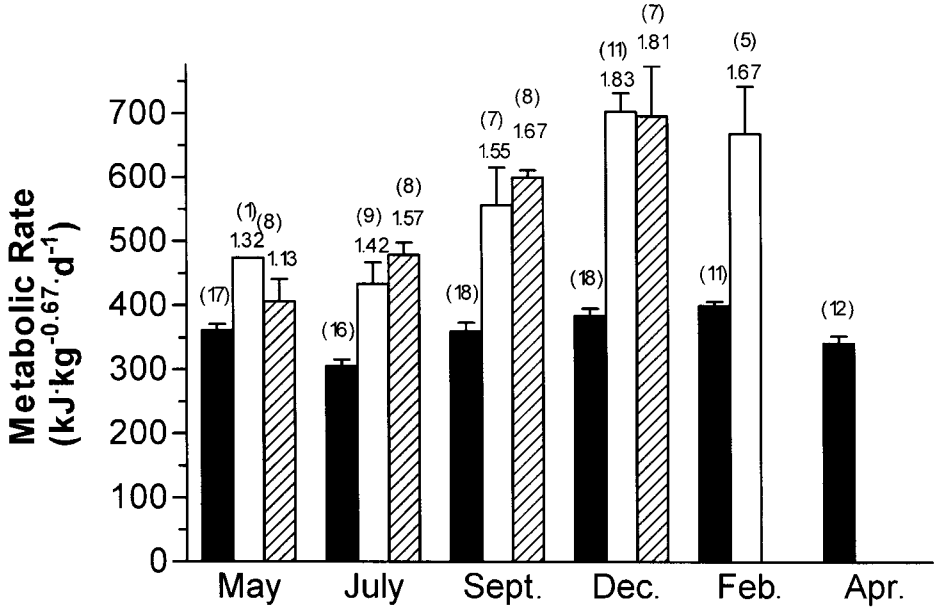


(Fig. 2). It should be noted that since we were studying seasonally acclimatized animals, we cannot conclusively isolate the effects of diet quality and energy intake from temperature, photoperiod, and other factors that may have contributed to the observed seasonal trends. Additionally, as no females were collected during the breeding season, the results presented herein exclude the energy costs associated with pregnancy and lactation.

Relationships between organ masses, BMR, and daily energy intake

Our finding that GEI tracked seasonal changes in stomach, caecum, large intestine, heart, and liver masses (Fig. 2) is consistent with the hypothesis that enlargement of these organs is associated with the need to absorb, metabolize, and transport the 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 demonstrated by their dominant effect in multiple regression models. The 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%), incur high maintenance costs. Body fat was also a significant predictor of BMR in both multiple regression and principal component (PC2) analyses (Tables 2 and 3).

Fig. 5. Relationship between seasonal changes in basal metabolic rate (BMR) of acclimatized muskrats (solid bars; this study) and metabolizable energy intake (MEI) of captive (hatched bars; Campbell and MacArthur 1996) and free-ranging (open bars; Campbell et al. 1998) muskrats. The numbers immediately above the bars indicate the mean MEI:BMR ratio and the numbers in parentheses show the sample size for each month. Vertical lines denote 1 SEM.



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 our body fat and BMR measurements (Fig. 2). It is conceivable that the relationship we observed between BMR and total body fat is linked to seasonal changes in brown adipose tissue and, therefore, to the 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. 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 allow disposal of the additional metabolic wastes associated with an increased rate of metabolism (Hammond et al. 1994; Konarzewski and Diamond 1994). However, our findings suggest that energy intake may not be the primary factor regulating kidney mass in 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 (Campbell and MacArthur 1997; Campbell et al. 1998).

If the masses of organs associated with nutrient assimilation are important determinants of both BMR and rate of food intake, the latter variables should be strongly correlated. However, few attempts have been made to collect both BMR and daily energy intake data 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). Our results clearly indicate that seasonal changes in metabolizable energy intake (MEI) of both captive and free-living muskrats (Campbell and MacArthur 1996; Campbell et al. 1998) closely follow seasonal adjustments in BMR (Fig. 5). Furthermore, in the 32 animals for which we had measurements of both BMR and GEI, we found that these variables were closely correlated ($P = 0.031$).

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). Our findings are consistent with these arguments and, in fact, variation in serum T_4 concentration in muskrats (Fig. 2) followed a similar trend to seasonal changes in forage quality (Fig. 1), energy intake (Campbell and MacArthur 1996; Campbell et al. 1998), organ masses, and BMR (Fig. 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 et al. 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 et al. 1977).

Muskrats do not appear to conform to this model, since they often increased body mass (Fig. 4), yet exhibited higher mass-specific $\dot{V}O_2$ and mass-independent BMR, beginning in late fall and continuing throughout winter (Table 1, Fig. 2). While a reduction in mass should theoretically lower basal energy requirements, it may also facilitate heat loss, owing to an increase

in the 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 the maximal metabolic rate (Wunder et al. 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 they are foraging in near-freezing water and daily energy expenditure appears to be greatest (Fig. 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

Our findings confirm the results of earlier studies indicating that muskrats accrue substantial lipid stores during winter (Aleksiuk and Frohlinger 1971; Jelinski 1989; Virgl and Messier 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). Our findings are not entirely consistent with this hypothesis. We found, for instance, that muskrats maintained lean muscle mass (Fig. 2) while increasing BMR (this study) and MEI (Campbell and MacArthur 1996; Campbell et al. 1998) from midsummer through late winter (Fig. 5). Moreover, most subadults for which we obtained recapture data gained mass and exhibited increases in TBW content from September through May (Fig. 4). As the absolute protein mass of muskrats is closely tied to water space (this study; Virgl and Messier 1993), these data strongly suggest that somatic growth continues during the winter months in our study population. Nagy et al. (1995) and Merritt (1986) reported similar seasonal changes in the body mass of collared lemmings (*Dicrostonyx groenlandicus*) and short-tailed shrews (*Blarina brevicauda*). Like the muskrat, these species have also been shown to have access to abundant, high-quality food sources and buffered microenvironments 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 et al. 1990; Weiner 1992). For terrestrial mammals, this ratio typically varies from 1.30 to 5.25 (Karasov 1992). The MEI:BMR ratios derived for penned, seasonally acclimatized (Campbell and MacArthur 1996) and free-ranging (Campbell et al. 1998) muskrats are close to the lower end of this range (mean = 1.55; Fig. 5). Interestingly, the MEI:BMR ratios of free-living muskrats in winter (1.67–1.83) are close to the ratio (1.7) reported for captive beaver (*Castor canadensis*) held in a simulated winter microhabitat (Dyck and MacArthur 1993). Both of these species must forage under ice during winter and, like muskrats, beaver accumulate substantial fat deposits during this period (Soprovich 1994).

As noted earlier, hyperphagia may induce hypertrophy of metabolically active organs and thus lead to a higher BMR. This hypothesis predicts that concurrent increases in MEI and BMR will result in the maintenance of a relatively constant MEI:BMR ratio (Speakman and McQueenie 1996). However, if energy intake increases at a faster rate than BMR, the MEI:BMR ratio should increase accordingly, and may indicate a surfeit of energy available for growth or storage. In fact, we found that the MEI:BMR ratio increased from summer to winter (Fig. 5) in concert with parallel changes in fat deposition (Figs. 2 and 3). Evidently, the increased energy intake observed during winter, perhaps in combination with reduced motor activity, more than compensated for the additional thermoregulatory costs incurred during winter foraging.

Lipid reserves accumulated during winter may be essential during spring break-up (April), when forage quality is minimal and access to rooted vegetation is often restricted by ice covering the marsh bottom (K.L. Campbell and R.A. MacArthur, unpublished observations). The continued mobilization of lipid stores from April through July (Virgl and Messier 1992*a*; this study) suggests that tissue reserves may contribute to the reproductive success of free-living muskrats. However, males and females appear to exploit different energy tactics during this period. Measurements collected during the breeding season (this study; Campbell et al. 1998), 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 the 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 energy demands of gestation and lactation (Hammond and Diamond 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 energy intake, 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 females. These data are vital to developing a fuller understanding of the seasonal allocation of energy and nutrients in this prominent North American rodent.

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