Seasonal and Interindividual Variation in Field Water Metabolism of Female Meadow Voles *Microtus pennsylvanicus*

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ABSTRACT

We analyzed variations in water flux rates on a large sample of meadow voles (*Microtus pennsylvanicus*) to quantify the effect of season on water metabolism of individuals and to examine patterns of intra- and interindividual variability. Voles were nonreproductive females maintained in outdoor enclosures where they fed on natural vegetation. They were injected one to three times with doubly labeled water, which resulted in one to six measures of daily water flux rate per individual. Summer water flux rates of voles were 258% of the predicted values for herbivorous eutherian mammals of similar size. To date, very few studies have focused on mammals with such high water flux rates. Body water volume of individuals was higher in summer than in winter (75.6% vs. 72.5%), and water flux rate of animals was 12.5% higher in the winter season (0.99 vs. 0.88 mL H_2O g^{-1} d^{-1}). Between-season differences in water fluxes were proportional to differences in energy expenditures, hence the water economy index remained constant across seasons (0.30 mL H_2O kJ^{-1}). Intraindividual variability of water flux rate was high compared to interindividual variability (repeatability, $r < 0.30$), which will make it difficult to study natural selection of water metabolism in a microevolutionary framework, at least in meadow voles.

Introduction

The greatest physiological challenge that animals face on land is probably the risk of dehydration (Schmidt-Nielsen 1990). For this reason, animals have to tightly regulate their exchanges of water with the environment to maintain a relatively constant body water content. It has been a long time since physiologists first elucidated the proximate routes that determine water balance in animals (e.g., Chew 1965). Only recently, however, did the use of radio- and stable isotopes, and particularly the doubly labeled water method (DLW), allow advances in our understanding of the ecological factors that influence water metabolism of animals in the field.

Between-species differences in water flux rates can be partly attributed to the availability of preformed water in the diet (Nagy and Peterson 1988). Lower levels of variability have received much less attention, however, although the understanding of physiological diversity at all levels is recognized as one major aim of physiological ecology (Feder et al. 1987). While it is clear that latitudinal and altitudinal gradients (Hayes and Shonkwiler 1996), as well as seasonal environmental changes, should induce variation in water metabolism through their effects on energy expenditure and food intake, it is unclear how intraindividual variation should compare to higher levels of variability.

Understanding variations that occur below the species level is important for at least two reasons. First, the comparison of groups of individuals within species has long been recognized as a powerful approach to test biological hypotheses (Garland and Adolph 1991). Second, interindividual variability is the raw material upon which natural selection acts, and a better understanding of the degree of individual variation in field water metabolism may help us understand the evolutionary pathways that shaped the metabolic capacities of organisms (Bennett 1987). To date, natural selection on metabolic rate or on water balance has not been studied in a microevolutionary framework (Hayes et al. 1998). If interindividual variation is to be used in an evolutionary context, it first has to be disentangled from intraindividual variation.

We used DLW to measure field water metabolism of a large sample of nonreproductive female meadow voles (*Microtus pennsylvanicus*). In light of the issues discussed above, we address three objectives in the hierarchy of sources of variation in field water metabolism. First, given that meadow voles live in hygric habitats (Getz 1961; Lewin 1968) and are one of the smallest herbivores known to rely only on a high-fiber, low-energy diet that is high in water content (Wunder 1985), we test the hypothesis that water flux rates in this species are higher than expected for a mammal of similar size. Meadow voles have been extensively used to test physiological, behavioral, and

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ecological hypotheses (Tamarin 1985), and indirect evidence (Getz 1961, 1963; Lewin 1968; Heisinger et al. 1973; Rhodes 1983) already suggests that they process larger amounts of water than most other small mammals. Many studies of body water regulation by small mammals have focused on the elaborate water conservation mechanisms used by species inhabiting arid regions, but much less attention has been directed toward establishing the patterns of water use exhibited by species from more mesic areas (Rhodes 1983). An important gap thus exists in the comparative database for water flux rates (Nagy and Peterson 1988).

Second, we quantify the effect of season (winter vs. summer) on water metabolism of meadow voles, in order to evaluate the effect of one general environmental factor on the within-species variability of water metabolism. Season should be a highly influential factor on field water metabolism of herbivore species from temperate regions because of yearly fluctuations in temperature, humidity, and food quality. Data on seasonal changes of field water metabolism will provide us with a reference against which to contrast other levels of variability. The hypothesis is that water flux will be higher in winter than in summer because cold winter temperatures result in high metabolic rates (Berteaux 1998).

Third, we partition the variation in water flux between the intra- and interindividual components. Whereas some attempts have been made recently to quantify the intraindividual component of several variables indicative of energy expenditure (Speakman et al. 1994; Chappell et al. 1995; Berteaux et al. 1996*b*; Hayes et al. 1998; Peterson et al. 1998), to our knowledge no study has ever partitioned field water metabolism into its intra- and interindividual components (see Peterson et al. 1998, however, for Pearson correlations for repeated measures of water influx and efflux rates). We hypothesize that intraindividual variability of water flux rates will be high compared to interindividual variability because water flux rate is partially dependent on metabolic rate, which we previously showed to be highly variable at the intraindividual level in meadow voles (Berteaux et al. 1996*b*).

Material and Methods

Field Procedures

Individuals were adult, nonreproductive, female meadow voles originating from a captive colony that was periodically outbred with wild voles (for details on rearing of voles, see Berteaux et al. 1994). Voles had similar past life history and were not related. We studied individuals in either 25- or 100-m^2 predator-free enclosures that were built in an old-field community located on the grounds of the Université de Sherbrooke, Québec, Canada (45°15′N, 27°00′W). The same enclosures were used in winter and summer, but animals were different. Animals fed on natural vegetation (forbs and grasses), and no supplementary food was added to the enclosures. The main plant species present in the enclosures, known to be preferred by meadow voles, were *Festuca rubra*, *Agropyron repens*, *Carex* spp., *Fragaria virginiana*, *Phleum pratense*, and *Poa* spp. (Bergeron and Jodoin 1989; Bergeron et al. 1990).

For the winter part of the study, we acclimatized voles to natural photoperiod and temperatures for 11–18 wk before individuals were released in the enclosures. Voles were released either alone in 25-m^2 enclosures or by groups of four in 100-m^2 enclosures. Social status did not affect water turnover or energy expenditures (Berteaux et al. 1996*a*). Individuals were allowed to habituate to the enclosures for 2 wk before they were captured with Sherman traps baited with apple slices and protected by trap shelters (Iverson and Turner 1969). Voles were transported within a few minutes after capture to a building located near the study site, weighed to the nearest 0.1 g, and given an intraperitoneal injection of 4 $\mu L\ g^{-1}$ body mass of distilled water containing 77.9 atoms of oxygen-18 (^{18}O) per 100 atoms of oxygen and 94 mCi of tritium mL^{-1}. After 1 h, a 150-μL blood sample was taken under light anesthesia (methoxyfluorane) from the suborbital sinus using heparinized glass capillary tubes. We released animals at their point of capture and tried to recapture them at the same time of day 24 and/or 48 h later for collection of second or third blood samples. Blood samples were taken before isotope injection in five individuals for measurement of natural abundance of ^{18}O. Measurements of daily water fluxes were obtained during six observation days that were staggered over a 51-d period from January 25 to March 16, 1994.

For the summer part of the study, animals were acclimatized to natural photoperiod and temperatures for ≥2 wk before being released singly into 25-m^2 enclosures. Voles were allowed to habituate to the enclosures for one wk before they were captured with Sherman traps baited with apple slices. Injection of DLW was performed as described above, except that all operations were performed in the field. Measurements of daily water fluxes were obtained during 12 observation days that were staggered over a 42-d period from July 12 to August 22, 1994.

In the field, thermoregulatory costs can greatly affect water turnover. They depend on the standard operative temperature, T_{es} (Bakken 1992), which combines shaded air temperature, radiation, and wind speed. We could not measure T_{es} in this study, so we used the daily mean temperature (average value of the nighttime minimum and the daytime maximum temperatures) at ground level as the independent variable most likely to reflect the influence of weather on water turnover. We consider this environmental temperature index to be adequate in the context of this study, since the influences of wind and solar radiation were minimal owing to the 40-cm snow layer (winter) and the dense vegetation cover (summer) under which animals lived.

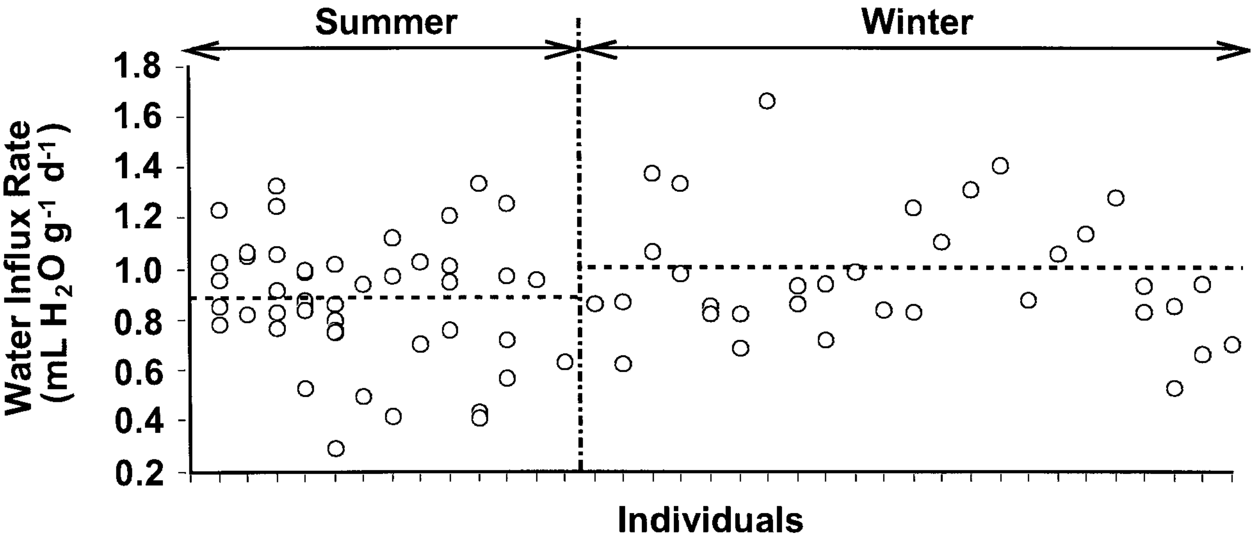

Figure 1. Point measurements of water influx rate in meadow voles. Data obtained by means of doubly labeled water on 36 adult female meadow voles ($i = 82$ measurements) maintained in enclosures during both the winter (23 individuals) and summer (13 individuals) seasons. Broken lines represent mean values (after averaging data per individual) for each season.

Determination of Water Flux Rates

Blood samples were kept refrigerated before being vacuum distilled in Pasteur pipettes to recover the water fraction (Nagy 1983). We analyzed for ^3H using liquid scintillation in a Beckman LS 6000 counter and for ^{18}O using the guanidine hydrochloride method to prepare CO_2 gas (Dugan et al. 1985; Wong et al. 1987) that was analyzed with a VG-Isogas Sira 12 isotope ratio mass spectrometer (see Thomas et al. 1995 for procedures). All samples were analyzed in duplicate and a triplicate sample was run when the coefficient of variation exceeded 2%.

Body water volume at the time of initial capture was estimated from the ^{18}O dilution space according to appendix 1 in Nagy (1983). We assumed that the percentage of body water content remained constant during the measurement period. When body mass changed between the first and subsequent recaptures, we assumed that the animal's body water volume increased or decreased linearly. Daily water influx and efflux were calculated according to equations (4) and (6) in Nagy and Costa (1980). We also calculated CO_2 production of voles using equation (1) in Nagy (1983) and estimated energy expenditure by assuming an energy equivalence of 21.7 J mL^{-1} (Nagy 1983). This allowed us to calculate the water economy index (in mL H_2O influx per kilojoule metabolized), which is the ratio of water influx rate (mL H_2O d^{-1}) to field metabolic rate (in kJ d^{-1}; Nagy and Peterson 1980). Note that using the equations of Nagy and Costa (1988) and Nagy (1983) led to a slight overestimation of water requirements and energy expenditures because their equations do not make any correction from fractionation. We chose to use these equations, however, on the grounds of comparability with previous studies.

Detailed analyses of energy expenditure data are presented elsewhere (Berteaux et al. 1996*a*, 1996*b*). We obtained a total of 82 measurements of water metabolism for 36 individuals. Thirty-five measurements (23 individuals) were obtained in winter and 47 measurements (13 individuals) were obtained in summer. Thus, 1.52 (±0.51 SD) and 3.62 (±1.80 SD) measurements were obtained per individual during the winter and summer seasons, respectively. It was necessary for us to repeat measurements on the same individuals in order to quantify the intraindividual component of variations in water flux rate.

Statistical Analyses

All parametric tests used in statistical analyses were performed after verification of data normality with Shapiro-Wilk *W* tests (SAS Institute 1994*a*). Test statistics are from Sokal and Rohlf (1981), except where otherwise stated. All regressions are simple linear regressions. Variability in the data sets was separated between intraindividual and interindividual components by calculating intraclass correlation coefficients (Sokal and Rohlf 1981; Lessells and Boag 1987). This parametric technique is based on variance components derived from Model II single-factor ANOVA and allows one to evaluate if a significant variance component is added at the interindividual level. A drawback of using the intraclass correlation coefficient for assessing repeatability is that it is sensitive to changes in the mean of repeated measures (Hayes and Jenkins 1997). Hence, we also

Table 1: Body mass, body mass change, and descriptors of water metabolism in adult female meadow voles living in field conditions in winter and summer 1994

Variable	Winter (n = 23)	Summer (n = 13)	$F_{1,34}$	P
Body mass (g)	34.6 ± 3.1 (28.9–41.0)	35.5 ± 3.5 (28.7–40.9)	.571	.455
Total body water (%)	72.5 ± 2.9 (65.9–78.0)	75.6 ± 3.1 (69.8–79.7)	8.645	.006
Daily mass change rate (%)	−1.0 ± 4.3 (−10.0 to 8.3)	−.7 ± 2.6 (−4.9 to 3.2)	.047	.830
Water influx rate (mL H_2O g^{-1} d^{-1})	1.00 ± .25 (.69–1.66)	.86 ± .12 (.63–1.03)	3.972	.05
Water efflux rate (mL H_2O g^{-1} d^{-1})	1.01 ± .25 (.72–1.66)	.86 ± .11 (.64–1.00)	4.243	.05
Field metabolic rate (kJ g^{-1} d^{-1})	3.40 ± .43 (2.82–4.74)	2.77 ± .42 (1.84–3.23)	17.464	.001
Water economy index (mL H_2O kJ^{-1})	.30 ± .02 (.18–.47)	.31 ± .01 (.26–.39)	.336	.566

Note. Minimum and maximum values (given in parentheses) and means and standard deviations ($\overline{X}$ ± SD) were calculated after averaging data per individual when multiple measurements were obtained on a given vole. Results of statistical tests refer to winter-summer comparisons; df = 1, 34. Data obtained by means of the doubly labeled water technique on 36 individuals maintained in outdoor enclosures 25–100 m^2.

computed the product-moment correlation between successive measurements, which does assess the consistency of a trait relative to the mean. Analyses were carried out with JMP version 3.0 (SAS Institute 1994*b*). Statistical significance was defined as $P < 0.05$. Results are reported as means and standard deviations, unless otherwise stated.

Results

Mean daily ground temperatures ranged from −3.4° to 1.7°C (−1.3° ± 1.4°C) during the six winter days of measurement, and from 9.2° to 32.0°C (19.7° ± 2.3°C) during the 12 summer days of measurement. Water influx and efflux rates (mL H_2O g^{-1} d^{-1}) were highly correlated with each other ($r^2 = 0.978$, $P < 0.001$, $n = 82$). Only water influx rates (Fig. 1) are used in subsequent analyses.

First, we compare water metabolism of voles studied in summer with that of voles studied in winter, after averaging data for individuals on which we obtained multiple measurements. Body mass of voles did not vary significantly by season (Table 1). Body water content of individuals, however, was 4.2% higher in summer than in winter (Table 1). Body mass did not vary significantly between first and second capture (paired *t*-tests; winter: $P = 0.126$, summer: $P = 0.567$; see Table 1 for average daily mass change rates). Water influx rate was significantly higher in winter than in summer (Table 1), but the water economy index remained remarkably constant at 0.30 mL H_2O kJ^{-1} because the between-season differences in water influx rates were exactly proportional to variations in field metabolic rates (Table 1).

In order to fully understand the dynamics of water metabolism of female meadow voles in the field, we now describe intraindividual variations for each variable. Because we used different individuals in winter and summer, variations within and between individuals are presented separately for the winter and summer data sets. Since the amount of water that an individual processed through its body each day was likely affected by its body mass and by ambient temperature, these two variables can confound the estimates of repeatability of water metabolism. As a consequence, we first evaluate their effect in order to factor them out if necessary.

Water influx (mL H_2O d^{-1}) was significantly related to body mass ($\log_{10}$ water influx $= -0.19 + 1.09$ $\log_{10}$ body mass, $F_{1,80} = 8.479$, $P = 0.005$, 95% confidence interval [CI] on the allometric scaling coefficient = 0.34–1.84). In subsequent analyses, we factor out the effect of body mass by dividing water flux by body mass ($g^{-1.09}$). However, since the CI on the allometric scaling coefficient (slope) includes 1.0, we present mass-specific (mL H_2O g^{-1}) values of flux rates in the tables and figures, in order to ease comparison of the results with those from previous publications (e.g., Nagy and Peterson 1988). Ambient temperature had no effect on summer water metabolism of voles (water influx rate: $F_{1,45} = 0.009$, $P = 0.926$; water economy index: $F_{1,45} = 0.337$, $P = 0.564$). During winter, however, temperature had a significant effect on water influx rate ($F_{1,33} = 31.958$, $P < 0.001$, $r^2 = 0.492$; Fig. 2*a*) and the water economy index ($F_{1,32} = 44.227$, $P < 0.0001$, $r^2 = 0.580$; Fig. 2*b*). The effect of temperature was thus factored out for winter values by using the residuals of the above regressions in the analyses of repeatability.

No significant variance component was added at the between-individual level when water influx rate and the water economy index were analyzed (*F* nonsignificant for both variables and seasons; Tables 2 and 3), which reflects the high intraindividual variability observed in water flux rates. Coefficients of repeatability of water flux rates were <0.30 in both seasons, indicating that >70% of the total variability observed in this variable originated from intraindividual differences. Likewise, the product-moment correlations always yielded nonsignificant results ($P > 0.1$). Body mass of individuals was highly repeatable in both seasons, since more than 75% of the variation registered for this variable came from interindividual differences (Tables 2 and 3). Body mass changes, however, showed great variations at the intraindividual level, and differences between voles were not significant (Tables 2 and 3).

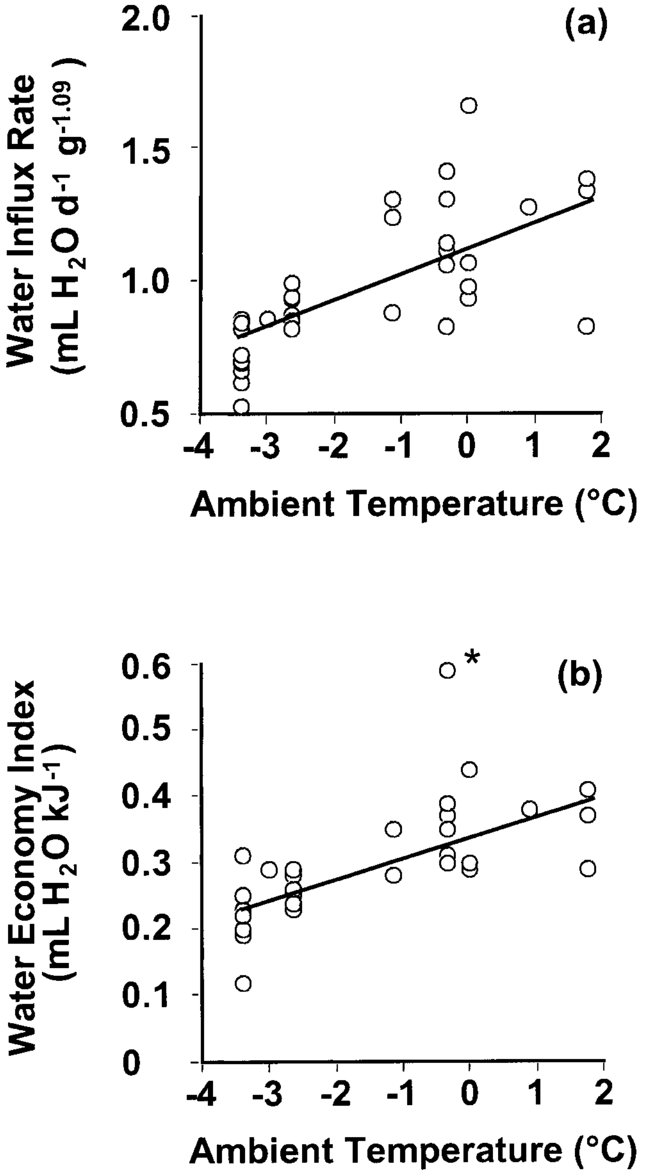

Figure 2. Daily mass-independent water influx rate (*a*) and water economy index (*b*) of wintering meadow voles as a function of mean daily ground temperature. In (*b*), the regression line excludes the outlier indicated by a star.

After averaging data for individuals on which multiple measurements had been performed, we regressed water influx rate on the daily body mass change rate (body mass change per day divided by body mass at first capture) in order to predict the mean rate of water flux when body mass change is constant. The equations of the regression lines were

$$\text{water influx rate (mL } H_2O \text{ g}^{-1} \text{ d}^{-1}) = 1.012 + 0.008 \text{ mass change rate (d}^{-1}),$$

and

$$\text{water influx rate (mL } H_2O \text{ g}^{-1} \text{ d}^{-1}) = 0.871 + 0.020 \text{ mass change rate (d}^{-1}),$$

for the winter and summer data sets, respectively. Slopes of the regression lines were not significantly different from 0 (winter: $F_{1,21} = 0.440$, $P = 0.514$, $r^2 = 0.02$, 95% CI = -0.018 to 0.034; summer: $F_{1,11} = 2.428$, $P = 0.147$, $r^2 = 0.18$, 95% CI = -0.008 to 0.048), indicating no effect of body mass change on water flux rates. The intercepts of the regression lines, however, were significantly different from 0 in both seasons (winter: *t*-ratio = 18.59, $P < 0.0001$, 95% CI = 0.899–1.125; summer: *t*-ratio = 26.28, $P < 0.0001$, 95% CI = 0.798–0.944 [*t*-ratios from SAS Institute; 1994*a*]). These intercepts represent water influx rate in a steady state situation. They are close to mean values reported in Table 1 since animals maintained, on average, a virtually constant body mass between initial and final captures (Table 1).

The water economy index was also regressed on daily body mass change rate in order to predict its value in a steady state situation. The equation of the regression line was

$$\text{water economy index (mL } H_2O \text{ kJ}^{-1}) = 0.308 + 0.003 \text{ mass change rate (d}^{-1}).$$

The slope of the regression line was not significantly different from 0, but the intercept was (slope: $F_{1,34} = 1.348$, $P = 0.254$, $r^2 = 0.04$, 95% CI = -0.003 to 0.009; intercept: *t*-ratio = 28.96, $P < 0.0001$, 95% CI = 0.286–0.329), indicating that voles processed, on average, 0.31 mL H_2O kJ^{-1} when maintaining constant body mass in the field.

We had predicted that water influx rates would show high intraindividual variability because (1) they are dependent on metabolic rates and (2) metabolic rates were known to be poorly repeatable within individuals. Here we use linear regression analyses to test the assumption that water flux rates are dependent on metabolic rates. Using individual measurements as data points, the dependence of daily water influx on daily energy expenditure was highly significant in both seasons (winter: $r^2 = 0.136$, $F_{1,32} = 5.018$, $P = 0.032$, Fig. 3*a*; summer: $r^2 = 0.543$, $F_{1,44} = 52.329$, $P < 0.0001$, Fig. 3*b*).

Discussion

Meadow Voles Compared to Other Mammals

Here we compare water flux rates of meadow voles with predictions from the allometric equations of Nagy and Peterson

Table 2: Results of single-classification ANOVAs (F, P, r_i) performed on the different variables used to analyze water metabolism of adult female meadow voles living in field conditions in winter 1994

Variable and Source of Variation	df	Mean Square	F	P	r_i
Body mass (g)			13.206	.001	.859
Among individuals	11	16.287			
Within individuals	12	1.233			
Daily mass change rate (%)			.307	.970	.530
Among individuals	11	11.509			
Within individuals	12	37.477			
Water influx rate (mL H_2O $g^{-1.09}$ d^{-1}) (Residuals)[a]			1.711	.185	.262
Among individuals	11	.020			
Within individuals	12	.012			
Water economy index (mL H_2O kJ^{-1}) (Residuals)[a]			2.620	.06	.447
Among individuals	11	.008			
Within individuals	12	.003			

Note. r_i refers to the intraclass correlation coefficient. Data obtained by means of the doubly labeled water technique on 12 individuals maintained in outdoor enclosures and for which more than one data point was obtained.

[a] Effect of temperature factored out after linear regression of the variable on mean daily temperature.

(1988). Their equation (2) predicts water flux rate of eutherian mammals in the field and is based on 115 data points from 42 species:

$$\text{water flux rate (mL H}_2\text{O d}^{-1}) = 0.326 \text{ body mass (g)}^{0.818}.$$

This formula predicts a water flux rate of 6.0 mL H_2O d^{-1} for a vole of 35.5 g (the average summer body mass in this study), and a water flux rate of 5.9 mL H_2O d^{-1} for an individual of 34.6 g (winter mass). Summer and winter flux rates of meadow voles (31.2 and 34.3 mL H_2O d^{-1}, respectively) thus correspond to 517% and 579% the predicted values. The hypothesis that meadow voles have higher water flux rates than other eutherian mammals of similar size is thus supported.

Nagy and Peterson (1988) computed a specific allometric equation for herbivorous eutherian mammals living in the field. Their equation (3) is based on 28 data points from eight species:

$$\text{water flux rate (mL H}_2\text{O d}^{-1}) = 0.708 \text{ body mass (g)}^{0.795}.$$

According to this equation, summer and winter body masses of voles yield water flux rates of 12.1 and 11.8 mL H_2O d^{-1}, respectively. Our measured flux rates thus represent 258% and 289% those predicted for a similar-sized herbivorous eutherian mammal.

Data from only two eutherian mammal herbivores living in hygric habitats had been published when Nagy and Peterson (1988) performed their review, thus no predictive equation exists that takes into account the wet habitat used by voles. These two species (the water vole, *Arvicola terrestris*, and the water buffalo, *Bubalus bubalis*; Williams and Ridpath 1982; Grenot et al. 1984), show water flux rates 210%–300% higher than predicted for eutherian mammal herbivores of similar size, which is a remarkable concordance with data from meadow voles.

The water economy index is expected to range between 0.15 and 0.26 mL H_2O kJ^{-1} for plant-eating animals consuming green forage without drinking (Fig. 18 in Nagy and Peterson 1988). Values obtained in our study (0.30 mL H_2O kJ^{-1}) are thus at the upper limit of what was expected. This indicates that voles not only had high absolute water flux rates, but also ingested high amounts of water relative to their rate of metabolism. Meadow voles are known to drink a lot in captivity when given dry food (D. Berteaux, unpublished data), but we do not know if they usually drink in the field.

Schmidt-Nielsen and O'Dell (1961) noted a strong correlation among mammals between the relative lengths of the renal papillae, the urine-concentrating ability, and the availability of water in the habitat of the different species. Virtually no data existed at that time, however, to test this information against actual water flux rates from wild animals living in the field (Chew 1965). Good information is now available as to the behavior, habitat use, anatomy, and physiology of meadow voles, which gives us the opportunity to test in a single species the degree of consistency between actual water flux rates of individuals measured in the field and existing knowledge on their physiology and ecology.

Anatomical data on *Microtus pennsylvanicus* show that the

Table 3: Results of single classification ANOVAs (F, P, r_i) performed on the different variables used to analyze water metabolism of adult female meadow voles living in field conditions in summer 1994

Variable and Source of Variation	df	Mean Square	F	P	r_i
Body mass (g)			12.117	.001	.733
Among individuals	10	35.320			
Within individuals	34	2.915			
Daily mass change rate (%)			1.078	.405	.019
Among individuals	10	27.913			
Within individuals	34	25.900			
Water influx rate (mL H_2O $g^{-1.09}$ d^{-1})			.680	.735	.086
Among individuals	10	.024			
Within individuals	34	.035			
Water economy index (mL H_2O kJ^{-1})			.301	.976	.209
Among Individuals	10	.003			
Within Individuals	34	.009			

Note. r_i refers to the intraclass correlation coefficient. Data obtained by means of the doubly labeled water technique on 11 individuals maintained in outdoor enclosures and for which more than one data point was obtained.

percentage medullary thickness of their kidneys is among the lowest found in the family Cricetidae (Heisinger et al. 1973), which indicates a poorly efficient kidney for water conservation. This poor efficiency was illustrated in laboratory studies by Getz (1963) and Heisinger et al. (1973). Both studies were based on water deprivation and salt-loading experiments, and demonstrated that meadow voles have a poor ability to concentrate urine and exhibit low survivorship on restricted water rations. In good agreement with these physiological and anatomical data, Getz (1961) and Lewin (1968) observed that meadow voles were mostly inhabitants of low, moist situations, and were more successful in wet than in dry habitats. The ecophysiological data presented in this study, which depict meadow voles as a species processing large amounts of water through their bodies each day, thus validate all the indirect conclusions on field water metabolism of the species previously inferred from laboratory or field studies.

Effect of Season on Water Metabolism

Water flux rates of meadow voles were 12% higher in winter than in summer. Much higher seasonal variations in water flux rates have been found in other species (see references in Nagy and Peterson 1988). For example, in the desert-dwelling kangaroo rat (*Dipodomys merriami*), mass-corrected water influx rates were over three times higher in spring than in autumn (Nagy and Gruchacz 1994). What were the immediate factors responsible for the seasonal changes in water flux rates of meadow voles? The reproductive state and body mass of individuals remained constant across seasons, so these factors cannot explain changes in water flux rates. Body water content of individuals was higher in summer than in winter (75.6% vs. 72.5%), indicating that body fats were lower in the summer season (Kodama 1971; Fleharty et al. 1973). The seasonal increase in the volume of body fluids thus cannot explain changes in water flux rates, since these two variables were inversely related. Free water was available in all seasons at the study site, either as surface water, dew, or snow. Therefore, no obvious causal link can be suspected between availability of free water and observed water flux rates.

Environmental temperature was the factor most likely to drive changes in water metabolism of individuals across seasons. Low winter temperatures increased thermoregulatory needs of individuals, which in turn inflated their energy demands (Berteaux 1998) and food consumption. Higher food consumption led to the formation of more metabolic water and to the ingestion of higher quantities of preformed water. This speculation is supported by our finding that daily water influx was dependent on daily metabolic rate, but it must remain qualitative because the caloric and water content of the food, together with the assimilation rate of plant parts consumed by voles, are unknown to us.

In summer, average ambient temperature (9.2°–32.0°C) had no effect on water influx rates of meadow voles. Rhodes (1983) obtained similar results with *Microtus pinetorum* maintained in laboratory conditions (15° vs. 30°C) or studied in the field (7.3°–21.7°C). These two *Microtus* species thus seem to be able to accurately regulate rates of body water turnover in the range of ambient temperatures mentioned above. In winter, however,

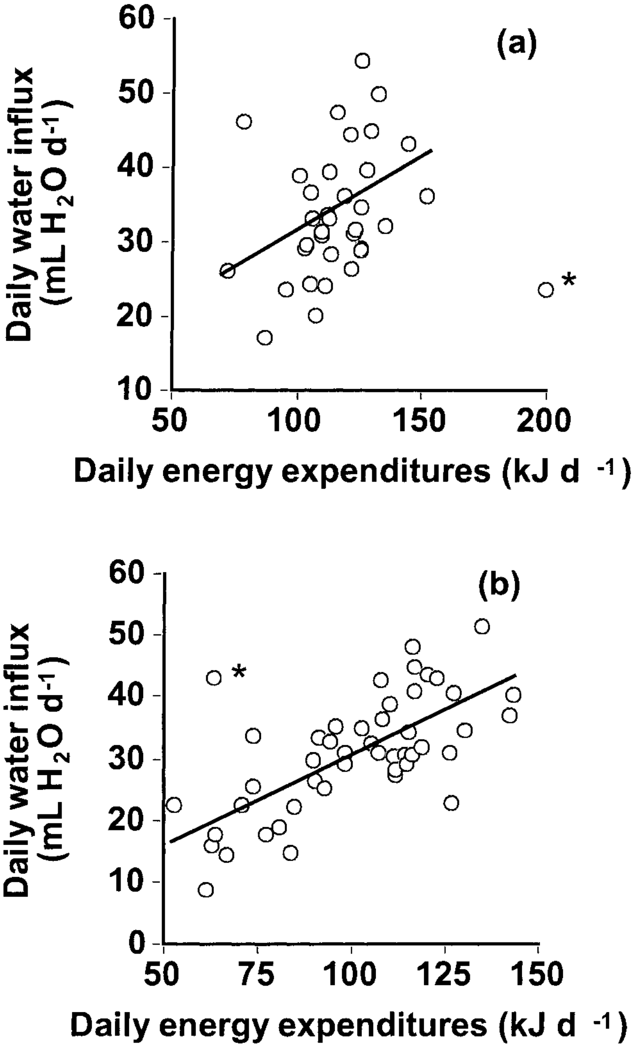

Figure 3. Daily water influx (mL H_2O d^{-1}) as a function of daily energy expenditure (kJ d^{-1}) in adult female meadow voles maintained in outdoor enclosures in winter (*a*) and summer (*b*). The regression lines exclude the outliers indicated by a star.

water influx rates of meadow voles increased with increasing average ambient temperature (−3.4° to +1.7°C), opposite to the trend predicted from the correlation between water flux and metabolic rate and the thermal dependence of metabolic rate. Several hypotheses may explain this pattern. First, since water influx rates were partly dependent on metabolic rates, it may simply be that, in winter, voles were less active when temperature decreased. Second, voles may have reduced the ingestion of preformed water at lower temperatures because most available water may have been frozen, and ingesting frozen water is energetically costly. Finally, water influx rates may have been affected by some unknown factors correlated with ambient temperatures.

Repeatability of Daily Water Flux Rates

Intraindividual variability of water flux rates was so high that there was no significant interindividual component to total variance. Daily water flux rate sometimes varied by >twofold within individuals, a huge variation when compared to the 12% seasonal variation of the average water flux rate. However, because no similar data on field water metabolism currently exists, we cannot compare our results with those from other mammalian species (Hayes et al. 1998). Any comparative attempt must thus rely on data of intraindividual variability observed on other physiological variables.

Values of repeatability obtained in summer ($r < 0.3$) are similar to those found by Speakman et al. (1994) in a study on daily energy expenditure of caged pouched mice, and to those given in Berteaux et al. (1996*b*) for daily energy expenditure of *M. pennsylvanicus* living in the field. They are much lower, however, than those of most other published physiological and performance measures, such as milk yield of British friesan cattle (Barker and Robertson, in Falconer 1981), maximum sprint speed of lizards (Huey and Dunham 1987), mass of fleece in domestic sheep (Morley in Falconer 1981), or maximum aerobic performance of Belding's ground squirrels (Chappell et al. 1995) or red jungle fowl (Chappell et al. 1996; see Hayes et al. 1998 for other examples). Coefficients of repeatability calculated from winter values were even lower than those from summer ones. One critical aspect of these comparisons is that most repeatability values cited here were either for characters resulting from strong artificial selection, or from animals pushed experimentally to maximum values of traits. This alone could explain why our measures show lower repeatabilities, if intraindividual variability of physiological measures decreases when animals work near their maximum physiological capacity (Berteaux et al. 1996*b*).

Two consequences emerge from the high intraindividual variability of water flux rate. First, this lack of repeatability decreases the probability that future studies will manage to detect any significant relation between individual water flux rates of meadow voles and other variables indicative of fitness. One should note, however, that measurements made over a longer time period (several days?) could possibly make a more adequate metric for analyzing individual differences. When the DLW technique can integrate longer periods of time (e.g., in ectotherms; Peterson et al. 1998), individual field metabolic rates may be more repeatable. Further research should investigate the effect of the length of the measurement period on the repeatability of field water flux rates or other metabolic measures.

If no repeatable measure of field water metabolism of voles can be found, the route by which water metabolism of *M.*

pennsylvanicus evolved will not be enlightened by studies of individual variation. This conclusion may be supported by the fact that no clear benefit or cost has yet been associated to variations in water flux rates of meadow voles. Rather, the amount of water that is processed through the body each day may largely be a by-product of other functions, such as energy needs, which may represent the actual target of natural selection. In good agreement with this reasoning, Deavers and Hudson (1979) suggested that no selective pressure exists for small mammals of mesic environments to possess highly efficient water conservation mechanisms.

Second, repeated measurements of water flux rates on some individuals should be viewed as a valuable addition of information when the aim of a study is to obtain an appreciation of the range of physiological performances animals achieve in the field. Ecologists and physiologists are rightly suspicious when several points in a data set come from the same individual because the risks associated with nonindependence of data are high when statistical tests are performed (Machlis et al. 1985). However, when intraindividual variability is large for a given variable, the benefits may be important of repeating measurements on some individuals and subsequently averaging these data (Hayes and Jenkins 1997). There are indeed two sources of variation that lead to errors in estimating the "true" value for any physiological trait: those inherent in the precision of each measurement and those due to sampling error. Repeating measurements on individuals decreases sampling error. When the doubly labeled water technique is used, a single injection may yield two or three measurements of daily water flux rate per individual, and repeated measurements can be performed at relatively low cost.

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