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Trophic Level Influence on Stable Hydrogen Isotopic Composition of Bat Fur

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Graduate Program in Biology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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TROPHIC LEVEL INFLUENCE ON STABLE HYDROGEN ISOTOPIC
COMPOSITION OF BAT FUR

(Thesis format: Monograph)

by

Leslie Erdman

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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Abstract

The effect of trophic level on the isotopic compositions of nitrogen ($\delta^{15}\text{N}$), carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) is well documented, but the effect that trophic level has on the stable hydrogen isotopic composition of bat fur ($\delta^2\text{H}_{\text{fur}}$) is not known. This can have implications when assigning the locations of origin of bats in migration studies that use $\delta^2\text{H}_{\text{fur}}$. I hypothesized that if there is an effect of trophic level on $\delta^2\text{H}_{\text{fur}}$, then $\delta^2\text{H}_{\text{fur}}$ would correlate positively with trophic level. I tested this possibility by correlating $\delta^2\text{H}_{\text{fur}}$ with $\delta^{15}\text{N}_{\text{fur}}$ and $\delta^{13}\text{C}_{\text{fur}}$, which both positively correlate with trophic level, and with $\delta^{18}\text{O}_{\text{fur}}$, which does not correlate with trophic level. My results show that $\delta^2\text{H}_{\text{fur}}$ increases by 15 to 40 ‰ per trophic level, which supports my hypothesis. Understanding the effect that trophic level has on $\delta^2\text{H}_{\text{fur}}$ can help researchers assign more accurate locations of origin for bats. Together, $\delta^{15}\text{N}_{\text{fur}}$, $\delta^{13}\text{C}_{\text{fur}}$ and $\delta^2\text{H}_{\text{fur}}$ can help determine what bats are eating at the individual or population level.

Keywords: *Artibeus jamaicensis*, Bat, *Carollia perspicillata*, Carbon, *Desmodus rotundus*, *Eptesicus fuscus*, Fur, *Glossophaga soricina*, Hydrogen, Isotope, Nitrogen, Oxygen, Size, Stable isotopic composition, Trophic level

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Chapter 1: Introduction

Several species of North American bats undertake short- or long-distance migrations to survive the winter. However, the timing, distance and routes of these migration events are not well understood. Traditional methods for studying migration are difficult to apply to many species of bats because of their nocturnal nature and small body size (Cryan et al., 2004). Tracking devices small enough for bats either have a short battery life or do not transmit over long enough distances. Mark-recapture methods are often not viable because many bats are solitary, making them difficult to capture in large numbers, and recapturing a marked bat is highly unlikely (Cryan et al., 2004; but see Dubois and Monson 2007). Measuring the stable isotopic composition of hydrogen in bat fur provides an alternative approach for studying bat migration events. The stable isotopic composition of bat tissue acts as an intrinsic marker of individual animal ecology, and requires capturing each bat only once (Britzke et al., 2009; Fraser et al., 2012; Popa-Lisseanu et al., 2012). Stable hydrogen isotope ratios of bat fur ($\delta^2\text{H}_{\text{fur}}$), in particular, can be useful for studying bat ecology, but little is known about the effect of trophic level on these compositions. Understanding the effect that trophic level has on $\delta^2\text{H}_{\text{fur}}$ can help researchers assign more accurate locations of origin for bats. It is becoming increasingly important to understand bat migration behaviours as bat populations face pressure from threats such as wind turbines (Arnett et al., 2008; Cryan and Barclay, 2009) and white nose syndrome (Frick et al., 2010).

1.1 Stable isotopes

The isotopic composition of an individual's tissues is determined by what that individual eats and drinks, as well as by metabolic processes. Changes in tissue isotopic composition occur when the amount of the heavier isotope changes relative to the amount of the lighter isotope. This process is known as fractionation. Fractionation is caused by the mass difference between the heavy and the light isotope of an element (Sulzman, 2007). Evaporation of water is an example of a process contributing to fractionation. Hydrogen atoms containing no neutrons (^1H) have a lower mass (i.e., are lighter) than those with one neutron (^2H). Consequently, on average, molecules containing ^1H enter

the vapour phase preferentially to molecules containing ^2H . The result of this fractionation is depletion of ^2H in the water vapour, and enrichment of ^2H in the remaining water. A similar fractionation process applies to oxygen isotopes in this example. Some examples of metabolic processes that can cause fractionation are nitrogenous excretion (Birchall et al., 2005) and respiration (DeNiro and Epstein, 1981a).

Bat $\delta^2\text{H}_{\text{fur}}$ values can indicate the occurrence and extent of migration of bats in North America. This is possible because of a latitudinal gradient in the $\delta^2\text{H}$ values of precipitation ($\delta^2\text{H}_{\text{precip}}$) across the continent. The $\delta^2\text{H}_{\text{precip}}$ values start near 0 per mil (‰) at equatorial latitudes and become increasingly negative towards higher latitudes, reaching as low as approximately -230 ‰ (Figure 1; Bowen, 2013; Bowen and Revenaugh, 2003).

Bats typically molt once per year during summer prior to migration (Constantine, 1957; Constantine, 1958; Cryan et al., 2004; Fraser et al., 2012). Local $\delta^2\text{H}_{\text{precip}}$ values are incorporated into their fur during this time. Once fur is grown, its isotopic composition is fixed. When a bat is captured after beginning migration, its $\delta^2\text{H}_{\text{fur}}$ value will be different than the $\delta^2\text{H}_{\text{precip}}$ value of the area where it is captured if the bat has moved far enough along the $\delta^2\text{H}_{\text{precip}}$ gradient. The size of this difference provides an estimate of the potential distance of migration, as each value of $\delta^2\text{H}_{\text{fur}}$ can typically be associated with a geographic range of origin (Britzke et al., 2009; Fraser, 2011). A limitation of this method is the potential for variation in the $\delta^2\text{H}_{\text{fur}}$ values of resident bats (Britzke et al., 2009; Fraser, 2011). Such variation can cause uncertainty when assigning a location of origin of a migrant bat. For example, a difference of 20 ‰ in $\delta^2\text{H}_{\text{fur}}$ values can cause the assigned location of origin to be off by hundreds of kilometres. Knowing the factors that contribute to variation can reduce uncertainty about the range of origin. Potential sources of variation include trophic level (Birchall et al., 2005; Reynard and Hedges, 2008) and body size (Betini et al., 2009; Whitley et al., 2006).

1.2 Trophic level and stable isotopic composition

Trophic level refers to the position an organism occupies in a food web. For the purpose of this study, I consider plants to be at the first trophic level. Bats that eat mainly plant

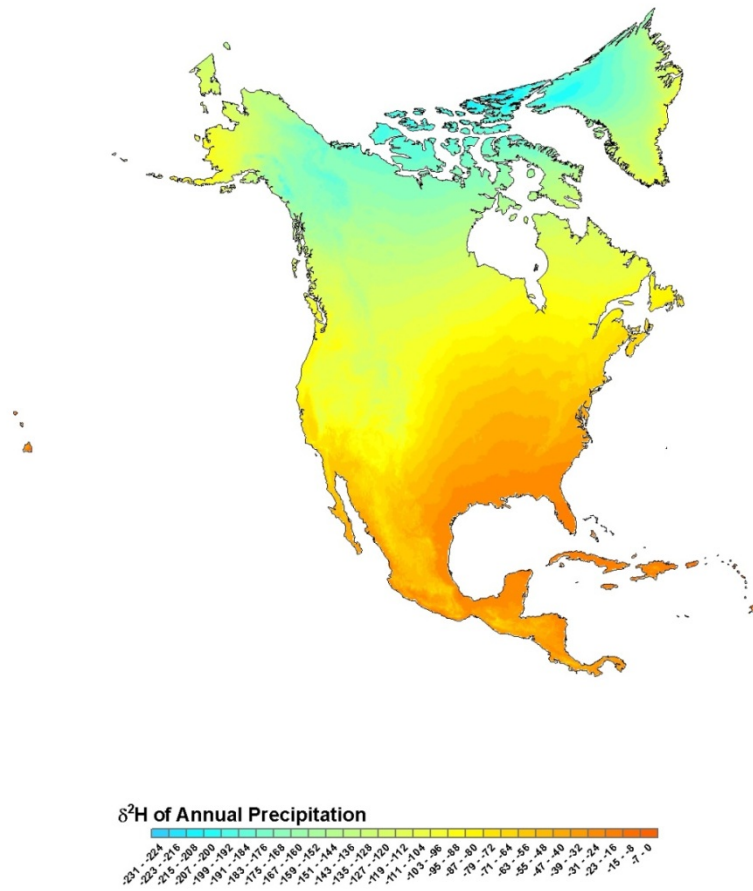


Figure 1: Gradient of $\delta^2\text{H}_{\text{precip}}$ (‰, VSMOW) values across North America (Bowen, 2013; Bowen and Revenaugh, 2003).

matter (e.g., fruit, nectar) represent the second trophic level. Bats that eat insects or blood represent the third trophic level when the insects or hosts are, in turn, only eating plants (Krebs, 2001).

The stable isotopic compositions of some elements can provide an indication of trophic level. Nitrogen isotopic composition ($\delta^{15}\text{N}$) is a very reliable indicator of trophic level, generally increasing by approximately 3 ‰ per trophic level (but this value can vary from

approximately 3 to 5 ‰; Blüthgen et al., 2003; DeNiro and Epstein, 1981a). This relationship is confounded in systems that have sources in both aquatic and terrestrial environments, because values of $\delta^{15}\text{N}$ are generally higher in aquatic than in terrestrial environments (DeNiro and Epstein, 1981a). It is also confounded in areas that have undergone organic nitrogen fertilization, as these fertilizers can cause enrichment in ^{15}N (Birchall et al., 2005; Szpak et al., 2012). For my study, I will use $\delta^{15}\text{N}$ values as a measure of trophic level.

Stable carbon isotopic composition ($\delta^{13}\text{C}$) can also positively correspond with trophic level, increasing by about 1 ‰ per trophic level (DeNiro and Epstein, 1978). That said, $\delta^{13}\text{C}$ values are not as strongly correlated with trophic level as $\delta^{15}\text{N}$ values (DeNiro and Epstein, 1978; Reynard and Hedges, 2008), and are more commonly used as indicators of the dietary source(s) of the organism whose tissues are being analysed (Fleming et al., 1993; Siemers et al., 2011). For example, food webs based on plants that use the C_3 photosynthetic pathway will have lower $\delta^{13}\text{C}$ values than food webs based on C_4 plants. Both photosynthetic pathways discriminate against ^{13}C during photosynthesis, but the discrimination is larger in C_3 plants. As a result, C_3 plants typically have $\delta^{13}\text{C}$ values that range from -35 to -20 ‰, and C_4 plants typically have $\delta^{13}\text{C}$ values that range from -15 to -7 ‰ (Kennedy and Krouse, 1990). For my study, I will use $\delta^{13}\text{C}$ values as a secondary measure of trophic level.

Both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are positively correlated with trophic level because they are derived from what an organism eats. Values of $\delta^{15}\text{N}$ increase because of the metabolic process of nitrogenous excretion, which preferentially incorporates ^{14}N over ^{15}N (McCutchan Jr. et al., 2003). This leaves the remaining nitrogen enriched in ^{15}N . Values of $\delta^{13}\text{C}$ increase because of the process of respiration. The ^{12}C is preferentially incorporated in the formation of CO_2 , leaving the remaining carbon slightly enriched in ^{13}C (DeNiro and Epstein, 1978). Unlike $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, the oxygen isotopic composition ($\delta^{18}\text{O}$) is derived from water. Therefore, $\delta^{18}\text{O}$ remains constant with increasing trophic level (Koch, 2007; Schimmelmann and DeNiro, 1986; van Hardenbroek et al., 2012).

The effects of trophic level on $\delta^{15}\text{N}$ (DeNiro and Epstein, 1981a), $\delta^{13}\text{C}$ (DeNiro and Epstein, 1978) and $\delta^{18}\text{O}$ (Schimmelmann and DeNiro, 1986) are quite well established. Less well-known is the effect of trophic level on $\delta^2\text{H}$ values. Migration studies that use $\delta^2\text{H}_{\text{fur}}$ values typically assume that trophic level has no effect (Britzke et al., 2009; Fraser, 2011; Popa-Lisseanu et al., 2012), but this assumption may not be correct. Several studies have reported an increase in $\delta^2\text{H}$ with trophic level. This increase has been reported for the bone collagen of terrestrial herbivores, omnivores and carnivores (Birchall et al., 2005; Reynard and Hedges, 2008) as well as in aquatic systems in the tissues phytoplankton, zooplankton (Stiller and Nissenbaum, 1980) and arthropods (Schimmelmann and DeNiro, 1986). The reported increase in $\delta^2\text{H}$ is usually between 20 and 50 ‰ (Birchall et al., 2005; Reynard and Hedges, 2008; Stiller and Nissenbaum, 1980). None of these studies accounted for physiological or behavioural differences among study species. Therefore, it remains unclear if the increase in $\delta^2\text{H}$ is caused by trophic level.

There are three potential sources of the H that is incorporated into an individual's tissues. The first is drinking water (DeNiro and Epstein, 1981b; Estep and Dabrowski, 1980). Migration studies generally assume that drinking water is the sole source of H for tissues (e.g., Britzke et al., 2013; Fraser et al., 2012). A second source of H is the water in food, also known as diet water (Birchall et al., 2005; Solomon et al., 2009). This source may be of particular importance in bats that eat foods with a high water content, such as fruit, nectar or blood (Carpenter, 1969; McFarland and Wimsatt, 1969; Studier et al., 1983). A third source of H is the amino acids in food tissue (Birchall et al., 2005; DeNiro and Epstein, 1981b; Estep and Dabrowski, 1980). Food has been considered to influence $\delta^2\text{H}_{\text{fur}}$ values by other researchers (Birchall et al., 2005; Stiller and Nissenbaum, 1980). Reynard and Hedges (2008) suggested that up to 60% of $\delta^2\text{H}$ in bone collagen can be derived from diet. Furthermore, food can contribute 29% of hydrogen in the body water of small mammals (Podlesak et al., 2008). For my study, I will refer to the H sources as “drinking water,” “diet water,” and “diet tissue,” respectively.

Three mechanisms could cause an increase in $\delta^2\text{H}$ values with increasing trophic level. The first mechanism, proposed by Solomon et al. (2009), is illustrated with an example of

an organism whose tissues contain 20% exchangeable H, and whose prey's tissues also contain 20% exchangeable H. In such a case, the organism will contain both the 20% of H it derived from diet water and the 20% the organism's prey derived from diet water. This is called the compounding effect of diet water contributions, and could cause the apparent trophic level increase in $\delta^2\text{H}$ values.

Birchall et al. (2005) suggested a second possible mechanism that could cause an increase in $\delta^2\text{H}$ values with increasing trophic level. Amino acid synthesis may incorporate body water, which is derived from both diet and drinking water. Processes such as respiration and transcutaneous evaporation leave the remaining body water enriched in ^2H (Schoeller et al., 1986). If this body water was subsequently used in fur formation, that would increase $\delta^2\text{H}_{\text{fur}}$ values. For both of the first and second mechanisms, the processes that cause ^2H enrichment should affect oxygen in the same way, causing a corresponding enrichment in ^{18}O in the remaining body water.

A third mechanism that could cause $\delta^2\text{H}$ values to increase with trophic level is the preferential catabolism of isotopically lighter amino acids from diet tissue (Birchall et al., 2005). This mechanism would cause enrichment in ^2H without a corresponding enrichment in ^{18}O . Values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ are usually highly correlated in drinking and diet water (Gat et al., 2001). Therefore, a measured increase in $\delta^2\text{H}$ values without a corresponding increase in $\delta^{18}\text{O}$ values supports the idea of a trophic effect on $\delta^2\text{H}$ values following the third mechanism. For my study, I will use $\delta^{18}\text{O}$ values in conjunction with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values to determine if changes in $\delta^2\text{H}$ values are caused by trophic level. A change in $\delta^2\text{H}$ values without a corresponding change in $\delta^{18}\text{O}$ values would support an effect of trophic level.

It is important to determine the role that trophic level plays in determining bat $\delta^2\text{H}_{\text{fur}}$ values so we can correct for the difference in $\delta^2\text{H}_{\text{fur}}$ values trophic level causes prior to using those $\delta^2\text{H}_{\text{fur}}$ values to track migration. A one trophic level increase in $\delta^2\text{H}_{\text{fur}}$ values of 20 ‰ could correspond to a geographic error of hundreds of kilometers if no correction was made (Bowen, 2013; Bowen and Revenaugh, 2003). Evaluating such effects is especially important for insectivorous bats. Insectivorous bats are more likely to

occupy a “trophic range,” as the insects they feed on may be herbivorous, insectivorous or sanguinivorous. If their trophic range influences their $\delta^2\text{H}_{\text{fur}}$ values, their location of origin will appear to span more kilometres than is actually the case.

1.3 Study species

In order to ascertain the effect of trophic level on $\delta^2\text{H}$ values, I sampled bats from two distinct trophic levels. These bats occupy the second and third trophic levels (as the first trophic level consists of plant matter). Representing the second trophic level, my species of study are *Artibeus jamaicensis*, *Carollia perspicillata* and *Glossophaga soricina*. Representing the third trophic level, my species of study are *Desmodus rotundus* and *Eptesicus fuscus*. For all five species I sampled captive bats, and for *Desmodus rotundus* and *Eptesicus fuscus* I also sampled wild bats. As the main difference between captive and wild bats is their diet, this allows me to compare the influence of diet on the N, C, H and O isotopic compositions.

Larger body sizes can be associated with higher $\delta^2\text{H}$ values among individuals (Betini et al., 2009; Soto et al., 2011), but this effect is usually only noticeable when the differences in body size are large (Soto et al., 2011; Whitley et al., 2006). To minimize the potential influence of size variations (as measured by bat forearm length) on $\delta^2\text{H}$ values, I sampled similarly sized bats for both of the trophic levels in my study (Table 1).

Table 1: Average forearm length (mm) and diets of species in my study. There are similar-sized species on each trophic level.

Species	Average forearm length (mm)	Diet in captivity	Trophic level
<i>Artibeus jamaicensis</i>	60 (Ortega and Castro-Arellano, 2001)	Fig, banana, sweet potato, mango, cantaloupe, Gala apple, marmoset mix, pear	Second
<i>Carollia perspicillata</i>	42 (Cloutier and Thomas, 1992)	Fig, banana, sweet potato, mango, cantaloupe, Gala apple, marmoset mix, pear	Second
<i>Glossophaga soricina</i>	36 (Alvarez et al., 1991)	Nectar, fig, banana, sweet potato, mango, cantaloupe, Gala apple, marmoset mix, pear	Second

<i>Desmodus rotundus</i>	58 (Greenhall et al., 1983)	Cow blood	Third
<i>Eptesicus fuscus</i>	47 (Kurta and Baker, 1990)	Mealworms	Third

1.4 Statement of Purpose

My objective was to ascertain the role that trophic level plays in determining the $\delta^2\text{H}$ values of bat fur. I hypothesized that if there is a trophic effect on $\delta^2\text{H}$ values of bat fur, then $\delta^2\text{H}_{\text{fur}}$ values should be positively correlated with trophic level. Knowing that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ correlate positively with trophic level, I used them as primary and secondary measures of trophic level, respectively. I then tested for a correlation between values of $\delta^2\text{H}$ and $\delta^{15}\text{N}$, and between values of $\delta^2\text{H}$ and $\delta^{13}\text{C}$ to determine if $\delta^2\text{H}$ values increase with trophic level. However, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ on their own do not definitively indicate if trophic level is the cause of the change in $\delta^2\text{H}$ values. Values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ are highly correlated in water. Knowing that $\delta^{18}\text{O}$ values do not correlate with trophic level, I also tested for a correlation between values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$. A change in $\delta^2\text{H}$ without a corresponding change in $\delta^{18}\text{O}$ would support an effect of trophic level. If my hypothesis was supported, I made the following three predictions:

- 1) $\delta^2\text{H}_{\text{fur}}$ and $\delta^{15}\text{N}_{\text{fur}}$ values will be positively correlated
- 2) $\delta^2\text{H}_{\text{fur}}$ and $\delta^{13}\text{C}_{\text{fur}}$ values will be positively correlated, but not as strongly as $\delta^2\text{H}$ and $\delta^{15}\text{N}$
- 3) $\delta^2\text{H}_{\text{fur}}$ and $\delta^{18}\text{O}_{\text{fur}}$ values will not be correlated

I also investigated whether there is a correlation between $\delta^2\text{H}_{\text{fur}}$ values and size, as measured by bat forearm length. If there is no size effect on $\delta^2\text{H}$ values of bat fur, then $\delta^2\text{H}_{\text{fur}}$ values would not be positively correlated with forearm length. As the size variance within each species is small (a few millimetres), I predicted there would be no intraspecific correlation of size with $\delta^2\text{H}_{\text{fur}}$ values. I had species of similar sizes on each trophic level, so I also predicted that inter-species variations in size would have no correlation with overall $\delta^2\text{H}_{\text{fur}}$ values.

If there is an effect of trophic level or size on $\delta^2\text{H}_{\text{fur}}$ values, these will be factors to account for in future migration studies when assigning the geographic location of origin of bats. If there is a trophic effect, values of $\delta^2\text{H}_{\text{fur}}$ could be used to provide information about trophic level when $\delta^{15}\text{N}$ values are ambiguous, such as areas that have undergone nitrogen fertilization. Knowing both the effects of trophic level and size on $\delta^2\text{H}_{\text{fur}}$ values can also help to make equations to relate $\delta^2\text{H}_{\text{fur}}$ values to $\delta^2\text{H}_{\text{precip}}$ values in migration studies applicable to multiple species. This would make stable hydrogen isotopic analysis a more useful tool for studying migration.

Chapter 2: Materials and Methods

2.1 Collection

I collected fur samples from captive populations of five species at three different locations. For two of the species, I also collected fur samples from wild populations at two locations (Table 2). I collected all fur samples from the bats' lower dorsum (see Appendix A for Animal Use Protocol). I collected fur samples from *A. jamaicensis* (n=13), *C. perspicillata* (n=43), *G. soricina* (n=20), captive *E. fuscus* (n=38), wild *E. fuscus* (n=38), captive *D. rotundus* (n=25) and wild *D. rotundus* (n=8). For the captive species I also collected samples of their diet and drinking water, with the exception of *A. jamaicensis* at the Havelock, ON location. For all bats except those from Belize, I recorded age (adult versus juvenile), sex and forearm length. I could not determine the exact age of the adults, but once the bats are fully developed I did not expect the stable isotopic compositions to be affected by differences in age (Hobson and Clark, 1992; Schimmelmann and DeNiro, 1986). Isotopic data for fur, diet and water are presented in Appendices B, C and D, respectively.

The diets of the captive *A. jamaicensis*, *C. perspicillata* and *G. soricina* consisted of Gala apple, cantaloupe, fig, pear, marmoset mix, sweet potato, papaya and banana imported from various locations throughout the year. The mealworms eaten by *Eptesicus fuscus* were fed a diet of bran and came from a supplier that collects them from a network of local growers. The cow blood eaten by captive *Desmodus rotundus* came from local cows slaughtered at a local butcher shop. The cows ate mainly a mixture of corn, oats, and grass. I collected samples of each of these diet items except for the corn, oats and grass on the same sampling trips as the fur. I placed each diet sample in a glass vial with a rubber stopper lid, then placed Parafilm® over the lid. This handling was necessary to prevent the loss of water from the partial drying of the sample, as drying would cause fractionation of the remaining water in the sample. This handling also ensured that there was no isotopic exchange between the diet sample and ambient water vapour outside the vial once the sample was removed from the collection site, as any water vapour inside the vial would be from the collection site. Only one diet type was placed in each vial.

Table 2: Sampling locations and dates for both captive and wild populations of the five study species.

Species	Captive or wild	Sampling location(s) and date(s)
<i>Artibeus jamaicensis</i>	Captive	Montreal, QC (15 May and 24 October, 2012) Havelock, ON (30 January, 2013)
<i>Carollia perspicillata</i>	Captive	Montreal, QC (15 May and 24 October, 2012)
<i>Glossophaga soricina</i>	Captive	Montreal, QC (15 May and 24 October, 2012)
<i>Desmodus rotundus</i>	Captive	Havelock, ON (30 January, 2013)
<i>Desmodus rotundus</i>	Wild	Lamanai, Belize (May 2012)
<i>Eptesicus fuscus</i>	Captive	Hamilton, ON (14 August and 1 November, 2012)
<i>Eptesicus fuscus*</i>	Wild	Windsor, ON (2 August, 2012) Hamilton, ON (1 November, 2012)

*At the time of the first sampling, the wild population of *Eptesicus fuscus* bats in Windsor, ON was taken into captivity by Paul Faure and placed in his colony in Hamilton, ON. The second time I sampled this wild population was while they were in captivity.

I stored all fur samples at room temperature and water samples at 4⁰C until analysis. I stored diet samples in the freezer until I extracted their water using vacuum distillation. Vacuum distillation was used to prevent isotopic exchange between the water in the sample and the ambient water vapour, and to avoid contamination of the sample water from atmospheric water vapour. It was also used to avoid fractionation as a result of evaporation of the water in the sample, as evaporation would cause the remaining water to be enriched in ²H and ¹⁸O. Lastly, it prevented loss of water from the extracted water sample. After distilling the diet samples, I freeze-dried them and then ground them into a powder-like consistency with a mortar and pestle. I then stored the powdered diet samples at room temperature until analysis.

2.2 Stable isotope analyses

All stable isotope analyses were completed in the Laboratory for Stable Isotope Science at the University of Western Ontario. Stable isotope compositions are presented in delta (δ) notation with units of per mil (‰). Isotopic ratios are standardized to air (AIR) and Vienna Pee Dee Belemnite (VPDB) for nitrogen and carbon, respectively, and to the Vienna Standard Mean Ocean Water (VSMOW) – Standard Light Antarctic Precipitation (SLAP) scale for both hydrogen and oxygen (Coplen, 1996; Coplen and Qi, 2012). All stable isotope results are calculated using the standard equation

Equation 1:

$$\delta = \left(\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right)$$

where R is the ratio of the heavy: light isotopes for any given element (e.g. $^2\text{H}/^1\text{H}$, $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$).

2.3 Fur analyses

For fur preparation I followed a procedure similar to that described by Fraser (2011). To clean the fur, I soaked the samples overnight in a 2:1 solution of chloroform: methanol. I then rinsed them in the same solution and left them in the fume hood for more than 48 hours to dry. My procedure differed slightly from Fraser (2011), in that I used scissors to chop my samples into a powder-like consistency after they were dry. I did this to mirror the procedure used for the standard preparation, following the Principle of Identical Treatment (Wassenaar and Hobson, 2003). The Principle of Identical Treatment insures that any procedure that may influence the isotopic composition of the samples will similarly influence the standards, thus preventing error that would have been introduced had the samples and standards been treated differently.

For stable carbon and nitrogen isotopic analyses, I weighed 0.395 ± 0.010 mg of fur into 3.5 x 5 mm tin capsules. I then loaded the samples into a Costech Elemental Analyser (EA; Costech Analytical Technologies Inc., Bremen, Germany) in continuous flow mode

where they were combusted. The CO₂ and N₂ gases produced by the combustion were measured using a Thermo Finnigan Delta^{plus} XL Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher Scientific Inc., Bremen, Germany), which was coupled to the EA. In order to determine the analytical precision of the IRMS, I used a 10% duplication rate for my samples. Isotopic data for all duplicates are presented in Appendix E. I calibrated the δ¹³C values to VPDB using USGS-40 (−26.39 ‰) and USGS-41 (+37.63 ‰; the results in parentheses are internationally accepted values). I used IAEA-CH-6 (−10.45 ‰) as an unknown to check the accuracy of the calibration curve, and I also placed a keratin internal standard (−24.04 ‰ for δ¹³C) approximately every 9-10 samples to check for analytical drift. For δ¹³C, I obtained an average value of −10.49 ± 0.13 ‰, n=12 for IAEA-CH-6, and an average value of −24.07 ± 0.05 ‰, n=31 for keratin. The average difference between duplicates of the same sample (± standard deviation) was 0.08 ± 0.06 ‰, n=14. I calibrated the δ¹⁵N values to AIR using USGS-40 (−4.52 ‰) and USGS-41 (+47.57 ‰), and used IAEA-N2 (+20.30 ‰) as an unknown to check the accuracy of the calibration curve. I also placed a keratin internal standard (+6.36 ‰ for δ¹⁵N) approximately every 9-10 samples to check for analytical drift. For δ¹⁵N I obtained an average value of +20.36 ± 0.19 ‰, n=14 for IAEA-N2, and an average value of +6.38 ± 0.17 ‰, n=36 for keratin. The average difference between duplicates of the same sample was 0.03 ± 0.02 ‰, n=14.

For oxygen isotopic composition analyses, I weighed 0.180 ± 0.010 mg of fur into 4 x 3.2 mm silver capsules. Samples were combusted in a ThermoFinnigan High Temperature Conversion Elemental Analyser (TC/EA; Thermo Fisher Scientific Inc., Bremen, Germany). The CO gas was then sent via continuous flow to a ThermoFinnigan Delta^{plus} XL mass spectrometer that was coupled to the TC/EA. In order to determine the analytical precision of the IRMS, I used a 16% duplication rate for my samples. I calibrated the oxygen isotopic results to VSMOW- SLAP using IAEA-CH-6 (+36.4 ‰) and USGS-LOW (−3.15 ‰), with the exception of the first analytical session, which was calibrated using IAEA-CH-6 and Ennadai (+22.2 ‰). All analytical sessions after the first one used Ennadai to verify the calibration curve. In each analytical session I also analyzed a *Lasiurus borealis* fur sample taken from the same bat approximately every 5 samples to check for analytical drift. I also included a Benzoic Acid internal standard in

each analysis to test the comparability of each analytical session to one another. For $\delta^{18}\text{O}$ I obtained an average value of $+20.4 \pm 0.6 \text{ ‰}$, $n=13$ for Ennadai and $+10.9 \pm 1.3 \text{ ‰}$, $n=41$ for *L. borealis*. The average difference between duplicates of the same sample was $0.3 \pm 0.3 \text{ ‰}$, $n=29$. The average value of Benzoic Acid across all $\delta^{18}\text{O}$ analyses was $+26.4 \pm 0.5 \text{ ‰}$, $n=8$, which is slightly lower than its previously reported range of $+27.1$ to $+27.8 \text{ ‰}$.

For stable hydrogen isotopic composition analyses, I weighed $0.165 \pm 0.010 \text{ mg}$ of fur into 4 x 3.2 mm silver capsules. Samples were combusted in the TC/EA and the resulting H_2 gas was sent via continuous flow to the IRMS. In order to determine the analytical precision of the IRMS, I used approximately a 16% duplication rate for my samples. Using the comparative equilibration technique (Wassenaar and Hobson, 2003), I calibrated them with a five-point curve using fur standards with known non-exchangeable $\delta^2\text{H}$ values (Lemming: -155 ‰ , Wolf: -118 ‰ , Dog: -88 ‰ , Jackrabbit: -59 ‰ and Skunk: -34 ‰). I also analyzed a *Lasiurus borealis* fur standard about every fifth analysis as a check for analytical drift. For $\delta^2\text{H}$, I obtained an average value of $-45 \pm 4 \text{ ‰}$, $n=39$ for *L. borealis*, and the average difference between duplicates of the same sample was $2 \pm 2 \text{ ‰}$, $n=30$. To ensure consistency between analyses I included the NBS-22 standard in each analytical session. Its average value from all analyses was $-134 \pm 6 \text{ ‰}$, $n=9$. The accepted value obtained for NBS-22, when calibrated using standards corrected for exchangeable hydrogen, is -136 ‰ (this is the corrected value taken from Fraser (2011)). The apparent value reported here for NBS-22 differs from its accepted value of -120 ‰ . This is because NBS-22 does not contain any exchangeable hydrogen, so when it is calibrated using standards that contain exchangeable hydrogen its apparent accepted value differs compared to when it is calibrated using standards that do not contain exchangeable hydrogen. The variability of the NBS-22 value among analytical sessions was likely due to absorption of atmospheric water.

2.4 Diet analyses

For stable carbon isotopic analyses, I weighed $0.400 \pm 0.010 \text{ mg}$ of each diet sample into 3.5 x 5mm tin capsules. I then placed them into the EA in continuous flow mode where

they were combusted. The CO₂ gas produced by the combustion was then measured using the IRMS. I used a 10% duplication rate and calibrated my samples to VPDB using USGS-40 (-26.39 ‰) and USGS-41 (+37.63 ‰). I used IAEA-CH-6 (-10.45 ‰) as an unknown to check the accuracy of the calibration curve, and placed a keratin internal standard (-24.04 ‰ for δ¹³C) approximately every 9-10 samples to check for analytical drift. I obtained an average δ¹³C value of -10.57 ± 0.23 ‰, n=3 for IAEA-CH-6 and an average δ¹³C value of -24.08 ± 0.06 ‰, n=9 for keratin. The average difference between duplicates of the same sample (± standard deviation) was 0.36 ± 0.29 ‰, n=2.

Due to the low nitrogen content of many of the diet samples, δ¹⁵N could not be measured simultaneously with δ¹³C for all samples. During the initial analysis, I determined the percentage of nitrogen contained in each sample and subsequently weighed an appropriately larger sample into 3.5 x 5mm tin capsules for δ¹⁵N analysis. The weights I used varied according to the nitrogen content of the sample (Table 3). The amount of N₂ gas produced by combustion was then sufficient for measurement using the IRMS. In order to determine the analytical precision of the IRMS, I used a 10% duplication rate for my samples. The δ¹⁵N values were calibrated using USGS-40 (-4.52 ‰) and USGS-41 (+47.57 ‰). I used IAEA-N2 (+20.30 ‰) as an unknown to check the accuracy of the calibration curve, and placed a keratin internal standard (+6.36 ‰ for δ¹⁵N) approximately every 9-10 samples to check for analytical drift. I obtained an average δ¹⁵N value of +20.31 ± 0.27 ‰, n=5 for IAEA-N2 and an average δ¹⁵N value of +6.42 ± 0.12 ‰, n=14 for keratin. The average difference between duplicates of the same sample (± standard deviation) was 0.29 ± 0.25 ‰, n=2 for δ¹⁵N.

2.5 Water analyses

To obtain δ²H and δ¹⁸O values for my diet samples, I analysed the distilled water drawn from the samples via vacuum distillation. These compositions represent the free water in the diet samples, but not the δ²H and δ¹⁸O values of the tissue portions of the food, which were not obtained.

Table 3: Weights used for $\delta^{15}\text{N}$ analysis of diet items with low nitrogen contents.

Diet type	Weight (mg)
Banana	5.00
Bran	1.58
Cantaloupe	2.27
Fig	5.46
Gala Apple	13.56
Marmoset Mix	1.16
Nectar	12.94
Papaya	6.42
Pear	7.73
Sweet Potato	2.41

Drinking water and distilled diet water samples were analyzed using Cavity Ring-Down Spectroscopy (CRDS) using an L1102-i Water Vapour Analyzer developed by Picarro, Inc. (Sunnyvale, CA, USA; hereafter referred to as the Picarro). We measured all samples using the high precision mode. The sample size for each water sample was 2 μL , and we analysed both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ simultaneously for each sample. We analysed twenty-three in-house standards alongside the samples in order to calculate accuracy and precision, and to calibrate samples to the VSMOW – SLAP scale. The Picarro cleaned the auto-sampler needle between each sample using either a N-methyl-2-pyrrolidinone rinse (all samples analysed prior to 24 January, 2013) or a Distilled Water rinse (all samples analysed after 24 January, 2013), followed by a rinse using water from the next sample, which was then sent to a waste tube. Each water sample was injected into the cavity six times, and the results from the last three injections were averaged to produce the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values. I calibrated the hydrogen and oxygen isotopic results to VSMOW – SLAP using Heaven (+88.7 ‰/–0.27 ‰ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively) and LSD (–161.8 ‰/–22.57 ‰ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively). I used MID (–108.1 ‰/–13.08 ‰ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively) as an unknown to check the accuracy of the calibration curve and placed an internal water sample, EDT (–56 ‰/–7.27 ‰ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively)

approximately every 9-10 samples to check for analytical drift. I obtained an average $\delta^2\text{H}$ value of -108.2 ± 0.8 ‰, $n=14$ and an average $\delta^{18}\text{O}$ value of -13.12 ± 0.09 ‰, $n=14$ for MID. For EDT, I obtained an average $\delta^2\text{H}$ value of -55.2 ± 0.8 ‰, $n=46$ and an average $\delta^{18}\text{O}$ value of -7.39 ± 0.13 ‰, $n=46$. In order to determine the analytical precision of the Picarro, I had an 18% duplication rate for both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for all water samples. For the drinking water samples, the average difference between duplicates of the same sample (\pm standard deviation) was 0.2 ± 0.1 ‰ for $\delta^2\text{H}$ and 0.05 ± 0.03 ‰ for $\delta^{18}\text{O}$. For distilled diet water samples, the average difference between duplicates of the same sample (\pm standard deviation) was 2.7 ± 0.4 ‰ for $\delta^2\text{H}$ and 0.44 ± 0.14 ‰ for $\delta^{18}\text{O}$.

2.6 Statistical analyses

I used R version 2.15.2 for all statistical analyses. I used Q-Q plots in conjunction with Shapiro-Wilk tests to determine if my data followed a normal distribution. I used parametric statistics when my data were close to a normal distribution. When my data could not be assumed to follow a normal distribution, I used non-parametric statistics for my analyses. To compare the average isotopic ratios among species or sampling trips I used either a Student's t-test (for data considered normal) or the Mann-Whitney U test (for all other data). I used a Levene's test to compare the variances in isotopic compositions among species or sampling trips. For correlations I used either a Pearson product-moment correlation (for data considered normal) or a Spearman's rank correlation (for all other data). When testing correlations involving $\delta^{13}\text{C}$ values, captive *D. rotundus* values were excluded as they were influenced by the presence of C_4 plants in the bats' diet. For species that I sampled over multiple trips (*A. jamaicensis*, *G. soricina*, *C. perspicillata*, *E. fuscus*) or over multiple locations (*A. jamaicensis*), I compared the results from each trip or location to one another. I pooled them when there were no significant differences in both the mean isotopic composition and the variance and then compared the fur $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values using species averages (for overall correlations) or individual data (for intra-trophic correlations). I also compared the average fur values of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for each species between trophic levels using one-tailed t-tests.

To ensure that differences in the fur hydrogen isotopic compositions are not just a reflection of variation in the drinking water, I calculated the difference between the $\delta^2\text{H}$ values of the fur and the $\delta^2\text{H}$ value of the drinking water ($\delta^2\text{H}_{\text{fur}} - \delta^2\text{H}_{\text{water}} = \Delta^2\text{H}$). As many of the bats that I sampled had completed their molt prior to my sampling period, and as not all bat species molt at the same time (Constantine, 1957; Constantine, 1958; Cryan et al., 2004; Fraser et al., 2012), the drinking water $\delta^2\text{H}$ value at the time of fur growth would not be the same for all species. In order to make the comparison consistent between species I needed to use $\delta^2\text{H}_{\text{water}}$ values that would reflect the $\delta^2\text{H}_{\text{water}}$ values at the time of fur growth. For the captive locations (Montreal, Hamilton, Havelock) I calculated the $\delta^2\text{H}_{\text{water}}$ value by taking the average $\delta^2\text{H}$ value of the water samples I collected at each site. The sources of drinking water for Montreal, Hamilton and Havelock are the St.Lawrence River, Lake Ontario, and groundwater wells, respectively. The $\delta^2\text{H}_{\text{water}}$ values of these sources remain relatively constant throughout the year (Huddart et al., 1999; Yang et al., 1996), so the $\delta^2\text{H}_{\text{water}}$ values I used should be representative of the actual values at the time of fur growth. I used the same procedure to calculate the difference between $\delta^{18}\text{O}_{\text{fur}}$ and $\delta^{18}\text{O}_{\text{water}}$ ($\Delta^{18}\text{O}$).

The source of drinking water for the wild populations of *E. fuscus* and *D. rotundus* is likely precipitation. To obtain values of $\delta^{18}\text{O}_{\text{water}}$ for the wild populations of *E. fuscus* and *D. rotundus*, I calculated the average $\Delta^{18}\text{O}$ values for the captive *E. fuscus* and *D. rotundus*. As $\delta^{18}\text{O}$ values are derived almost entirely from drinking water and are not influenced by trophic level (Schimmelmann and DeNiro, 1986; van Hardenbroek et al., 2012) or physiology (Bowen et al., 2009), the difference between $\delta^{18}\text{O}_{\text{fur}}$ and $\delta^{18}\text{O}_{\text{water}}$ ($\delta^{18}\text{O}_{\text{fur}} - \delta^{18}\text{O}_{\text{water}}: \Delta^{18}\text{O}$) should be approximately equal for the captive and wild populations of the same species. I therefore set the average $\Delta^{18}\text{O}$ value for each wild population to be equal to that of the corresponding captive population (e.g., the average $\Delta^{18}\text{O}$ value for wild *E. fuscus* is equal to the average $\Delta^{18}\text{O}$ value of captive *E. fuscus*).

Values of $\delta^2\text{H}_{\text{precipitation}}$ are highly correlated with $\delta^{18}\text{O}_{\text{precipitation}}$ values, following the general expression:

Equation 2:

$$\delta^2\text{H} = 8.0 * \delta^{18}\text{O} + 10$$

This is called the Global Meteoric Water Line (Craig, 1961; Gat et al., 2001). However, this relationship can vary in slope and intercept by region depending on parameters such as latitude, altitude, humidity, temperature, evaporation, transpiration and vapour recycling (Gat et al., 2001). Using the Online Isotopes in Precipitation Calculator (OIPC) version 2.2 (<http://www.waterisotopes.org>; Bowen, 2013; Bowen et al., 2005) I calculated the Local Meteoric Water Line (LMWL) for each wild sampling location (Appendix F). The OIPC allows a user to input geographic coordinates and obtain the monthly $\delta^{18}\text{O}$ and $\delta^2\text{H}$ precipitation values for those coordinates. The locations of the wild populations included Windsor, Ontario and Lamanai, Belize.

Using $\Delta^{18}\text{O}$ and the calculated average $\delta^{18}\text{O}_{\text{fur}}$ value, I was able to calculate the corresponding $\delta^{18}\text{O}_{\text{water}}$ value for each of the wild populations. The calculated $\delta^{18}\text{O}_{\text{water}}$ value is a reflection of the $\delta^{18}\text{O}_{\text{water}}$ value at the time of fur growth. Having a $\delta^{18}\text{O}_{\text{water}}$ value allowed me to calculate the corresponding $\delta^2\text{H}_{\text{water}}$ values using the LMWL for each sampling location, which in turn allowed me to calculate $\Delta^2\text{H}$ for each individual (see Appendix F for sample calculations; see Appendix G for $\delta^2\text{H}_{\text{water}}$ and $\delta^{18}\text{O}_{\text{water}}$ values for each sampling of the wild populations).

To compare the average value of forearm length among species I used a Mann-Whitney U test. I tested for correlations between forearm length and $\Delta^2\text{H}$ values for all samples as well as within each species using a Spearman's rank correlation. To determine the significance of statistical tests I used $\alpha=0.05$.

Chapter 3: Results

3.1 Isotopic compositions of fur, diet and drinking water

Values of $\delta^{15}\text{N}_{\text{fur}}$ increased from the second to the third trophic level. On the second trophic level, values of $\delta^{15}\text{N}$ ranged from +5.80 to +7.65 ‰ with a standard deviation of 0.43 ‰. On the third trophic level, values ranged from +6.27 to +13.9 ‰, with a standard deviation of 1.01 ‰ (Figure 2).

Values of $\delta^{13}\text{C}_{\text{fur}}$ were not as distinctly different with trophic level as $\delta^{15}\text{N}_{\text{fur}}$, but still increased from the second trophic level to the third level (Figure 2). On the second trophic level, values ranged from -23.04 to -21.04 ‰, with a standard deviation of 0.44 ‰. On the third trophic level, values ranged from -24.27 to -14.68 ‰, with a standard deviation of 2.15 ‰. If captive *D. rotundus* values are excluded, values ranged from -24.27 to -15.27 ‰, with a standard deviation of 1.45 ‰.

Values of $\Delta^2\text{H}$ also increased from the second trophic level to the third level (Figures 3 and 4). On the second trophic level, values ranged from -40 to +11 ‰, with a standard deviation of 11 ‰. On the third trophic level, values ranged from +4 to +61 ‰, with a standard deviation of 10 ‰.

Values of $\Delta^{18}\text{O}$ showed no relationship with trophic level (Figure 4). On the second trophic level, values ranged from +14.6 to +23.9 ‰ with a standard deviation 2.1 ‰. On the third trophic level, values ranged from +16.8 to +22.1 ‰ with a standard deviation of 1.1 ‰.

The equation of the line for the values of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in the diet water was

$$\delta^2\text{H} = 5.8 * \delta^{18}\text{O} - 12.8 \text{ (Figure 5).}$$

This is similar to the equation for the values of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ given by the drinking water samples, which was

$$\delta^2\text{H} = 5.1 * \delta^{18}\text{O} - 14.2 \text{ (Figure 6).}$$

Both of these equations differed from the Local Meteoric Water Lines of any of the sampling locations (Appendix F).

The presence of C₄ plants was noticeable in the significantly higher $\delta^{13}\text{C}$ value of blood compared to the $\delta^{13}\text{C}$ values of the other diet samples (Mann-Whitney U test, $W = 1.5$, $Z = -2.128$, $p\text{-value} = 0.020$; Figure 7). The fruit and bran samples generally had lower $\delta^{15}\text{N}$ values than the samples of blood and mealworms, but the difference was not significant (Mann-Whitney U test, $W = 20.5$, $Z = -1.511$, $p\text{-value} = 0.138$; Figure 7).

3.2 Correlations

In a comparison of all species' means, there was a trend of $\delta^{13}\text{C}$ values increasing with $\delta^{15}\text{N}$ values. There was also a trend of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values increasing with $\Delta^2\text{H}$ values. There was no trend between values of $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$. This lack of a trend was also present between the values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ that were not corrected for drinking water. Values of $\delta^{18}\text{O}$ also did not show a trend of increasing with values of $\delta^{15}\text{N}$. However, these were not significant correlations due to the small sample size (Table 4).

The correlations within individual trophic levels differed from the overall correlations. On the second trophic level, there were no significant correlations between values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, $\delta^{15}\text{N}$ and $\Delta^2\text{H}$ or $\delta^{13}\text{C}$ and $\Delta^2\text{H}$. However, there were significant positive correlations between values of $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ as well as $\delta^2\text{H}$ and $\delta^{18}\text{O}$. There was also a very weak significant correlation between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ (Table 5). On the third trophic level, values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were significantly positively correlated, but less strongly than in the overall values. There was no significant correlation between values of $\delta^{15}\text{N}$ and $\Delta^2\text{H}$. Values of $\delta^{13}\text{C}$ and $\Delta^2\text{H}$ showed a significant very weak negative correlation. Values of $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ as well as $\delta^2\text{H}$ and $\delta^{18}\text{O}$ showed a significant positive correlation, but unlike the second trophic level and the overall values, the correlation was much stronger between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ than between $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$. Values of $\Delta^{18}\text{O}$ and $\delta^{15}\text{N}$ showed a significant medium positive correlation, which was also different from both the second trophic level and the overall values (Table 6).

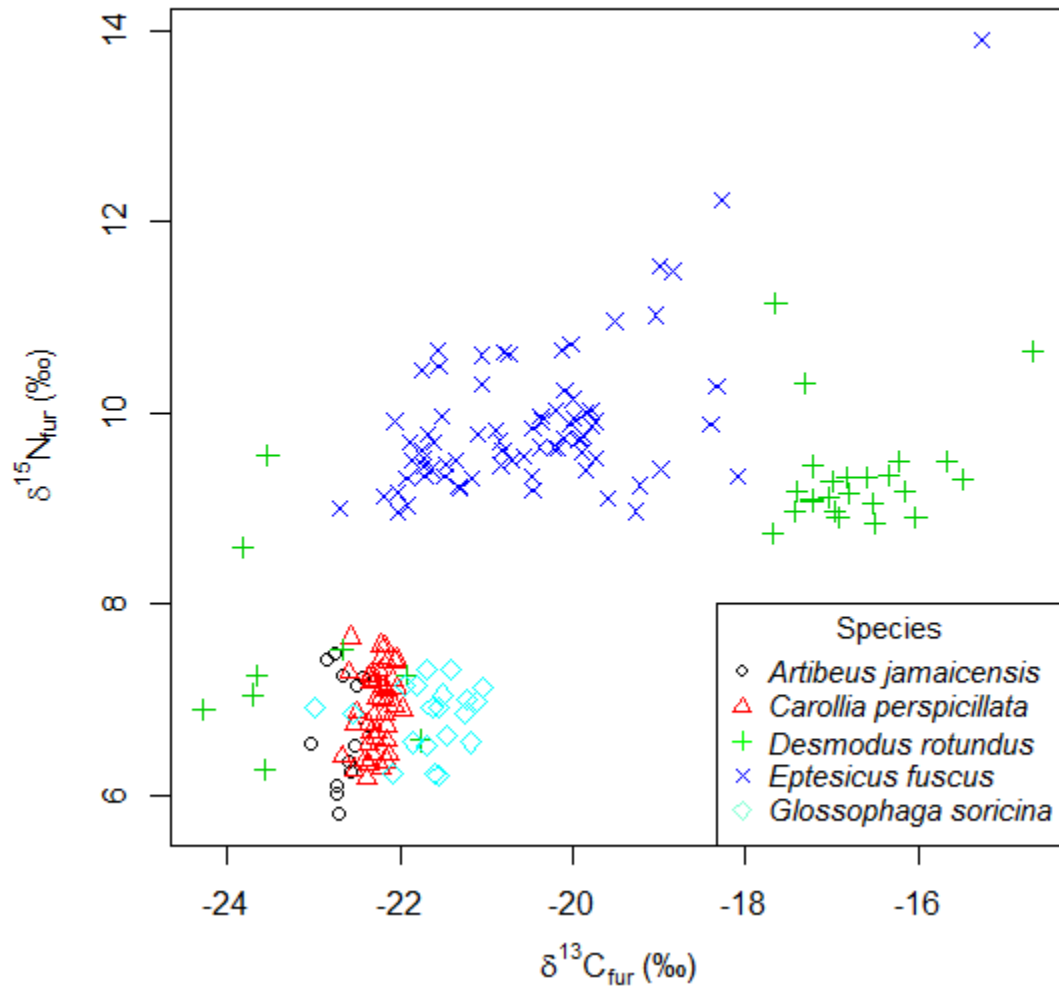


Figure 2: Values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for all fur samples. Captive *D. rotundus* samples had higher $\delta^{13}\text{C}$ values because their diet is based on both C_3 and C_4 plants, whereas the diets of all other populations are based solely on C_3 plants. Species from the second trophic level (*A. jamaicensis*, *C. perspicillata*, *G. soricina*) had lower $\delta^{15}\text{N}$ values than species on the third trophic level (*D. rotundus*, *E. fuscus*). The Spearman's rank correlation coefficient for species' mean values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ is $R=0.800$ (Table 4).

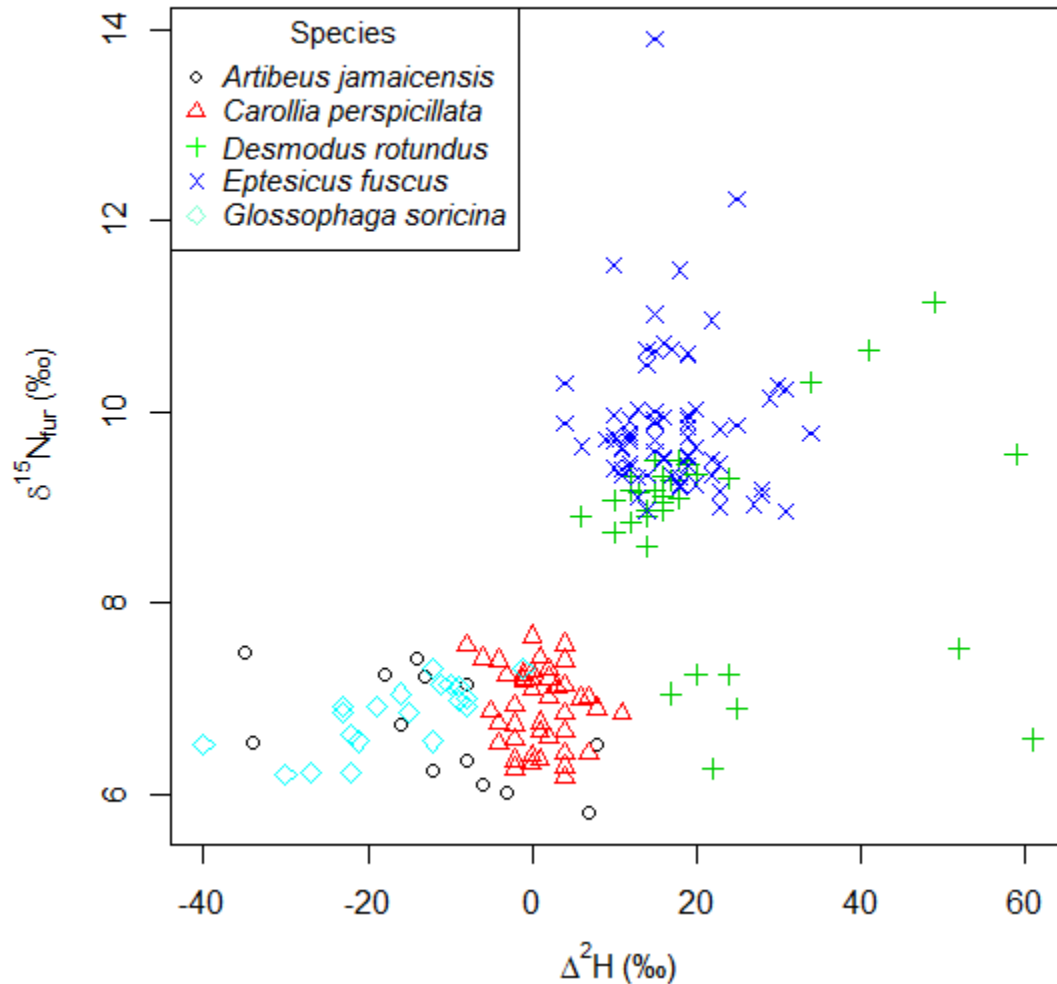


Figure 3: Values of $\delta^{15}\text{N}$ and $\Delta^2\text{H}$ for all fur samples. Species from the second trophic level (*A. jamaicensis*, *C. perspicillata*, *G. soricina*) had lower $\Delta^2\text{H}$ values than species on the third trophic level (*D. rotundus*, *E. fuscus*). The Spearman's rank correlation coefficient for species' mean values of $\delta^{15}\text{N}$ and $\Delta^2\text{H}$ is $R=0.800$ (Table 4).

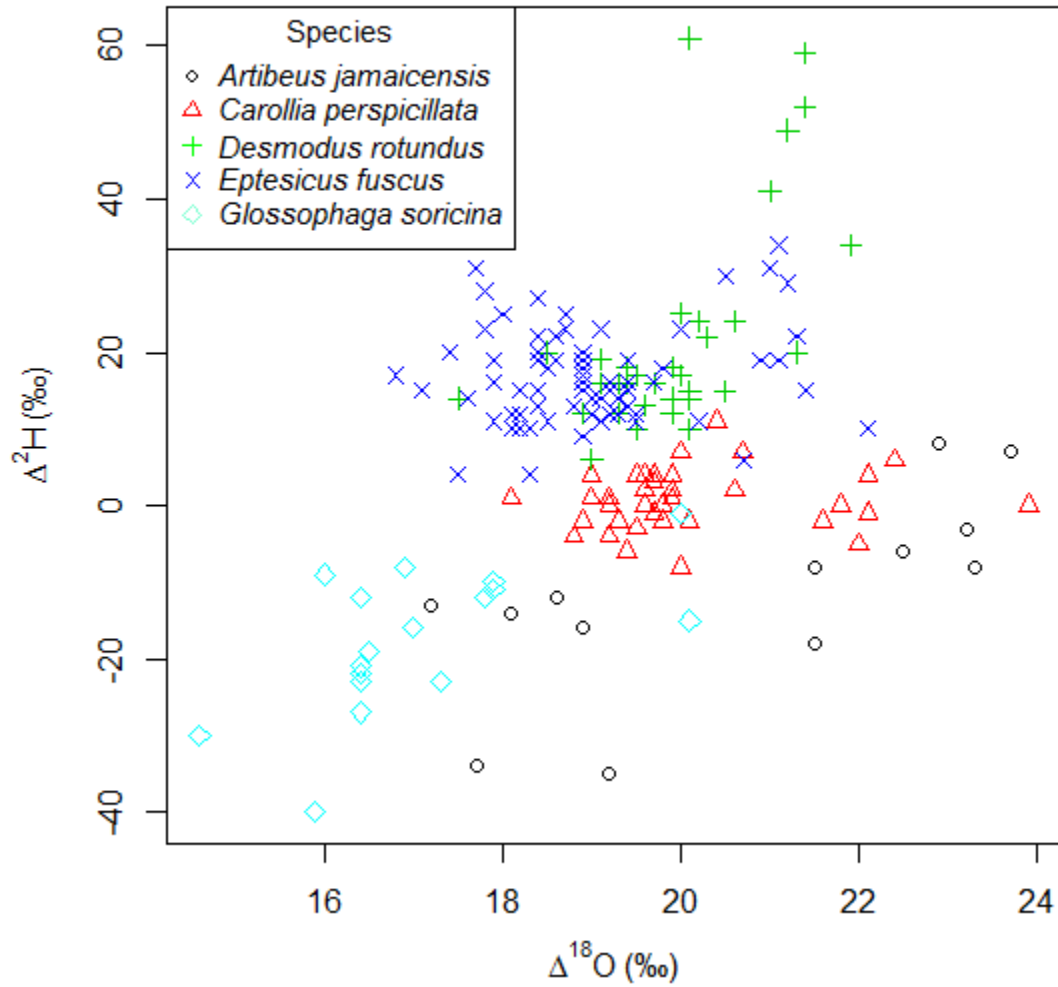


Figure 4: Values of $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ for all fur samples. Values of $\Delta^{18}\text{O}$ did not differ between the second and third trophic level. The Spearman's rank correlation coefficient for species' mean values of $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ is $R=0.300$ (Table 4).

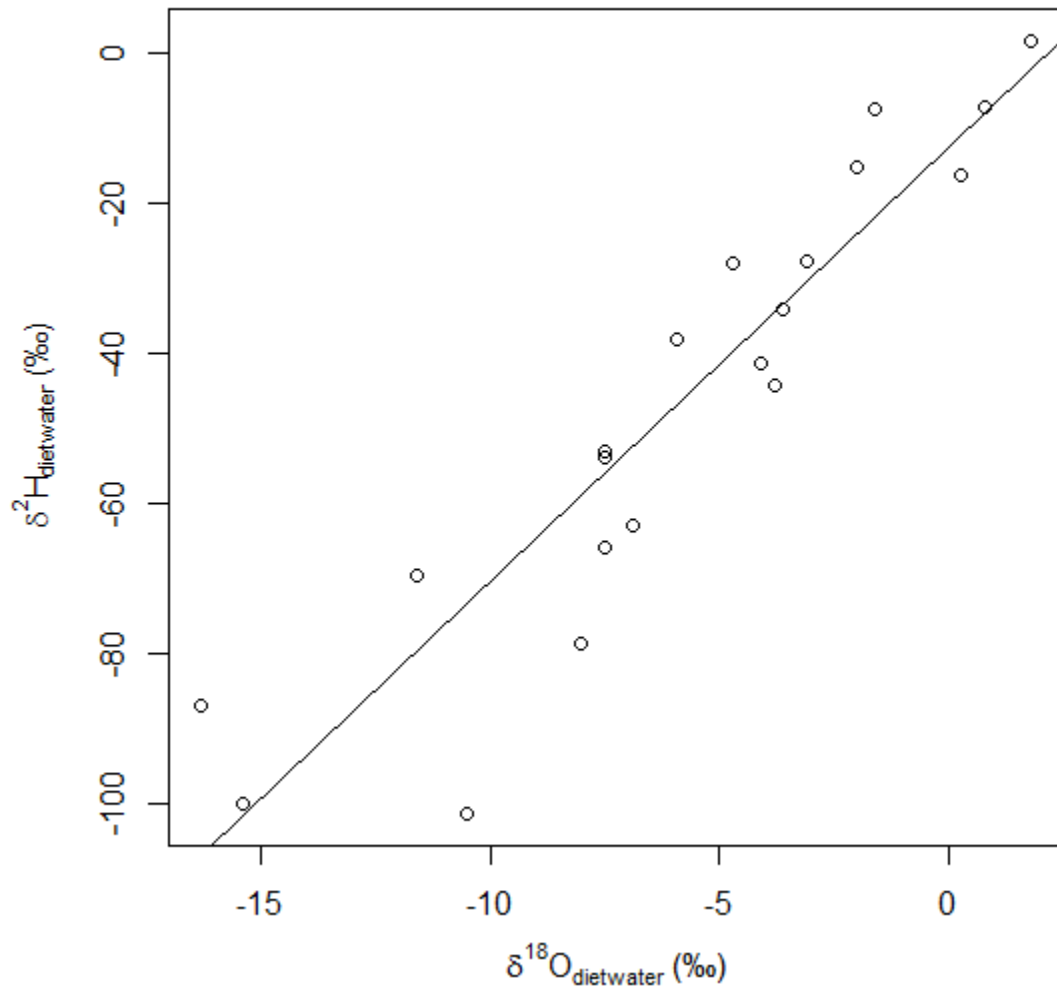


Figure 5: Values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for all water extracted from diet samples (called “diet water” in the text). The equation of this line was

$$\delta^2\text{H} = 5.8 * \delta^{18}\text{O} - 12.8$$

This differed from the Local Meteoric Water Lines of any of the sampling locations (Appendix F) but was similar to the equation given by the drinking water samples (Figure 6).

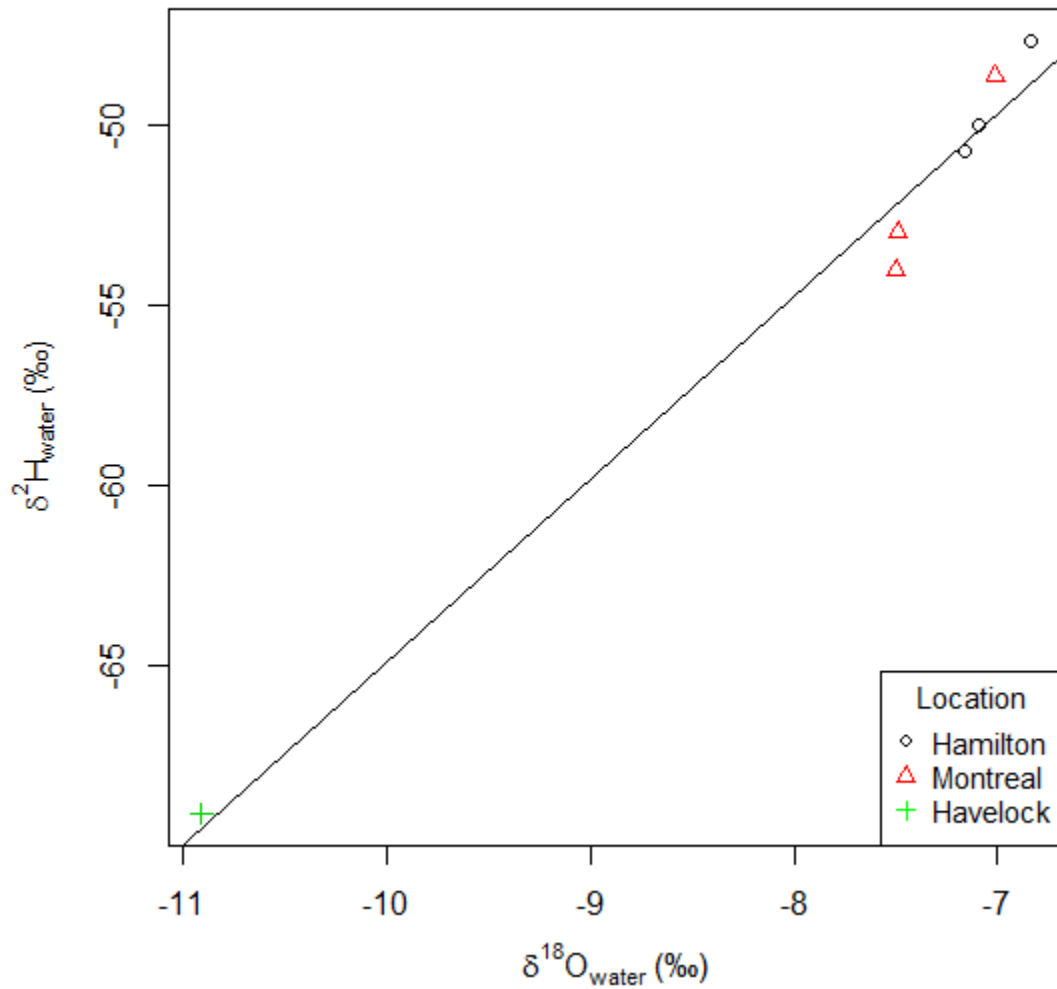


Figure 6: Values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for all drinking water samples. The equation of this line was

$$\delta^2\text{H} = 5.1 * \delta^{18}\text{O} - 14.2$$

This equation had a shallower slope than the Local Meteoric Water Line for any of the three captive population sampling locations (Appendix F), but was very similar to the equation of the line given by the diet water samples (Figure 5).

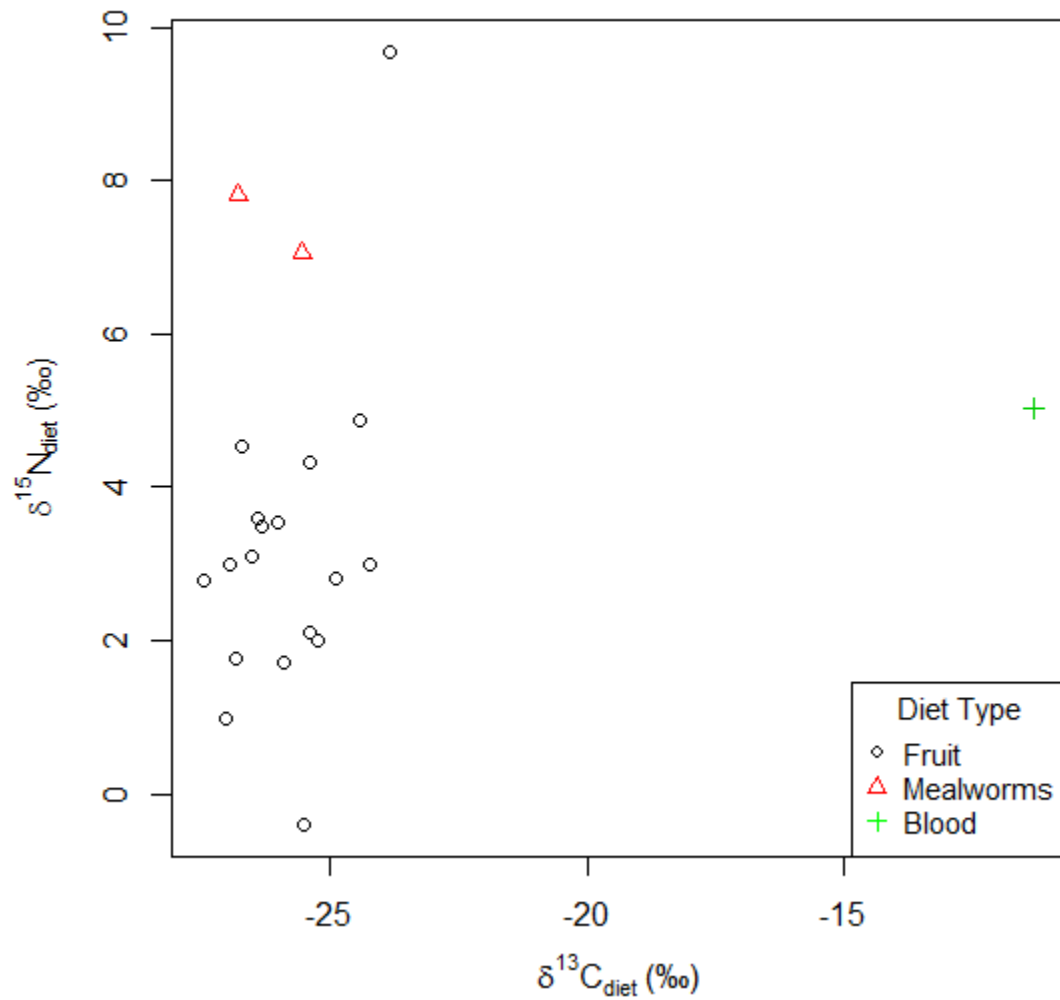


Figure 7: Values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for all diet tissue samples. The sample with the very high $\delta^{13}\text{C}$ value is cow blood. It was enriched in carbon-13 because corn, which is a C_4 plant, comprises the main part of the cows' diet.

Unlike the overall fur samples, diet samples showed no significant correlation between values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ or values of $\delta^{13}\text{C}$ and $\delta^2\text{H}$. However, there was a significant medium positive correlation between values of $\delta^{15}\text{N}$ and $\delta^2\text{H}$ and values of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$. There was a significant very strong positive correlation between values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ (Table 7). This significant very strong positive correlation between values of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ was also present in the drinking water (Table 8).

Table 4: Spearman's rank correlation statistics for pairwise comparisons of each species' mean stable isotope compositions. Captive *D. rotundus* mean $\delta^{13}\text{C}$ values were not included in these correlations as their diet contained C_4 plants while all other diets were based solely on C_3 plants.

Comparison	n	R	p-value
$\delta^{13}\text{C} - \delta^{15}\text{N}$	4	0.800	0.133
$\delta^{15}\text{N} - \Delta^2\text{H}$	5	0.800	0.133
$\delta^{13}\text{C} - \Delta^2\text{H}$	4	0.700	0.233
$\delta^2\text{H} - \delta^{18}\text{O}$	5	-0.300	0.683
$\Delta^2\text{H} - \Delta^{18}\text{O}$	5	0.300	0.683
$\delta^{18}\text{O} - \delta^{15}\text{N}$	5	-0.300	0.683

Table 5: Spearman's rank correlation statistics for pairwise comparisons of stable isotope compositions of fur samples from the second trophic level (*A. jamaicensis*, *C. perspicillata*, and *G. soricina*).

Comparison	n	R	p-value
$\delta^{13}\text{C} - \delta^{15}\text{N}$	76	0.129	0.265
$\delta^{15}\text{N} - \Delta^2\text{H}$	76	0.014	0.906
$\delta^{13}\text{C} - \Delta^2\text{H}$	76	-0.119	0.306
$\delta^2\text{H} - \delta^{18}\text{O}$	71	0.595	<0.001
$\Delta^2\text{H} - \Delta^{18}\text{O}$	71	0.607	<0.001
$\delta^{18}\text{O} - \delta^{15}\text{N}$	71	0.236	0.047

Table 6: Spearman's rank correlation statistics for pairwise comparisons of stable isotope compositions of fur samples from the third trophic level (*D. rotundus* and *E. fuscus*). Captive *D. rotundus* $\delta^{13}\text{C}$ values were not included in these correlations as their diet contained C_4 plants while all other diets were based solely on C_3 plants.

Comparison	n	R	p-value
$\delta^{13}\text{C} - \delta^{15}\text{N}$	84	0.519	<0.001
$\delta^{15}\text{N} - \Delta^2\text{H}$	108	-0.028	0.775
$\delta^{13}\text{C} - \Delta^2\text{H}$	83	-0.240	0.029
$\delta^2\text{H} - \delta^{18}\text{O}$	106	0.731	<0.001
$\Delta^2\text{H} - \Delta^{18}\text{O}$	106	0.243	0.012
$\delta^{18}\text{O} - \delta^{15}\text{N}$	106	0.366	<0.001

Table 7: Pearson product-moment correlation statistics for pairwise comparisons of stable isotope compositions of all diet samples. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values are from the water extracted from the diet samples, and the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are from the tissues of the diet samples.

Comparison	n	R	t	df	p-value
$\delta^{13}\text{C} - \delta^{15}\text{N}$	21	0.213	0.950	19	0.354
$\delta^{15}\text{N} - \delta^2\text{H}$	20	0.510	2.517	18	0.022
$\delta^{13}\text{C} - \delta^2\text{H}$	20	0.187	0.808	18	0.430
$\delta^{18}\text{O} - \delta^2\text{H}$	20	0.926	10.407	18	<0.001
$\delta^{18}\text{O} - \delta^{15}\text{N}$	20	0.478	2.306	18	0.033

Table 8: Pearson product-moment correlation statistics for pairwise comparison of stable oxygen and hydrogen isotope compositions of all drinking water samples (does not include any diet water samples).

Comparison	n	R	t	df	p-value
$\delta^{18}\text{O} - \delta^2\text{H}$	7	0.992	17.716	5	<0.001

3.3 Interspecific variation

Values of $\delta^{15}\text{N}_{\text{fur}}$ showed a significant increase of 2.71 ‰ from the second to the third trophic level (One-tailed t-test, $t = -11.216$, $df = 3$, $p\text{-value} = <0.001$; Figure 8). This was similar to the expected increase of 3 ‰ from the second to the third trophic level. The $\delta^{13}\text{C}$ values varied significantly among species in the same trophic group as well as between trophic groups. Values of $\delta^{13}\text{C}_{\text{fur}}$ showed a significant increase of 2.29 ‰ from the second trophic level to the third level (One-tailed t-test, $t = -2.512$, $df = 3$, $p\text{-value} = 0.043$), which was slightly higher than the 1 ‰ increase I expected. However, the presence of C_4 plants in the diet of captive *D. rotundus* raised their $\delta^{13}\text{C}_{\text{fur}}$ values relative to the rest of the bats (Figure 9). If captive *D. rotundus* are excluded, there was an increase of 1.40 ‰ from the second to the third trophic level (One-tailed t-test, $t = -2.936$, $df = 2$, $p\text{-value} = 0.050$), which was closer to the 1 ‰ increase I expected.

The $\Delta^2\text{H}$ values varied significantly between species in the same trophic group, but also showed a significant increase from the second to the third trophic level. Values of $\Delta^2\text{H}$ showed a significant increase of 23 ‰ from the second trophic level to the third level (One-tailed t-test, $t = -3.869$, $df = 3$, $p\text{-value} = 0.015$; Figure 10). This was within the 20 to 25 ‰ increase I expected from the second trophic level to the third level. As expected, values of $\Delta^{18}\text{O}$ showed no increase from the second trophic level to the third trophic level (One-tailed t-test, $t = -0.483$, $df = 3$, $p\text{-value} = 0.331$; Figure 11). Results of all pairwise Mann-Whitney U tests comparing species for Figures 8-11 are presented in Appendix H.

3.4 Sampling date variation

A power analysis indicated that the sample sizes for *A. jamaicensis* were too small for a comparison of the means and variances across sampling trips and locations ($h = 0.5$, $n1 = 5$, $n2 = 5$, significance level = 0.05, power = 0.12; $h = 0.5$, $n1 = 3$, $n2 = 5$, significance level = 0.05, power = 0.11), so I pooled *A. jamaicensis* samples for all analyses. The

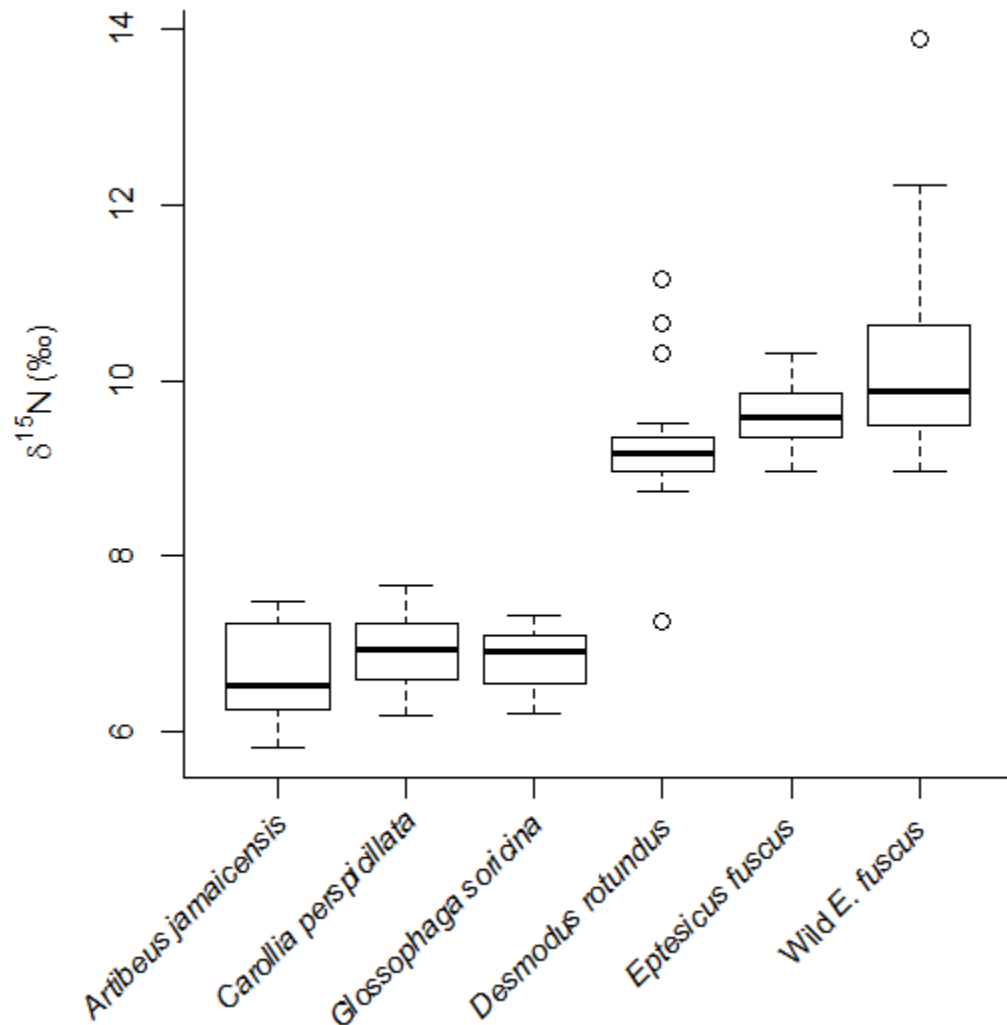


Figure 8: Box-and-whisker plot representing the $\delta^{15}\text{N}_{\text{fur}}$ values for *A. jamaicensis* (n=13), *C. perspicillata* (n=43), *G. soricina* (n=20), captive *D. rotundus* (n=25), captive *E. fuscus* (n=37) and wild *E. fuscus* (n=38). The band inside the box represents the median. The top and bottom lines of the box represent the first and third quartiles of the data, respectively. The whiskers extending from the top and bottom of the box represent the maximum and minimum of all data, respectively, that are not outliers. Circles represent outliers. Outliers are defined as any data point that falls further than 1.5 times the interquartile range. Species at the third trophic level (*D. rotundus* and *E. fuscus*) had significantly higher $\delta^{15}\text{N}$ values than those on the second trophic level (*A. jamaicensis*, *C. perspicillata* and *G. soricina*).

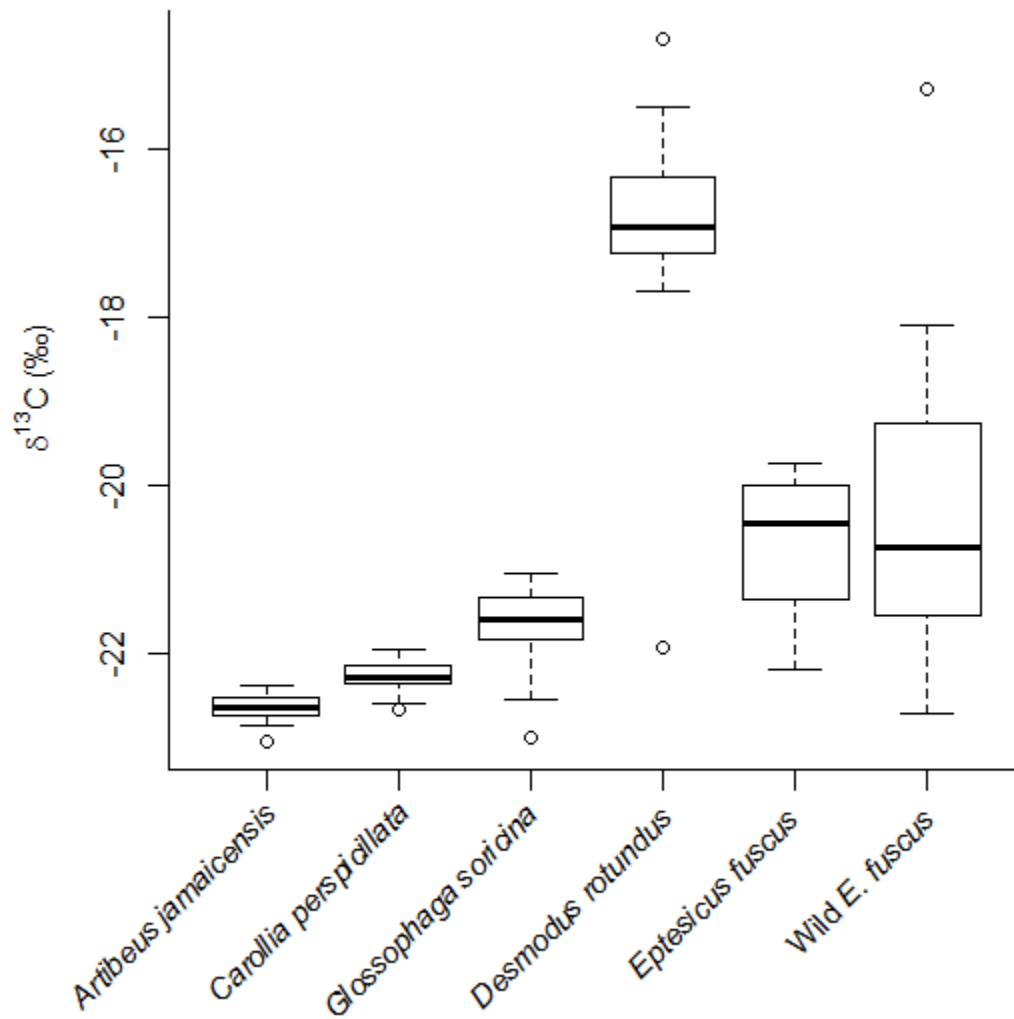


Figure 9: Box-and-whisker plot representing $\delta^{13}\text{C}_{\text{fur}}$ values for *A. jamaicensis* (n=13), *C. perspicillata* (n=43), *G. soricina* (n=20), captive *D. rotundus* (n=25), captive *E. fuscus* (n=37) and wild *E. fuscus* (n=39). Values of $\delta^{13}\text{C}_{\text{fur}}$ were significantly different among species. Overall, species at the third trophic level (*D. rotundus* and *E. fuscus*) had higher $\delta^{13}\text{C}_{\text{fur}}$ values than species on the second trophic level (*A. jamaicensis*, *C. perspicillata* and *G. soricina*). *Desmodus rotundus* $\delta^{13}\text{C}_{\text{fur}}$ values were much higher because of the presence of C_4 plants in the source of their diet (Kennedy and Krouse, 1990; Siemers et al., 2011).

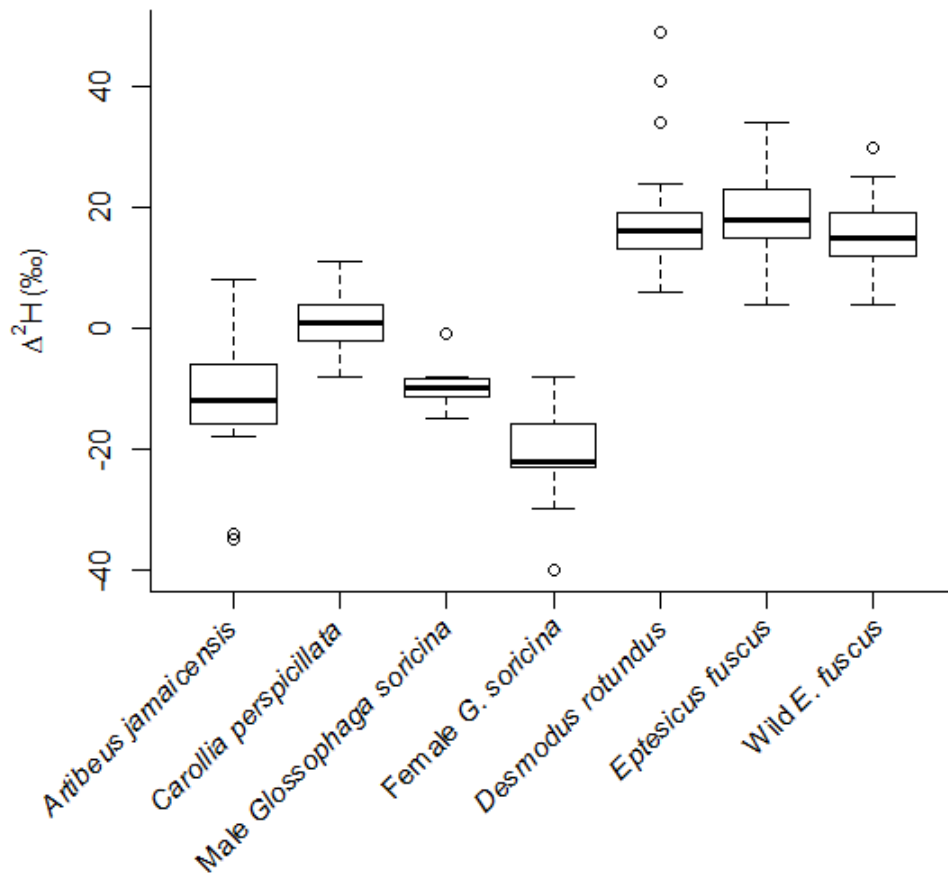


Figure 10: Box-and-whisker plot representing $\Delta^2\text{H}$ values for *A. jamaicensis* (n=13), *C. perspicillata* 1 (n=43), male *G. soricina* (n=7), female *G. soricina* (n=13), captive *D. rotundus* (n=25), captive *E. fuscus* (n=37) and wild *E. fuscus* (n=38). As the median $\Delta^2\text{H}$ values differed significantly for male and female *G. soricina*, they are shown separately. Species at the third trophic level (*D. rotundus* and *E. fuscus*) had significantly higher $\Delta^2\text{H}$ values than species on the second trophic level (*A. jamaicensis*, *C. perspicillata* and *G. soricina*). The smallest difference in $\Delta^2\text{H}$ values between trophic levels was approximately 15 ‰ (between *C. perspicillata* and *E. fuscus*). Within the third trophic level, $\Delta^2\text{H}$ values did not significantly differ from each other. Within the second trophic level, the $\Delta^2\text{H}$ value of *C. perspicillata* was significantly higher than the other three groups. Values of $\Delta^2\text{H}$ for *A. jamaicensis* were not significantly different from male or female *G. soricina*.

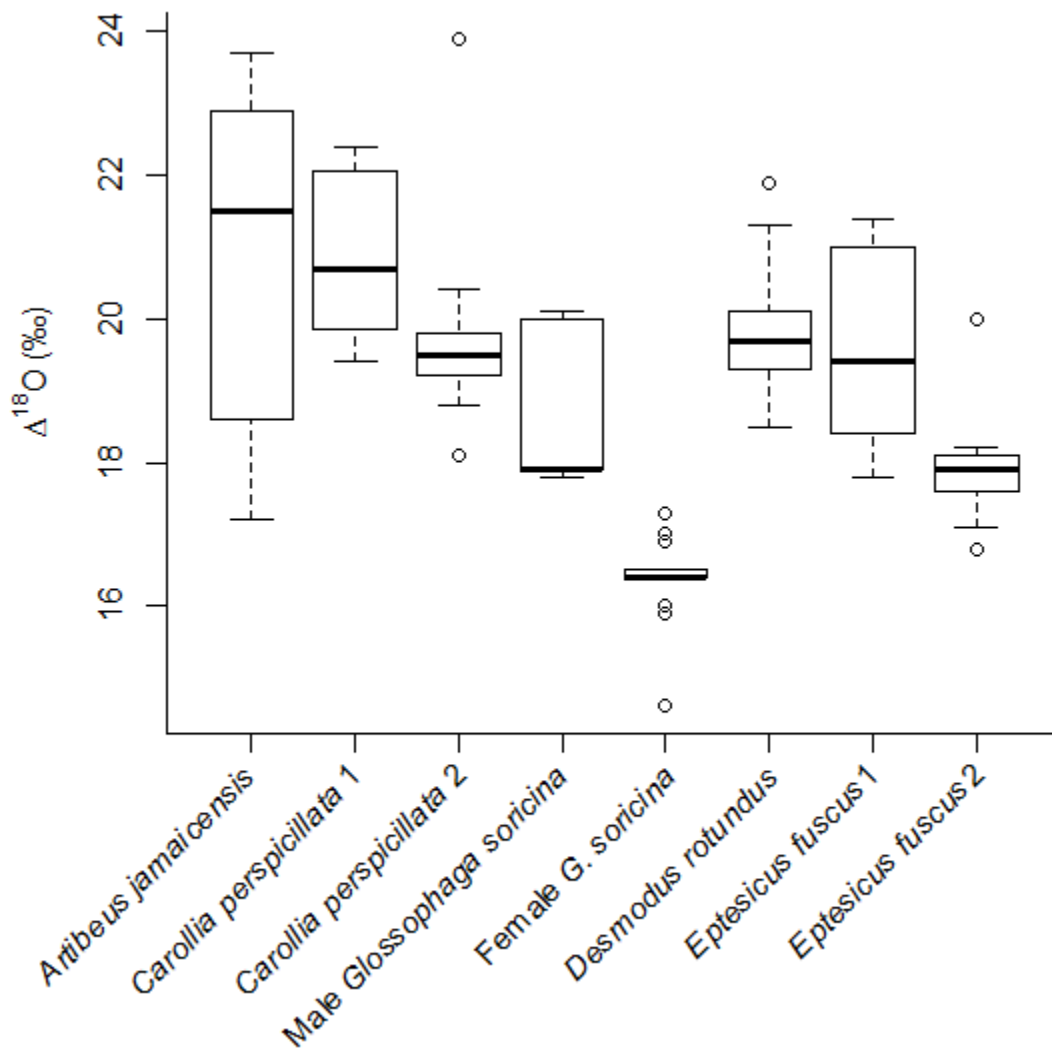


Figure 11: Box-and-whisker plot representing $\Delta^{18}\text{O}$ values for *A. jamaicensis* (n=13), *C. perspicillata* from the first sampling trip (n=15), *C. perspicillata* from the second sampling trip (n=25), male *G. soricina* (n=7), female *G. soricina* (n=13), captive *D. rotundus* (n=25), captive *E. fuscus* from the first sampling trip (n=22) and captive *E. fuscus* from the second sampling trip (n=13). Values of $\Delta^{18}\text{O}$ showed no pattern with trophic level.

means and variances of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\Delta^2\text{H}$ did not differ between sampling trips for *C. perspicillata*, captive *E. fuscus* or wild *E. fuscus* (Tables 9 and 10). The mean $\Delta^2\text{H}$ value for *G. soricina* was significantly lower on the second sampling trip than the first trip (Table 9). The variance of $\Delta^{18}\text{O}$ was significantly lower for *C. perspicillata* and captive *E. fuscus* on the second sampling trip compared to the first, but was significantly higher on the second sampling trip compared to the first for *G. soricina* (Table 10). The comparison of means and variances indicated that I could pool $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\Delta^2\text{H}$ values for all species sampled over two trips except for *G. soricina*, where I could not combine $\Delta^2\text{H}$ values across sampling trips. These analyses also indicated that I could not pool values of $\Delta^{18}\text{O}$ for *C. perspicillata*, *G. soricina* or captive *E. fuscus* across sampling trips.

3.5 Captive versus wild

I found no significant differences between the median $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\Delta^2\text{H}$ values of captive and wild populations of *E. fuscus* (Appendix H). I found significant differences between the variance of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ values, but no significant difference in the variance of $\Delta^2\text{H}$ values (Table 11).

3.6 Size differences

I attempted to minimize the chances that size would influence the $\delta^2\text{H}_{\text{fur}}$ values by having species of approximately the same size on each trophic level (Table 12). For species on the second trophic level, I found a weak positive correlation between forearm length and $\Delta^2\text{H}$ values, but for species on the third trophic level I found no correlation between forearm length and $\Delta^2\text{H}$ values (Table 13). Within each species, I found no correlation between forearm length and $\Delta^2\text{H}$ values for any species (Table 14).

Table 9: Comparison of the mean/median $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ values between sampling trips. Comparisons were conducted using either a Mann-Whitney U test (comparing medians) or a Student's t-test (comparing means). W, Z and p-values are presented for Mann-Whitney U tests, and t, df and p-values are presented for Student's t-tests. Values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are not significantly different between sampling trips for any species. The $\Delta^2\text{H}$ values were significantly different between sampling trips for *G. soricina*. The $\Delta^{18}\text{O}$ values differed significantly between sampling trips for each species.

Comparison	W or t	Z or df	p-value
<i>Carollia perspicillata</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	-0.531	43	0.598
$\delta^{13}\text{C}$	0.3123	43	0.756
$\Delta^2\text{H}$	0.126	43	0.901
$\Delta^{18}\text{O}$	319	3.681	<0.001
<i>Glossophaga soricina</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	-0.069	20	0.946
$\delta^{13}\text{C}$	66	0.683	0.514
$\Delta^2\text{H}$	2.864	20	0.010
$\Delta^{18}\text{O}$	65	3.265	<0.001
Captive <i>Eptesicus fuscus</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	0.410	37	0.684
$\delta^{13}\text{C}$	143.5	-0.922	0.365
$\Delta^2\text{H}$	0.847	37	0.403
$\Delta^{18}\text{O}$	262.5	4.086	<0.001
Wild <i>Eptesicus fuscus</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	190.5	0.041	0.973
$\delta^{13}\text{C}$	147.5	-1.141	0.260
$\Delta^2\text{H}$	-0.508	38	0.615

Table 10: Comparison of the variance of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ values between sampling trips. Comparisons were conducted using a Levene's test. Variance of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\Delta^2\text{H}$ values was not significantly different between sampling trips for any species. Variance of $\Delta^{18}\text{O}$ values differed significantly between sampling trips for captive *E. fuscus*.

Comparison	F-value	df	p-value
<i>Carollia perspicillata</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	0.368	41	0.547
$\delta^{13}\text{C}$	0.0001	41	0.991
$\Delta^2\text{H}$	0.414	41	0.523
$\Delta^{18}\text{O}$	3.756	38	0.060
<i>Glossophaga soricina</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	1.151	18	0.298
$\delta^{13}\text{C}$	0.134	18	0.719
$\Delta^2\text{H}$	1.807	18	0.196
$\Delta^{18}\text{O}$	1.733	16	0.207
Captive <i>Eptesicus fuscus</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	0.625	35	0.435
$\delta^{13}\text{C}$	1.027	35	0.318
$\Delta^2\text{H}$	1.268	35	0.268
$\Delta^{18}\text{O}$	6.36	33	0.017
Wild <i>Eptesicus fuscus</i>			
Trip 1 - Trip 2			
$\delta^{15}\text{N}$	0.241	37	0.627
$\delta^{13}\text{C}$	0.320	37	0.258
$\Delta^2\text{H}$	0.890	36	0.352
$\Delta^{18}\text{O}$	2.346	37	0.134

Table 11: Comparison of the variances of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ between captive and wild populations. Comparisons were conducted using a Levene's test. The variances of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were higher in the wild population than the captive one. The variance of $\Delta^{18}\text{O}$ values was lower in the wild population than the first sampling of the captive one.

Comparison	F-value	df	p-value
$\delta^{15}\text{N}$	10.543	74	0.002
$\delta^{13}\text{C}$	8.655	74	0.004
$\Delta^2\text{H}$	2.696	73	0.105
$\Delta^{18}\text{O}$ Wild – Captive 1	11.522	59	0.001
$\Delta^{18}\text{O}$ Wild – Captive 2	0.012	50	0.912

Table 12: Average forearm length \pm 1 SD (mm) of each of the five species included in my study. The average forearm length differed significantly between each species with the exception of *A. jamaicensis* and *D. rotundus*.

Species	Forearm length \pm SD (mm)
<i>Artibeus jamaicensis</i>	61 \pm 2
<i>Carollia perspicillata</i>	45 \pm 2
<i>Glossophaga soricina</i>	38 \pm 1
<i>Desmodus rotundus</i>	63 \pm 3
<i>Eptesicus fuscus</i>	47 \pm 2

Table 13: Spearman's rank correlation statistics for correlation of forearm length with $\Delta^2\text{H}$ values within the second and third trophic levels.

Trophic level	n	R	p-value
Second	76	0.292	0.011
Third	98	-0.083	0.416

Table 14: Spearman’s rank correlation statistics for correlation of forearm length with $\Delta^2\text{H}$ values within each species.

Species	n	R	p-value
<i>Artibeus jamaicensis</i>	13	0.235	0.440
<i>Carollia perspicillata</i>	43	-0.029	0.855
Male <i>Glossophaga</i>	7	-0.179	0.701
Female <i>G. soricina</i>	13	0.297	0.324
<i>Desmodus rotundus</i>	25	-0.372	0.067
<i>Desmodus rotundus</i> *	22	-0.072	0.750
<i>Eptesicus fuscus</i>	73	-0.095	0.424

*In this correlation, only adult *Desmodus rotundus* were included. Two juveniles and one sub-adult were excluded.

Chapter 4: Discussion

I hypothesized that if there is a trophic effect on $\delta^2\text{H}_{\text{fur}}$ values, then $\delta^2\text{H}_{\text{fur}}$ values would positively correlate with trophic level. I used $\delta^{15}\text{N}_{\text{fur}}$ as a measure of trophic level and $\delta^{13}\text{C}_{\text{fur}}$ as a secondary measure of trophic level. Due to the low statistical power, the overall correlations were not statistically significant. My results suggested a trend of $\delta^2\text{H}_{\text{fur}}$ values and $\delta^{15}\text{N}_{\text{fur}}$ values increasing together. They also suggested that $\delta^2\text{H}_{\text{fur}}$ and $\delta^{13}\text{C}_{\text{fur}}$ values increased together. Because $\delta^{15}\text{N}_{\text{fur}}$ and $\delta^{13}\text{C}_{\text{fur}}$ on their own do not definitively indicate if trophic level is the cause of the change in $\delta^2\text{H}_{\text{fur}}$ values, I compared $\delta^2\text{H}_{\text{fur}}$ to another parameter related to $\delta^2\text{H}_{\text{fur}}$ but unaffected by trophic level. The $\delta^{18}\text{O}_{\text{fur}}$ value was used as this parameter because $\delta^2\text{H}$ and $\delta^{18}\text{O}$ are highly positively correlated in water. Therefore, a change in $\delta^2\text{H}_{\text{fur}}$ without a corresponding change in $\delta^{18}\text{O}_{\text{fur}}$ supports an effect of trophic level. There was no trend of $\delta^2\text{H}_{\text{fur}}$ and $\delta^{18}\text{O}_{\text{fur}}$ values increasing together. While these trends suggest a possible trophic level increase in $\delta^2\text{H}_{\text{fur}}$ values, it is not possible to draw conclusions without a larger sample size. I did find that values of $\delta^2\text{H}_{\text{fur}}$, $\delta^{15}\text{N}_{\text{fur}}$, and $\delta^{13}\text{C}_{\text{fur}}$ increased significantly from the second trophic level to the third trophic level, while $\delta^{18}\text{O}_{\text{fur}}$ values did not increase. These results support the idea of a trophic level effect on $\delta^2\text{H}_{\text{fur}}$ values.

My results are consistent with those of other researchers who found a 3 ‰ difference in $\delta^{15}\text{N}$ values between trophic levels (DeNiro and Epstein, 1981a; Peters et al., 2012). The $\delta^{13}\text{C}$ values clearly distinguish between the presence of C_4 plants in the captive *D. rotundus* diet and the presence of only C_3 plants in the diets of the other species (Kennedy and Krouse, 1990; Siemers et al., 2011). The differences between the $\delta^{13}\text{C}_{\text{fur}}$ values of *A. jamaicensis*, *C. perspicillata* and *G. soricina* likely indicate dietary preferences between species, as I also found variation in the $\delta^{13}\text{C}$ values of the different fruits and vegetables they had available to eat. Otherwise, my results are consistent with the results of other researchers who have found an increase of 0.5 to 3 ‰ in $\delta^{13}\text{C}$ values with trophic level (DeNiro and Epstein, 1978; Reynard and Hedges, 2008). Knowing $\delta^{15}\text{N}_{\text{fur}}$ and $\delta^{13}\text{C}_{\text{fur}}$ consistently demonstrate a distinction between trophic levels, I can use an approach similar to that used by Reynard and Hedges (2008) and Birchall et al.

(2005). This approach involves using $\delta^{13}\text{C}_{\text{fur}}$ and $\delta^{15}\text{N}_{\text{fur}}$ as trophic indicators and comparing them with $\Delta^2\text{H}$, which will determine if $\Delta^2\text{H}$ also increases with trophic level.

It is possible that some bats meet most of their metabolic needs for water with water from food. This is especially true in the case of bats eating nectar, fruit and blood (Carpenter, 1969; McFarland and Wimsatt, 1969; Studier et al., 1983). Therefore, the contribution of diet water versus drinking water may be very different among my study species. Even if this is the case, there should still be a high correlation between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in the fur, as it would reflect the strong positive correlation of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in both the drinking water and diet water.

Within each trophic level the correlations were very different compared to the overall trends. The trends indicated by the comparison of species' means were not significant due to the low sample size, but they suggest that $\delta^2\text{H}_{\text{fur}}$ values are as strongly influenced by trophic level as $\delta^{13}\text{C}_{\text{fur}}$ values. The lack of correlations of $\delta^{15}\text{N}_{\text{fur}}$ and $\delta^{13}\text{C}_{\text{fur}}$ with $\Delta^2\text{H}$ in the absence of a trophic difference further support the idea that trophic level is the cause of the increase in $\Delta^2\text{H}$ values from the second trophic level to the third one. The moderate to strong relationship between $\delta^{18}\text{O}_{\text{fur}}$ and $\delta^2\text{H}_{\text{fur}}$ within each trophic level could explain why several bat migration studies (Cryan et al., 2004; Fraser et al., 2012; Popa-Lisseanu et al., 2012) found a high correlation between $\delta^2\text{H}_{\text{fur}}$ and $\delta^2\text{H}_{\text{precip}}$ values. If $\delta^{18}\text{O}_{\text{fur}}$ and $\delta^2\text{H}_{\text{fur}}$ values are largely determined by drinking water, but trophic level is fractionating $\delta^2\text{H}_{\text{fur}}$ values and not $\delta^{18}\text{O}_{\text{fur}}$ values, then comparing across trophic levels would cause the correlation to become weak or non-existent. However, within one trophic level the positive correlation between $\delta^{18}\text{O}_{\text{fur}}$ and $\delta^2\text{H}_{\text{fur}}$ values should still be present. This is supported by my results. The intra-trophic correlations also suggest that the $\delta^2\text{H}_{\text{fur}}$ values are not sensitive to small changes in trophic level. This may be why Birchall et al. (2005) were not able to distinguish between herbivores and omnivores using $\delta^2\text{H}$. Overall, my results are similar to those of other researchers (Birchall et al., 2005; Reynard and Hedges, 2008) who found a positive correlation between $\delta^2\text{H}$ and $\delta^{15}\text{N}$ and $\delta^2\text{H}$ and $\delta^{13}\text{C}$. Although these correlations were not statistically significant in my study, they suggest that a larger sample size would support the idea that ^2H experiences trophic enrichment.

My results support the idea that diet tissue plays a role in determining $\delta^2\text{H}_{\text{fur}}$ values (Birchall et al., 2005; Podlesak et al., 2008; Solomon et al., 2009). The $\delta^2\text{H}_{\text{fur}}$ values must be influenced by diet for a trophic effect to occur. Otherwise, changes that occur on one trophic level would not be passed on to the next trophic level. If both $\delta^2\text{H}_{\text{fur}}$ and $\delta^{18}\text{O}_{\text{fur}}$ values increase together, then their increase can be attributed to the influence of either drinking or diet water, or a combination of both (Birchall et al., 2005; Solomon et al., 2009). If values of $\delta^2\text{H}_{\text{fur}}$ increase without a corresponding increase in $\delta^{18}\text{O}_{\text{fur}}$ values, the increase can be attributed to the influence of diet tissues (Birchall et al., 2005).

Evidence of the influence of diet tissue on $\delta^2\text{H}_{\text{fur}}$ values can be seen in the comparison of *A. jamaicensis* to *C. perspicillata*. *Artibeus jamaicensis* and *C. perspicillata* are kept in the same enclosure and are fed the same diet. Both species experience the same temperature and humidity conditions. To account for the lower $\Delta^2\text{H}$ values of *A. jamaicensis*, the $\delta^2\text{H}_{\text{fur}}$ values of *A. jamaicensis* must have been influenced independently of their $\delta^{18}\text{O}_{\text{fur}}$ values. As $\delta^{18}\text{O}_{\text{fur}}$ values are influenced mainly by water, they should remain unchanged by any differences in the diet tissues (Bowen et al., 2009; Schimmelmann and DeNiro, 1986; van Hardenbroek et al., 2012). If $\delta^2\text{H}_{\text{fur}}$ values are influenced by each of diet tissue, diet water and drinking water, then it is possible that $\delta^2\text{H}$ values in the diet tissue changed the $\delta^2\text{H}_{\text{fur}}$ values without influencing $\delta^{18}\text{O}_{\text{fur}}$ values. In this case, the diet tissue appears to have lowered the $\delta^2\text{H}_{\text{fur}}$ values. Fig and pear both have particularly low $\delta^2\text{H}$ diet water values compared with the other diet items. Since the $\delta^2\text{H}$ of diet water is often related to the $\delta^2\text{H}$ of diet tissue (Estep and Hoering, 1980; Leaney et al., 1985), this could indicate that the $\delta^2\text{H}$ tissue values are also lower in these fruits compared to the other diet items. It is possible that *A. jamaicensis* bats may be eating a larger proportion of fig and pear than *C. perspicillata*. This would cause *A. jamaicensis* $\Delta^2\text{H}$ values to be lower relative to *C. perspicillata*. Wild *A. jamaicensis* bats have a noticeable preference for eating figs (Ortega and Castro-Arellano, 2001; Studier et al., 1983) so it is likely that they would show a preference for this food in captivity. To add further support, the $\delta^{13}\text{C}$ values of fig and pear are lower than those of the other diet items. Therefore, preferential consumption of fig and pear is compatible with both the lower $\Delta^2\text{H}$ values and the lower $\delta^{13}\text{C}_{\text{fur}}$ values of *A. jamaicensis* compared to *C. perspicillata*. This explanation is also compatible with the mechanism of trophic

enrichment proposed by Birchall et al. (2005), which suggests that the H in diet tissues influences the H in consumer tissues. This differs from the results of Solomon et al., (2009) that suggested the mechanism of trophic enrichment is the compounding effect of diet water contributions.

The comparison of captive and wild *E. fuscus* supports the idea that $\delta^2\text{H}_{\text{fur}}$ values are not sensitive to small changes in trophic level. The main difference between the captive and wild *E. fuscus* is in their diet. Captive *E. fuscus* only have access to herbivorous mealworms. Moosman et al. (2012) and Cryan et al. (2012) found that beetles and moths make up the majority of the diet of wild *E. fuscus*, but in general wild *E. fuscus* are opportunistic feeders (Cryan et al., 2012). Wild *E. fuscus* have access to a variety of prey insects that may be herbivorous, carnivorous, insectivorous or sanguivorous (Cryan et al., 2012). Therefore, wild *E. fuscus* are more likely to occupy a “trophic range.” The higher $\delta^{15}\text{N}_{\text{fur}}$ value of the wild *E. fuscus* compared to the captive *E. fuscus* provides evidence that some of the insects eaten by the wild bats are not herbivorous. That said, the increase in $\delta^{15}\text{N}_{\text{fur}}$ values is small, suggesting that the diet of the wild *E. fuscus* is mainly derived from herbivorous insects with only a small addition of insects from a higher trophic level. The small increase in $\delta^{15}\text{N}_{\text{fur}}$ values is not reflected in the $\Delta^2\text{H}$ values. This could explain why no trophic effect was found in Fraser (2011), because there were no distinct trophic levels in that study.

Evidence of the larger variety of prey available to the wild bats is also found in the greater variance of $\delta^{15}\text{N}_{\text{fur}}$ and $\delta^{13}\text{C}_{\text{fur}}$ values in the wild *E. fuscus* compared to the captive *E. fuscus*. I am unsure why the variance of $\Delta^{18}\text{O}$ values is greater in the first sampling of captive *E. fuscus*. If they are incorporating a wider range of $\delta^{18}\text{O}_{\text{water}}$ values from their food or drinking water, this would create a larger range of both $\delta^2\text{H}_{\text{water}}$ and $\delta^{18}\text{O}_{\text{water}}$ values to be incorporated into their fur, as $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of diet water are highly correlated. I would expect to see a corresponding increase in the variation in $\Delta^2\text{H}$ values if this was the case, but it is possible that the naturally higher variance of $\delta^2\text{H}$ values made the increase difficult to detect. The greater variance of $\Delta^{18}\text{O}$ values in the first sampling of captive *E. fuscus* could be due to the effects of evaporation on their drinking water. Their drinking water is provided in a shallow dish, and evaporation over the course

of a day would cause the $\delta^{18}\text{O}_{\text{water}}$ values to increase. It is possible that more evaporation was occurring in August than in October, causing a wider range of $\delta^{18}\text{O}_{\text{water}}$ values to be incorporated into the bats' fur.

Given that diet water may be a significant influence on $\delta^2\text{H}_{\text{fur}}$ values of bats, and many migration studies focus on insectivorous bats (Cryan et al., 2004; Fraser, 2011; Popa-Lisseanu et al., 2012), there is the potential for insect $\delta^2\text{H}$ values to significantly influence the $\delta^2\text{H}_{\text{fur}}$ values of migrant bats. Bortolotti et al. (2013) determined that variation of insect $\delta^2\text{H}$ values may increase variation in $\delta^2\text{H}$ feather values of tree swallows, but the variation was not enough to alter the predicted range of origin of the tree swallows. Van Hardenbroek et al. (2012) found that $\delta^2\text{H}$ values in beetle chitin reflected the $\delta^2\text{H}$ precipitation values of their collection site, but that $\delta^2\text{H}$ values were 33 ‰ higher in *Hydroporus* beetles than *Helophorus* beetles, indicating trophic level enrichment. Having a better understanding of bats' insect prey items and knowing the variation present in the $\delta^2\text{H}$ values of those prey items could help us understand the sources of variation in $\delta^2\text{H}_{\text{fur}}$ values of resident bats. That knowledge would then allow researchers to make better estimates of the location of origin of migrant bats.

The 15 to 40 ‰ trophic effect I found is consistent with the results of other researchers who also found a trophic effect on $\delta^2\text{H}$ values. Birchall et al. (2005) found a 46 ‰ difference between the bone collagen of carnivores and the combined herbivore/omnivore group. A 40-50 ‰ difference between bone collagen of herbivores and humans was found by Reynard and Hedges (2008), although they noted that the water difference was 25 ‰, so the true difference between trophic levels was 15-25 ‰. Peters et al. (2012) found that tissues of caterpillars and moths were enriched in ^2H by approximately 45 and 23 ‰ compared to their diet, respectively. As these studies included a variety of carnivores, herbivores and omnivores, it is possible that organism size had an influence on the tissue $\delta^2\text{H}$ values (Betini et al., 2009; Soto et al., 2011). These size differences could have contributed to the wide range of values given for the trophic effect in the literature.

If size is positively correlated with $\delta^2\text{H}_{\text{fur}}$ values, it is possible that a size difference between the two trophic levels could confound the correlation between trophic level and $\delta^2\text{H}_{\text{fur}}$ values. Betini et al., (2009) found an increase in $\delta^2\text{H}$ values of songbird nestling feathers with an increase in the size of the nestling. However, they noted that larger nestlings often had higher quality nests made with more feathers. These nests would have higher temperatures, increasing the amount of evaporative water loss experienced by the nestlings. This would result in higher $\delta^2\text{H}$ values in their feathers. Soto et al. (2011) found a positive correlation between $\delta^2\text{H}$ and size in fish, but noted the correlation only existed when the size difference was large. Whitley et al. (2006) found no correlation between size and $\delta^2\text{H}$ values in fish.

I reject that the correlation I found for bats between trophic level and $\Delta^2\text{H}$ values was confounded by size. Both trophic levels contained species that were very similar in size. The suggestion that size and $\delta^2\text{H}$ values are positively correlated (Betini et al., 2009; Soto et al., 2011) was not supported by my results. However, the size differences among species may not have been large enough to cause a noticeable difference in $\delta^2\text{H}$ values (Soto et al., 2011; Whitley et al., 2006).

If $\delta^2\text{H}_{\text{fur}}$ values continue to be used to study migration in bats, knowing the influence of trophic level on $\delta^2\text{H}_{\text{fur}}$ values will be essential for interpreting stable isotope results to estimate the location of origin of migrant bats. My results indicate that an increase in trophic level causes a significant increase in $\delta^2\text{H}_{\text{fur}}$ values. Since most migratory bats are insectivorous (Cryan et al., 2004; Fraser et al., 2012; Popa-Lisseanu et al., 2012), this trophic effect plays a significant role in studies using $\delta^2\text{H}_{\text{fur}}$ values to determine the occurrence and extent of migration of bats in North America (e.g., Cryan et al., 2004; Fraser et al., 2012). It is known that the equations used in migration studies to calculate $\delta^2\text{H}_{\text{precip}}$ values from $\delta^2\text{H}_{\text{fur}}$ values cannot be applied to multiple species (Britzke et al., 2009; Fraser, 2011; but see Popa-Lisseanu et al., 2012). Understanding the influence of trophic level can help researchers refine those equations and make stable hydrogen isotopic analysis a more useful tool for studying bat migration. Presently, if an equation for one species was applied to another species on a higher trophic level, researchers

would obtain origin ranges that are potentially offset by hundreds of kilometres from the actual range of origin (Bowen, 2013; Bowen and Revenaugh, 2003).

Based on the knowledge gained from this study, I make four recommendations for future studies:

- 1) Studies using $\delta^2\text{H}$ values to determine the occurrence and extent of migration should use caution when relating tissue $\delta^2\text{H}$ values to $\delta^2\text{H}_{\text{precip}}$ values. It may be more useful to use $\delta^{18}\text{O}$, even if the scale is not as refined as the $\delta^2\text{H}$ scale, as it reduces the possibility of error caused by a trophic effect. At the very least, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values should be compared to help determine if there are additional factors skewing the $\delta^2\text{H}$ values.
- 2) Migration studies using $\delta^2\text{H}$ should consider making a trophic level correction to their $\delta^2\text{H}$ values. This could be done by measuring the $\delta^{15}\text{N}$ values and using those to determine which trophic level the study organism occupies. Based on my results, the correction value would be difficult to determine without knowledge of the diet of the organism under study. I would suggest using a minimum correction of +15 ‰ per trophic level.
- 3) The $\delta^2\text{H}$ values could be used in conjunction with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values to learn more about the diet of a population or an individual.
- 4) Measurement of $\delta^2\text{H}$ and, if possible, $\delta^{18}\text{O}$ values of diet tissue could be used to determine how closely they are related to the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of diet water. Measurement of $\delta^2\text{H}$ of diet tissue could also help determine if the diet tissue is the source of trophic enrichment for ^2H .

Conclusions

- 1) Values of $\delta^2\text{H}_{\text{fur}}$ are positively correlated with trophic level
- 2) Values of $\delta^2\text{H}_{\text{fur}}$ and $\delta^{18}\text{O}_{\text{fur}}$ are not correlated across trophic levels
- 3) Values of $\delta^2\text{H}_{\text{fur}}$ and $\delta^{18}\text{O}_{\text{fur}}$ maintain their positive correlation within a trophic level
- 4) Values of $\delta^2\text{H}_{\text{fur}}$ are influenced by $\delta^2\text{H}$ values of diet tissues
- 5) Values of $\delta^2\text{H}_{\text{fur}}$ increase by 15 to 40 ‰ per trophic level
- 6) Values of $\delta^2\text{H}$ can be used in conjunction with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values to provide information about the diet of an individual or a population

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Appendix A: Animal Use Protocol Form



2008-003-04::3:

AUP Number: 2008-003-04
AUP Title: Behavioural Ecology of Bats

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2008-003-04 has been approved.

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Thompson, Sharla H
on behalf of the Animal Use Subcommittee

The University of Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, • London, Ontario • CANADA – N6A 5C1
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Appendix B: Stable Isotopic Data for Fur

ID	Date	Age	Sex	Species	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\Delta^2\text{H}^a$	$\Delta^{18}\text{O}^b$
					‰	‰	‰	‰	‰	‰
Montreal										
MB-1	15-May-12	A	M	<i>Glossophaga soricina</i>	-21.6	+6.9	-60		-8	
MB-2	15-May-12	A	M	<i>Glossophaga soricina</i>	-21.9	+7.1	-62	+10.6	-10	+17.9
MB-3	15-May-12	A	M	<i>Glossophaga soricina</i>	-21.8	+7.2	-63	+10.6	-11	+17.9
MB-4	15-May-12	A	M	<i>Glossophaga soricina</i>	-21.2	+6.9	-67	+12.8	-15	+20.1
MB-5	15-May-12	A	M	<i>Glossophaga soricina</i>	-21.7	+7.3	-53	+12.7	-1	+20.0
MB-6	15-May-12	A	M	<i>Glossophaga soricina</i>	-21.2	+6.6	-64	+10.5	-12	+17.8
MB-7	15-May-12	A	M	<i>Glossophaga soricina</i>	-21.0	+7.1	-61		-9	
MB-12	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.4	+6.2	-48	+12.3	+4	+19.6
MB-9	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.1	+6.4	-45	+13.4	+7	+20.7
MB-8	15-May-12	A	M	<i>Artibeus jamaicensis</i>	-22.4	+6.7	-68	+11.6	-16	+18.9
MB-10	15-May-12	A	M	<i>Artibeus jamaicensis</i>	-22.5	+7.1	-60	+14.2	-8	+21.5
MB-11	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.1	+6.6	-54	+12.8	-2	+20.1
MB-14	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.6	+7.3	-52	+14.5	+0	+21.8
MB-15	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.0	-46	+15.1	+6	+22.4
MB-13	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.3	+6.6	-50	+12.6	+2	+19.9
MB-25	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.1	+6.9	-54	+14.3	-2	+21.6
MB-24	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.3	+7.2	-53	+14.8	-1	+22.1
MB-16	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.5	+6.9	-57	+14.7	-5	+22.0
MB-21	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.3	+7.2	-50	+13.3	+2	+20.6
MB-20	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.3	+7.3	-53	+14.8	-1	+22.1
MB-23	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.0	+6.9	-44		+8	
MB-27	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.2	+6.7	-54	+12.5	-2	+19.8
MB-30	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.3	+6.4	-48	+14.8	+4	+22.1
MB-17	15-May-12	A	M	<i>Artibeus jamaicensis</i>	-22.7	+7.3	-70	+14.2	-18	+21.5
MB-29	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.0	+7.4	-58	+12.1	-6	+19.4
MB-28	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.3	+7.2	-53	+12.4	-1	+19.7
MB-22	15-May-12	A	M	<i>Carollia perspicillata</i>	-22.2	+6.3	-48		+4	
Windsor										
WI-2	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-18.3	+10.3	-26	+12.0	+30	+20.5

WI-3	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.4	+10.0	-37	+9.9	+19	+18.4
WI-4	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.0	+11.5	-46	+9.6	+10	+18.1
WI-5	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.3	+9.2	-38	+11.3	+18	+19.8
WI-9	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.7	+9.4	-37	+10.1	+19	+18.6
WI-11	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.7	9.5	-40	+11.2	+16	+19.7
WI-12	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.8	+10.6	-41	+10.4	+15	+18.9
WI-14	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.7	+9.8	-44	+10.8	+12	+19.3
WI-15	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.9	+9.7	-47	+10.4	+9	+18.9
WI-16	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.9	+9.7	-44	+10.7	+12	+19.2
WI-17	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.6	+10.7	-42	+10.8	+14	+19.3
WI-18	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-22.7	+9.0	-33	+10.2	+23	+18.7
WI-20	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.6	+9.1	-43	+9.9	+13	+18.4
WI-25	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-18.4	+9.9	-52	+9.0	+4	+19.1
WI-26	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.9	+9.3	-43	+10.3	+13	+17.5
WI-27	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.5	+11.0	-34	+10.1	+22	+18.8
WI-29	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.5	+9.5	-44	+10.5	+12	+18.6
WI-30	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.0	+10.7	-40	+10.4	+16	+19.0
WI-32	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-18.3	+12.2	-31	+10.2	+25	+18.9
WI-33	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.7	+9.6	-45	+11.0	+11	+18.7
WI-34	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.0	+9.4	-46	+9.8	+10	+19.5
WI-35	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-15.3	+13.9	-41	+9.9	+15	+18.3
WI-36	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.5	+10.0	-46	+9.7	+10	+18.4
WI-37	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.6	+9.7	-46	+13.6	+10	+18.2
WI-38	2-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.5	+10.5	-42	+10.6	+14	+22.1
Hamilton										
B46	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.7	+9.3	-27	+11.4	+22	+18.4
YEL028	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.0	+9.9	-34	+12.4	+15	+19.4
B48	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.9	+10.0	-34	+14.4	+15	+21.4
RED004	14-Aug-12	A	M	<i>Eptesicus fuscus</i>	-21.9	+9.0	-22	+11.4	+27	+18.4
YEL027	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.2	+9.6	-43	+13.7	+6	+20.7
B42	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.7	+9.5	-30		+19	
RED005	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.5	+9.2	-21	+10.8	+28	+17.8

B43	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.7	+9.5	-33	+12.2	+16	+19.2
S50	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.9	+9.8	-39	+11.2	+10	+18.2
YEL045	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.3	+9.5	-33	+12.4	+16	+19.4
Y44	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.8	+9.9	-24	+11.0	+25	+18.0
Y40	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.9	+9.5	-27	+14.3	+22	+21.3
Y43	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-22.0	+9.0	-18	+14.0	+31	+21.0
YEL037	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-22.2	+9.1	-21		+28	
B49	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.7	+9.9	-30	+14.1	+19	+21.1
S46	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.0	+10.1	-20	+14.2	+29	+21.2
YEL006	14-Aug-12	A	M	<i>Eptesicus fuscus</i>	-20.5	+9.8	-30	+13.9	+19	+20.9
YEL029	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.6	+9.5	-30	+12.4	+19	+19.4
YEL011	14-Aug-12	A	M	<i>Eptesicus fuscus</i>	-21.3	+9.2	-31	+12.8	+18	+19.8
B47	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.1	+9.8	-15	+14.1	+34	+21.1
YEL040	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.2	+9.3	-31	+11.9	+18	+18.9
YEL010	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-19.8	+10.0	-36	+12.4	+13	+19.4
YEL038	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-21.1	+10.3	-45	+11.3	+4	+18.3
B45	14-Aug-12	A	F	<i>Eptesicus fuscus</i>	-20.4	+9.6	-38	+13.2	+11	+20.2
Montreal										
MO-1	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.7	+6.4	-52	+12.5	+0	+19.8
MO-2	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.5	+6.3	-54	+12.0	-2	+19.3
MO-3	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.4	+6.3	-52		+0	
MO-4	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.4	+6.4	-51	+11.9	+1	+19.2
MO-5	24-Oct-12	A	M	<i>Artibeus jamaicensis</i>	-22.4	+7.2	-65	+9.9	-13	+17.2
MO-6	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.3	+7.2	-55	+12.2	-3	+19.5
MO-7	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.3	+6.7	-48	+12.2	+4	+19.5
MO-8	24-Oct-12	A	M	<i>Artibeus jamaicensis</i>	-22.6	+6.2	-64	+11.3	-12	+18.6
MO-9	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.0	-45	+12.7	+7	+20.0
MO-10	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.1	+7.4	-48	+12.4	+4	+19.7
MO-11	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.4	+6.7	-51	+11.7	+1	+19.0
MO-12	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.6	-48	+12.6	+4	+19.9
MO-13	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.1	+7.2	-52	+16.6	+0	+23.9
MO-14	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.6	+7.6	-52	+12.3	+0	+19.6

MO-15	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.4	+6.5	-56	+11.9	-4	+19.2
MO-16	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.0	+7.1	-49	+12.4	+3	+19.7
MO-17	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+6.8	-41	+13.1	+11	+20.4
MO-18	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.3	+7.0	-50	+12.3	+2	+19.6
MO-19	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.3	+6.8	-48	+12.2	+4	+19.5
MO-20	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.4	-51	+12.6	+1	+19.9
MO-21	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.1	-52	+11.9	+0	+19.2
MO-22	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.1	-48	+11.7	+4	+19.0
MO-23	24-Oct-12	A	M	<i>Artibeus jamaicensis</i>	-23.0	+6.5	-86	+10.4	-34	+17.7
MO-24	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.1	+7.4	-56	+11.5	-4	+18.8
MO-25	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.1	+6.3	-54	+11.6	-2	+18.9
MO-26	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.3	+6.7	-51	+10.8	+1	+18.1
MO-27	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.3	-50	+12.3	+2	+19.6
MO-28	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.5	+6.7	-56	+11.9	-4	+19.2
MO-29	24-Oct-12	A	M	<i>Artibeus jamaicensis</i>	-22.9	+7.4	-66	+10.8	-14	+18.1
MO-30	24-Oct-12	A	M	<i>Artibeus jamaicensis</i>	-22.8	+7.5	-87	+11.9	-35	+19.2
MO-31	24-Oct-12	A	M	<i>Carollia perspicillata</i>	-22.2	+7.6	-60	+12.7	-8	+20.0
MO-32	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.5	+6.9	-71	+9.2	-19	+16.5
MO-33	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.1	+7.0	-61	+8.7	-9	+16.0
MO-34	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.4	+7.3	-64	+9.1	-12	+16.4
MO-35	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.5	+7.1	-68	+9.7	-16	+17.0
MO-36	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-22.5	+6.9	-75	+9.1	-23	+16.4
MO-37	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.7	+6.5	-92	+8.6	-40	+15.9
MO-38	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-23.0	+6.9	-75	+10.0	-23	+17.3
MO-39	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.6	+6.2	-74	+9.1	-22	+16.4
MO-40	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.2	+7.0	-60	+9.6	-8	+16.9
MO-41	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.5	+6.6	-74	+9.1	-22	+16.4
MO-42	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-22.1	+6.2	-79	+9.1	-27	+16.4
MO-43	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.8	+6.6	-73	+9.1	-21	+16.4
MO-44	24-Oct-12	A	F	<i>Glossophaga soricina</i>	-21.6	+6.2	-82	+7.3	-30	+14.6
Hamilton										
HM-1	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.1	+9.7	-37	+11.1	+12	+18.1

HM-2	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-21.6	+9.4	-37	+11.2	+12	+18.2
HM-3	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.3	+9.9	-34	+10.1	+15	+17.1
HM-4	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.1	+10.2	-18	+10.7	+31	+17.7
HM-5	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.0	+10.0	-33	+10.9	+16	+17.9
HM-6	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-22.0	+9.2	-26	+13.0	+23	+20.0
HM-7	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-19.9	+9.7	-30	+10.9	+19	+17.9
HM-8	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.8	+9.5	-26	+10.8	+23	+17.8
HM-9	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-19.9	+9.6	-34	+11.2	+15	+18.2
HM-10	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-21.5	+9.3	-32	+9.8	+17	+16.8
HM-11	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.5	+9.3	-35	+10.6	+14	+17.6
HM-12	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-19.8	+9.4	-38	+10.9	+11	+17.9
HM-13	1-Nov-12	A	M	<i>Eptesicus fuscus</i>	-20.2	+9.6	-29	+10.4	+20	+17.4
HM-14	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.9	+9.8	-37	+10.1	+23	+19.1
HM-15	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-19.0	+11.0	-45	+10.2	+15	+19.2
HM-16	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.1	+10.6	-43	+9.9	+17	+18.9
HM-17	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-18.8	+11.5	-42	+9.5	+18	+18.5
HM-18	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-18.1	+9.3	-49	+9.5	+11	+18.5
HM-19	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-21.1	+10.6	-41	+10.4	+19	+19.4
HM-20	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-19.3	+9.0	-46	+10.0	+14	+19.0
HM-21	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.7	+10.6	-41	+9.9	+19	+18.9
HM-22	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.8	+9.6	-49	+10.1	+11	+19.1
HM-23	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-22.1	+9.9	-48	+10.5	+12	+19.5
HM-24	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.2	+10.0	-40	+9.9	+20	+18.9
HM-25	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-20.8	+9.7	-45	+10.4	+15	+19.4
HM-26	1-Nov-12	A	F	<i>Eptesicus fuscus</i>	-19.2	+9.2	-40	+9.4	+20	+18.4
Havelock										
A1	30-Jan-13	A	M	<i>Artibeus jamaicensis</i>	-22.7	+6.1	-86	+11.6	-6	+22.5
A2	30-Jan-13	A	M	<i>Artibeus jamaicensis</i>	-22.6	+6.3	-88	+12.4	-8	+23.3
A3	30-Jan-13	A	M	<i>Artibeus jamaicensis</i>	-22.5	+6.5	-72	+12.0	+8	+22.9
A4	30-Jan-13	A	M	<i>Artibeus jamaicensis</i>	-22.7	+5.8	-73	+12.8	+7	+23.7
A5	30-Jan-13	A	M	<i>Artibeus jamaicensis</i>	-22.7	+6.0	-70	+12.3	-3	+23.2
V1	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-15.5	+9.3	-43	+9.7	+24	+20.6

V2	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.2	+9.1	-57	+8.6	+10	+19.5
V3	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.8	+9.3	-56	+9.0	+12	+19.9
V4	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.0	+8.9	-61	+8.1	+6	+19.0
V5	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.5	+8.8	-56	+8.0	+12	+18.9
V6	30-Jan-13	SA	M	<i>Desmodus rotundus</i>	-14.7	+10.7	-26	+10.1	+41	+21.0
V7	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.0	+9.0	-53	+9.0	+14	+19.9
V8	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.5	+9.1	-52	+8.5	+16	+19.4
V9	30-Jan-13	J	M	<i>Desmodus rotundus</i>	-17.7	+11.2	-20	+10.3	+49	+21.2
V10	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.6	+9.3	-51	+8.2	+16	+19.1
V11	30-Jan-13	A	M	<i>Desmodus rotundus</i>	-15.7	+9.5	-48	+8.5	+18	+19.4
V12	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.2	+9.5	-53	+9.6	+15	+20.5
V13	30-Jan-13	J	M	<i>Desmodus rotundus</i>	-17.3	+10.3	-33	+11.0	+34	+21.9
V14	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.2	+9.2	-56	+8.4	+12	+19.3
V15	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.2	+9.1	-51	+9.0	+18	+19.9
V16	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.7	+8.7	-57	+9.2	+10	+20.1
V17	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-21.9	+7.3	-47	+10.4	+20	+21.3
V18	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.4	+9.0	-51	+8.8	+16	+19.7
V19	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.8	+9.1	-53	+8.7	+13	+19.6
V20	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.2	+9.4	-49	+8.2	+19	+19.1
V21	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.4	+9.2	-65	+9.2	+15	+20.1
V22	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.9	+8.9	-66	+9.2	+14	+20.1
V23	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.0	+9.1	-64	+8.4	+16	+19.3
V24	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-16.3	+9.3	-60	+7.6	+20	+18.5
V25	30-Jan-13	A	F	<i>Desmodus rotundus</i>	-17.0	+9.3	-52	+8.6	+17	+19.5
Belize										
BZ-1	Apr-12		M	<i>Desmodus rotundus</i>	-23.7	+7.3	-59	+9.3	+24	+20.2
BZ-2	Apr-12		F	<i>Desmodus rotundus</i>	-24.3	6.9	-56	+9.1	+25	+20.0
BZ-3	Apr-12		M	<i>Desmodus rotundus</i>	-21.8	+6.6	-20	+9.2	+61	+20.1
BZ-4	Apr-12		M	<i>Desmodus rotundus</i>	-23.7	+7.0	-77	+9.1	+17	+20.0
BZ-5	Apr-12		M	<i>Desmodus rotundus</i>	-22.7	+7.5	-42	+10.5	+52	+21.4
BZ-6	Apr-12		M	<i>Desmodus rotundus</i>	-23.8	+8.6	-80	+6.6	+14	+17.5
BZ-7	Apr-12		M	<i>Desmodus rotundus</i>	-23.5	+9.6	-35	+10.5	+59	+21.4

BZ-8	Apr-12	M	<i>Desmodus rotundus</i>	-23.6	+6.3	-72	+9.4	+22	+20.3
<hr/>									
^a $\delta^2\text{H}_{\text{fur}} - \delta^2\text{H}_{\text{water}}$									
^b $\delta^{18}\text{O}_{\text{fur}} - \delta^{18}\text{O}_{\text{water}}$									

Appendix C: Stable Isotopic Data for Diet

Sample ID	Location	Date	$\delta^2\text{H}_{\text{dietwater}}^*$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}_{\text{dietwater}}^*$
			‰	‰	‰	‰
Banana	Montreal, QC	15-May-12	-8	-24.4	4.9	-1.6
Figs	Montreal, QC	15-May-12	-87	-27.0	3.0	-16.3
Gala Apple	Montreal, QC	15-May-12	-102	-25.5	-0.4	-10.5
Marm Mix	Montreal, QC	15-May-12	-54	-25.2	2.0	-7.5
Melon	Montreal, QC	15-May-12	-7	-26.4	3.6	0.8
Papaya	Montreal, QC	15-May-12	2	-25.4	4.3	1.8
Pear	Montreal, QC	15-May-12	-66	-27.5	2.8	-7.5
Sweet Potato	Montreal, QC	15-May-12	-53	-26.7	4.5	-7.5
Nectar	Montreal, QC	Apr-13			2.2	
Banana	Montreal, QC	24-Oct-12	-28	-26.3	3.5	-3.1
Cantaloupe	Montreal, QC	24-Oct-12	-44	-23.8	9.7	-3.8
Fig	Montreal, QC	24-Oct-12	-100	-25.9	1.7	-15.4
Gala Apple	Montreal, QC	24-Oct-12	-34	-24.2	3.0	-3.6
Marm Mix	Montreal, QC	24-Oct-12	-41	-25.4	2.1	-4.1
Papaya	Montreal, QC	24-Oct-12	-28	-27.0	1.0	-4.7
Pear	Montreal, QC	24-Oct-12	-79	-26.8	1.8	-8.0
Sweet Potato	Montreal, QC	24-Oct-12	-38	-26.5	3.1	-5.9
Bran	Hamilton, ON	14-Aug-12	-70	-26.0	3.5	-11.6
Mealworms	Hamilton, ON	14-Aug-12	-15	-25.5	7.1	-2.0
Bran	Hamilton, ON	1-Nov-12	-63	-24.9	2.8	-6.9
Mealworms	Hamilton, ON	1-Nov-12	-16	-26.8	7.8	0.3
Blood	Havelock, ON	30-Jan-12		-11.3	5.0	

*water extracted from the diet sample

Appendix D: Stable Isotopic Data for Drinking Water

Sample ID	Location	Collection Date	$\delta^2\text{H}$ ‰	$\delta^{18}\text{O}$ ‰
Hamilton 1	Hamilton, ON	14-Aug-12	-51	-7.2
Hamilton 2	Hamilton, ON	1-Nov-12	-50	-7.1
Hamilton 3	Hamilton, ON	25-Apr-13	-48	-6.8
Montreal 1	Montreal, QC	15-May-12	-54	-7.5
Montreal 2	Montreal, QC	24-Oct-12	-49	-7.0
Montreal 3	Montreal, QC	30-Mar-13	-53	-7.5
Havelock	Havelock, ON	30-Jan-13	-69	-10.9

Appendix E: Results for Duplicate Isotopic Analyses

ID	Species	$\delta^{13}\text{C}_{\text{fur}} \text{‰}$	Absolute difference
			‰
B48	<i>Eptesicus fuscus</i>	-19.8	
B48dup	<i>Eptesicus fuscus</i>	-20.0	0.20
HM-15	<i>Eptesicus fuscus</i>	-19.1	
HM-15dup	<i>Eptesicus fuscus</i>	-19.0	0.06
HM-2	<i>Eptesicus fuscus</i>	-21.6	
HM-2dup	<i>Eptesicus fuscus</i>	-21.7	0.12
MB-22	<i>Carollia perspicillata</i>	-22.1	
MB-22dup	<i>Carollia perspicillata</i>	-22.3	0.13
MB-27	<i>Carollia perspicillata</i>	-22.1	
MB-27dup	<i>Carollia perspicillata</i>	-22.2	0.05
MO-1	<i>Carollia perspicillata</i>	-22.6	
MO-1dup	<i>Carollia perspicillata</i>	-22.7	0.05
MO-12	<i>Carollia perspicillata</i>	-22.2	
MO-12dup	<i>Carollia perspicillata</i>	-22.2	0.05
MO-17	<i>Carollia perspicillata</i>	-22.3	
MO-17dup	<i>Carollia perspicillata</i>	-22.1	0.16
MO-26	<i>Carollia perspicillata</i>	-22.2	
MO-26dup	<i>Carollia perspicillata</i>	-22.3	0.05
MO-35	<i>Glossophaga soricina</i>	-21.5	
MO-35dup	<i>Glossophaga soricina</i>	-21.5	0.02
WI-20	<i>Eptesicus fuscus</i>	-19.6	
WI-20dup	<i>Eptesicus fuscus</i>	-19.5	0.13
WI-27	<i>Eptesicus fuscus</i>	-19.5	
WI-27dup	<i>Eptesicus fuscus</i>	-19.5	0.01
WI-37	<i>Eptesicus fuscus</i>	-21.6	
WI-37dup	<i>Eptesicus fuscus</i>	-21.6	0.05
WI-9	<i>Eptesicus fuscus</i>	-21.7	

WI-9dup	<i>Eptesicus fuscus</i>	-21.7	0.00
		Average	0.08
		Standard Deviation	0.06

ID	Species	$\delta^{15}\text{N}_{\text{fur}} \text{‰}$	Absolute Difference ‰
B48	<i>Eptesicus fuscus</i>	+10.03	
B48dup	<i>Eptesicus fuscus</i>	+9.99	0.04
HM-15	<i>Eptesicus fuscus</i>	+11.05	
HM-15dup	<i>Eptesicus fuscus</i>	+10.99	0.06
HM-2	<i>Eptesicus fuscus</i>	+9.41	
HM-2dup	<i>Eptesicus fuscus</i>	+9.41	0.00
MB-22	<i>Carollia perspicillata</i>	+6.28	
MB-22dup	<i>Carollia perspicillata</i>	+6.30	0.03
MB-27	<i>Carollia perspicillata</i>	+6.75	
MB-27dup	<i>Carollia perspicillata</i>	+6.72	0.04
MO-1	<i>Carollia perspicillata</i>	+6.41	
MO-1dup	<i>Carollia perspicillata</i>	+6.40	0.02
MO-12	<i>Carollia perspicillata</i>	+7.54	
MO-12dup	<i>Carollia perspicillata</i>	+7.60	0.06
MO-17	<i>Carollia perspicillata</i>	+6.85	
MO-17dup	<i>Carollia perspicillata</i>	+6.84	0.01
MO-26	<i>Carollia perspicillata</i>	+6.65	
MO-26dup	<i>Carollia perspicillata</i>	+6.67	0.02
MO-35	<i>Glossophaga soricina</i>	+7.05	
MO-35dup	<i>Glossophaga soricina</i>	+7.06	0.01
WI-20	<i>Eptesicus fuscus</i>	+9.10	
WI-20dup	<i>Eptesicus fuscus</i>	+9.12	0.02
WI-27	<i>Eptesicus fuscus</i>	+10.92	
WI-27dup	<i>Eptesicus fuscus</i>	+11.00	0.07

WI-37	<i>Eptesicus fuscus</i>	+9.68	
WI-37dup	<i>Eptesicus fuscus</i>	+9.70	0.01
WI-9	<i>Eptesicus fuscus</i>	+9.45	
WI-9dup	<i>Eptesicus fuscus</i>	+9.44	0.02
		Average	0.03
		Standard Deviation	0.02

ID	Species	$\delta^2\text{H}_{\text{fur}}$ ‰	Absolute Difference ‰
MB-8	<i>Artibeus jamaicensis</i>	-66	
MB-8 dup	<i>Artibeus jamaicensis</i>	-70	3
A2	<i>Artibeus jamaicensis</i>	-75	
A2 dup	<i>Artibeus jamaicensis</i>	-78	3
MB-20	<i>Carollia perspicillata</i>	-52	
MB-20 dup	<i>Carollia perspicillata</i>	-54	2
MB-28	<i>Carollia perspicillata</i>	-55	
MB-28 dup	<i>Carollia perspicillata</i>	-51	4
MB-22	<i>Carollia perspicillata</i>	-46	
MB-22 dup	<i>Carollia perspicillata</i>	-50	3
MB-21	<i>Carollia perspicillata</i>	-50	
MB-21 dup	<i>Carollia perspicillata</i>	-52	1
MO-10	<i>Carollia perspicillata</i>	-51	
MO-10 dup	<i>Carollia perspicillata</i>	-46	4
MO-28	<i>Carollia perspicillata</i>	-53	
MO-28 dup	<i>Carollia perspicillata</i>	-58	5
MO-31	<i>Carollia perspicillata</i>	-61	
MO-31 dup	<i>Carollia perspicillata</i>	-58	3
MO-18	<i>Carollia perspicillata</i>	-49	
MO-18 dup	<i>Carollia perspicillata</i>	-50	0
MO-26	<i>Carollia perspicillata</i>	-50	

MO-26 dup	<i>Carollia perspicillata</i>	-51	1
BZ-4	<i>Desmodus rotundus</i>	-67	
BZ-4 dup	<i>Desmodus rotundus</i>	-66	1
BZ-1	<i>Desmodus rotundus</i>	-59	
BZ-1 dup	<i>Desmodus rotundus</i>	-59	0
V17	<i>Desmodus rotundus</i>	-49	
V17 dup	<i>Desmodus rotundus</i>	-49	0
V16	<i>Desmodus rotundus</i>	-56	
V16 dup	<i>Desmodus rotundus</i>	-63	7
V13	<i>Desmodus rotundus</i>	-34	
V13 dup	<i>Desmodus rotundus</i>	-37	3
B48	<i>Eptesicus fuscus</i>	-34	
B48 dup	<i>Eptesicus fuscus</i>	-34	0
WI-29	<i>Eptesicus fuscus</i>	-45	
WI-29 dup	<i>Eptesicus fuscus</i>	-43	2
WI-17	<i>Eptesicus fuscus</i>	-43	
WI-17 dup	<i>Eptesicus fuscus</i>	-42	1
WI-26	<i>Eptesicus fuscus</i>	-43	
WI-26 dup	<i>Eptesicus fuscus</i>	-43	0
YEL028	<i>Eptesicus fuscus</i>	-33	
YEL028 dup	<i>Eptesicus fuscus</i>	-35	2
HM-4	<i>Eptesicus fuscus</i>	-18	
HM-4 dup	<i>Eptesicus fuscus</i>	-19	0
HM-10	<i>Eptesicus fuscus</i>	-32	
HM-10 dup	<i>Eptesicus fuscus</i>	-31	1
HM-8	<i>Eptesicus fuscus</i>	-25	
HM-8 dup	<i>Eptesicus fuscus</i>	-27	2
HM-17	<i>Eptesicus fuscus</i>	-42	
HM-17 dup	<i>Eptesicus fuscus</i>	-42	0
HM-19	<i>Eptesicus fuscus</i>	-42	
HM-19 dup	<i>Eptesicus fuscus</i>	-40	2

WI-27	<i>Eptesicus fuscus</i>	-34	
WI-27 dup	<i>Eptesicus fuscus</i>	-34	0
WI-32	<i>Eptesicus fuscus</i>	-29	
WI-32 dup	<i>Eptesicus fuscus</i>	-33	4
MO-44	<i>Glossophaga soricina</i>	-82	
MO-44 dup	<i>Glossophaga soricina</i>	-83	1
MO-41	<i>Glossophaga soricina</i>	-74	
MO-41 dup	<i>Glossophaga soricina</i>	-74	1
		Average	2
		Standard Deviation	2

ID	Species	$\delta^{18}\text{O}_{\text{fur}} \text{‰}$	Absolute Difference ‰
A1	<i>Artibeus jamaicensis</i>	+11.8	
A1 dup	<i>Artibeus jamaicensis</i>	+11.5	0.2
A5	<i>Artibeus jamaicensis</i>	+12.3	
A5 dup	<i>Artibeus jamaicensis</i>	+12.3	0.0
MO-23	<i>Artibeus jamaicensis</i>	+10.5	
MO-23 dup	<i>Artibeus jamaicensis</i>	+10.3	0.2
MO-5	<i>Artibeus jamaicensis</i>	+9.8	
MO-5 dup	<i>Artibeus jamaicensis</i>	+9.9	0.1
MB-20	<i>Carollia perspicillata</i>	+14.7	
MB-20 dup	<i>Carollia perspicillata</i>	+14.9	0.2
MB-21	<i>Carollia perspicillata</i>	+13.2	
MB-21 dup	<i>Carollia perspicillata</i>	+13.3	0.0
MB-30	<i>Carollia perspicillata</i>	+15.2	
MB-30 dup	<i>Carollia perspicillata</i>	+14.4	0.8
MO-19	<i>Carollia perspicillata</i>	+12.4	
MO-19 dup	<i>Carollia perspicillata</i>	+12.0	0.4
MO-2	<i>Carollia perspicillata</i>	+12.2	

MO-2 dup	<i>Carollia perspicillata</i>	+11.7	0.5
MO-20	<i>Carollia perspicillata</i>	+13.0	
MO-20 dup	<i>Carollia perspicillata</i>	+12.1	0.9
MO-24	<i>Carollia perspicillata</i>	+12.2	
MO-24 dup	<i>Carollia perspicillata</i>	+10.7	1.5
MO-25	<i>Carollia perspicillata</i>	+11.4	
MO-25 dup	<i>Carollia perspicillata</i>	+11.7	0.3
MO-4	<i>Carollia perspicillata</i>	+11.7	
MO-4 dup	<i>Carollia perspicillata</i>	+12.0	0.2
BZ-1	<i>Desmodus rotundus</i>	+9.2	
BZ-1 dup	<i>Desmodus rotundus</i>	+9.3	0.1
BZ-2	<i>Desmodus rotundus</i>	+9.1	
BZ-2 dup	<i>Desmodus rotundus</i>	+9.0	0.1
BZ-8	<i>Desmodus rotundus</i>	+9.2	
BZ-8 dup	<i>Desmodus rotundus</i>	+9.6	0.4
V6	<i>Desmodus rotundus</i>	+10.1	
V6 dup	<i>Desmodus rotundus</i>	+10.2	0.1
B45	<i>Eptesicus fuscus</i>	+14.6	
B45 dup	<i>Eptesicus fuscus</i>	+14.4	0.2
B49	<i>Eptesicus fuscus</i>	+14.0	
B49 dup	<i>Eptesicus fuscus</i>	+14.1	0.0
HM-14	<i>Eptesicus fuscus</i>	+9.9	
HM-14 dup	<i>Eptesicus fuscus</i>	+10.2	0.3
HM-21	<i>Eptesicus fuscus</i>	+9.8	
HM-21 dup	<i>Eptesicus fuscus</i>	+9.8	0.0
HM-23	<i>Eptesicus fuscus</i>	+10.7	
HM-23 dup	<i>Eptesicus fuscus</i>	+10.3	0.4
HM-3	<i>Eptesicus fuscus</i>	+10.1	
HM-3 dup	<i>Eptesicus fuscus</i>	+10.1	0.0
WI-26	<i>Eptesicus fuscus</i>	+10.3	
WI-26 dup	<i>Eptesicus fuscus</i>	+10.4	0.0

WI-3	<i>Eptesicus fuscus</i>	+10.0	
WI-3 dup	<i>Eptesicus fuscus</i>	+9.9	0.1
WI-32	<i>Eptesicus fuscus</i>	+10.3	
WI-32 dup	<i>Eptesicus fuscus</i>	+10.0	0.3
WI-9	<i>Eptesicus fuscus</i>	+10.1	
WI-9 dup	<i>Eptesicus fuscus</i>	+10.1	0.0
YEL006	<i>Eptesicus fuscus</i>	+13.9	
YEL006 dup	<i>Eptesicus fuscus</i>	+13.9	0.0
YEL029	<i>Eptesicus fuscus</i>	+12.2	
YEL029 dup	<i>Eptesicus fuscus</i>	+12.5	0.4
Average			0.3
Standard Deviation			0.3

Diet Type	Location and Sampling	$\delta^2\text{H}_{\text{dietwater}}$ * ‰	Absolute Difference ‰
Gala Apple	Montreal, QC Trip 1	-102	0
Gala Apple dup	Montreal, QC Trip 1	-102	
Canteloupe	Montreal, QC Trip 2	-44	0
Canteloupe dup	Montreal, QC Trip 2	-44	
Gala Apple	Montreal, QC Trip 2	-34	1
Gala Apple dup	Montreal, QC Trip 2	-35	
Average			1
Standard Deviation			1

* $\delta^2\text{H}$ value of water extracted from the diet sample

Diet Type	Location and Sampling	$\delta^{13}\text{C}$ ‰	Absolute Difference ‰
Melon	Montreal, QC Trip 1	-26.3	0.2
Melon DUP	Montreal, QC Trip 1	-26.5	
Bran	Hamilton, ON Trip 1	-26.3	0.6
Bran dup	Hamilton, ON Trip 1	-25.8	
		Average	0.4
		Standard Deviation	0.3

Diet Type	Location and Sampling	$\delta^{15}\text{N}$ ‰	Absolute Difference ‰
Canteloupe	Montreal, QC Trip 2	+9.7	0.1
Canteloupe dup	Montreal, QC Trip 2	+9.6	
Bran	Hamilton, ON Trip 2	+2.3	0.5
Bran dup	Hamilton, ON Trip 2	+2.8	
		Average	0.3
		Standard Deviation	0.3

Diet Type	Location and Sampling	$\delta^{18}\text{O}_{\text{dietwater}}$ * ‰	Absolute Difference ‰
Gala Apple	Montreal, QC Trip 1	-10.5	0.06
Gala Apple dup	Montreal, QC Trip 1	-10.5	
Canteloupe	Montreal, QC Trip 2	-3.9	0.06
Canteloupe dup	Montreal, QC Trip 2	-3.8	
Gala Apple	Montreal, QC Trip 2	-3.6	0.09
Gala Apple dup	Montreal, QC Trip 2	-3.6	
		Average	0.07

	Standard Deviation	0.02
* $\delta^{18}\text{O}$ value of water extracted from the diet sample		

$\delta^2\text{H}$ drinking water		Absolute
Sample ID	‰	Difference ‰
Hamilton 1	-51	0.1
Hamilton 1 dup	-51	
Montreal 1	-54	0.2
Montreal 1 dup	-54	
Montreal 1a	-54	0.2
Montreal 1a dup	-54	
Montreal 3	-53	0.3
Montreal 3 dup	-53	
Average		0.2
Standard Deviation		0.1

$\delta^{18}\text{O}$ drinking water		Absolute
Sample ID	‰	Difference ‰
Hamilton 1	-7.1	0.08
Hamilton 1 dup	-7.2	
Montreal 1	-7.4	0.03
Montreal 1 dup	-7.5	
Montreal 1a	-7.5	0.02
Montreal 1a dup	-7.6	
Montreal 3	-7.5	0.06
Montreal 3 dup	-7.5	
Average		0.05
Standard Deviation		0.03

Appendix F: Local Meteoric Water Lines and Sample Calculations

Hamilton, ON: $\delta^2\text{H} = 7.1 * \delta^{18}\text{O} + 3.8$

Montreal, QC: $\delta^2\text{H} = 7.4 * \delta^{18}\text{O} + 7.3$

Havelock, ON: $\delta^2\text{H} = 7.3 * \delta^{18}\text{O} + 5.7$

Windsor, ON: $\delta^2\text{H} = 7.2 * \delta^{18}\text{O} + 4.8$

Lamanai, Belize: $\delta^2\text{H} = 8.8 * \delta^{18}\text{O} + 11.3$

Sample calculation of the Hamilton LMWL:

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
$\delta^{18}\text{O}$	-15.1	-14.7	-10.5	-7.6	-6.3	-5.8	-5.4	-5.7	-7.4	-9.4	-10.2	-13.3
$\delta^2\text{H}$	-108	-99	-67	-52	-43	-38	-35	-36	-49	-62	-66	-91

(www.waterisotopes.org; Bowen, 2013; Bowen et al., 2005)

Equation of the line:

$$\delta^2\text{H} = 7.1 * \delta^{18}\text{O} + 3.8$$

Average $\Delta^{18}\text{O}$ value for captive *E. fuscus*: +19.0 ‰

Average value of $\delta^{18}\text{O}_{\text{fur}}$ for the first sampling of wild *E. fuscus*: +10.5 ‰

$$\delta^{18}\text{O}_{\text{water}} = \delta^{18}\text{O}_{\text{fur}} - \Delta^{18}\text{O} = +10.5 - (+19.0) = -8.5 \text{ ‰}$$

The value of -8.5‰ is the $\delta^{18}\text{O}_{\text{water}}$ value that was used for the first sampling of wild *E. fuscus*.

Sample calculation of $\delta^2\text{H}_{\text{water}}$ for the first sampling of wild *E. fuscus*:

The LMWL for Windsor, ON is

$$\delta^2\text{H} = 7.2 * \delta^{18}\text{O} + 4.8$$

Substituting the value of -8.5 for $\delta^{18}\text{O}$ gives:

$$\delta^2\text{H} = 7.2 * (-9.0) + 4.8 = -56\text{‰}; \text{ this is the } \delta^2\text{H}_{\text{water}} \text{ value.}$$

The $\Delta^2\text{H}$ value for any bat fur sample from the first sampling of wild *E. fuscus* can now be obtained using the equation:

$$\Delta^2\text{H} = \delta^2\text{H}_{\text{fur}} - \delta^2\text{H}_{\text{water}}$$

For example, sample WI-11 has a $\delta^2\text{H}_{\text{fur}}$ of -40‰ :

$$\Delta^2\text{H} = (-40) - (-56) = +24\text{‰}; \text{ this is the } \Delta^2\text{H} \text{ value for sample WI-11.}$$

Appendix G: Calculated Water Isotopic Compositions for Wild Populations

Date	Species	Location	Water $\delta^2\text{H}$ ‰	Water $\delta^{18}\text{O}$ ‰
02-Aug-12	<i>Eptesicus fuscus</i>	Windsor, ON	-60	-9.0
01-Nov-12	<i>Eptesicus fuscus</i>	Windsor, ON	-64	-9.5
Apr-12	<i>Desmodus rotundus</i>	Lamanai, Belize	-79	-10.3

Appendix H: P-values for Pairwise Mann-Whitney U Tests for Figures 8-11

P-values of pairwise Mann-Whitney U test for $\delta^{15}\text{N}$ (Figure 8).

	<i>Artibeus jamaicensis</i>	<i>Carollia perspicillata</i>	<i>Desmodus rotundus</i>	<i>Eptesicus fuscus</i>	Wild <i>Eptesicus fuscus</i>
<i>Artibeus jamaicensis</i>	NA	NA	NA	NA	NA
<i>Carollia perspicillata</i>	1.000	NA	NA	NA	NA
<i>Desmodus rotundus</i>	<0.001	<0.001	NA	NA	NA
<i>Eptesicus fuscus</i>	<0.001	<0.001	0.005	NA	NA
Wild <i>Eptesicus fuscus</i>	<0.001	<0.001	<0.001	0.200	NA
<i>Glossophaga soricina</i>	1.000	1.000	<0.001	<0.001	<0.001

P-values of pairwise Mann-Whitney U test for $\delta^{13}\text{C}$ (Figure 9).

	<i>Artibeus jamaicensis</i>	<i>Carollia perspicillata</i>	<i>Desmodus rotundus</i>	<i>Eptesicus fuscus</i>	Wild <i>Eptesicus fuscus</i>
<i>Artibeus jamaicensis</i>	NA	NA	NA	NA	NA
<i>Carollia perspicillata</i>	<0.001	NA	NA	NA	NA
<i>Desmodus rotundus</i>	<0.001	<0.001	NA	NA	NA
<i>Eptesicus fuscus</i>	<0.001	<0.001	<0.001	NA	NA
Wild <i>Eptesicus fuscus</i>	<0.001	<0.001	<0.001	1.000	NA
<i>Glossophaga soricina</i>	<0.001	<0.001	<0.001	0.004	0.010

P-values of pairwise Mann-Whitney U test for $\Delta^2\text{H}$ (Figure 10).

	<i>Artibeus jamaicensis</i>	<i>Carollia perspicillata</i>	<i>Desmodus rotundus</i>	<i>Eptesicus fuscus</i>	<i>Female Glossophaga soricina</i>	<i>Male Glossophaga soricina</i>
<i>Carollia perspicillata</i>	<0.001	NA	NA	NA	NA	NA
<i>Desmodus rotundus</i>	<0.001	<0.001	NA	NA	NA	NA
<i>Eptesicus fuscus</i>	<0.001	<0.001	1.000	NA	NA	NA
<i>Female Glossophaga soricina</i>	1.000	<0.001	<0.001	<0.001	NA	NA
<i>Male Glossophaga soricina</i>	0.671	0.003	0.001	<0.001	0.164	NA
<i>Wild Eptesicus fuscus</i>	<0.001	<0.001	1.000	0.577	<0.001	<0.001

P-values of pairwise Mann-Whitney U test for $\Delta^{18}\text{O}$ (Figure 11).

	<i>Artibeus jamaicensis</i>	<i>Carollia perspicillata</i> 1	<i>Carollia perspicillata</i> 2	<i>Desmodus rotundus</i>	<i>Eptesicus fuscus</i> 1	<i>Eptesicus fuscus</i> 2	<i>Female Glossophaga soricina</i>
<i>C. perspicillata</i> 1	1.000	NA	NA	NA	NA	NA	NA
<i>C. perspicillata</i> 2	1.000	0.007	NA	NA	NA	NA	NA
<i>D. rotundus</i>	1.000	0.106	1.000	NA	NA	NA	NA
<i>E. fuscus</i> 1	1.000	0.155	1.000	1.000	NA	NA	NA
<i>E. fuscus</i> 2	0.081	<0.001	<0.001	<0.001	0.001	NA	NA
<i>Female G. soricina</i>	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	NA
<i>Male G. soricina</i>	1.000	0.572	1.000	1.000	1.000	1.000	0.037

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Erdman, Leslie. Effect of trophic level on stable hydrogen isotopic composition of bat fur. Bat-Moth Research Conference, University of Havana, Havana, Cuba, August 2012.