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Bioenergetics and thermal physiology of American water shrews (*Sorex palustris*)

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Abstract Rates of O_2 consumption and CO_2 production, telemetered body temperature (T_b) and activity level were recorded from adult and subadult water shrews (*Sorex palustris*) over an air temperature (T_a) range of 3–32°C. Digesta passage rate trials were conducted before metabolic testing to estimate the minimum fasting time required for water shrews to achieve a postabsorptive state. Of the 228 metabolic trials conducted on 15 water shrews, 146 (64%) were discarded because the criteria for inactivity were not met. Abdominal T_b of *S. palustris* was independent of T_a and averaged 38.64 ± 0.07°C. The thermoneutral zone extended from 21.2°C to at least 32°C. Our estimate of the basal metabolic rate for resting, postabsorptive water shrews (96.88 ± 2.93 J g^{-1} h^{-1} or 4.84 ± 0.14 ml O_2 g^{-1} h^{-1}) was three times the mass-predicted value, while their minimum thermal conductance in air (0.282 ± 0.013 ml O_2 g^{-1} h^{-1}) concurred with allometric predictions. The mean digesta throughput time of water shrews fed mealworms (*Tenebrio molitor*) or ground meat was 50–55 min. The digestibility coefficients for metabolizable energy (ME) of water shrews fed stickleback minnows (*Culaea inconstans*) and dragonfly nymphs (*Anax* spp. and *Libellula* spp.) were 85.4 ± 1.3% and 82.8 ± 1.1%, respectively. The average metabolic rate (AMR) calculated from the gas exchange of six water shrews at 19–22°C (208.0 ± 17.0 J g^{-1} h^{-1}) was nearly identical to the estimate of energy intake (202.9 ± 12.9 J g^{-1} h^{-1}) measured for these same animals during digestibility trials (20°C). Based on 24-h activity trials and our derived ME coefficients, the minimum daily energy requirement of an adult (14.4 g) water shrew at T_a = 20°C is 54.0 kJ, or the energetic equivalent of 14.7 stickleback minnows.

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Abbreviations BMR: Basal metabolic rate · C: Thermal conductance · DE: Digestible energy · HIF: Heat increment of feeding · MAD: Motion activity detector · ME: Metabolizable energy · N: Number of animals · n: Number of samples · RER: Respiratory exchange ratio · T_a: Ambient (chamber) temperature · T_b: Body temperature · T_{lc}: Lower critical temperature · TNZ: Thermal neutral zone · VCO_2: Rate of carbon dioxide production · VO_2: Rate of oxygen consumption

Introduction

The American water shrew (*Sorex palustris*) is an opportunistic aquatic predator found in close proximity to a diverse range of aquatic habitats, but preferring the banks of fast flowing streams and rivers (Conaway 1952; Sorenson 1962). Weighing 12–14 g, *S. palustris* is the world's smallest mammalian diver (Calder 1969), making its physiological attributes of special theoretical interest. Unfortunately, little is known regarding the rate of metabolism, thermoregulatory competence, or bioenergetics of this diminutive diver. These data are essential to develop an understanding of how this intriguing insectivore is able to meet its energetic requirements while exploiting the adverse thermal conditions inherent in a semi-aquatic lifestyle.

Among the smallest of mammalian endotherms, soricine or red-toothed shrews are generally recognized as exhibiting higher mass-specific basal metabolic rates (BMR), body temperatures (T_b) and lower thermal conductance coefficients (C) than other similarly sized mammals (Kleiber 1961; McNab 1991). These attributes are often linked to the elevated rates of heat loss and risks of hypothermia inherent to species inhabiting cool north-temperate climates (Vogel 1980; McNab 1991). However, the extent to which the BMR of red-toothed

shrews exceeds allometric predictions has been hard to evaluate, as there is much discrepancy in the literature concerning rates of energy expenditure by shrews, both within (Hindle et al. 2003) and between species (Sparti and Genoud 1989; McNab 1991). This inconsistency has arisen at least in part from the use of non-standardized experimental techniques in earlier studies.

As minimum requirements for estimating BMR, animals should be mature, postabsorptive, resting in darkness in the thermal neutral zone (TNZ) and not reproductively active (Kleiber 1961). To date, accurate BMR determinations from shrews have proven especially challenging since two of the key criteria, inactivity and a postabsorptive state, are often difficult to achieve and monitor concurrently (McDevitt and Andrews 1995). This situation arises from the rapid throughput time and short fasting endurance of soricine shrews, traits dictating they can only remain postabsorptive for a relatively short period (few hours) before succumbing to starvation (Genoud 1988). Consequently, fasting tends to increase the level of animal activity (McDevitt and Andrews 1995), leading to an overall elevation in metabolic rate (MR), T_b and C. It is common practice in indirect calorimetry to assume that the lowest resting rates of oxygen consumption ($\dot{V}O_2$) recorded during metabolic trials are equivalent to 'resting' or 'basal' values. However, without an independent means for assessing motor activity, there is no reliable way to accurately determine periods of inactivity. Further, published MR values are routinely expressed as $\dot{V}O_2$ (ml g^{-1} h^{-1}) or are converted to units of heat production using a pre-determined conversion factor (e.g. 20.1 J ml^{-1} O_2) with the implicit assumption that protein catabolism is negligible. Unfortunately, this method may overestimate true metabolic heat production by 10–18% in animals like shrews that consume meals high in protein immediately prior to metabolic testing, because the thermal equivalent of protein catabolism (17.71 J ml^{-1} O_2; Jungas et al. 1992) is substantially lower than for fat or carbohydrate catabolism (Campbell et al. 2000). Consequently, reliable BMR data for shrews meeting all of the above criteria are sparse in the literature, with most energetic studies to date utilizing values from active and/or replete animals, often at subthermoneutral ambient temperatures (T_a) (Morrison et al. 1959; McNab 1991).

In an effort to address the methodological problems encountered in previous studies of shrew energetics, we measured $\dot{V}O_2$, rate of carbon dioxide production ($\dot{V}CO_2$), T_b and activity level of 15 fasted American water shrews (*Sorex palustris*) over a range of air temperatures likely to be experienced by these animals in nature. Digesta passage rate trials were conducted before metabolic testing in order to establish the minimum time shrews must be fasted to qualify as postabsorptive. Information garnered from metabolic experiments was combined with data collected on the diel activity/T_b patterns of captive water shrews to estimate their daily energy expenditure. Our final objective was to determine the metabolizable energy (ME) intake and digestive efficiency of shrews fed insect and meat-based diets. From these data, we calculated the minimum daily food requirements for this species to stay in energy balance in an exclusively terrestrial environment. This is the first in a series of studies directed at the foraging energetics and thermal/diving physiology of this remarkable but little known soricid shrew.

Materials and methods

Animal collection and maintenance

A total of 17 water shrews were trapped in Nopiming and Whiteshell Provincial Parks, Manitoba, Canada, using Sherman live traps (256×76×76 mm) baited with frozen fish. Traps were checked at 2-h intervals to minimize fatalities due to stress and/or starvation. On the morning following capture, shrews were transported to the University of Manitoba Animal Holding Facility where they were acclimated for at least 3 weeks before initiating trials. Two additional shrews born and raised in captivity (Gusztak and Campbell 2004) also completed metabolic testing (see below). Animals were aged as either subadult (young of the year; ca. 3–6 months of age) or adult (>1 year) following Gusztak and Campbell (2004).

Housing and care of water shrews are described in detail elsewhere (Gusztak and Campbell 2004). Briefly, shrews were housed individually in modified glass aquaria (88×50×60 cm) in a controlled environment room set at 20 ± 1°C with a photoperiod of 12L:12D. Each holding tank consisted of an aquatic section and an adjoining terrestrial compartment. The aquatic section, filled with 24 cm of water, comprised ca. one quarter of the tank volume and was fitted with an access ramp that allowed the shrew to swim and dive voluntarily. The terrestrial section was provided with ca. 10 cm of soil substrate together with small logs, rocks, bark, nesting materials, and a nest box.

Shrews were maintained on a mixture of beef and chicken heart, pig and beef liver, ground beef, fish fillets and canned dog food enriched with vitamin and calcium supplements (Campbell et al. 1999). Ground dry cat food and sunflower seeds were also provided ad libitum in separate feeding dishes. Diets were supplemented with mealworms (*Tenebrio molitor*), dragonfly nymphs (*Anax* spp. and *Libellula* spp.), leeches, crickets, sow bugs, crayfish and frozen brook stickleback (*Culaea inconstans*), when available.

Digesta passage rate

Throughput times were calculated to determine the length of time water shrews must be fasted prior to metabolic trials in order to achieve a postabsorptive state. Shrews held at 22°C were fed either a 100%

mealworm ration ($N = 7$) or a ration consisting of a ground meat mixture ($N = 8$). Shrews were fasted for 1 h before throughput trials and then provided a ration mixed with indigestible Microtracer iron/nickel alloy particles (Micro Tracers Inc., San Francisco, USA). Following the initial feeding period of 1–2 min, shrews were momentarily placed in a container with ca. 2–3 cm of water to rinse off any tracer adhering to the body. Animals were then transferred to a clean 76-l container furnished with an open-floor nest box and supplied with the same ration (minus tracer) and water ad libitum for the duration of the 6-h fecal collection period.

Following each defecation, the time was recorded, and the feces collected and frozen at −20°C. The recovery of alloy markers was subsequently quantified following the procedure of Campbell et al. (2000). Mean retention time (t_m) was calculated from the equation (Warner 1981): $t_m = \sum m_i \times t_i / \sum m_i$, where m_i = number of tracer particles recovered from the ith defecation and t_i = minutes into the trial. The times to 50% (t_{50}) and 90% (t_{90}) marker recovery were also calculated.

Metabolic trials

Metabolic responses of 15 water shrews (ten adult, five subadult) to T_as varying from 3 to 32°C were assessed using a negative-pressure, open-flow respirometry system similar to Fig. 1B of Withers (1977). However, the inlet air was dried and scrubbed of CO_2 by passing through a Drierite soda lime/Drierite train prior to entering the animal chamber. Shrews were fasted for 60 min before initiating trials and then placed into either a 250- or a 560-ml metabolic chamber. The results of the throughput trials (see below) established that this was a sufficient fast to achieve a postabsorptive state in *S. palustris*. Exhaust gas from the outlet port was drawn through a Drierite column then metered (500 ml min^{-1}) with a TR-SS2 gas analysis sub-sampler (Sable Systems Inc., Las Vegas, USA) calibrated using the bubble meter technique (accuracy: ±2%, Levy 1964). A portion of this gas (ca. 250 ml min^{-1}) was passed sequentially through a Qubit S153 infrared CO_2 analyzer and an Applied Electrochemistry S-3A oxygen analyzer. The inside walls of the metabolic chamber were painted flat black to lower radiative heat transfer, and a 3–4 mm depth of dry, sterilized soil was provided on the chamber floor to minimize animal activity (Campbell et al. 1999; Campbell and Hochachka 2000). The metabolic chamber was mounted on a motion-activity detector (MAD-1, Sable Systems Inc.) situated in a controlled environment room to ensure a constant T_a. The fractional excurrent O_2 and CO_2 content, relative animal activity, flow rate and T_a were recorded every 2 s for 75 min using the Datacan V data acquisition software (Sable Systems Inc.). Trials at T_as >30°C were limited to 60 min to minimize the possibility of hyperthermia-induced mortality (Campbell and Hochachka 2000). $V\!CO_2$ was determined by multiplying the fractional CO_2 content of the outlet air by the flow rate ($V\!e$; ml h^{-1}) of dry expired air. $V\!O_2$ (ml O_2 g^{-1} h^{-1}) was calculated as $[V\!e \times (FiO_2 - FeO_2) - V\!CO_2 \times FiO_2]/(1 - FiO_2)$, where FiO_2 and FeO_2 are inlet and outlet fractional O_2 contents, respectively (Withers 1977). $V\!O_2$ was converted to units of heat production (J g^{-1} h^{-1}) using the equation (Campbell et al. 2000): $MR = [16.218 + 4.716(RER)] \times V\!O_2$, where RER is the respiratory exchange ratio. This equation is based on the premise that protein catabolism by postabsorptive shrews is negligible and assumes carbohydrate and lipid catabolism yield 20.93 and 19.55 J of heat per ml of O_2 consumed, respectively (Jungas et al. 1992).

The first 30 min of each trial was excluded from analyses in order to allow for system equilibration and to permit animals to adjust to the chamber. The resting MR of postabsorptive shrews was calculated as the average of the lowest one to three stable measurement(s), each lasting a minimum of 2 min (range: 2–5 min). Resting status of shrews was verified with the MAD. Shrews were weighed to the nearest 0.01 g before and immediately after metabolic trials. Body mass corresponding to periods of minimal MR was linearly interpolated for mass-specific metabolic calculations. To minimize handling stress, animals were never directly handled and recording of rectal temperatures was not attempted. Instead, shrews were provided access to a blind-ending, pre-weighed 45-cm length of ABS tubing. Shrews readily entered the darkened tube, which was capped for weighing and transferring the animal to and from the metabolic chamber. T_b of radio-implanted shrews (see below) was monitored continually throughout each trial. Coefficients of thermal conductance (C) were calculated from the equation: $C = V\!O_2/(T_b - T_a)$, and by forcing the regression of $V\!O_2$ on T_a (below TNZ) to intersect the abscissa at T_b (McNab 1980).

A continuous two-phase regression model (Nickerson et al. 1989) was used to analyse the relationship between MR and T_a, and statistically determine the lower limit of the TNZ (Campbell and Hochachka 2000). Mean BMR values calculated for adult and subadult shrews were compared using a Student's t-test to determine whether these variables could be pooled. Summary statistics are presented as mean ±1 SE.

Body temperature

Abdominal T_b recordings were obtained from five water shrews implanted with 1.0-g model X-M transmitters (Mini-mitter Inc., Bend, OR, USA). To minimize the size of each unit, the plastic casing provided by the manufacturer was removed and transmitter + battery inserted into a 1-cm section of drinking straw (0.6-cm diameter) that was then sealed with a thin coating of Parafilm-Elvax (Mini-mitter Inc.). Procedures for calibrating transmitters are described elsewhere (Dyck and MacArthur 1992).

Shrews were anesthetized with an Isoflurane/oxygen mixture administered at 1 l min^{-1} via a C-Pram circuit connected to a Tec III Isoflurane vaporizer (Benson

Medical Industries, Inc., Markham, ON, Canada). Induction was achieved by placing animals in a purpose-built acrylic induction chamber (8×9×11.5 cm) equipped with inlet and outlet gas ports for administration of 3% Isoflurane from the anesthetic machine. Once anesthetized, shrews were removed from the chamber, immediately placed on a heated surgical table and connected to a C-pram circuit via a modified rodent mask. A surgical plane of anesthesia was maintained with 2–3% Isoflurane. The abdominal incision site was shaved and scrubbed with a bacterial soap (4% Hibitane) followed by 70% isopropyl alcohol. A 0.9- to 1.2-cm midline incision was made through the skin followed by a 0.7- to 0.9-cm incision through the body wall, along the *linea alba*. A sterilized transmitter was inserted into the abdominal cavity, and the body wall and skin wounds closed with 4-0 catgut and 5-0 silk sutures, respectively. Following surgery, the animal was transferred to a disinfected 48-l plastic container furnished with a nest box, sterile nesting material and fresh food and water. Shrews were returned to their holding tanks after 48 h and metabolic trials were initiated 5 days following surgery.

Digestibility trials

Following the total balance trial method (Robbins 1993), in vivo digestibility trials were conducted at 20 ± 1°C on water shrews consuming either brook stickleback ($N = 6$) or dragonfly nymph diets ($N = 2$). To adjust shrews to the rodent metabolic cages (Nalgene Co., Rochester, USA), animals were placed in these cages on three separate days during the week preceding digestibility trials. Each metabolic cage was fitted with a 0.3-cm wire mesh floor and an open-bottomed resting shelter. Trials were limited to 24 and 16 h for stickleback and dragonfly nymph diets, respectively. All trials were divided into two equal sessions: a pre-trial period (12-h stickleback diet; 8-h nymph diet) and a data collection period (12-h stickleback diet; 8-h nymph diet). Metabolic cages were placed on the MAD and relative animal activity recorded on a computer every 15 s using the Datacan V data acquisition software (Sable Systems Inc.). Following the pre-trial, shrews were transferred to a clean metabolic cage with ad libitum water and fed an excess amount of pre-weighed ration every 4 h. A ration sample of equal mass was frozen at −20°C for energy and chemical analyses. A few drops of concentrated HCl were added to urine collection vials to inhibit the conversion of nitrogen products to ammonia (Robbins 1993). Urine was collected from the vials every 4 h and feces gathered at the end of each trial; both were stored at −20°C for later analyses. Following each trial, metabolic cages were rinsed with distilled water and the washings pooled with the urine samples. Body mass was measured to the nearest 0.01 g before and after each trial.

Ration samples, feces, urine and uneaten food items were lyophilized separately for 48 h, and the gross energy content of each sample determined in duplicate using a Parr 1241 adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, USA). A known amount of mineral oil was added to each urine sample to ensure complete combustion (Campbell et al. 2000). Ration and fecal subsamples were ashed at 600°C for 6 h. A portion of each stickleback ration sample was sent to a feed analysis laboratory (Norwest Labs, Winnipeg, Canada) for protein (Kjeldahl $N \times 6.25$) and lipid determinations. Digestibility coefficients for apparent dry matter, ash-free dry matter, digestible energy (DE) and ME were calculated for each shrew following the method of Robbins (1993).

Daily activity trials

A total of ten 36- to 48-h activity recordings were obtained from eight water shrews held at 20 ± 1°C with a 12L:12D photoperiod. $T_b$was recorded concurrently during four of these trials. Shrews were placed in a mesh-covered, 38-l plastic container containing 2–3 cm of soil, a nest box, logs and rocks, and allowed 24 h to adjust to the set-up prior to initiating recordings. Food and water were provided ad libitum. Following this adjustment period, the container was set on the MAD and the relative movement of the shrew recorded every 15 s for the duration of the trial. T_b was recorded at 15-min intervals on analog tape using an automated recording system similar to that described by Dyck and MacArthur (1992).

Results

Digesta passage rate

The mean cumulative rate of marker clearance was similar for shrews consuming both the meat and insect rations (Fig. 1). Inorganic tracer from the mealworm diet was first recovered 15 min post-ingestion (range: 8–25 min). The t_m of shrews fed this diet was 50 ± 3 min and 90% of the marker was collected 66 ± 3 min post-feeding. Tracer was first detected 16 min (range: 19–24 min) following consumption of the meat mixture. On this diet, t_m was 55 ± 5 min and 90% of the marker was collected at 78 ± 8 min post-feeding (Fig. 1).

Metabolic trials

Of the 228 metabolic trials conducted, 146 (64%) were discarded because shrews did not meet the specified criterion of remaining inactive for at least 2 min. In all trials, including those at T_as > 30°C, shrews were active over 90% of the time. Animals were rarely inactive longer than 2–3 min and in most cases only one rest period ≥2-min duration was obtained per 75-min trial. The majority (ca. 80%) of resting MR values were recorded during the last 15 min of each trial.

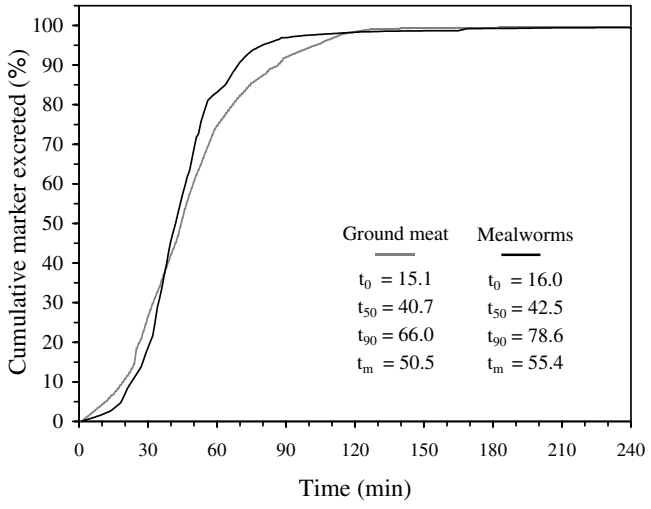

Fig. 1 Digesta passage rate of *S. palustris* consuming either mealworms ($N=7$) or a mixture of ground meat ($N=8$). *Lines* denote mean cumulative percent excretion of the particulate tracer in relation to time following ingestion. t_0 = time of first tracer appearance in feces (min); t_{50} = time to 50% excretion of tracer; t_{90} = time to 90% excretion of tracer; t_m = mean retention time of tracer

Because several test animals adapted better to the metabolic chamber, we obtained an unequal number of resting measurements from each animal—a factor that may bias the mean. To correct this, we first determined mean values for each animal and then used these values to determine the overall mean. Over a T_a range of 7–32°C, the mean abdominal T_b of *S. palustris* was independent of T_a and averaged 38.6 ± 0.1°C ($N=4$; $n=21$). Three resting T_b measurements for one shrew were unusually high (open squares Fig. 2), averaging 40.2°C, and tended to strongly bias the relationships of T_b and C to T_a. Consequently, these T_b values were omitted from analyses. A lower critical temperature (T_{lc}) of 21.2°C was calculated from the relationship observed between MR and T_a (Fig 2). Below this temperature, the inverse relationship between MR and T_a was described by the least-squares regression: MR (J g^{-1} h^{-1}) = 215.34 − 5.64×T_a ($r^2=0.81$, $n=24$, $P<0.0001$). Substituting VO_2 (ml O_2 g^{-1} h^{-1}) for MR, the equation becomes: $VO_2 = 10.663 - 0.276\times T_a$ ($r^2=0.82$, $P<0.0001$). The mean BMRs of resting, postabsorptive adult (98.52 ± 3.53, $N=9$; $n=25$) and subadult (93.93 ± 5.53; $N=5$; $n=33$) shrews were not significantly different ($P=0.46$), and therefore pooled. Thus, the estimated BMR of water shrews averaged 96.88 ± 2.93 J g^{-1} h^{-1}, or 4.84 ± 0.14 ml O_2 g^{-1} h^{-1} ($N=14$; $n=58$). The latter value is 3.0 times that predicted for a 14.4-g placental mammal ($VO_2 = 3.45$ $Mass^{-0.287}$ or 1.60 ml O_2 g^{-1} h^{-1} ; McNab 1988). Mean thermal conductance below thermoneutrality (0.282 ± 0.013 ml O_2 g^{-1} h^{-1} °C^{-1}) was similar to the C estimate (0.274 ml O_2 g^{-1} h^{-1} °C^{-1}) derived by forcing the regression of VO_2 on T_a to intersect the abscissa at 38.7°C, and was close to the value predicted from allometry (0.263 ml O_2 g^{-1} h^{-1} °C^{-1}; McNab and Morrison 1963). The RER of resting, fasted shrews averaged 0.81 and varied inversely with T_a (RER = 0.8394 − 0.0013×T_a, $r^2=0.05$, $n=82$, $P=0.045$; Fig. 2).

Intake and partitioning of energy

The mean gross energy content of sticklebacks (21.67 kJ g^{-1} dry matter) was similar to that calculated for dragonfly nymphs (22.23 kJ g^{-1} dry matter; Table 1). Apparent dry matter, DE and ME digestibility coefficients of six water shrews consuming stickleback were 79.1 ± 0.6, 90.4 ± 0.3 and 85.4 ± 1.3%, respectively. Though too few data were obtained for statistical testing, the corresponding values for the two shrews fed dragonfly nymphs were within 6% of the values derived for the stickleback diet (Table 2). ME intake for water shrews on the stickleback and dragonfly nymph rations averaged 202.9 ± 12.9 and 290.6 ± 4.0 J g^{-1} h^{-1}, respectively.

Activity trials

Though motor activity did not exhibit an obvious diel rhythm, episodes of activity were accompanied by matching increases in abdominal T_b (Fig. 3). During these 24-h trials, the abdominal T_b of radio-implanted water shrews averaged 38.5 ± 0.3°C with mean minimum and maximum values of 37.5 ± 0.5 and 39.6 ± 0.2°C, respectively. On average, shrews were active for 12.2 ± 0.8 h and inactive for 11.8 ± 0.8 h of each 24-h interval.

Discussion

Metabolic trials

The unavoidable liberation of heat arising from the digestion, biochemical transformation and excretion of food (termed the heat increment of feeding, or HIF) has recently been shown to cause a protracted increase in the resting MR of insectivorous mammals at thermoneutrality (Campbell et al. 2000; Hindle et al. 2003). As the mean throughput time of *S. palustris* is only 50–55 min (Fig. 1), the mechanical component of HIF (i.e. physical costs associated with mastication, nutrient absorption and peristalsis) in this species should be negligible following a 1-h fast. However, a heightened metabolic state arising from the biochemical HIF (i.e. postabsorptive transformation and storage of lipids, proteins and carbohydrates) may persist longer. Indeed, in both short-tailed shrews, *Blarina brevicauda*, and star-nosed moles, *Condylura cristata*, this obligatory increase in MR lasted ca. 120 min following the consumption of 3.5-g meals that corresponded to 10 and 6.5% of body mass, respectively (Campbell et al. 2000, Hindle et al. 2003). In the present study, most resting intervals occurred during

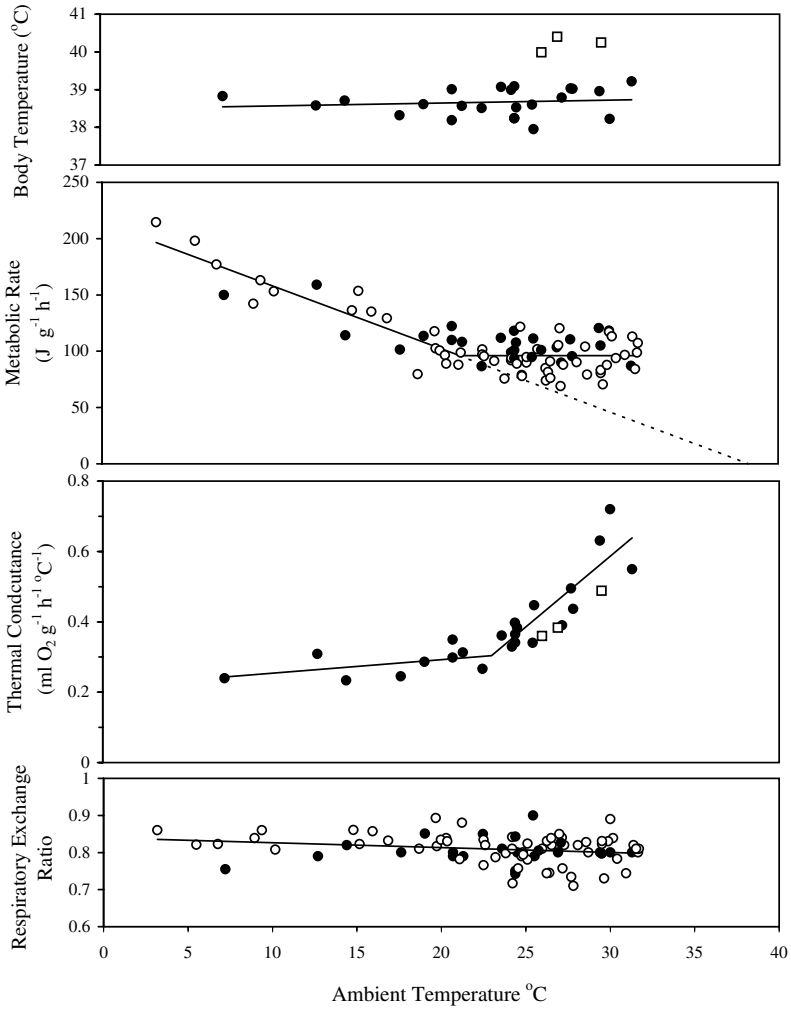

Fig. 2 Body temperature (T_b), rate of metabolism, thermal conductance (C) and RER of 14 fasted American water shrews in relation to ambient temperature (T_a). *Closed* and *open circles* denote measurements from radio-implanted and non-implanted animals, respectively. The *dotted line* is an extension of the linear regression of MR on T_a below thermoneutrality, and intersects the abscissa at a predicted T_b of 38.2°C. Note: three resting T_b measurements from one individual (*open squares*) were notably higher than values recorded for other shrews, and therefore metabolic data for these trials were omitted from subsequent analyses (see text for details)

Table 1 Mean (±SE) dry matter content and composition of brook stickleback, *C. inconstans*, and dragonfly nymphs, *Anax* (Aeshnidae) and *Libellula* (Libellulidae), fed to water shrews during digestibility trials

	Stickleback	Nymphs
Dry matter (%)	22.7 ± 0.3 (6)[b]	25.6 ± 0.1 (2)[b]
Gross energy (kJ g^{-1} dry matter)	21.7 ± 0.2 (6)[b]	22.2 ± 0.2 (2)[b]
Ash (%)[a]	13.7 ± 0.0 (6)[b]	–
Protein (%)[a]	65.6 (76.0)[c]	–
Lipid (%)[a]	15.1 (17.5)[c]	–
Carbohydrate (%)[a]	5.6 (6.5)[c]	–

[a]Values presented as percentage of dry matter content
[b]Sample size
[c]Percentage composition on an ash-free basis

the final 15 min of each trial, by which point animals had fasted for >2 h. This observation, together with the fact that meals offered in the aforementioned studies were substantially larger than those (ca. 0.2 g or 1.4% of body mass) consumed by water shrews immediately preceding the pre-trial period, suggests that HIF likely contributed negligibly, if at all, to our metabolic estimates.

Despite efforts to minimize pre-trial handling stress and provision of a quiet, stable environment during experimentation, all individuals exhibited high levels of activity throughout most of the 60- or 75-min metabolic trials. In fact, stable resting $\dot{V}O_2$ recordings exceeding 2-min duration were recorded in only 36% (82/228) of trials. Similarly, Sparti and Genoud (1989) obtained resting $\dot{V}O_2$ measurements in only 20–30% of metabolic trials performed on crowned shrews, *S. coronatus*, and Eurasian pygmy shrews, *S. minutus*, underscoring the inherently active nature of soricine species. Given this attribute, it is perhaps not surprising that initial estimates of BMR of red-toothed shrews substantially exceeded allometric predictions (Morrison and Pearson 1946; Pearson 1948). However, subsequent re-examinations of shrew energetics (see McNab 1991; Sparti and Genoud 1989) yielded mass-specific BMRs ranging from 2.5 to 3.7 times higher than the values predicted from allometry ($\dot{V}O_2 = 3.45\ \text{Mass}^{-0.287}$; McNab 1988). Though some values included in these reviews were obtained from fed and potentially active animals, our conservative estimate of BMR for *S. palustris* (96.88 J g^{-1} h^{-1} or 4.84 ml O_2 g^{-1} h^{-1}) is still 302% of the mass-predicted value. It is noteworthy that McIntyre

Table 2 Body mass, apparent digestibility coefficients and metabolizable energy intake (MEI) of American water shrews fed brook stickleback and dragonfly nymph rations at 20 ± 1°C. Also included are average metabolic rates (AMRs) of these same individuals collected during gas exchange trials at 19–22°C

Animal	Stickleback ration							Nymph ration		
	1	2	3	4	5	6	Mean ± SE	4	5	Mean ± SE
Mean body mass (g)	15.05	13.51	14.39	13.87	14.55	15.32	14.55 ± 0.30	15.19	14.29	14.74 ± 0.63
Mass change (g)	−0.41	−0.16	−0.45	0.10	0.00	−0.34	−0.21 ± 0.10	1.11	0.44	0.77 ± 0.47
Digestibility (%)										
Dry matter	78.8	79.4	77.2	81.1	80.0	78.0	79.1 ± 0.6	79.6	81.7	80.7 ± 1.5
Ash-free dry matter	86.9	87.9	84.2	86.0	87.5	87.1	86.6 ± 0.0	–	–	–
Digestible energy	90.6	90.2	89.7	91.5	90.9	89.6	90.4 ± 0.3	84.6	85.4	85.0 ± 0.6
Metabolizable energy	89.5	84.7	83.9	87.5	85.7	81.3	85.4 ± 1.3	82.1	83.6	82.8 ± 1.1
MEI (J g^{-1} h^{-1})	210.9	209.3	171.6	229.5	237.9	158.3	202.9 ± 12.9	284.2	296.9	290.6 ± 4.0
AMR (J g^{-1} h^{-1})	205.5	234.9	153.4	207.4	262.6	184.2	208.0 ± 17.0	–	–	–

(2000), following procedures identical to those outlined in this study, obtained an estimate of basal VO_2 for two American water shrews (4.75 ml O_2 g^{-1} h^{-1}) that was virtually identical to ours. Thus, data from these studies strengthen the tenet (McNab 1991) that soricine shrews exhibit BMRs that are inherently higher than those of similar-sized endotherms.

An elevated BMR combined with low thermal conductance and high T_b are common attributes of semi-aquatic mammals (Grant and Dawson 1978; Costa and Kooyman 1982; Doncaster et al. 1990; Campbell et al. 1999). Unfortunately, reliable BMR data for strictly terrestrial soricines with a body mass similar to American water shrews are unavailable for intra-generic comparisons. However, it is noteworthy that the estimated daily energy expenditure of *S. palustris* at 20°C (54.0 kJ day^{-1}, see below) is 9% greater than that predicted for captive terrestrial insectivores maintained at similar air temperatures (kJ day^{-1} = 12.967 Mass$^{0.50}$ or 49.2 kJ day^{-1}; Grodzinski and Wunder 1975). Conversely, *S. palustris* exhibit a T_{lc} (21.2°C) well below that of other soricids and substantially lower than the value (32.0°C) predicted on the basis of mass ($T_{lc} = T_b - 3.35$ Mass$^{0.26}$; Sparti and Genoud 1989). In addition, the

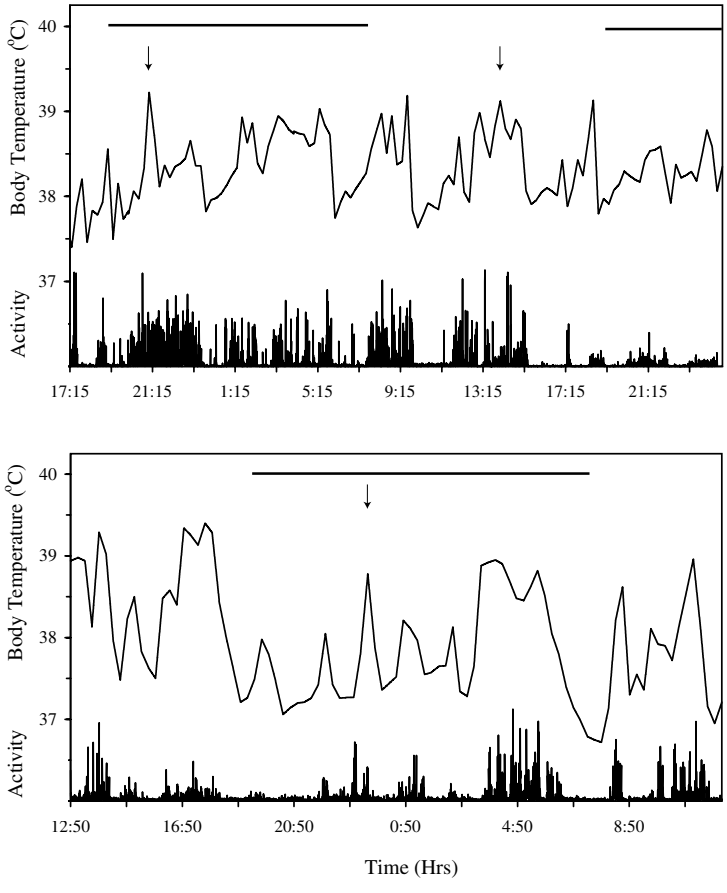

Fig. 3 Twenty-four hour activity and body temperature patterns of two water shrews housed at an air temperature of 20°C. *Vertical bars* denote relative levels of activity, *horizontal bars* specify hours of darkness and *arrows* indicate feeding times (see text for details)

resting T_b of American water shrews (38.6°C) is 0.2–1.8°C higher than for other soricids examined to date (see Sparti and Genoud 1989). However, this latter finding could reflect discrepancies in methodology since Sparti and Genoud (1989) reported only rectal T_bs that may be lower than intra-abdominal measurements recorded telemetrically (this study).

Sorex palustris strictly defended T_b during the 75-min trials at all temperatures tested (Fig. 2), implying a high degree of thermoregulatory competence in air. Maintenance of a high BMR and T_b may be advantageous to delay immersion hypothermia or expedite rewarming, as *S. palustris* apparently cools quickly in the aquatic medium (Calder 1969; R.W. Gusztak, unpublished data). Calder (1969) further remarked that American water shrews are not especially adapted to cold exposure, as this species possessed 'typical' insulation for its size. This conclusion is consistent with our finding that the thermal conductance of *S. palustris* (0.274–0.282 ml O_2 g^{-1} h^{-1} $°C^{-1}$) is 1.04–1.07 times the mass-predicted value for Eutherians and is within the range reported for terrestrial shrews (0.99–1.34 times the mass-predicted value; Sparti and Genoud 1989). In this context, it is relevant to note that the thermal conductance of American water shrews is substantially higher than that calculated for European water shrews, *Neomys fodiens* (0.71–0.84 times the mass predicted value; Nagel 1985; Sparti 1990). The slightly larger *N. fodiens* also exhibits a lower abdominal T_b (37.3–37.9°C) and basal $\dot{V}O_2$ (2.9–3.2 ml g^{-1} h^{-1}), but higher T_{lc} (27.5–30°C; Sparti 1990; but see Nagel 1985) than the American water shrew. These observations highlight the distinct physiological phenotypes expressed by these independently derived semi-aquatic shrews. Additional data on the dive performance and aquatic energetics of *S. palustris* are clearly needed to evaluate and compare the thermal and metabolic attributes of these species in water.

Intake and partitioning of energy

To our knowledge, this study is the first to report ME digestibility coefficients for any species of shrew. Calculated ME digestibilities for *S. palustris* consuming brook stickleback (85.4%) and dragonfly nymphs (82.8%) were higher than for *C. cristata* eating earthworms (68.0%; Campbell et al. 2000). However, the calculated DE digestibility of water shrews on the stickleback diet (90.4%) was within the range previously reported for four European *Sorex* species consuming minnows (range: 86–93%; Hanski 1984) and comparable to the DE digestibility of *B. brevicauda* maintained on a diet of canned dog food (92%; Buckner 1964). Likewise, the DE digestibility of water shrews fed exclusively a diet of dragonfly nymphs (84.9%) was comparable to that of other *Sorex* species fed sawfly cocoons (76.5–85.3%; Hanski 1984), and short-tailed shrews consuming mealworms (89.5%; Barrett and Stueck 1976) and sawfly larvae (78%; Buckner 1964). These DE efficiencies are generally ca. 5% lower than for larger carnivores consuming invertebrates (90.5%) or meat and fish diets (95.5%; Robbins 1993), and presumably relate to the shorter digesta passage rates of soricine shrews.

Our estimates of energy turnover (Table 2) and relative animal activity (data not shown) during metabolic and digestibility trials were similar for each animal tested. Additionally, the mean ME intake for water shrews held at 20°C and consuming the brook stickleback ration (203 J g^{-1} h^{-1}) was very close to the average MR calculated for these same six individuals at 19–22°C (208 J g^{-1} h^{-1}; Table 2). We utilized the latter value together with data obtained from our 24-h activity trials to estimate the mean daily energy requirements of *S. palustris* in captivity. Assuming that shrews are active for an average of 12.2 h day^{-1} (Fig. 3) and that resting and active MRs at 20°C are 103 and 208 J g^{-1} h^{-1}, respectively, the average energy requirement of a 14.4-g water shrew is 156.2 J g^{-1} h^{-1} or 54.0 kJ day^{-1}. Based on the average mass (0.93 g) and caloric content (4.92 kJ g^{-1} wet mass) of individual brook stickleback (Table 1), and assuming ME digestibility = 85.4% and HIF = 6% of gross energy intake (Campbell et al. 2000; Hindle et al. 2003), water shrews must consume 14.7 minnows, or 0.95 g minnow g^{-1} day^{-1} in order to meet this daily energy requirement. This estimate is virtually identical to those previously derived for captive water shrews by Sorenson (1962; 0.95 g meat g^{-1} day^{-1}) and Conaway (1952; 1.03 g fish/meat g^{-1} day^{-1}). However, it should be cautioned that all of these values are based on studies of American water shrews maintained in exclusively terrestrial settings. Consequently, they are best viewed as minimum estimates only, since they make no allowances for the appreciable thermoregulatory cost of foraging in cold water. To address this issue, studies examining the thermal biology, energetics and physiology of diving by this remarkable insectivore are currently ongoing in our laboratory.

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