Relating $\Delta^{17}O$ Values of Animal Body Water to Exogenous Water Inputs and Metabolism

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The dynamics of animal body water and metabolism are integral aspects of biological function but are difficult to measure, particularly in free-ranging individuals. We demonstrate a new method to estimate inputs to body water via analysis of $\Delta^{17}O$, a measure of $^{17}O/^{16}O$ relative to $^{18}O/^{16}O$. Animal body water is primarily a mixture of drinking or food water (meteoric water; $\Delta^{17}O \approx 0.030$ per mille [‰]) and metabolic water synthesized from atmospheric oxygen ($\Delta^{17}O \approx -0.450$‰). Greater drinking or food water intake should increase $\Delta^{17}O$ toward 0.030‰, whereas greater metabolic rate should decrease $\Delta^{17}O$ toward –0.450‰. We found that wild mammal $\Delta^{17}O$ values generally increased with body mass, consistent with both a decline in mass-specific metabolic rate and an increase in water intake. Captive mouse (Peromyscus maniculatus) $\Delta^{17}O$ values were higher than predicted but exhibited the expected relative change based on metabolic rate and water intake. Measurements of $\Delta^{17}O$ may enable novel ecophysiological studies.

Keywords: hibernation, stable oxygen isotope, water balance

Water is critical for all life, and understanding how organisms obtain and retain water is important in physiology, ecology, and evolution. However, measuring source contributions to organism water pools is difficult, especially outside of laboratory conditions. Terrestrial animals generally lose water in waste products (e.g., feces or urine), cutaneous evaporation, and exhaled breath vapor while gaining water via three sources (Hill et al. 2008). The first source is water ingested in drinking water and food, most of which is ultimately derived from meteoric water precipitation. The second source is endogenous synthesis of metabolic water, in which oxygen inhaled from the air (i.e., atmospheric oxygen, O_2) is the terminal acceptor in the mitochondrial electron transport chain and is combined with hydrogen, producing water (H_2O). The third but relatively minor source is water produced by condensation reactions that use oxygen bound in food molecules to create H_2O during metabolic processes such as glycolysis. Although the latter two sources are sometimes grouped as *metabolic water* (Kohn 1996), in the present article, this term is used only in reference to water synthesized from atmospheric oxygen in the mitochondria.

In the present article, we show that the triple oxygen isotope system (^{16}O, ^{17}O, ^{18}O) provides a means to estimate the fractional inputs of different sources into body water from a single sample (Pack et al. 2013). Although our focus is on animal body water, the concepts apply to all living things that primarily use aerobic oxidation to obtain energy. This approach is based on measurement of $\Delta^{17}O$, defined as the positive or negative deviation (i.e., residual) from the tight correlation that naturally exists between $\delta^{17}O$ and $\delta^{18}O$ (figure 1). This correlation, known as the *terrestrial fractionation line*, has a slope of approximately .528 for nearly all terrestrial materials (Pack and Herwartz 2014). Analytical advances have enabled accurate measurement of $\Delta^{17}O$ values with a precision of 0.005 per mille (‰; Sharp et al. 2016).

For most animals, metabolic water and ingested drinking or food water together provide 80%–99% of their body water (Bryant and Froelich 1995, Kohn 1996). Importantly, the $\Delta^{17}O$ values of these two sources are relatively constant but strikingly different from each other (table 1). The oxygen in the first source, metabolic water, is derived exclusively from atmospheric oxygen, which has a uniquely negative $\Delta^{17}O$ value of approximately –0.450‰ (Young et al. 2014). The oxygen in the second source, ingested water, comes from drinking water and water molecules in food. This water is primarily derived from meteoric water, which has a $\Delta^{17}O$ value that averages approximately 0.030‰ with a range of 0.000‰–0.050‰ (Sharp et al. 2018). Slight variations in this value are driven by processes such as precipitation in

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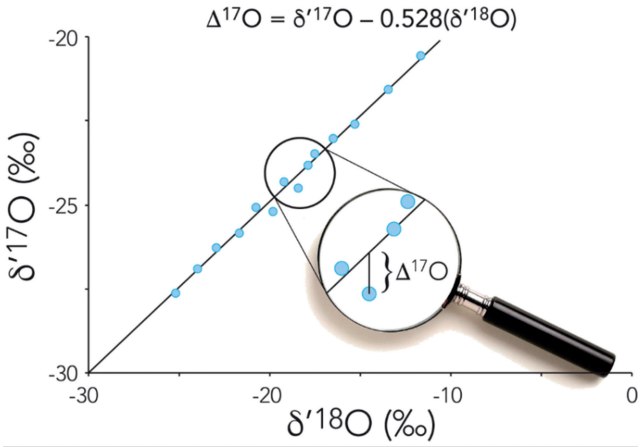

Figure 1. $\delta^{17}O$ and $\delta^{18}O$ express the abundance of ^{17}O and ^{18}O relative to the abundance of ^{16}O. Most fractionation processes are mass dependent; therefore, when fractionation affects one isotope (e.g., ^{18}O), the effects on other isotopes (e.g., ^{17}O or ^{16}O) are predictable. This predictability is represented by the terrestrial fractionation line (the black line). Deviation from this line is expressed as $\Delta^{17}O$. Note that because of curvilinearity in the relationship between $\delta^{17}O$ and $\delta^{18}O$, $\delta^{x}O$ is recalculated as $\delta^{x}O = 1000 \times \ln((\delta^{x}O/1000)+1)$, where $x = {}^{17}O$ or ^{18}O.

the extreme cold of polar regions (Landais et al. 2012). For meteoric water with very low (below –55‰) or high (above –20‰) values of $\delta^{18}O$, the value of $\Delta^{17}O$ approaches 0‰; otherwise, it is closer to 0.030‰ (Li et al. 2015, Sharp et al. 2018). Importantly, even though the $\delta^{18}O$ value of meteoric water can vary by more than 50‰, the mass-dependent fractionation that occurs during typical evaporation and condensation alters $\delta^{17}O$ and $\delta^{18}O$ values in tandem, which moves them both along the terrestrial fractionation line (figure 1). This results in little change in the value of $\Delta^{17}O$. If the $\Delta^{17}O$ values of atmospheric oxygen and meteoric water are treated as fixed endpoints of –0.450‰ and 0.030‰, their difference is 0.480‰, almost two orders of magnitude larger than the current precision of $\Delta^{17}O$ measurements (less than 0.010‰).

The third and relatively minor source of water, condensation reactions, contributes 1%–20% of animal body water (Bryant and Froelich 1995, Kohn 1996). This source includes the oxygen that is bound in dietary carbohydrates, proteins, and lipids, which likely have similar $\Delta^{17}O$ values as meteoric water (see methods descriptions). The water produced by condensation reactions mixes with ingested water and metabolic water to form the body water pool. Losses from this pool likely have minimal effect on animal body water $\Delta^{17}O$ ($\Delta^{17}O_{BW}$) values because liquid losses contain representative contributions from each input, and evaporative losses generally cause fractionation that moves $\delta^{17}O$ and $\delta^{18}O$ values along the terrestrial fractionation line in figure 1.

Current methods to estimate fractional inputs into animal body water require injecting a tracer (e.g., isotopically labeled water) into the animal, allowing it to equilibrate with the body water pool, then collecting at least two samples of body water (e.g., distilled from blood plasma) in events separated by enough time for the tracer to measurably decline (Holleman and Dieterich 1973, Nagy and Gruchacz 1994). Although this approach has led to invaluable insights into the physiology of free-ranging animals, it is often not feasible, particularly for highly mobile or cryptic animals—hence the relative paucity of ecophysiological data on such species. In contrast, $\Delta^{17}O_{BW}$ values can be measured in a single sample, eliminating the need for a second capture. If these values can support inferences regarding inputs to animal body water, this measurement has the potential to be an important new tool with applications in ecology, ecophysiology, and paleoecology.

Using observational, experimental, and modeled data to understand $\Delta^{17}O_{BW}$

In the present article, three approaches were used to evaluate the hypothesis that $\Delta^{17}O_{BW}$ values can be used to quantify the fractional contributions to animal body water. First, we measured $\Delta^{17}O_{BW}$ values in water distilled from the blood plasma of wild and captive mammals ranging across six orders of magnitude in body mass. Increasing body mass correlates with declines in mass-specific metabolic rate (White and Seymour 2005) and, to a lesser extent, water flux (Nagy and Peterson 1988). Therefore, as body mass increases, metabolic rate and metabolic water production should decline more than water intake, which should reduce the fractional contribution of metabolic water to body water. Second, we tested whether $\Delta^{17}O_{BW}$ values of captive deer mice (*Peromyscus maniculatus*) responded as predicted to changes in body water inputs. Housing temperature of deer mice was decreased to alter their metabolic rate and water intake. Their oxygen consumption and drinking or food water intake were monitored, and their predicted and measured values of $\Delta^{17}O_{BW}$ were compared. Third, to complement the observational and experimental data, we modeled $\Delta^{17}O_{BW}$ values for a generic herbivore and carnivore over a range of water intake and metabolic rates using data from previous studies, illustrating the dependence of $\Delta^{17}O_{BW}$ values on these two key variables. The herbivore model was also used to test sensitivity of $\Delta^{17}O_{BW}$ values to analytical error.

$\Delta^{17}O_{BW}$ versus body mass

In the first approach to study $\Delta^{17}O_{BW}$, archived serum or plasma samples were obtained for 19 individuals of nine mammal species (table 2). The individuals were sampled once, except for the hibernating black bears (URAM), which were sampled in December 2013 and in March 2014 (early and late hibernation). The sampling of the black bears occurred on days 32 and 114 (URAM-1) and days 56 and 136 (URAM-2) of hibernation, based on GPS locations; locations were not available for URAM-3.

The measured $\Delta^{17}O_{BW}$ values of all of the individuals were compared with a trend that was predicted solely by the

Table 1. Inputs into animal body water and their fractional contributions (F) and $\Delta^{17}O$ values.

Input	Ultimate source of oxygen	$F^{a,b}$	$\Delta^{17}O$ (per mille)
Preformed H_2O			
Drinking water	Meteoric water[c]	0.00–0.60	approximately 0.030
Food water	Meteoric water, animal body water	0.00–0.60	?–0.030
Metabolic H_2O			
From mitochondria	Atmospheric oxygen[d]	0.20–0.40	approximately –0.450
Bound H_2O			
In carbohydrates	Meteoric water, atmos. CO_2[e]	0.00–0.10	–0.200–0.030
In proteins	Meteoric water, animal body water	0.00–0.10	?–0.030
In lipids	Carbohydrates and proteins	0.00–0.10	?–0.030

Note: F values are generalized across mammals with a body mass of up to 1000 kilograms. Question marks indicate unknown values. $\Delta^{17}O$ is versus the standard VSMOW. [a]Kohn 1996. [b]Bryant and Froelich 1995. [c]Sharp et al. 2018. [d]Young et al. 2014. [e]Luz and Barkan 2010.

Table 2. Mammals for which samples of blood plasma or serum were measured for $\Delta^{17}O$.

Species	n	Habitat	Drinking water
Lab mouse, *Mus musculus*; MUMU	2	Captive; University of New Mexico	*Ad libitum*
Silky pocket mouse, *Perognathus flavus*; PGFV	3	Wild; Sevilleta National Wildlife Refuge, New Mexico	Arid desert; annual precipitation 25 centimeters (cm); 0.1 millimeters over the 11 days before sampling
Merriam's kangaroo rat, *Dipodomys merriami*; DIME	1		
Banner-tailed kangaroo rat, *Dipodomys spectabilis*; DISP	2		
White-footed mouse, *Peromyscus leucopus*; PELE	1		
River otter, *Lontra canadensis*; LOCA	3	Wild; coastal Kenai Fjords National Park, Alaska	Mesic; >50 cm annual precipitation
Mule deer, *Odocoileus hemionus*; ODHE	2	Wild; Wyoming Range and Wind River Range	Semiarid plains, high-elevation forest; annual precipitation from 15–100 cm by elevation
American black bear, *Ursus americanus*; URAM	3	Wild (hibernating); northern Minnesota	Mesic mixed coniferous and deciduous forest and developed lands; annual precipitation 60–80 cm
African elephants, *Loxodonta africana*; LOAF	2	Captive; San Diego Zoo, San Diego, California	*Ad libitum*

allometric scaling of metabolic rate and water flux with body mass. A mixing model was used in which the $\Delta^{17}O_{BW}$ value was predicted by the fractional contributions of atmospheric oxygen in metabolic water (F_A) and of oxygen in ingested drinking or food water ($1 - F_A$) that we assumed had the $\Delta^{17}O$ value of meteoric water while ignoring the minor contribution from water produced by condensation reactions:

$$\Delta^{17}O_{BW} = F_A(-0.450‰) + (1 - F_A) \times (0.030‰) \quad (1)$$

The scaling of metabolic rate with body mass (M, in grams) for free-ranging mammals was calculated following White and Seymour (2005):

$$O_2\ ml \times hr^{-1} = 4.53 \times M^{0.75} \quad (2)$$

The total amount of O_2 (in milliliters) consumed in 24 hours was calculated and converted to the amount of metabolic water produced (also in milliliters), assuming that each milliliter of O_2 contained 5.38×10^{19} oxygen atoms (i.e., 4.46×10^{-5} moles of O_2, treating O_2 as an ideal gas) and that each oxygen atom was incorporated into a molecule of metabolic water. The scaling of total water flux with body mass for free-ranging mammals was calculated following Nagy and Peterson (1988):

$$\log(H_2O\ ml \times day^{-1}) = -0.487 + 0.818 \times \log(M) \quad (3)$$

Total water flux (in milliliters) for 24 hours was calculated, then metabolic water input (in milliliters) was subtracted, and the remainder was assumed to represent ingested meteoric water input. These values were then used to calculate the fractional contributions of metabolic water (F_A) and ingested drinking or food water ($1 - F_A$) over a 24-hour span, and those values were inserted into equation 1 for the range of body masses of the 19 mammals in the data set.

Deer mice experiment

Adult female deer mice ($n = 4$) were purchased from the *Peromyscus* Genetic Stock Center, at the University of South Carolina, and housed individually in custom-made flow-through metabolic chambers (Lexan, GSI Outdoors,

Spokane, Washington) in a controlled-temperature environment (Darwin Chambers, St. Louis, Missouri) at the University of Massachusetts. The chambers (28 × 22 × 13 centimeters) contained absorbent floor material, bedding, domes, and *ad libitum* water (from a glass bottle) and food pellets containing 22% protein, 12% fat, 52% carbohydrates, and 9% water (Prolab 5P75, St. Louis, Missouri).

Oxygen consumption was measured with multiplexed, open-flow respirometry, with each mouse and a baseline sampled every 8–10 minutes (Lighton and Halsey 2011). Using a scroll air compressor (Atlas-Copco, Springfield, Massachusetts) with a membrane air dryer, dry fresh air was pushed through the chambers (approximately 750 milliliters [ml] per minute), and the excurrent air was subsampled at approximately 300 ml per minute. O_2 (Sable Systems, Las Vegas, Nevada), carbon dioxide (CO_2), and H_2O (Licor 840A, Lincoln, Nebraska) were recorded once per second. O_2, $\dot{V}CO_2$, $\dot{V}H_2O$ were calculated using standard equations (Lighton 2008).

The experiment was started after 9 days of acclimation. On day 1, masses were recorded for the mice and their water and food pellets. During days 1–10, the temperature was 25°C. On day 4, the water and food masses were recorded. On day 10, the mice, water, and food masses were recorded, and blood samples were collected from the saphenous vein into capillary tubes. From day 10 to day 11, the temperature was gradually decreased to 5°C. Over approximately 24 hours prior to day 17, the temperature drifted up to 13°C because of a sensor error; on day 17, the masses were recorded, blood samples were collected from the contralateral limb, and the temperature was gradually returned to 5°C. On day 21, the masses were recorded, and the final blood samples were collected.

Expanding equation 1, the deer mice's $\Delta^{17}O_{BW}$ values were predicted on the basis of all of the primary inputs into their body water:

$$\Delta^{17}O_{BW} = F_A(\Delta^{17}O_A) + F_{DW}(\Delta^{17}O_{DW}) + F_{FW}(\Delta^{17}O_{FW}) + F_{DC}(\Delta^{17}O_{DC}) + F_{DP}(\Delta^{17}O_{DP}) + F_{DL}(\Delta^{17}O_{DL}) \quad (4)$$

In the present article, F is the fractional contribution to body water (Luz and Kolodny 1985) from sources including atmospheric O_2 ($_A$), ingested drinking water ($_{DW}$) and food water ($_{FW}$), and bound oxygen in dietary carbohydrates ($_{DC}$), protein ($_{DP}$), and lipids ($_{DL}$). The following assumptions were made: $\Delta^{17}O_A$ = –0.450‰; $\Delta^{17}O_{DW}$ and $\Delta^{17}O_{FW}$ = 0.030‰; $\Delta^{17}O_{DC}$ = –0.085‰ (the midpoint between the $\Delta^{17}O$ values of the two contributors to carbohydrate oxygen, which are meteoric water [0.030‰] and CO_2 from the atmosphere [$\Delta^{17}O \approx$ –0.200‰]; Liang and Mahata 2015, Hofmann et al. 2017); $\Delta^{17}O_{DP}$ = 0.030‰ (oxygen in amino acids likely originates from meteoric water, although it may exchange with animal body water; Stewart et al. 2001, Ehleringer et al. 2008); and $\Delta^{17}O_{DL}$ = –0.028‰ (the midpoint between values for $\Delta^{17}O_{DC}$ and $\Delta^{17}O_{DP}$, because lipids may contain oxygen from carbohydrates or protein; Nelson and Cox 2008). Although $\Delta^{17}O_{DC}$, $\Delta^{17}O_{DP}$, and $\Delta^{17}O_{DL}$ are estimates, this likely did not bias our predictions because of their relatively small contributions to the total body water pool.

For F values, the amount of water contributed to body water from each input was calculated for the 72 hours prior to sampling, adequate time for complete body water turnover (Holleman and Dieterich 1973). For F_A, the total amount of consumed oxygen was converted to milliliters of metabolic water as was described above (O_2 was corrected to standard temperature and pressure). For F_{DW} and F_{FW}, the fraction of mass loss of the water bottles and food water were calculated for 72 hours. F_{DC}, F_{DP}, and F_{DL} were assumed to contribute a total of 0.14 to body water (Kohn 1996), and this contribution was divided by the relative proportions of these macromolecules in the diet.

Analytical methods

For all of the samples, whole blood was centrifuged, and serum or plasma was stored (supplement S1) at 4°C–8°C (deer mice, lab mice, wild rodents) or at –20°C to –40°C (other species) until their analysis at the University of New Mexico Center for Stable Isotopes. The samples (1–2 microliters) were admitted into a vacuum line and cryogenically distilled, and the resulting H_2O was reacted with BrF_5 at approximately 300°C for 5 minutes to quantitatively convert H_2O to HF+O_2. The O_2 was purified using liquid nitrogen traps then passed through a GC column to remove traces of NF_3 and other contaminants. The O_2 was admitted into a Thermo 253 isotope ratio mass spectrometer, and the $\delta^{17}O$ and $\delta^{18}O$ values were analyzed in dual inlet mode using 20 cycles (26 seconds integration). The value of $\Delta^{17}O$ was calculated as is described in figure 1.

Before each analytical session, a local water standard was measured (NM2: $\delta^{18}O$ = –13.1‰, $\delta^{17}O$ = –6.919‰) that had been calibrated against the international water standards VSMOW2 ($\delta^{17}O = \delta^{18}O$ = 0.000‰) and SLAP2 ($\delta^{18}O$ = –55.5‰, $\delta^{17}O$ = –29.699‰; Schoenemann et al. 2013, Sharp et al. 2016). The NM2 $\Delta^{17}O$ values associated with each measurement were used to calculate a correction factor that was applied to the raw $\Delta^{17}O$ values of samples to yield corrected values. Because of logistical constraints, NM2 measurements could not be made in association with three samples (PGFV-3, PELE-1, DISP-2; see supplemental table S1), and unfortunately, no sample remained after the analysis. These three samples were analyzed alongside a different sample (DIME-1; table S1) that was later analyzed again along with NM2 as was described above. The difference in values for DIME-1 between the measurements was then considered the correction factor for the three samples analyzed earlier. Details for this correction are provided in table S1.

Out of 30 total samples, 18 were measured in replicate to assess precision; the intensive analytical nature of this technique prevented replication for all of the samples. The replicate standard deviation (SD) ranged from 0.000‰ to 0.015‰ (table S1). Precision was consistent across time,

as was indicated by two samples from URAM-1, both measured in December 2016 and again in May 2017. The $\Delta^{17}O_{BW}$ value of the first sample was –0.095‰ in December (single injection) and –0.104‰ in May (two injections, SD = 0.006‰), and the second sample was –0.163‰ in December (single injection) and –0.159‰ in May (two injections, SD = 0.002‰).

Modeling animal $\Delta^{17}O_{BW}$

In the third approach of this study, $\Delta^{17}O_{BW}$ values were calculated for a generic mammalian herbivore and carnivore using equation 4. The *F* values (Bryant and Froelich 1995, Kohn 1996, Podlesak et al. 2008) and $\Delta^{17}O$ values of water sources were from models and published measurements that were realistic for a 100–500-gram herbivore and a 10–20-kilogram carnivore (supplemental table S2). For the herbivore, the $\Delta^{17}O$ value of bound oxygen in dietary carbohydrates was assumed to be –0.085‰. For the carnivore, its food was the herbivore. Therefore, the ingested water in its food had the $\Delta^{17}O_{BW}$ value of the herbivore, and the $\Delta^{17}O$ value of bound oxygen in the dietary proteins and lipids was assumed to be the midpoint between the herbivore's body water and meteoric water.

For both models, the $\Delta^{17}O_{BW}$ value was calculated that would be reached at a steady state after changing the metabolic rate to 50%–500% of the initial value, representing extremes from hibernation (Hellgren 1998, Tøien et al. 2011) to the maximum sustained metabolic scope (Peterson et al. 1990). It was assumed that changes in metabolic rate had the same effect on the rates of input into body water from metabolic water and from condensation reactions. The calculations were repeated across a plausible range of values of the initial input rate of ingested water: 50%, 75%, 125%, 150%, and 200% (Little and Shaw 1978, Bachmanov et al. 2002; example values appear in supplemental table S3). Finally, to assess the propagation of measurement error, the $\Delta^{17}O$ values of inputs to body water for the herbivore model were altered to reflect plausible analytical variation in each input, and $\Delta^{17}O_{BW}$ was recalculated.

Observational data support the physiological interpretation of $\Delta^{17}O_{BW}$

Based solely on the allometric scaling of metabolic rate and water flux (equations 1–3), $\Delta^{17}O_{BW}$ values were expected to increase with body size (the gray line in figure 2a). Consistent with this expectation, there was a general trend toward increased $\Delta^{17}O_{BW}$ values with increased body mass (figure 2a). The measured $\Delta^{17}O_{BW}$ values of all 19 mammals (table S1) were between the assumed endpoints of meteoric water (0.030‰) and atmospheric O_2 (–0.450‰).

The metabolic rates and drinking or food water intake rates for these mammals were unknown. However, plausible constraints can be applied to some of these inputs. The wild rodents were sampled at a single location that had received 0.1 millimeters of precipitation during the prior 11 days, a period long enough for complete body water turnover (Mullen 1970, 1971). If they all ingested similarly low amounts of drinking or food water, differences in their $\Delta^{17}O_{BW}$ values would primarily reflect variation in metabolic water input, and accordingly, these differences correlated with body mass (the black line in figure 2a). The black bears likely had essentially no access to drinking or food water between sampling events, because they were fasting in winter dens (Hellgren 1998). Assuming that metabolic water was the sole input to their body water during that period, the change in $\Delta^{17}O_{BW}$ values between early and late hibernation indicates that the bears replaced 0.2% (URAM-1, URAM-2) to 0.4% (URAM-3) of their body water per day. If these turnover rates were also representative of the first 1–2 months of hibernation, the $\Delta^{17}O_{BW}$ values on the day of den entry would have been –0.074‰ for URAM-1 and –0.081‰ for URAM-2.

Applying the mixing model in which only metabolic water and meteoric water contributed to body water (equation 1) led to realistic calculated F_A values for selected species (figure 2b). In addition, a $\delta^{18}O$ value for the drinking or food water ingested by these selected species was calculated on the basis of their $\Delta^{17}O_{BW}$ and $\delta^{18}O_{BW}$ values using this equation:

$$\delta^{18}O_{\text{Drinking/Food Water}} = (\delta^{18}O_{BW} - (F_A) \times (\delta^{18}O_{Air})) / (1-F_A) \quad (5)$$

In equation 5, the value of $\delta^{18}O_{Air}$ was assumed to be 17.8‰ because of the fractionation that occurs when inhaled oxygen is incorporated into the blood (supplement S2; Epstein and Zeiri 1988, Zanconato et al. 1992). There was variable agreement between the calculated $\delta^{18}O$ value of ingested meteoric water and local water sources (figure 2b). The water ingested by URAM (early hibernation sample) had a mean estimated value of –19‰, similar to the precipitation in Minnesota from October to December (Bowen 2017), when bears probably last accessed water. The calculated $\delta^{18}O$ value of water ingested by LOAF was –8‰, in the range expected for their location in southern California (–9 to –5‰; Bowen 2017). However, the water ingested by DISP and PGFV had mean calculated values of $\delta^{18}O$ of –4‰ and –19‰, respectively, which were outside of the range of surface waters in their habitat (–15 to –3‰; Bowen 2017). The water ingested by LOCA had a $\delta^{18}O$ value of –10‰, higher than local freshwater sources (–20‰ to –15‰; Bowen 2017).

Variation in metabolic rate, water intake, and $\Delta^{17}O_{BW}$ were related in individual deer mice

A decreasing ambient temperature caused the mice to increase oxygen consumption and water and food intake (supplemental table S4). On the basis of the model of all inputs to body water (equation 4), these changes were predicted to cause the mouse $\Delta^{17}O_{BW}$ values (figure 3) to either decline between sampling events (mice 1–3) or remain unchanged (mouse 4). The measured values of $\Delta^{17}O_{BW}$ were consistently higher than the predicted values,

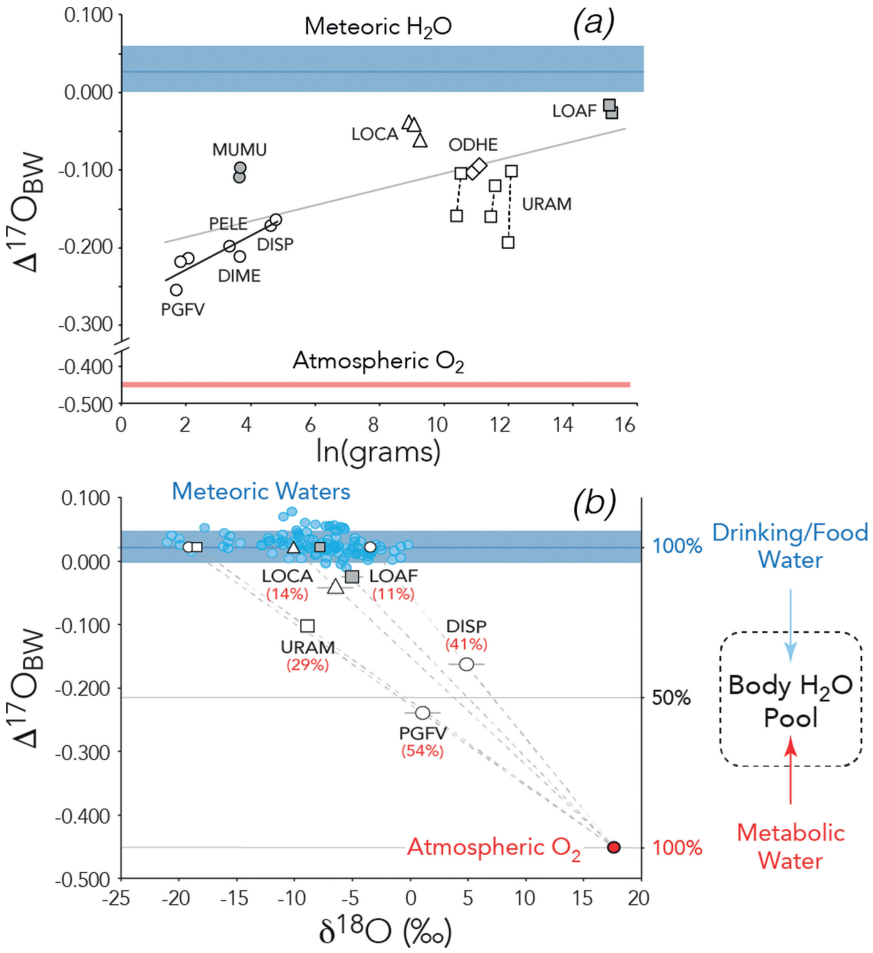

Figure 2. The body water $\Delta^{17}O$ values ($\Delta^{17}O_{BW}$) of 19 mammals increased with increasing body mass. Panels show the general range of values for $\Delta^{17}O$ of meteoric water and atmospheric oxygen. (a) $\Delta^{17}O_{BW}$ values (error measurements are available in supplemental table S1) of captive animals (house mice, the gray circles; elephants, the gray squares) and wild animals (rodents, the white circles; river otters, the white triangles; mule deer, the white diamonds; black bears, the white squares). See table 2 for the abbreviations. The symbols represent one individual; black bears were sampled in early hibernation (the upper symbols) and in late hibernation (the lower symbol). The solid gray line represents values predicted solely by the allometric scaling of water flux and metabolic rate (see the text and equations 1–3 for details). The solid black line is a regression among rodents ($R^2 = .75$, $p < .01$). (b) On the y-axis, atmospheric O_2 (the red circle) has a negative $\Delta^{17}O$ value compared with meteoric waters (the upper blue shading). On the x-axis, the $\delta^{18}O$ of inhaled O_2 is fractionated to approximately 17.8‰. Meteoric waters (i.e., potential sources of drinking and food water) have variable $\delta^{18}O$ values (the blue circles). The symbols show the mean $\Delta^{17}O$ and $\delta^{18}O$ of body water of selected species from (a); for visual clarity, only a subset is presented. Below each species in red text is the estimated percentage of body water derived from metabolic water (F_A; y-axis on right). On the basis of these F_A and $\delta^{18}O_{BW}$, the $\delta^{18}O$ of the meteoric water ingested by the animal was calculated using the relationship in equation 5 (the dashed gray line); this calculated value is indicated by the symbol at the intersection of each dashed gray line and the blue meteoric water line.

except for mouse 1 on day 10. However, supporting expectations, the measured and predicted values of $\Delta^{17}O_{BW}$ exhibited similar direction and magnitude of changes.

Modeled data illustrate physiological influences on animal $\Delta^{17}O_{BW}$

Increasing the metabolic rate from 100% to 500% of normal values caused the modeled $\Delta^{17}O_{BW}$ values to decrease for both the generic herbivore and carnivore, reflecting the greater input of metabolic water (figure 4). This decrease was greatest up to approximately 200% of normal metabolic rate; beyond that, the $\Delta^{17}O_{BW}$ values were less sensitive to metabolic rate, especially for the herbivore. As we expected, increasing and decreasing ingestion of drinking or food water respectively increased and decreased the $\Delta^{17}O_{BW}$ values. In an assessment of sensitivity to analytical error, altering the $\Delta^{17}O$ values of inputs into animal body water in equation 4 changed the predicted value of the herbivore $\Delta^{17}O_{BW}$ from –0.148‰ to a maximum of –0.127‰ and a minimum of –0.178‰ (table 3). These new values covered a total range of 0.051‰, or 11% of the total plausible range of $\Delta^{17}O$ values (0.480‰).

Interpreting variation in the relationship between $\Delta^{17}O_{BW}$ and body mass

The data from wild and captive mammals generally supported the hypothesis that $\Delta^{17}O_{BW}$ values can be used to quantify relative contributions to animal body water. Although metabolism and drinking or food water intake could not be measured for these species, the combined effects of the allometric scaling of metabolic rate and water flux were predicted to cause $\Delta^{17}O_{BW}$ values to increase with body size (the gray line in figure 2a). This trend was observed among the wild desert rodents, all of which were sampled at the same time in a habitat that had little water availability. Assuming a similarly low intake of drinking or food water among these mostly granivorous

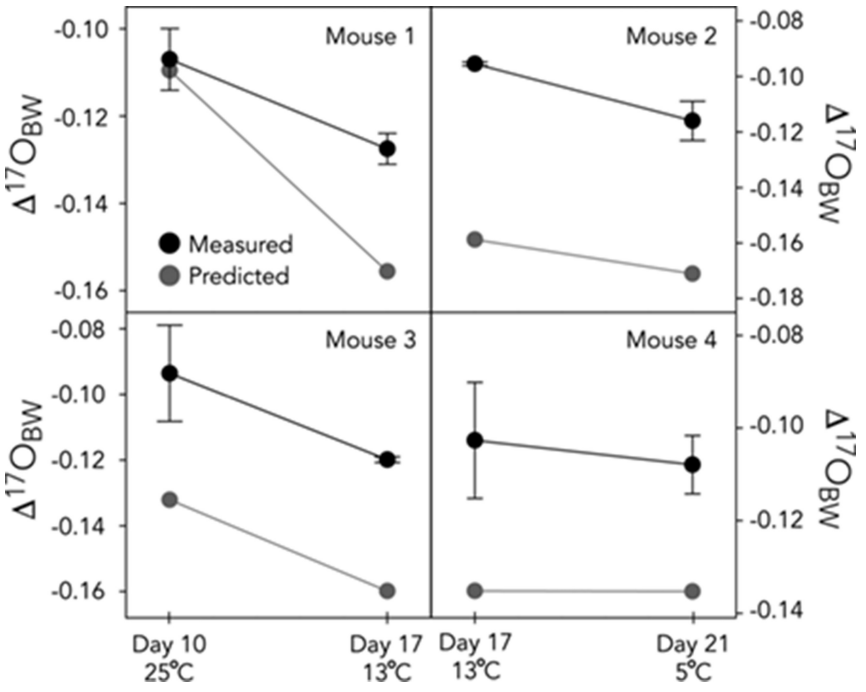

Figure 3. Predicted (the gray lines) and measured (the black lines) $\Delta^{17}O_{BW}$ value of deer mice on days 10, 17, and 21 of an experiment in which temperature was decreased, causing changes in rates of oxygen consumption and water and food intake. The error bars represent the standard deviation. Not all of the mice were analyzed on all days because of logistical constraints.

rodents, their positive correlation between body size and $\Delta^{17}O_{BW}$ values would be consistent with decreasing mass-specific metabolic rate and decreasing contributions of metabolic water into body water (Hayward 1965, Hinds and MacMillen 1985, White and Seymour 2005).

The large wild mammals in this study (river otters, mule deer, black bears) had higher $\Delta^{17}O_{BW}$ values than the wild rodents, except for the black bears in late winter hibernation (see below). This difference is also consistent with the larger animals receiving smaller fractional contributions of metabolic water into body water. However, these larger animals were not desert adapted like the rodents and therefore had likely consumed more water, presenting a confounding factor. Future studies should compare these influences by also sampling small animals from mesic habitats and large animals from xeric habitats. Of the large mammals, river otters had the highest value of $\Delta^{17}O_{BW}$, despite being known to have the greatest mass-specific metabolic rates (Silver et al. 1969, Williams et al. 2002, Tøien et al. 2011). This could have been caused by high intake rates of drinking or food water associated with living in an aquatic habitat; see below for a discussion of the potential water sources based on $\delta^{18}O$ values. The mule deer had lower $\Delta^{17}O_{BW}$ values than the river otters. Although the reason for this difference is unknown, this pattern could have been caused by low intake rates of drinking or food water by the mule deer because their habitat has less water availability than the habitat of the river otters (table 2).

The data from the black bears clearly demonstrate the influence of drinking or food water intake on $\Delta^{17}O_{BW}$. The black bears had lower $\Delta^{17}O_{BW}$ values in late hibernation than in early hibernation. This trend indicates that between sampling events the input into their body water had a strongly negative $\Delta^{17}O$ value, which could feasibly only be provided by the atmospheric oxygen in metabolic water. This source was likely the only input during this time because black bears do not drink or eat while hibernating. The calculated water flux rates for these hibernating bears were approximately 2%–5% of the predicted turnover rates based on body mass (Nagy and Peterson 1988). Body water turnover has not been measured in hibernating bears. However, respiratory water loss falls to approximately 1% of normal in hibernating ground squirrels (*Ictidomys tridecemlineatus*; Deavers and Musacchia 1980), implying that body water turnover is also approximately 1% of expected values because respiration is the primary route of water loss during hibernation. The $\Delta^{17}O_{BW}$ values calculated for the black bears at their time of den entry likely reflect normal activity, and accordingly, these values were close to those of the similar size mule deer.

The lab mice and elephants analyzed in this study can be used to consider the effects of a dramatic difference in body size, given *ad libitum* access to water. The elephants had higher $\Delta^{17}O_{BW}$ values than the mice, supporting the expectation that a low mass-specific metabolic rate of a large animal (White and Seymour 2005) can cause a reduction in the metabolic water contribution to body water. Importantly, in comparison with the lab mice, the elephants likely had lower mass-specific intake rates of drinking or food water because of the allometric scaling of water flux (Nagy and Peterson 1988). Nevertheless, this potentially lower intake of meteoric water apparently did not substantially reduce their $\Delta^{17}O_{BW}$ values, indicating that, as predicted, the decline in metabolic rate is more influential than the decline in water flux as body size increases.

Several of the calculated $\delta^{18}O$ values of ingested meteoric water were similar to local water sources, but some were not. This may reflect that the modeling was incomplete (i.e., it did not include all the terms in equation 4), as well as the influence of abiotic (evaporation) and biotic (evapotranspiration) processes that can alter the $\delta^{18}O$ of meteoric water. The water ingested by the kangaroo rats and river otters had higher $\delta^{18}O$ values than expected. This may be because kangaroo rats consume leaf water, which typically has higher $\delta^{18}O$ values than local meteoric water because

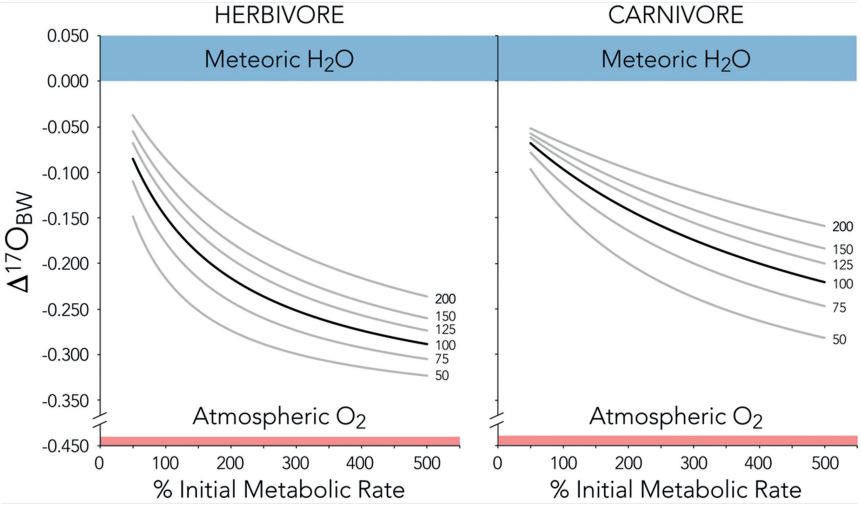

Figure 4. The predicted $\Delta^{17}O$ values of animal body water ($\Delta^{17}O_{BW}$) of a generic herbivore and carnivore based on variable rates of metabolism and ingestion of drinking and food water. Also shown are the range of values for $\Delta^{17}O$ of meteoric water (the upper blue shading) and atmospheric oxygen (the lower red shading). The x-axis indicates relative percentages of normal metabolic rate. For example, a value of 200 on the x-axis indicates that the animal has doubled its metabolic rate (i.e., 200% of normal). The black line depicts the predicted value of $\Delta^{17}O_{BW}$ as the animal varies its metabolic rate while holding its ingestion of water constant. The gray lines depict the predicted value of $\Delta^{17}O_{BW}$ as the animal simultaneously varies its metabolic rate and its water ingestion (e.g., 50 indicates the animal has reduced its water ingestion to 50% of normal).

of evapotranspiration (Flanagan and Ehleringer 1991), and because coastal river otters consume marine prey (Stenson et al. 1984) that is in osmotic balance with seawater, which has a $\delta^{18}O$ value near zero in the northeast Pacific Ocean (McMahon et al. 2013). In contrast, the water ingested by the pocket mice had a surprisingly low estimated $\delta^{18}O$ value of –19‰. Models show that the mean $\delta^{18}O$ value of precipitation at this field site varies among months from –3‰ to –15‰, with 95% confidence intervals ranging from –20‰ to 0‰ (Bowen 2017). We speculate that the mice may have fed on seeds and leaves of larger shrubs that use water from deeper within the soil, which is more influenced by winter precipitation that has lower $\delta^{18}O$ values than soil water closer to the surface (Schulze et al. 1996). Overall, estimating the $\delta^{18}O$ value of ingested water would be beneficial because it can be used to infer geographic origins (Rubenstein and Hobson 2004) and climate patterns, such as seasonality (Rivera-Araya and Pilaar Birch 2018). Such information could be used in paleoclimate reconstruction, where $\Delta^{17}O_{BW}$ values can be assessed in skeletal or tooth enamel apatite from fossil animals (Pack et al. 2013, Passey et al. 2014, Gehler et al. 2016); see "Applications of $\Delta^{17}O_{BW}$" below.

Assessing the predictability of $\Delta^{17}O_{BW}$ in individuals

The data from the captive deer mice indicated that the direction and magnitude of changes in $\Delta^{17}O_{BW}$ values can be predicted on the basis of rates of oxygen consumption and intake of drinking or food water. However, the predicted values were lower for almost all of our samples, indicating that our current model for predicting $\Delta^{17}O_{BW}$ values is incomplete. Overall, these results are encouraging and suggest that $\Delta^{17}O_{BW}$ values enable physiological inferences regarding inputs to animal body water, although further studies are required. As was expected, the $\Delta^{17}O_{BW}$ values declined in response to elevated metabolic rate (mice 1–3) and remained steady (mouse 4) when the metabolic rate didn't increase (figure 3). The changes in $\Delta^{17}O_{BW}$ values were small but measurable. Inferring changes in the combination of water inputs and metabolic rate from a single blood sample represents a substantial advance in animal biology, ecophysiology, and potentially paleoecology (see "Applications of $\Delta^{17}O_{BW}$" below).

On the basis of equation 4, the offset between the predicted and measured values of $\Delta^{17}O_{BW}$ for the captive deer mice would be eliminated if the mean rates of drinking water intake had been 1.4 times higher than was measured. The measured intake may indeed have been inaccurate, because it was based on changes in water bottle mass between days 4, 10, 17, and 21, but the predicted $\Delta^{17}O_{BW}$ value only considered the 72 hours prior to sampling, the period for full body water turnover in deer mice (Holleman and Dieterich 1973). Although a similar inaccuracy could have occurred with food intake, eliminating the offset between the predicted and measured $\Delta^{17}O_{BW}$ values would require an implausibly high intake rate of food, 7.1 times greater than measured. Conversely, this offset between the predicted and measured $\Delta^{17}O_{BW}$ values would be eliminated if the mean inputs of metabolic water had been 0.75 of our calculated values. Although metabolic water production is often predicted by the mass of oxidized foodstuffs (Nelson and Cox 2008), our calculations were based on the biological path of inhaled oxygen from the atmosphere into metabolic water. Other complicating factors could include formation of water with oxygen atoms cleaved from inorganic phosphate during ADP phosphorylation (Horiike et al. 1996). Further controlled studies of $\Delta^{17}O$ values in biological systems are needed to clarify these poorly constrained variables.

Comparing modeled and measured $\Delta^{17}O_{BW}$

The modeled $\Delta^{17}O_{BW}$ values of a generic herbivore and carnivore (–0.323‰ to –0.037‰; figure 4) were similar to the empirical measurements of the wild and captive mammals (–0.255‰ to –0.016‰, figure 2). For the herbivore, $\Delta^{17}O_{BW}$ values declined steeply as metabolic rate increased. At high

Table 3. Modeled $\Delta^{17}O$ values of animal body water ($\Delta^{17}OBW$), as predicted using equation 4 (see main text).

Change in input (‰)	Reasoning	$\Delta^{17}OBW$ (‰)
None	Base model	–0.148
$\Delta^{17}O_A$ from –0.450 to –0.410	Modeled value[a]	–0.134
$\Delta^{17}O_{DW}$ and $\Delta^{17}O_{FW}$ from 0.030 to 0.000	Min. for most meteoric water[b]	–0.165
$\Delta^{17}O_{DW}$ and $\Delta^{17}O_{FW}$ from 0.030 to 0.050	Max. for most meteoric water[b]	–0.137
$\Delta^{17}O_{DC}$ from –0.085 to 0.030	Plausible max. for carbohydrates	–0.138
$\Delta^{17}O_{DC}$ from –0.085 to –0.200	Plausible min. for carbohydrates	–0.159
$\Delta^{17}O_{DP}$ from 0.030 to –0.260	Observed min. for body water	–0.151
$\Delta^{17}O_{DL}$ from 0.030 to –0.260[c]	Observed min. for body water	–0.151
All $\Delta^{17}O$ except $\Delta^{17}O_A$ set to min above	Multiple simultaneous errors	–0.178
All $\Delta^{17}O$ except $\Delta^{17}O_A$ set to max above	Multiple simultaneous errors	–0.127

Note: Here, the base model used the same input values as for the model herbivore assuming 100% of normal metabolic rate and water intake rate (see figure 4). The $\Delta^{17}O$ value of each input was then changed on the basis of the reasoning listed in the table, and the $\Delta^{17}O_{BW}$ value was recalculated. Note that the minimum and maximum calculated values for $\Delta^{17}O_A$ values reflect different assumptions about the slope and intercept of the relationship between $\delta^{17}O$ and $\delta^{18}O$, as well as a comparison with different standards. [a]Young et al. 2014. [b]Sharp et al. 2018. [c]F_{DL} also changed from 0.00 to 0.01, and F_{DP} changed from 0.01 to 0.00.

metabolic rates this decline was tempered by a simultaneous increase in the contribution of water from carbohydrate condensation reactions, which was assumed to have a higher $\Delta^{17}O$ value (–0.085‰) than metabolic water. The decline in $\Delta^{17}O_{BW}$ values was more linear for the carnivore because of a lack of dietary carbohydrates (Kohn 1996). These results illuminate how changes in metabolic rate and ingestion of drinking or food water can independently affect $\Delta^{17}O$ values.

$\Delta^{17}O_{BW}$ research needs

The empirical and modeled results indicate that $\Delta^{17}O_{BW}$ values have measurable, predictable relationships to the fractional contributions to body water from metabolism and from drinking or food water. These results encourage further validation experiments that combine serial sampling of $\Delta^{17}O_{BW}$ values with continuous and precise measurements of oxygen consumption, as well as food and water intake. In addition, assumptions regarding the values of $\Delta^{17}O_{DC}$, $\Delta^{17}O_{DP}$, and $\Delta^{17}O_{DL}$ should be tested. Researchers should also assess plant water $\Delta^{17}O$ values (i.e., $\Delta^{17}O_{FW}$ for herbivores), because evapotranspiration may affect the relationship between $\delta^{17}O$ and $\delta^{18}O$ values differently than the evaporation and condensation encompassed by the terrestrial fractionation line (Landais et al. 2006). In addition, researchers may increase model precision by refining their meteoric water value on the basis of measurements for specific locations (Sharp et al. 2018). However, our results suggest that the current uncertainties regarding $\Delta^{17}O$ values of body water inputs have relatively small effects on $\Delta^{17}O_{BW}$ values. Similarly, the SDs of measured samples (up to 0.015‰) represented up to 3% of the potential total range of values.

In our study, $\Delta^{17}O$ values were measured by fluorinating water to convert H_2O to O_2 for isotope analysis. This is the most precise method that is presently available, but it requires customized, complex instrumentation. Alternatively, widespread adoption of $\Delta^{17}O_{BW}$ measurements can be enabled by use of commercially available, laser-spectroscopy instruments. These devices now allow measurements of $\Delta^{17}O$ directly from liquid water with precision up to 0.010‰ (Schauer et al. 2016), substantially reducing the time, cost, and required expertise. Concomitant with further testing of the biological influences on $\Delta^{17}O_{BW}$ values, biologists should continue to improve techniques for laser spectroscopy and disseminate the detailed methodology alongside experimental results.

Another frontier in animal studies is the measurement of $\Delta^{17}O$ in inorganic and organic tissues. Bone and tooth enamel apatite form in equilibrium with body water and record $\Delta^{17}O_{BW}$ (Pack et al. 2013, Passey et al. 2014, Gehler et al. 2016). Measurements of enamel from bone and teeth would enhance physiological studies of extinct species, which often must rely instead on macroecological correlations between morphology and metabolism (e.g., Grady et al. 2014). Estimating the $\delta^{18}O$ value of ingested meteoric water on the basis of bones and teeth would also contribute to paleoclimate research because this variable is an important climate proxy. Proteinaceous tissues may also record $\Delta^{17}O_{BW}$ values in the carboxyl oxygen of amino acids (Stewart et al. 2001, Nelson and Cox 2008, Ehleringer et al. 2008). Analyzing $\Delta^{17}O$ of tissues with different isotopic incorporation rates (Martínez del Rio and Carleton 2012) would enable ecophysiological studies across varying timescales. In addition, because some tissues can be collected noninvasively (e.g., molted fur or feathers), it may be possible to complete such studies without animal capture.

Applications of $\Delta^{17}O_{BW}$

If further studies support the physiological interpretation of $\Delta^{17}O_{BW}$ values in the present article, this variable will have at least three broadly defined scenarios for application. In the first scenario, no contributions to body water are known or constrained. However, equation 1 can be used to estimate

F_A on the basis of only inputs of ingested drinking or food water and metabolic water. For example, estimated F_A of wild rodents ranged from 0.41 to 0.54 (figure 2b), which are similar to previously published values from 0.36 for a generic herbivorous rodent (Kohn 1996) to 0.51 for wild Merriam's kangaroo rats (Nagy and Gruchacz 1994). Such F_A values estimate the extent to which animals rely on metabolic water, an important physiological trait.

In the second scenario, contributions to body water from drinking or food water and from water from condensation reactions can be constrained, enabling inferences regarding metabolic water production and by extension metabolic rate. For example, differences in $\Delta^{17}O_{BW}$ values between two animal populations could primarily reflect variation in metabolic rate, provided other conditions were met (e.g., similar access to drinking or food water and comparable values of hydration markers such as hematocrit; Senay and Christensen 1965). In the third scenario, if the contributions to body water from metabolic water production can be constrained, hypotheses can be tested regarding the intake of drinking or food water. In addition to these three scenarios, $\delta^{18}O$ values of ingested drinking or food water may be estimated (figure 2b). Because $\delta^{18}O$ values vary in predictable geographic patterns that are linked to hydrological processes, this variable is useful in studies of climate (e.g., precipitation cycles) and animal ecology (e.g., migration; Rubenstein and Hobson 2004).

In conclusion, patterns in $\Delta^{17}O_{BW}$ values of wild and captive mammals and of deer mice in a controlled experiment were consistent with expectations based on the fractional inputs of metabolic water versus drinking or food water. Additional studies are encouraged relating $\Delta^{17}O_{BW}$ values to body water inputs. If further validated, $\Delta^{17}O_{BW}$ values will provide novel data from a single sample, substantially broadening the scope of information available for a wide variety of biological applications.

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Supplemental material

Supplemental data are available at *BIOSCI* online.

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