

Diet–tissue discrimination factors of carbon and nitrogen stable isotopes in blood of Snowy Owl (*Bubo scandiacus*)

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Abstract: Analysis of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotope ratios (hereafter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) in animal tissues is a powerful tool in food-web studies. However, isotopic ratios of prey are not transmitted directly to a consumer, as a diet–tissue discrimination factor (denoted Δ) occurs between sources and consumer’s tissues. An accurate assessment of the diet of a consumer with stable isotopes thus requires that the $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ of the studied species are known. Our aim was to establish $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values in the Snowy Owl (*Bubo scandiacus* (L., 1758)). Moreover, we assessed the potential effect of ethanol preservation of blood samples on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. We kept four captive adult Snowy Owls on a pure diet of mice for ≥ 6 weeks. We then collected mouse muscle and blood samples from the owls and analyzed their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values (mean \pm SE) for owl blood were $+0.3\text{‰} \pm 0.2\text{‰}$ and $+1.9\text{‰} \pm 0.1\text{‰}$, respectively. These values are the first discrimination factors ever reported in Strigiformes and are lower, for $\Delta^{15}\text{N}$, than those obtained in terrestrial carnivores and other bird species, including falcons. Preservation in ethanol did not significantly affect $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Résumé : L’analyse des ratios isotopiques de carbone ($^{13}\text{C}/^{12}\text{C}$) et d’azote ($^{15}\text{N}/^{14}\text{N}$) (symbolisés par $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ respectivement) dans les tissus animaux est un outil puissant pour l’étude des réseaux trophiques. Cependant, les ratios isotopiques des proies ne sont pas transmis directement au consommateur et un facteur de discrimination (noté Δ) est observé entre les ratios des sources et ceux du consommateur. La détermination exacte du régime alimentaire d’un consommateur à l’aide des isotopes stables demande donc une connaissance des $\Delta^{13}\text{C}$ et $\Delta^{15}\text{N}$ chez l’espèce à l’étude. Notre objectif était de déterminer les valeurs de $\Delta^{13}\text{C}$ et $\Delta^{15}\text{N}$ chez le harfang des neiges (*Bubo scandiacus* (L., 1758)). De plus, nous voulions déterminer l’impact potentiel de la conservation d’échantillons sanguins dans une solution d’éthanol 70 % sur les valeurs de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$. Nous avons gardé quatre harfangs des neiges en captivité, nourris exclusivement de souris pendant ≥ 6 semaines. Nous avons ensuite récolté des échantillons sanguins des oiseaux et des muscles de souris et nous avons déterminé leur signature de $\delta^{13}\text{C}$ et de $\delta^{15}\text{N}$. Les valeurs de $\Delta^{13}\text{C}$ et $\Delta^{15}\text{N}$ (moyenne \pm ES) du sang chez le harfang des neiges sont de $+0,3 \text{‰} \pm 0,2 \text{‰}$ et $+1,9 \text{‰} \pm 0,1 \text{‰}$, respectivement. Ces valeurs de discrimination sont les premières rapportées à ce jour chez les strigiformes et elles sont plus faibles, pour l’azote, que celles observées chez les carnivores terrestres et d’autres espèces d’oiseaux, incluant les faucons. La conservation des échantillons sanguins dans une solution d’éthanol 70 % n’as pas affecté significativement les valeurs de $\delta^{13}\text{C}$ et de $\delta^{15}\text{N}$.

Introduction

Naturally occurring stable isotopes of carbon (^{13}C , ^{12}C) and nitrogen (^{15}N , ^{14}N) are present in every living organism and they occur under specific ratios (denoted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) depending on the trophic position and diet of an animal. Based on the assumption that “you are what you eat”, analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in animal tissues allows the determination of the relative contributions of isotopically distinct sources to the diet of consumers (Hobson and Wassenaar 1999; Kelly 2000; Wolf et al. 2009). In many situations, this technique provides time-integrated dietary information

and allows measuring the contribution of assimilated food to tissue protein, thereby avoiding several biases commonly found with conventional techniques such as stomach content analyses or the dissection of regurgitated pellets or scats (Hobson and Wassenaar 1999).

However, isotopic ratios of sources are not transmitted directly to a consumer, as a diet–tissue discrimination factor occurs between sources and consumer’s tissues for both carbon and nitrogen isotopes (Tieszen et al. 1983; Hobson and Clark 1992a). Those diet–tissue discrimination values (also called fractionation and referred to as Δ , i.e., the change in isotope ratio between diet and consumer’s tissues) are

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influenced by several factors such as trophic position (Vanderklift and Ponsard 2003), tissue type and composition, or metabolic activity (Bearhop et al. 2002). Those values may thus be unique for each species and tissue (DeNiro and Epstein 1977; Tieszen et al. 1983; Hobson and Clark 1992a). An accurate assessment of the diet of a given consumer with stable isotopes thus requires that the $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ of the studied species are known, otherwise substantial errors and misinterpretation of results are possible (McCutchan et al. 2003; Caut et al. 2008a; Wolf et al. 2009).

In wild animals, blood is commonly used to assess diet with stable isotopes (e.g., Hobson and Clark 1993; Inger et al. 2006). Because blood isotope ratios integrate assimilated diet isotope ratios over a few weeks, this tissue offers a higher temporal resolution of an animal's diet than the analysis of feather or claw isotope ratios, while being less invasive than biopsies. However, preservation of blood samples can be problematic at remote field situations where freezers are not readily available and 70% ethanol is commonly used to preserve samples in such situations (Hobson et al. 1997; Chérel et al. 2005). Ethanol has been recommended for preservation of blood samples because it did not affect stable isotope ratios (Hobson et al. 1997), but a recent experiment revealed that this may not be the case in all situations (Bugoni et al. 2008).

Our first aim was to establish $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values in an avian predator, the Snowy Owl (*Bubo scandiacus* (L., 1758)). To our knowledge, no published values of discrimination factors exist in Strigiformes. As Snowy Owl is a key top predator of the tundra food web (Gauthier et al. 2004), such estimates are needed to untangle the trophic dynamic of this ecosystem with stable isotopes. Our second aim was to assess the potential effect of blood samples stored in 70% ethanol on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, a method well suited for studies taking place in remote field sites such as in the Arctic.

Materials and methods

Experimental procedures

We used four captive adult Snowy Owls held in a raptor rehabilitation centre affiliated with the Veterinary College of Université de Montréal in Saint-Hyacinthe, Quebec, Canada. These birds were either found injured in the wild and taken to the centre for treatment (1 male and 1 female of at least 18 months old) or born at the rehabilitation centre from captive parents (2 males of 7 and 11 years old). Sample size was limited by the number of owls available at the rehabilitation centre. Individuals had been in captivity for at least 2 months prior to the start of our experiment. As wild Snowy Owls are known to prey mainly on small rodents (Parmelee 1992), all individuals kept at the rehabilitation centre were fed with a diet of mice. Blood isotopic ratios of carbon and nitrogen are known to stabilize within 30 days after a diet switch in birds (Hobson and Clark 1992b; Bearhop et al. 2002; Evans Ogden et al. 2004). To ensure such stabilization in blood of the Snowy Owls, we kept all birds on the same pure diet of mice for at least 6 weeks before any sampling occurred. Mice came from a single source (Charles River Canada, Saint-Constant, Quebec, Canada) and were reared on standard commercial feed (Purina 50–75). All the mice used in this experiment came from the same batch and were kept fro-

zen at the rehabilitation centre until offered to the birds. Birds ate 4–6 mice every day and ingested individual mice whole. Throughout the experiment, all birds kept a constant body mass (mean = 1.5 kg, range = 1.4–1.7). Those values were close to the mean mass of wild Snowy Owls (Parmelee 1992). We randomly picked six mice in the frozen batch used throughout the experiment and sampled their leg muscles, as muscles represent the bulk of the digestible and thus assimilable portion of an ingested mouse. At the end of the experiment, we sampled 1 mL of blood from the ulnar vein of Snowy Owls, just before they received their daily food ration. All animal manipulations were in accordance with the Animal Care Committee of the Faculté de médecine vétérinaire of Université de Montréal (CÉUA project #10-Rech-1572).

We separated blood samples in duplicates, which were then either frozen at $-20\text{ }^{\circ}\text{C}$ within 2 h or preserved in 70% ethanol. Five weeks following their collection, all samples were freeze-dried for 48 h and ground to a fine powder. Because $\delta^{13}\text{C}$ values typically differ between lipid and nonlipid tissues (Tieszen et al. 1983), it is often recommended to chemically remove lipids from tissues with a high concentration in lipids (like muscles) before submitting samples for isotopic analyses. Failure to do so can potentially introduce significant biases in the determination of the diet (Post et al. 2007; Logan and Lutcavage 2008; Tarroux et al. 2010). However, chemical lipid extraction may also affect the nonlipid fraction of tissues and thus bias the $\delta^{15}\text{N}$ values as well (Kojadinovic et al. 2008; Logan et al. 2008; Logan and Lutcavage 2008). It is therefore suggested to analyze $\delta^{13}\text{C}$ of samples after lipid extraction and $\delta^{15}\text{N}$ with original samples (non-extracted) to avoid any biases. We thus separated each mouse muscle sample in two and extracted lipids from one of the duplicates by boiling them for 20 min in a chloroform:methanol (2:1) solution with a Soxhlet apparatus following Bligh and Dyer (1959). Lipid content was measured as the mass difference of a sample before and after lipid extraction. Because lipid content of whole blood is usually very low and hence does not affect carbon and nitrogen isotopic ratios (Bearhop et al. 2000), we did not extract lipids from whole blood samples.

Stable isotopes analysis

We encapsulated approximately 0.220 mg of all samples into tin capsules and sent them to the Stable Isotopes in Nature Laboratory (SINLab), University of New Brunswick, Fredericton, New Brunswick, Canada, for analyses. Samples were flash combusted at $1100\text{ }^{\circ}\text{C}$ and the resulting gases delivered via continuous flow for analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a DELTA^{plus} isotope ratio mass spectrometer. Stable isotope ratios are measured as parts per thousand (‰) differences relative to international standards:

$$\delta X = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000$$

where X is ^{13}C or ^{15}N , and R is the corresponding ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. PeeDee Belemnite carbonate (Craig 1957) and atmospheric nitrogen (Mariotti 1983) were used as standards arbitrarily set at 0‰ for ^{13}C and ^{15}N , respectively. A greater δ value indicates enrichment of the heavier isotope component, whereas a smaller δ value indicates depletion of the heavier isotope. Measurements of commercially available reference material (acetanilide) were both

Table 1. Whole blood values of carbon and nitrogen stable isotopic ratios (denoted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and diet–tissue discrimination factors (denoted $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) of Snowy Owls (*Bubo scandiacus*) fed with mice at the Saint-Hyacinthe Rehabilitation Centre, Quebec, Canada.

ID No.	Sex	Preserved frozen				Preserved in 70% ethanol			
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$
1914	Male	-19.1	9.7	0.5	1.9	-19.4	9.8	0.3	2.0
3427	Male	-19.2	9.7	0.4	1.9	-19.2	9.9	0.4	2.1
5454	Male	-19.9	9.4	-0.3	1.6	-19.6	9.3	0.0	1.5
5692	Female	-19.2	9.9	0.4	2.1	-19.3	9.9	0.3	2.1

Note: Values from samples preserved either frozen or in 70% ethanol are displayed.

accurate and precise with values (mean \pm SD) of $-27.8\text{‰} \pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $-2.0\text{‰} \pm 0.1\text{‰}$ for $\delta^{15}\text{N}$ ($n = 7$). Maximum variation value in sample repeats ($n = 2$) were 0.2‰ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{15}\text{N}$.

Statistical analysis

We assessed the effect of lipid extraction and preservation technique using paired t tests. We evaluated if diet–tissue discrimination values were significantly different from 0 using Student's t tests. We performed all analysis with SAS release 9.2 (SAS Institute Inc. 2008). Value are reported as means \pm SE unless mentioned otherwise.

Results

Mouse muscle samples contained $4.4\text{‰} \pm 1.1\%$ lipids. The $\delta^{13}\text{C}$ values of lipid-free mouse muscles were $0.6\text{‰} \pm 0.2\text{‰}$ higher than for whole tissues (paired t test, $t = -3.52$, $n = 6$, $p = 0.02$). The $\delta^{15}\text{N}$ value did not differ following lipid extraction (mean difference: $0.0\text{‰} \pm 0.1\text{‰}$; paired t test, $t = 0.21$, $n = 6$, $p = 0.85$). We therefore used $\delta^{13}\text{C}$ values determined on lipid-free muscles and $\delta^{15}\text{N}$ from whole tissues to assess diet–tissue discrimination factors. Mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of frozen samples were, respectively, $-19.3\text{‰} \pm 0.2\text{‰}$ and $9.7\text{‰} \pm 0.1\text{‰}$ for whole blood from Snowy Owls ($n = 4$) and showed little variation among individuals (Table 1). For mouse muscles, $\delta^{13}\text{C} = -19.6\text{‰} \pm 0.1\text{‰}$ (range = -19.9‰ to -19.3‰) (lipid-free) and $\delta^{15}\text{N} = 7.8\text{‰} \pm 0.1\text{‰}$ (range = 7.6‰ to 8.0‰ ; $n = 6$). Diet–tissue discrimination thus occurs in blood of Snowy Owl, though not significantly so for carbon (Student's t test: $t_{[8]} = 1.53$, $p = 0.1$ for $\delta^{13}\text{C}$; $t_{[8]} = 16.2$, $p < 0.001$ for $\delta^{15}\text{N}$). Discrimination factors were $+0.3\text{‰} \pm 0.2\text{‰}$ for carbon and $+1.9\text{‰} \pm 0.1\text{‰}$ for nitrogen.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of blood of Snowy Owls preserved in 70% ethanol were not significantly different from those of frozen samples (mean difference: $\delta^{13}\text{C} = -0.1\text{‰} \pm 0.1\text{‰}$; paired t test, $t = -0.73$, $n = 4$, $p = 0.50$; $\delta^{15}\text{N} = +0.1\text{‰} \pm 0.1\text{‰}$, $t = 2.07$, $n = 4$, $p = 0.10$). Although our small sample size limits the power of our analysis, the mean differences of -0.1‰ and $+0.1\text{‰}$ are very small and represent less than one-fifth and one-third of the mean variation observed among individuals for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Moreover, results were consistent among our four samples (Table 1).

Discussion

Our study provides the first diet–tissue discrimination factors ever reported in Strigiformes. Differences in discrimination values between carbon and nitrogen mainly come from the differ-

ent metabolic pathways of those atoms (DeNiro and Epstein 1977; Minagawa and Wada 1984). In most organisms, $\delta^{13}\text{C}$ usually increases by 0.4‰ – 1.0‰ and $\delta^{15}\text{N}$ increases by about 2.5‰ – 3.5‰ as we go up one trophic level (DeNiro and Epstein 1978; Minagawa and Wada 1984; reviewed by Post 2002; Caut et al. 2009). Our discrimination values are therefore close to the mean $\Delta^{13}\text{C}$ values reported from a wide variety of terrestrial carnivores (reviewed by Post 2002) but appear lower for $\Delta^{15}\text{N}$. Compared with the mean discrimination values reported by Post (2002), those of Snowy Owl differ by 0.0‰ for $\Delta^{13}\text{C}$ but by -1.5‰ for $\Delta^{15}\text{N}$. Our diet–tissue discrimination values are also similar for $\Delta^{13}\text{C}$ but lower for $\Delta^{15}\text{N}$ than those measured on the same tissue in another avian predator, the Peregrine Falcon (*Falco peregrinus* Tunstall, 1771) (compared with the Peregrine Falcon, discrimination factors for Snowy Owl are higher by 0.1‰ for $\Delta^{13}\text{C}$ and lower by 1.4‰ for $\Delta^{15}\text{N}$) (Hobson and Clark 1992a). Considering the interspecific variation observed in diet–tissue discrimination factors obtained experimentally (reviewed by McCutchan et al. 2003; Dalerum and Angerbjorn 2005), our results thus reinforce the need to determine those values for each studied species and tissue because using inaccurate diet–tissue discrimination values could lead to misinterpretation of diet results and food-web relationships (Vanderklift and Ponsard 2003).

At least two hypotheses may explain the differences observed in diet–tissue discrimination values for $\Delta^{15}\text{N}$ among raptor families and with other terrestrial carnivores. First, the observed differences between blood of Snowy Owl (this study) and blood of Peregrine Falcon (Hobson and Clark 1992a) may be the consequence of differences in the diet used in those experimental studies. Caut et al. (2008b) attributed differences in diet–tissue discrimination factors of consumers to differences in experimental diet used, as their observed differences in the value of discrimination factors were inversely proportional to the differences in isotopic ratios among diets. Similarly, mice used in our study had higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures than the quails used to feed Peregrine Falcons (see Hobson and Clark 1992a; difference of $+4.7\text{‰}$ for $\delta^{13}\text{C}$ and $+2.3\text{‰}$ for $\delta^{15}\text{N}$, respectively), which was associated with lower diet–tissue discrimination factors in owls. Moreover, it has been recently reported that specific amino acid routing in protein synthesis could influence stable isotope ratios of a consumer's tissue, and thus potentially the diet–tissue discrimination values, if they are present in different proportion among experimental diets (Lorrain et al. 2009; McMahan et al. 2010). Further studies of the mechanism underlying this phenomenon, however, are needed. Second, Vanderklift and Ponsard (2003) showed that differences in diet–tissue discrimination factors observed among species

can be caused by the singular biochemical form of nitrogen excretion in the different taxonomic groups. Unlike Falconiformes and other terrestrial carnivores, the digestive tract of Strigiformes has a well-developed caecum (Clench and Mathias 1995). Because the caecum is known to play a role in nitrogen assimilation and excretion in birds (Clench and Mathias 1995), the lower $\Delta^{15}\text{N}$ value in Snowy Owls compared with Peregrine Falcons and other terrestrial carnivores may be a consequence of differential nitrogen assimilation and excretion rates by the digestive tract.

Various methods exist to preserve samples in the field and 70% ethanol is often used when freezing is not possible (Cherel et al. 2005). Recently, concerns have been raised regarding blood preservation in absolute (100%) ethanol for isotopic analyses (Bugoni et al. 2008). However, as previously found by Hobson et al. (1997), our study showed that preservation in 70% ethanol did not markedly affect $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of blood of Snowy Owls after 5 weeks of preservation, which is encouraging. We cannot exclude, however, the possibility that a higher alcohol concentration (70% vs. 100%) or a longer preservation period could still affect isotopic ratios, and thus a more thorough investigation using larger sample sizes is warranted.

Recent advances in stable isotope mixing models now allow researchers to assess the proportion of different sources in consumers diet while coping with variability in sources and diet–tissue discrimination factors, and to include a priori information on diet coming from scat and (or) pellet analyses (Parnell et al. 2010). Because discrimination factors can have a large impact on diet estimates (Caut et al. 2008a), it is critical to obtain the best possible values. Further studies are needed to assess the variability in diet–tissue discrimination values among raptor species, but until values are known for a broader diversity of owl species and diet types, we recommend that future studies using stable isotopes of carbon and nitrogen in Strigiformes rely on the diet–tissue discrimination values obtained in this study.

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