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Isotopic discrimination and indications for turnover in hair and wing membranes of the temperate bat *Nyctalus noctula*

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Abstract Stable isotope ratios, especially of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), are often used to make predictions of an animal's diet. Next to the isotope ratios of the studied animal and its diet, two factors are important for the interpretation of stable isotope data: the discrimination factor and the turnover rate. Both parameters are species- and tissue-specific but sparsely reported, especially for insectivorous bats. We determined the diet-tissue discrimination factors ($\Delta^{13}C_{tissue}$ and $\Delta^{15}N_{tissue}$) for the insectivorous common noctule bat (*Nyctalus noctula*) in hairs and wing membranes. No sex-related differences in discrimination of ^{13}C and ^{15}N could be detected, but wing membranes were significantly less enriched in ^{13}C (4.0±0.6‰) than hairs (5.9±1.3‰). However, tissues were not significantly different in $\Delta^{15}N_{tissue}$ ($\Delta^{15}N_{wing}$ 3.7±0.6‰ and $\Delta^{15}N_{hair}$ 3.4±0.6‰). Furthermore, we compared $\delta^{15}N$ and $\delta^{13}C$ of wing membranes and hairs from individuals feeding on a mealworm diet for 7 weeks (♀♀$_{short}$ and ♂♂$_{short}$) and for an average of 124 weeks (range 27–298; ♀♀$_{long}$ and ♂♂$_{long}$). As for ♀♀$_{short}$ and ♂♂$_{short}$, no molting occurred after the dietary switch; we assumed that hairs still reflect the isotopic signature of their natural diet. The metabolically more active wing membranes, however, should have incorporated, at least partly, the isotopic signature of the mealworms. Values of $\delta^{15}N$ and $\delta^{13}C$ indicate that a dietary switch after 7 weeks is reflected in wing membranes but not in hair. This provides further evidence that the turnover rate of wing membranes of insectivorous bats is only a few weeks.

Keywords Carbon · Nitrogen · SEA$_c$ · Hair · Wing membrane

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Introduction

Stable isotope analysis is a useful tool to study the ecology of species (Peterson and Fry 1987) and has been used to study species' migration (Hobson and Wassenaar 2008), diet (Kelly 2000), social behavior (Voigt et al. 2011), and foraging behavior (Sullivan et al. 2006). Stable nitrogen (^{14}N and ^{15}N; $\delta^{15}N$) and carbon (^{12}C and ^{13}C; $\delta^{13}C$) isotope ratios provide meaningful information about dietary aspects (Kelly 2000). For both isotopes, the heavy isotope (^{15}N and ^{13}C) accumulates in a characteristic way within a food web providing information on the food source (DeNiro and Epstein 1976, 1981). Nitrogen isotope ratios differ between consumers on different trophic positions while carbon isotope ratios are suitable to differentiate between feeding grounds, e.g., C_3 vs. C_4 plants (Kelly 2000). Carbon and nitrogen isotopes are related to different parameters of an animals' diet, and therefore, by combining both isotopes, different aspects of the diet can be described. The combination of both isotopes can be used to estimate the isotopic niche of an individual or a population. The isotopic niche is closely correlated to the trophic niche and provides information on the isotopic space that is occupied (Jackson et al. 2011). Therefore, individuals feeding on different diets should occupy different isotopic niches which should become more similar if individuals start feeding on the same diet. For a reliable assessment of a species' diet, two species- and tissue-specific factors must be considered: the discrimination factor and the turnover rate.

The stable isotope ratios in tissues of individuals reflect an enriched signal of all isotopic sources that were consumed by this individual during the time span that is reflected by the respective tissue. This diet to tissue accumulation is described by the discrimination factor ($\Delta^{15}N_{tissue}$ and $\Delta^{13}C_{tissue}$). To quantify this enrichment or discrimination, individuals should have fed on a diet with known stable isotope ratios over a long period (at least longer than the tissue-specific time span). Carbon and nitrogen discrimination factors are known to vary according to an organisms' taxonomy, its habitat, the nitrogen content of the diet, its nitrogen metabolism, its nutrition, the analyzed tissue, and the individual fitness (Caut et al. 2009; Gannes et al. 1998; Mirón et al. 2006; Vanderklift and Ponsard 2003; Voigt and Matt 2004). Discrimination factors are essential in diet reconstruction studies and the validity of mixing models might be highly influenced by the accuracy of the applied discrimination factors (Caut et al. 2009). Nonetheless, discrimination factors are only rarely determined experimentally (Martínez del Rio et al. 2009).

The turnover rate reflects the retrospective time frame over which isotopes of a specific tissue are integrated (Tieszen et al. 1983). Tissue-specific turnover rates are related to the metabolic rate of the respective tissue (Tieszen et al. 1983). Concerning isotopic turnover, two different types of tissues can be distinguished: metabolic active tissues (e.g., blood or wing membrane) and metabolic inert tissues (e.g., hair or feathers). Isotope ratios of metabolic active tissues are incorporated continuously and reflect the isotopic source of a time span. Isotope ratios of metabolic inert tissues are incorporated only at the time of synthesis and reflect the isotopic source at the time point of synthesis (Hobson 1999). As hairs are metabolically inert, turnover depends on the time of synthesis. The turnover rate of a tissue can be measured by changing the isotopic source (i.e., the diet) of an animal and afterwards taking tissue samples in definite time intervals (e.g., Hobson and Clark 1992; Tieszen et al. 1983). The turnover rate of this tissue can then be statistically determined using one-compartment or multi-compartment models (Cerling et al. 2007; Martínez del Rio and Anderson-Sprecher 2008).

Stable nitrogen and carbon isotope ratios are often used to assess the diet of insectivorous bats using wing membrane and/or hair samples (Painter et al. 2009; Roswag et al. 2014; Siemers et al. 2011). However, discrimination factors and turnover rates are only rarely reported for these species. For insectivorous bats, only Siemers et al. (2011) described discrimination factors for carbon and nitrogen isotopes in hair samples of *Myotis myotis* and *Myotis nattereri*. To correctly interpret isotopic signatures in hair molting behavior has to be considered. The molting behavior of bats varies among species and with sex and age classes (for review, see Fraser et al. 2013). Nonetheless, for temperate bat species, molting mainly occurs once a year during summer/fall (Cryan et al. 2004; Fraser et al. 2013). It seems that in male bats, molting occurs earlier in the year compared to reproducing females. Turnover rates of wing membranes ($\delta^{15}N$ and $\delta^{13}C$), hair ($\delta^{13}C$), and blood ($\delta^{15}N$ and $\delta^{13}C$) are known only for nectarivorous bat species (Mirón et al. 2006; Voigt and Matt 2004; Voigt et al. 2003). Based on these turnover rates, values for the metabolically active wing membranes of insectivorous species are extrapolated to "a few weeks" (Roswag et al. 2014; Siemers et al. 2011). However, a reliable and definite value is essential especially for dietary studies of migratory species. In the temperate zone, bats appear in the summer site during spring (March/April; Encarnação et al. 2010; Weid 2002). Studies that assess the summer diet of bats using stable isotope analysis started approximately 1.5–4 months after the bats' arrival in the summer site (Roswag et al. 2014; Siemers et al. 2011; Sullivan et al. 2006). We asked if 7 weeks are enough time for the winter diets' stable isotope signal to change. Too long turnover rates would lead to misinterpretations as the $\delta^{15}N$ and $\delta^{13}C$ signal might reflect the diet on migration or at winter sites rather than the diet at summer sites. The aim of this study was to contribute knowledge about nitrogen and carbon discrimination factors for wing membranes ($\Delta^{15}N_{wing}$ and $\Delta^{13}C_{wing}$) and hairs ($\Delta^{15}N_{hair}$ and $\Delta^{13}C_{hair}$) and to provide further evidence for the short turnover rates of wing membranes.

As it is known that the discrimination factor depends on the analyzed tissue (Caut et al. 2009), we hypothesize that both hair and membrane tissues should have tissue-specific $\Delta^{15}N$ and $\Delta^{13}C$. As the isotopic signal of hairs can only change during the molting period, we hypothesize that the isotopic signature of hair samples differs between individuals with or without a molting event after a dietary switch. Based on the fact that wing membranes are metabolically active, we hypothesize that 7 weeks after a dietary switch, the isotopic signature of wing membranes should be similar regardless of the time span individuals fed on mealworms.

Methods

Study species

As study species, we chose the common noctule (*Nyctalus noctula*), a long-distance migrating species (Hutterer et al. 2005; Voigt et al. 2012; Weid 2002). The molting behavior of this species is described as one single-molting period per year between mid-July and mid-August (Blohm and Heise 2008; Fraser et al. 2013; Spitzenberger 2007). Besides its regular activity period (summer, migration), this species is known to interrupt its hibernation period to forage even at temperatures below 0 °C (Avery 1986; Kaňuch et al. 2005). However, they have different foraging grounds and diet compositions during winter and summer (Kaňuch et al. 2005; Vaughan 1997) probably leading to distinct $\delta^{15}N$ and $\delta^{13}C$ in their

tissues. Therefore, it is essential to be aware of the turnover rates to correctly interpret stable isotope results. All bats were sampled at a bat caretaking facility in Hanover (Germany). We took hair from the upper tips of the dorsal fur and wing membrane tissue from the plagiopatagium using a biopsy punch (diameter 3.5 mm) of 33 *N. noctula*. Of those, 13 individuals (7 females, afterwards "♀♀$_{long}$"; 6 males, afterwards "♂♂$_{long}$") were held in captivity for an average of 124 (27–298) weeks and their diet consisted exclusively of mealworms (*Tenebrio molitor*). This allowed on average for three (one–six) molting events (Blohm and Heise 2008; Fraser et al. 2013; Spitzenberger 2007). Therefore, the isotopic signature in hairs should have changed. The isotopic turnover of wing membranes is assumed to be "a few weeks" (Roswag et al. 2014; Siemers et al. 2011; Voigt et al. 2003). Thus, a minimum of 27 weeks should be enough to change the isotopic signature in wing membranes. Based on these facts, even though these individuals originated from different habitats, the isotopic signature of their hair and wing membrane tissues can be considered as reflecting the exclusive mealworm diet. The other individuals (11 females, afterwards "♀♀$_{short}$"; 9 males, afterwards "♂♂$_{short}$") arrived at the facility in January after their hibernacula (tree cavity) was destroyed by felling. Since then, they stayed active and were on a mealworm diet for 7 weeks, without a molting event. No information about the catchment area of their hibernacula or the individual summering grounds is available. Thus, no assumption of their prior natural diet is possible. Samples from ♀♀$_{long}$ and ♂♂$_{long}$ were taken once per individual between March and August when they met our sampling criteria. We took samples from all ♀♀$_{short}$ and ♂♂$_{short}$ once on 15th March after the 7-week period. To ensure the release of completely healthy individuals, repeated invasive sampling (e.g., wing membrane) was not supported. As a diet sample, we took 15 mealworms of two different deliveries. Wing membranes were transferred into 96 % ethanol, and all samples were stored at −20 °C. Research followed the guidelines of the American Society of Mammalogists. The study was ethically and methodically approved by the relevant Nature Conservation and the Animal Care Authorities (permit numbers: V54—19 c 20—15(1) GI 15/8 Nr. 03/2010, V/53.2—R 22-3(2) and 42508_F/60233_2).

Stable isotope analysis

Mealworm samples were milled to a fine powder prior to analysis. Whole wing membrane and hair samples were washed three times in a 1:1 chloroform/methanol solution. All samples were dried to constant mass. On average, 0.15 mg of wing membrane, 0.67 mg hair, and 0.49 mg mealworm were weighed into tin capsules for stable nitrogen and carbon analysis. All samples were pyrolized at 1020 °C and analyzed using elemental analyzer isotope ratio mass spectrometry (EA-IRMS). The configuration consisted of an autosampler AS 300 II, an elemental analyzer Flash-EA 2000, a technical interface ConFlo IV, and the mass spectrometer Delta V Advantage (all devices: Thermo Fisher Scientific GmbH, Dreieich, Germany). Wing membranes and hairs were analyzed once and mealworms in triplicate. We used acetanilide and L-phenylalanine that were calibrated to air-N_2 and PDB as laboratory standards every 20 samples or between different sample types. We calculated $\delta^{13}C$ and $\delta^{15}N$ based on Eq. 1. Precision of measurements was 0.09‰ for $\delta^{13}C$ and 0.12‰ for $\delta^{15}N$. One hair sample produced an unexpected high $\delta^{13}C$ value (−6.78‰) which was probably due to the low mass of this sample (0.30 mg). We classified this sample as biased and excluded it from further analyses. $\Delta^{15}N$ and $\Delta^{13}C$ were calculated using ♀♀$_{long}$ and ♂♂$_{long}$ based on Eq. 2.

$$\delta X = \left(R_{sample}/R_{reference} - 1\right) \times 1000 \quad (1)$$

where R_{sample} and $R_{reference}$ represent the ratio between the heavy and the light isotope of the element X of the sample (wing membrane, hair, or mealworm) and the international standard, respectively.

$$\Delta X_{tissue} = \delta X_{tissue} - \delta X_{mealworm} \quad (2)$$

where X represents ^{13}C or ^{15}N; δX_{tissue} and $\delta X_{mealworm}$ represents measured stable nitrogen or carbon ratios of wing membrane ($\delta^{15}N_w$ and $\delta^{13}C_w$), hair ($\delta^{15}N_h$ and $\delta^{13}C_h$), or mealworms ($\delta^{15}N_m$ and $\delta^{13}C_m$), respectively.

Data analysis

The Shapiro-Wilk test was used to check for normal distribution, and data were tested for significant differences either by a *t* test, Mann-Whitney *U* test, or for multiple groups by one-way ANOVA followed by a Fisher least significant difference (Fisher LSD) test (Statistica 12; StatSoft Inc). All values are given in mean±SD.

For each tissue and group, we calculated the standard ellipse area corrected for small sample size (SEA_c) using the Stable Isotope Analysis in R (SIAR) package (Parnell and Jackson 2011) with the included SIBER metrics (Jackson et al. 2011). Standard ellipse areas are a measure for the isotopic niche, i.e., the used isotopic sources. The SEA_c is based on $\delta^{13}C$ and $\delta^{15}N$ and represents 40 % of all data regardless of the sample size. Based on this, we calculated the overlap of SEA_c between all groups to compare if the isotopic signal of tissues converged after the 7-week period.

Results

We checked the discrimination factors $\Delta^{15}N_{wing}$, $\Delta^{15}N_{hair}$, $\Delta^{13}C_{wing}$, and $\Delta^{13}C_{hair}$ for differences between sexes. There

were no significant differences in the discrimination of ^{15}N and ^{13}C in wing membrane or hair samples between ♀♀$_{long}$ and ♂♂$_{long}$ ($\Delta^{15}N_{wing}$: U=11.0, p>0.05; $\Delta^{15}N_{hair}$: U=15.0, p>0.05; $\Delta^{13}C_{wing}$: t=−1.3, p>0.05; $\Delta^{13}C_{hair}$: t=0.9, p>0.05). Therefore, ♀♀$_{long}$ and ♂♂$_{long}$ were pooled for the tissue comparison of the discrimination factors. The δ^{13}C and δ^{15}N of the mealworms averaged −28.0±0.9‰ for carbon and 5.4±0.3‰ for nitrogen and was generally lower than the tissues of *N. noctula* (Fig. 1). Discrimination of ^{15}N for the long-time individuals did not differ significantly between $\Delta^{15}N_{wing}$ (3.7±0.6‰) and $\Delta^{15}N_{hair}$ (3.4±0.6‰; U=48.0, p>0.05). Discrimination of ^{13}C for the long-time individuals was significantly lower for $\Delta^{13}C_{wing}$ (4.0±0.6‰) than for $\Delta^{13}C_{hair}$ (5.9±1.3‰; t=−4.69, d.f.=24, p<0.001).

Regarding isotopic changes in wing tissues, we did not find any significant differences in the $\delta^{13}C_w$ and $\delta^{15}N_w$ values of ♀♀$_{long}$, ♂♂$_{long}$, ♀♀$_{short}$, and ♂♂$_{short}$ ($\delta^{13}C_w$: $F_{3,29}$=2.51, p>0.05; $\delta^{15}N_w$: $F_{3,29}$=2.73, p>0.05, Table 1). For hair, however, both $\delta^{13}C_h$ and $\delta^{15}N_h$ of ♀♀$_{long}$, ♂♂$_{long}$, ♀♀$_{short}$, and ♂♂$_{short}$ exhibited significant differences ($\delta^{13}C_h$: $F_{3,28}$=10.77, p<0.001; $\delta^{15}N_h$: $F_{3,28}$=7.20, p<0.001, Table 1). In detail, for $\delta^{13}C_h$ ♀♀$_{long}$ had significantly lower values than ♀♀$_{short}$ (Fisher LSD, p<0.001) and ♂♂$_{short}$ (Fisher LSD, p<0.01) and ♂♂$_{long}$ were significantly depleted in ^{13}C compared to ♀♀$_{short}$ (Fisher LSD, p<0.001) and ♂♂$_{short}$ (Fisher LSD, p<0.05). No further differences could be detected for $\delta^{13}C_h$ (Fisher LSD, p>0.05). Values of $\delta^{15}N_h$ were significantly higher in ♀♀$_{short}$ than in ♂♂$_{short}$ (Fisher LSD, p<0.001) and ♀♀$_{long}$ (Fisher LSD, p<0.05). The other sampling groups did not differ in their $\delta^{15}N_h$ (Fisher LSD, p>0.05).

For wing membranes, the SEA$_c$ of ♀♀$_{long}$, ♂♂$_{long}$, ♀♀$_{short}$, and ♂♂$_{short}$ covered a small area and showed comparable overlap to each other (Fig. 2a). For hairs, the SEA$_c$ of ♀♀$_{short}$ and ♂♂$_{short}$ covered a greater area and less or even no overlap neither to each other nor to ♀♀$_{long}$ nor ♂♂$_{long}$. In contrast, the SEA$_c$ for hairs of ♀♀$_{long}$ overlapped almost completely (98 %) with that of ♂♂$_{long}$ (Fig. 2b, Table 1).

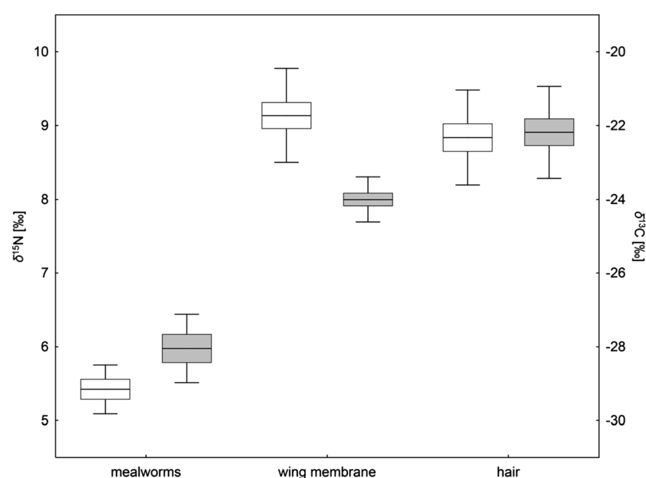

Fig. 1 Measured δ^{15}N (*white boxes*) and δ^{13}C (*gray boxes*) in per mille of mealworms (*Tenebrio molitor*), hair, and wing membranes (only ♀♀$_{long}$ and ♂♂$_{long}$) for the calculation of $\Delta^{15}N_{tissue}$ and $\Delta^{13}C_{tissue}$. *Boxes* represent mean±SE and *whiskers* the standard deviation

Discussion

In this study, we determined the discrimination factors for two tissues of *N. noctula*. As hypothesized Δ^{13}C differed significantly between wing membrane and hair samples, but this could not be observed for Δ^{15}N. Furthermore, we assessed if a period of 7 weeks is sufficient to study the summer diet for migrating bat species by stable isotope analysis. We did not directly measure the turnover rate of wing membranes or hair samples of our study species. Therefore, our results do not provide detailed information about the time span that is reflected by the two tissues. Nonetheless, we found further evidence that the turnover rate of wing membranes is only a few weeks since after 7 weeks $\delta^{15}N_w$ and $\delta^{13}C_w$ of *N. noctula* showed no significant differences between a short-time and long-time mealworm diet. Hair $\delta^{15}N_h$ and $\delta^{13}C_h$, however, differed significantly between the two groups. This confirms that the hair signal of individuals on a short-time mealworm diet, based on the lacking molting event, still reflect their natural diet which was isotopic different to the mealworm diet reflected by the hairs of the individuals on a long-term mealworm diet that had molting event(s).

Discrimination factors in hairs of *M. myotis* and *M. nattereri* were comparable to our results although, $\Delta^{13}C_{hair}$ of *N. noctula* was higher compared to the *Myotis* species (Table 2; Siemers et al. 2011). Furthermore, carbon and nitrogen discrimination factors for wing membrane and blood of nectarivorous bats (*Glossophaga soricina* and *Leptonycteris curasoae*) are described in the literature (Mirón et al. 2006; Voigt and Matt 2004; Voigt et al. 2003). Discrimination of ^{15}N seems to be similar in nectarivorous and insectivorous bat species while ^{13}C enrichment seems to be higher in insectivorous compared to nectarivorous bats (Table 2). Caut et al. (2009) reviewed Δ^{13}C and Δ^{15}N values described in the literature and examined some factors potentially influencing discrimination factors. They could show that taxonomic classes differ in their Δ^{13}C. For mammals, the mean Δ^{13}C and Δ^{15}N were 0.5 and 3.0‰, respectively. The Δ^{15}N of bats are comparable to that of other mammals while Δ^{13}C is higher than the mammalian average. Discrimination factors are highly variable and several factors influencing ^{13}C enrichment are described in the literature (for a comprehensive review, see Caut et al. 2009). Based on the current data, it is difficult to examine what might cause such high Δ^{13}C in bats. Therefore, this should be addressed in further studies. As expected, the Δ^{13}C for *N. noctula* was tissue-specific. However, this could not be observed for Δ^{15}N. Voigt and Matt (2004) studied discrimination of ^{15}N in blood and wing membrane

Table 1 δ^{15}N±SD [‰], δ^{13}C±SD [‰] and SEA$_c$ [‰^{2}] of wing membranes and hairs of the four groups and the overlap of SEA$_c$ [%] between the groups

Group	δ^{15}N [‰]		δ^{13}C [‰]		SEA$_c$ [‰^{2}]		Overlap [%]			
	Wing	Hair	Wing	Hair	Wing	Hair	♀♀$_{short}$	♂♂$_{short}$	♀♀$_{long}$	♂♂$_{long}$
♀♀$_{short}$	**9.4±1.1**	*9.7±0.8*	**−24.6±0.6**	*−24.9±1.4*	**1.70**	*3.83*		*34/29*	*47/64*	*20/21*
♂♂$_{short}$	**8.3±0.9**	*8.1±0.8*	**−24.3±0.7**	*−24.0±0.7*	**2.01**	*1.79*	**5/5**		*41/66*	*19/23*
♀♀$_{long}$	**9.0±0.6**	*8.7±0.4*	**−24.2±0.5**	*−21.9±0.8*	**1.25**	*1.33*	**0/0**	**0/0**		*56/43*
♂♂$_{long}$	**9.3±0.6**	*9.0±0.8*	**−23.8±0.7**	*−22.5±1.6*	**1.63**	*3.71*	**15/33**	**0/0**	**35/98**	

The first number indicates the overlap from the group of corresponding row to the group of the corresponding column and the second number indicates the overlap from the group of corresponding column to the group of the corresponding row. Bold values correspond to wing membranes, italic values to hairs

samples of the nectarivorous bats *L. curasoae* and *G. soricina*. They found significant differences for Δ^{15}N for *L. curasoae* but not for *G. soricina*. Therefore, tissue specific discrimination might be a species-specific pattern.

The comparison of stable isotope ratios and their resulting SEA$_c$ in hair and wing membranes of ♀♀$_{long}$, ♂♂$_{long}$, ♀♀$_{short}$, and ♂♂$_{short}$ might provide further information about the isotopic turnover. For hairs, $\delta^{13}C_h$ differed significantly between ♀♀$_{long}$, ♂♂$_{long}$, ♀♀$_{short}$, and ♂♂$_{short}$, especially individuals that fed for a long time on mealworms differed from those feeding for a short time on this diet. Similar to this, $\delta^{15}N_h$ differed significantly between sampling groups, i.e., between ♀♀$_{short}$, ♂♂$_{short}$, and ♀♀$_{long}$ but not between ♀♀$_{long}$ and ♂♂$_{long}$. We observed a high overlap in SEA$_c$ for hair between ♀♀$_{long}$ and ♂♂$_{long}$ but not between ♀♀$_{short}$ and ♂♂$_{short}$ or in any other combination, respectively. This

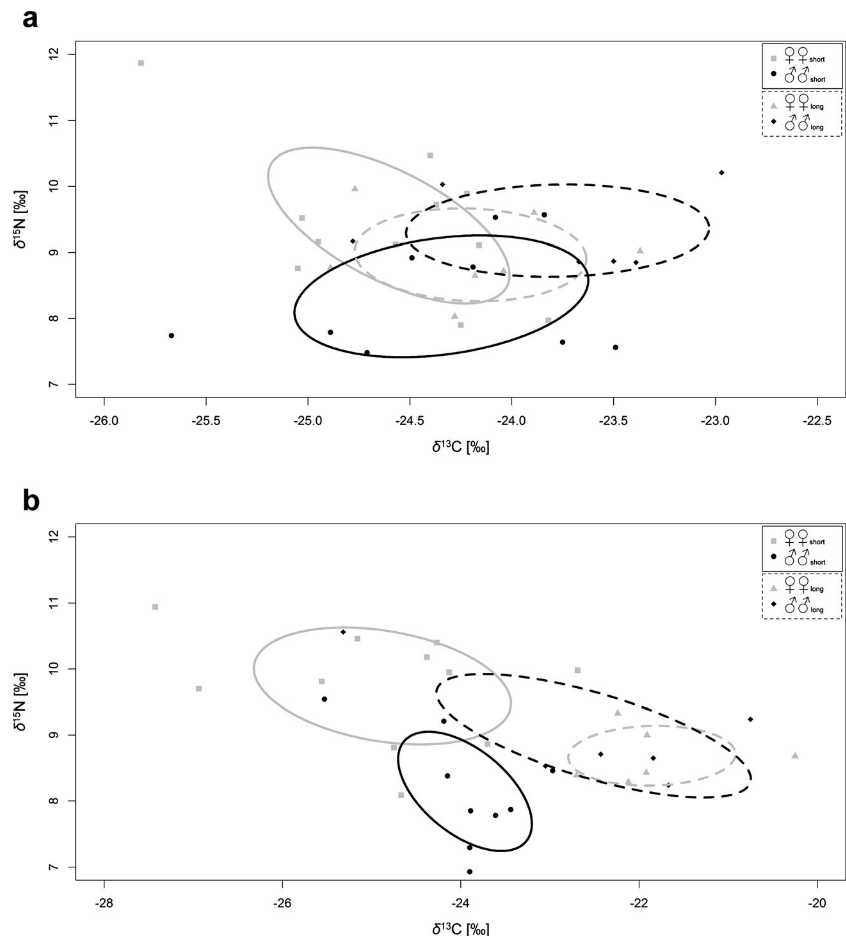

Fig. 2 SEA$_c$ in ‰^{2} of **a** wing membrane and **b** hair samples of ♀♀$_{short}$ (*squares*, *gray line*), ♂♂$_{short}$ (*circles*, *black line*), ♀♀$_{long}$ (*triangles*, *gray-dotted line*), and ♂♂$_{long}$ (*diamonds*, *black-dotted line*)

Table 2 Nitrogen and carbon discrimination factors measured in different tissues of insectivorous and nectarivorous bat species obtained from the literature and from the present study

Species	Diet	Tissue	Δ^{15}N [‰]	Δ^{13}C [‰]	Reference
Nyctalus noctula	Insectivorous	Wing	3.7	4.0	Present study
Nyctalus noctula	Insectivorous	Hair	3.4	5.9	Present study
Myotis myotis	Insectivorous	Hair	2.6	3.6	Siemers et al. 2011
Myotis nattereri	Insectivorous	Hair	3.2	3.2	Siemers et al. 2011
Glossophaga soricina	Nectarivorous	Blood	4.4	2.0	Mirón et al. 2006
Glossophaga soricina	Nectarivorous	Blood	3.3	0.1	Mirón et al. 2006
Leptonycteris curasoae	Nectarivorous	Hair/wing/blood		2.8	Voigt et al. 2003
Glossophaga soricina	Nectarivorous	Hair/wing/blood		2.6	Voigt et al. 2003
Leptonycteris curasoae	Nectarivorous	Blood	3.0		Voigt and Matt 2004
Glossophaga soricina	Nectarivorous	Blood	3.2		Voigt and Matt 2004
Leptonycteris curasoae	Nectarivorous	Wing	4.7		Voigt and Matt 2004
Glossophaga soricina	Nectarivorous	Wing	4.0		Voigt and Matt 2004

confirms that the same diet is reflected by similar $\delta^{15}N_h$ and $\delta^{13}C_h$ of ♀♀$_{long}$ and ♂♂$_{long}$ as well as similar isotopic niches. These niches differed, however, from the ones of ♀♀$_{short}$ and ♂♂$_{short}$. Based on the fact that for ♀♀$_{short}$ and ♂♂$_{short}$, no molting occurred after the dietary switch, these hair samples reflect the natural diet that was isotopically distinct from the mealworm diet. Additionally, the low overlap in the SEA$_c$ between ♀♀$_{short}$ and ♂♂$_{short}$ shows that their natural diet comprised different isotopic sources. The signal in the hair might represent a short and relatively constant time point of a species' diet as bats molt only once per year (Cryan et al. 2004; Fraser et al. 2013). The differences between ♀♀$_{short}$ and ♂♂$_{short}$ could be due to different molting periods because females are known to molt later the year than males (Fraser et al. 2013). For wing tissues, there were no significant differences for either $\delta^{15}N_w$ or $\delta^{13}C_w$ between ♀♀$_{long}$, ♂♂$_{long}$, ♀♀$_{short}$, and ♂♂$_{short}$. The SEA$_c$ was comparably high for all groups (♀♀$_{long}$, ♂♂$_{long}$, ♀♀$_{short}$, and ♂♂$_{short}$), indicating that they occupy similar-sized isotopic niches. The combination of the results of δ^{15}N and δ^{13}C of hair and wing membrane samples suggests a distinct isotopic signature of the natural and mealworm diet. This distinctness was not reflected in the wing membrane samples. It seems that a period of 7 weeks might be suitable to reflect a dietary switch in wing membranes of insectivorous bats. As we did not collect a timeline of the changing isotopic signals of these tissues, it is possible that the turnover rate of wing membranes is much faster but not longer than 7 weeks. The knowledge of the turnover rate of different tissues of insectivorous bats will highly improve the interpretation of stable isotope data, but more studies of different tissues and species are needed. This study added the carbon and nitrogen discrimination factors for two tissues to the literature and could confirm that wing membranes are suitable to study the diet of insectivorous bats in early summer.

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