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RESEARCH ARTICLE

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Discrimination factors of carbon and nitrogen stable isotopes from diet to hair in captive large Arctic carnivores of conservation concern

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Rationale: Stable isotope analysis is widely used to reconstruct diet, delineate trophic interactions, and determine energy pathways. Such ecological inferences are based on the idea that animals are, isotopically, what they eat but with a predictable difference between the isotopic ratio of a consumer and that of its diet, coined as the discrimination factor. Providing correct estimates of diet-consumer isotopic discrimination in controlled conditions is key for a robust application of the stable isotopes technique in the wild.

Methods: Using a Finnigan Mat Delta Plus isotope-ratio mass spectrometer, we investigated isotopic discrimination of carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) in guard hairs of four Arctic predators; the wolf ($n = 7$), the wolverine ($n = 2$), the grizzly bear ($n = 2$), and the polar bear ($n = 3$). During a 3-month trial, carnivores were fed a mixed diet. The $\delta^{13}C$ and $\delta^{15}N$ values, and the mass (g) of diet items, were monitored weekly for each individual to determine their Total Diet Average ratios.

Results: Diet-hair isotopic discrimination (Δx) varied according to species, ranging [1.88 ± 0.69‰: 3.2 ± 0.69‰] for $\delta^{13}C$ values, and [1.58 ± 0.17‰: 3.81 ± 0.22‰] for $\delta^{15}N$ values. Adult wolves $\Delta^{13}C$ average (2.03 ± 0.7‰) was lower than that of young wolves (2.60 ± 0.8‰) and any other species (combined average of 2.59 ± 0.28‰), except for the wolverine (2.12 ± 0.23‰). Wolves $\Delta^{15}N$ averages (juveniles: 3.51 ± 0.34‰, adults: 3.68 ± 0.28‰) were higher than those of any other species (combined average: 2.50 ± 0.58‰).

Conclusions: The discrimination factors for $\delta^{13}C$ and $\delta^{15}N$ values calculated in this study could be used in ecological studies dealing with free-ranging animals, with implications for non-invasive research approaches. As in other controlled discrimination studies, we recommend caution in applying our discrimination factors when the population structure is heterogeneous.

1 | INTRODUCTION

Stable isotope analysis (SIA) is widely used in ecology to reconstruct diet, delineate trophic interactions, and determine energy pathways.[1-3] Such ecological inferences are based on the general idea that animals are, isotopically, what they eat but with a predictable difference, i.e. the difference in the isotopic ratio between a consumer and its diet, which is coined as the discrimination factor. Correct estimates of discrimination are a prerequisite to describe trophic interactions and diet reconstruction in a robust manner since models are sensitive to the uncertainty in discrimination estimates.[4] For example, increasing uncertainty in diet reconstruction models might overestimate or underestimate the contribution of a given prey species to the diet of predators. Several reviews[5,6] and experimental studies[4,7,8] have highlighted the need for more validation with experimental studies under controlled conditions, and the importance of species-based estimates.

Past ecological studies conducted on wild species typically relied on 'borrowing' discrimination factors experimentally derived from related species. For instance, farmed fox discrimination factors[9] are commonly used in other wild carnivores.[10-12] Recent experimental studies have produced robust species- and tissue-specific discrimination estimates[4,13-15] enabling more accurate modelling in field studies.[16,17] However, species- and tissue-specific discrimination factors are still lacking for many species with conservation concerns such as large carnivores (but see[13,14]). Moreover, intra-population variation in isotopic discrimination is still poorly understood.[4]

In the Canadian Arctic, wolves, wolverines, grizzly bears, and polar bears are large carnivores of conservation importance. These top-predators can feed on various prey and all play important ecological roles, such as regulating prey populations.[12,18-21] The wolverine, the grizzly bear, and the polar bear are listed as species of special concern by the Committee on the Status of Endangered Wildlife in Canada.[22,23] Despite their ecological importance and status, field studies on carnivore trophic interactions are still very scarce, largely because of the research challenges associated with their low density and wide-ranging behaviour. However, several indirect monitoring methods, such as hair snagging and carcass collection, are now increasingly used by Arctic biologists to monitor population size and structure,[24,25] health and reproductive status (Lecomte, unpublished data), and trophic interactions (L'Hérault, unpublished data). Taking advantage of non-invasive techniques, particularly the use of hair tissue for stable isotopes analyses, could provide a cost-effective avenue for inferring trophic interactions and resource use in these sensitive species. In this context, quantifying species-specific diet-consumer isotopic discrimination for hair tissue is essential.

We ran an experiment with captive animals to determine diet-hair discrimination estimates of carbon and nitrogen isotope ratios in wolverine, wolf, grizzly bear, and polar bear. Diet items fed to individuals matched the isotopic range of diet items potentially encountered in the wild. Following the recommendations of Lecomte et al[4] we explored intra-population variation in isotopic discrimination allowed by sample sizes.

2 | EXPERIMENTAL

2.1 | Hair and diet samples

The wolverines (n = 2: F,M adults), wolves (n = 7: F,M adults; 3F,2M juveniles), grizzly bears (n = 2: F,M adults), and polar bears (n = 3: F adult; F,M juveniles) lived at the Zoo Sauvage de St-Félicien (48°68' N, 72°51' W), located in the boreal ecosystem of Quebec, Canada. The experiment ran from 1 August 2011 to mid-November 2011, for a total duration of ca 105 days. The control diet fed to animals during that period was specific to each species, following veterinarian standards developed by the Canadian Zoos and Aquarium Association (CAZA). The diet incorporated a similar range of isotopic composition to natural food. In wolverines, a CAZA meat mix for terrestrial carnivores (fresh horse meat, liver, vegetal oil, vitamins, and dry supplement from commercial mix for foxes) was provided (Table 1; Table S2, supporting information). Polar bears ate a CAZA meat mix for marine carnivores (fresh horse meat, liver, fish oil, and vitamins), fresh herring, and dry supplements from commercial dog food (Table 1; Table S2, supporting information). In addition, polar bear cubs, which were in their weaning phase, fed from maternal milk at least once a day. In grizzly bears, a mix of fresh herring, vegetal sources (bread, fresh apples and fresh carrots) and dry supplements from commercial dog food were provided (Table 1; Table S1, supporting information). Finally, wolves consumed dry commercial dog food with occasional fresh horse meat (<1% diet). A similar proportion of each food item was provided to all individuals within a given species, except for polar bears where the proportion of each diet item was adjusted to age and sex based on veterinarian standards. The mass of diet items provided to animals was monitored weekly and 10 g of each diet item were stored at −20°C for subsequent measurements of $\delta^{13}C$ and $\delta^{15}N$ values.

Guard hairs of wolves and wolverines were pulled out of their necks using tweezers during routine captures performed at the end of the experiment. Guard hair samples were collected opportunistically on grizzly bears and polar bears using snag wires deployed near the feeding area in the captive habitat. Underfur samples were also collected on animals as back-up tissue (when the number of guard hairs sampled was not sufficient). Hair samples were labelled and stored at −20°C until lab analysis.

2.2 | Stable isotope analysis (SIA)

Diet item samples were rinsed in 70% ethanol and cut into small pieces, stored at −80°C for 24 h, desiccated by vacuum lyophilization, and reduced to powder using a grindmill (Retsch©, Eragny sur Oise, France). Diet sources (0.4 mg for animals and 1.2 mg for vegetal materials) were loaded in tin cups (precision ±0.01 mg) for SIA. Guard hairs and underfur samples were manually brushed and rinsed in

TABLE 1 Contribution (% total mass) of different food items to the diets of large carnivores

Carnivores	Mix #1[a]	Mix #2[a]	Mix #3[a]	Mix #4[a]	CAZA-tc[b]	CAZA-mc[b]	Horse meat	Herring[c]	Bread	Apple	Carrot
Grizzly	4.5	3.4	26.2	21.3	0	0	0	22.4	5.3	13.2	3.7
Wolf	11.6	9.7	46.5	32.0	0	0	0.3	0	0	0	0
Wolverine	0	0	0	0	100	0	0	0	0	0	0
Polar bear F Ad.	4.1	3.1	18.9	11.6	0	14.3	14.4	33.5	0	0	0
Polar bear F Juv.	3.0	2.7	18.9	11.6	0	18.4	20.9	24.3	0	0	0
Polar bear M Juv.	2.8	2.7	19.9	15.6	0	17.0	19.4	22.5	0	0	0

[a]Bulk commercial dog diet mixed with various ingredients. For detailed description of diet content, see Table S1 (supporting information).

[b]Canadian Association of Zoos and Aquarium's food for terrestrial carnivores (tc) and marine carnivores (mc). See Table S2 (supporting information).

[c]Herring provided to polar bears were supplemented with fish oil.

chloroform/methanol (2:1) to remove dirt and lipid traces.[26] We subsampled 40-mm sections from the base of each guard hair to capture the specific 3-month trial with a controlled diet. The use of 40-mm sections is a conservative estimate when we assume a constant late summer/fall growth rate of 0.63 mm/day,[27] for a total of ca 60 mm of total growth during the 105 days of the experiment. The underfur tissue was not subsampled because this tissue typically starts to grow in late summer,[28] after our experiment had started. Guard hair (40-mm sections) and whole underfur tissue were ground to fine powder using a cryomill (Retsch©) at −196°C. Hair powder (0.4 mg) was loaded in tin capsules for SIA.

Diet and hair samples were combusted in either a Carlo Erba NC2500 (ThermoFinnigan, Bremen, Germany) or a Costech 4010 elemental analyzer (Costech, Valencia, CA, USA) connected via continuous flow to a Finnigan Mat Delta Plus isotope-ratio mass spectrometer (ThermoFinnigan) at the Stable Isotope in Nature Laboratory (SINLAB: Fredericton, NB, Canada). The instrument was calibrated against international reference standards from the International Atomic Energy Agency (IAEA, Vienna, Austria). Isotope ratios are represented as permil (‰) ratios referenced against Peedee Belemnite carbonate (PDB) for $\delta^{13}C$ values and atmospheric nitrogen (AIR) for $\delta^{15}N$ values: $\delta^{13}C = [(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{PDB}) - 1]$, $\delta^{15}N = [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{AIR}) - 1])$, respectively. The precision across spectrometer runs was measured at SINLAB using an internal small mouth bass muscle standard ($\delta^{13}C = -23.39 \pm 0.11$‰ SD, $\delta^{15}N = 12.28 \pm 0.12$‰ SD, $n = 12$). The accuracy was estimated at SINLAB using a commercially available nicotinamide standard (Elemental Microanalysis Ltd, Okehampton, UK), where $\delta^{13}C = -34.51 \pm 0.13$‰ and $\delta^{15}N = -1.72 \pm 0.08$‰ SD ($n = 14$) as target ratios.

2.3 | Data analyses

Prior to calculating discrimination factors, we corrected the carbon isotopic ratio of diet items for lipid content because lipid-rich tissues are typically depleted in ^{13}C, thus showing lower $\delta^{13}C$ values than lipid-free tissues.[29,30] Typical procedures to account for the lipid-induced bias in $\delta^{13}C$ values involve chemically removing lipids from samples, or applying mathematical normalization to standardize $\delta^{13}C$ values among diet types with various lipid contents.[31-33] We used the latter method following Post et al.[32] We first determined the % of lipids in diet items (Table S3, supporting information) and calculated lipid correction factors ($\Delta\delta^{13}C$) using Equation 5 in Post et al[32] ($\Delta\delta^{13}C = -0.81 + 0.11$*% lipid) for animal food sources, and Equation 7 ($\Delta\delta^{13}C = 0.20 + 0.07$ * % lipid) for vegetal food sources. We also used Equation 13 in Post et al[32] ($\Delta\delta^{13}C = -5.83 + 0.14$ * % carbon) for fresh vegetable items (bread, apple, carrot) as the % lipid was not available. For diet items with a mixed content of animal and vegetal sources, such as dry commercial dog food mix, $\Delta\delta^{13}C$ was calculated as the average of $\Delta\delta^{13}C_{Animal}$ and $\Delta\delta^{13}C_{Vegetal}$ (Table S3, supporting information). The $\Delta\delta^{13}C$ value was then applied to the bulk $\delta^{13}C$ values to obtain $\delta^{13}C_{LN}$ values (lipid-normalized carbon ratios, Table S3, supporting information). Given their low lipid content, no correction was needed for guard hairs and underfur.[32]

We calculated the discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$ values) by subtracting the Total Diet Average ratio ($\delta X_{Total\ Diet\ Average}$) from the isotopic ratio of individual animal hair ($\delta X_{consumer}$): ΔX (±SD) = $\delta X_{consumer} - \delta X_{Total\ Diet\ Average}$ (±SD). The Total Diet Average ratio (±SD) was calculated as the weighted sum of the isotopic ratio of each diet item, as follows: $\delta X_{Total\ Diet\ Average}$ (±SD) = {($\delta X_{item\ 1}$ * % $_{item\ 1}$) + ($\delta X_{item\ 2}$ * % $_{item\ 2}$) + …} (Table S4, supporting information). $\delta X_{consumer}$ was based on guard hairs or on underfur tissue when the former was not available. To confirm that this method did not introduce any bias in the calculation of discrimination factors, we tested for statistical differences in $\delta^{13}C$ and $\delta^{15}N$ values among guard hair and underfur tissues. We ran linear mixed models with individual ID as random effect. We also tested for the fixed effects species, age, and sex. We used the *nlme* library[34] in the open access software R 3.1.1.[35] The results of the linear mixed effect models indicated how to cluster the isotopic data to calculate discrimination factors valid for groups of consumers with more than one data point. However, we did not run statistical analyses on the discrimination factors *per se* due to the small sample sizes. We nonetheless provide 95% confidence intervals (CI 95%), which effectively describe ranges of variation in data (see the review of Nakagawa et al[36]). We also provide averages and standard deviations to describe the variation in the discrimination factors among species and age classes. For group discrimination factors, the standard deviation was given as the sum of SD Total Diet Average and SD group.

3 | RESULTS

3.1 | Carbon and nitrogen isotope ratios in fur

The isotopic ratios of individuals' fur ranged from −20.22‰ to −17.36 (−18.48 ± 0.98‰, CI 95% [−19.04: −17.91‰]), and from 7.01 to 9.48‰ (7.89 ± 0.73‰, CI 95% [7.47: 8.31‰]), for $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Table 2). The results from the linear mixed effect model showed significant differences in $\delta^{13}C$ values among species and age classes. The average $\delta^{13}C$ values for wolves and wolverines were 1.08‰ (CI 95% [0.53: 1.63‰]) higher and 1.03‰ (CI 95% [−1.65:-0.42‰]) lower, respectively, than those of grizzly bears (intercept). The average $\delta^{13}C$ value for juveniles was 0.40‰ (CI 95% [0.08: 0.73‰]) higher than for adults (Table S5, supporting information). We found significant differences in $\delta^{15}N$ values among species only. The average $\delta^{15}N$ value for polar bears was 1.42‰ (CI 95% [0.94: 1.90‰]) higher than that for grizzly bears (intercept) (Table S5, supporting information). Sex had no significant effect on carbon and nitrogen ratios. We found no significant differences in carbon and nitrogen ratios between guard hair and underfur, which justifies the use of both guard hairs and underfur (back-up) in the calculation of the discrimination factors (Table 2).

3.2 | Carbon and nitrogen isotope ratios in diet items

The $\delta^{13}C$ lipid normalization factors calculated on diet items ranged from −0.28‰ to 3.47‰ (0.64 ± 0.77‰, CI 95% [0.49: 0.79‰]) (Table S3, supporting information). CAZA meat for marine carnivores was the diet item for which the lipid normalization factors were the highest (2.09 ± 0.5‰, CI 95% [1.82: 2.36‰]), followed by

TABLE 2 Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) of fur and diet, and discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$ values), for individuals of various species, sex and age classes used in the feeding experiment. The $\Delta^{13}C$ value is based on lipid-extracted diet. Averages ± SD are presented for group discrimination factors

					Animal fur		Total Diet Average			Discrimination factors	
Species[a]	ID	Sex	Age (years)	Mass (kg)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C_{Bulk}$	$\delta^{13}C_{LN}$[b]	$\delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$
Gb	960053	M	22.0	na	−18.63	7.78	−22.19 ± 0.39	−21.61 ± 0.37	5.53 ± 0.22	2.97 ± 0.37	2.24 ± 0.22
Gb	950197	F	20.0	na	−19.38	7.48	_	_	_	2.23 ± 0.37	1.95 ± 0.22
Wf	980151	M	15.0	43.0	−18.25	7.68	−20.90 ± 0.69	−20.14 ± 0.69	3.87 ± 0.22	1.89 ± 0.69	3.81 ± 0.22
Wf	A70000	F	7.0	33.0	−17.96	7.43	_	_	_	2.19 ± 0.69	3.56 ± 0.22
Wf	B00038	M	1.0	52.5	−17.55	7.50	_	_	_	2.59 ± 0.69	3.63 ± 0.22
Wf	B00037	F	1.0	39.0	−17.36	7.01	_	_	_	2.78 ± 0.69	3.14 ± 0.22
Wf	B00040	M	1.0	57.0	−17.58	7.52	_	_	_	2.56 ± 0.69	3.65 ± 0.22
Wf	B00042	F	1.0	37.5	−18.26	7.65	_	_	_	1.88 ± 0.69	3.78 ± 0.22
Wf	B00055	F	1.0	42.5	−16.95	7.20	_	_	_	3.2 ± 0.69	3.33 ± 0.22
Wv	B00095	M	12.0	15.2	−20.09	7.82	−22.78 ± 0.21	−22.27 ± 0.21	4.87 ± 0.25	2.18 ± 0.21	2.95 ± 0.25
Wv	950142	F	18.0	8.4	−20.22	8.01	_	_	_	2.05 ± 0.21	3.15 ± 0.25
Pb	A90107	M	1.5	216.0	−18.93	9.16	−22.36 ± 0.43	−21.51 ± 0.42	6.42 ± 0.21	2.58 ± 0.42	2.74 ± 0.21
Pb	A90108	F	1.5	162.0	−18.83	9.48	−22.41 ± 0.46	−21.56 ± 0.45	6.58 ± 0.21	2.72 ± 0.45	2.90 ± 0.21
Pb	A4006	F	8.5	219.0	−18.68	8.76	−21.95 ± 0.43	−21.14 ± 0.40	7.19 ± 0.19	2.46 ± 0.40	1.58 ± 0.17
Gb					−19.01 ± 0.53	7.63 ± 0.21	_	_	_	2.60 ± 0.65	2.10 ± 0.30
$Wf_{Juv.}$					−17.54 ± 0.48	7.37 ± 0.26	_	_	_	2.60 ± 0.84	3.51 ± 0.34
$Wf_{Ad.}$					−18.11 ± 0.21	7.55 ± 0.18	_	_	_	2.04 ± 0.72	3.68 ± 0.28
Wv					−20.15 ± 0.09	7.91 ± 0.14	_	_	_	2.12 ± 0.23	3.05 ± 0.28

[a]Gb: Grizzly bear, $Wf_{Juv.}$: Wolf juvenile, $Wf_{Ad.}$: Wolf Adult, Wv: Wolverine, Pb: Polar bear.

[b]$\delta^{13}C_{LN}$ diet signatures were normalized for lipid content following the equations in Post et al.[32] For calculation details, see Table S3 (supporting information).

the dry commercial dog food (0.68 ± 0.24‰, CI 95% [0.56: 0.80‰]), bread (0.63 ± 0.15‰, CI 95% [0.52:0.74‰]), CAZA meat for terrestrial carnivores (fixed at 0.51‰), horse and herring (0.35 ± 0.55‰, CI 95% [0.12: 0.58‰]), and vegetal sources (−0.12 ± 0.08‰, CI 95% [−0.16: −0.08]) (Table S3, supporting information). In terms of the Total Diet Average, the differences between the $\delta^{13}C_{Lipid\ Normalized}$ and the $\delta^{13}C_{Bulk}$ values ranged from 0.51‰ (wolverines) to 0.85‰ (polar bear cubs) (Table 2).

The lipid-normalized carbon ratios and nitrogen ratios of diet items were summed according to their respective proportion in the diet to calculate the Total Diet Average isotopic ratios. Table 2 shows the Total Diet Average $\delta^{13}C_{Bulk}$ and the Total Diet Average $\delta^{13}C_{Lipid\ Normalized}$ values, as well as the $\delta^{15}N$ (±SD) values of diets used in the experiment. The Total Diet Average of wolves (−20.14 ± 0.69‰) and the adult female polar bear (7.19 ± 0.19‰) had the highest $\delta^{13}C_{Lipid\ Normalized}$ and $\delta^{15}N$ values, respectively. In contrast, the Total Diet Average of wolverines (−22.27 ± 0.21‰) and wolves (3.87 ± 0.22‰) had the lowest $\delta^{13}C_{Lipid\ Normalized}$ and $\delta^{15}N$ values, respectively.

3.3 | Diet-hair discrimination in carbon and nitrogen ratios

At the individual level, the $\Delta^{13}C$ values ranged from 1.88 ± 0.69‰ to 3.2 ± 0.69‰ (the combined average of all individuals was 2.45 ± 0.40‰, CI 95% [2.22: 2.68‰]) and the $\Delta^{15}N$ values ranged from 1.58 ± 0.17‰ to 3.81 ± 0.22‰ (the combined average was 3.03 ± 0.70‰, CI 95% [2.63: 3.43‰]) (Table 2). Although small sample sizes precluded statistical tests, the average $\Delta^{13}C$ value in adult wolves (2.03 ± 0.7‰) appeared lower than in young wolves (2.60 ± 0.8‰, CI 95% [1.56:3.64‰]) and any other species (combined average 2.59 ± 0.28‰, CI 95% [2.24: 2.94‰]) except wolverine (2.12 ± 0.23‰) (Table 2, Figure 1). However, overlapping variance among groups precludes any firm conclusion. On the other hand, variation in $\Delta^{15}N$ values was more pronounced across species (and variance did not overlap), with wolves showing higher average values (juveniles: 3.51 ± 0.34‰, adults: 3.68 ± 0.28‰) than any other species (combined average of 2.5 ± 0.58‰, CI 95% [1.96: 3.04‰]; Table 2, Figure 1). For polar bears, the variance in $\Delta^{15}N$ values prevents any clear patterns from emerging (adult female: 1.58 ± 0.17‰, M cub: 2.74 ± 0.21‰, F cub: 2.90 ± 0.21‰) (Table 2, Figure 1).

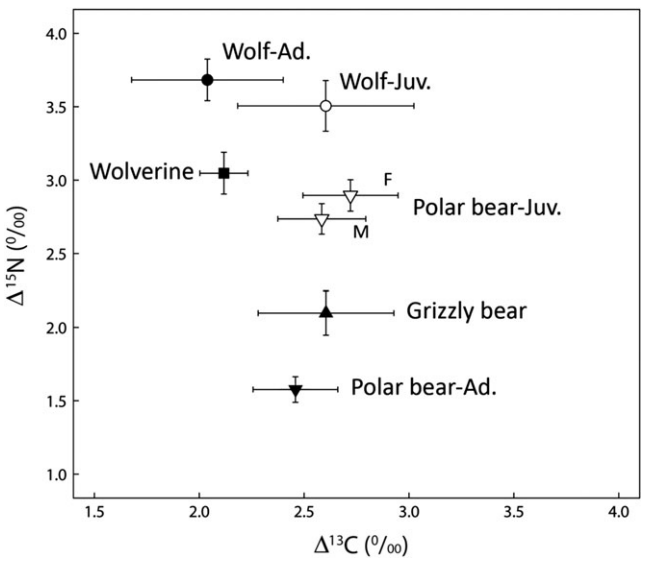

FIGURE 1 Mean and standard deviation of diet-hair discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$ values) for four species of carnivores fed in captivity (open symbols: juveniles, closed symbols; adults). Discrimination factors for $\Delta^{13}C$ were calculated on lipid-normalized values (see section 2)

4 | DISCUSSION

Incomplete understanding of the sources of variation in diet-consumer isotopic discrimination and the lack of experimental validation of discrimination factors of $\delta^{13}C$ and $\delta^{15}N$ values are common in field wildlife studies.[4] Our study provides experimentally derived diet-hair discrimination factors applicable to free-ranging Arctic carnivore species, with implication for conservation methodologies. Experimental diet-hair $\Delta^{13}C$ and $\Delta^{15}N$ values are provided for the first time in wolverines and can serve as a comparison basis in grizzly and polar bears[15,37] and in wolves[27,38] for which published values are also available. No discrimination values had yet been published for juvenile wolves, and our results suggest that the age effect can probably generate variation in $\Delta^{13}C$ values (Figure 1). Although this requires confirmation, given that observed trends could not be statistically validated, this is coherent with previous studies on canids.[4]

4.1 | Comparison with published discrimination factors

The overall average diet-hair discrimination factors for $\delta^{13}C$ (2.45 ± 0.52‰) and $\delta^{15}N$ (3.03 ± 0.22‰) values observed in our study were higher than those reviewed by Caut et al[6] for mammals ($\Delta^{13}C$: 0.5 ± 0.75‰, n = 21 studies, $\Delta^{15}N$: 2.59 ± 0.41, n = 23 studies). This is not surprising given that carnivores typically show higher $\Delta^{13}C$ and $\Delta^{15}N$ values than species from other mammalian orders.[39,40] Recent experiments on carnivore isotopic discrimination provide further support to this trend.[4,13,14]

The $\Delta^{13}C$ values obtained in adult wolves (2.03 ± 0.7‰) were very similar to those (1.97‰, n = 10, calculated on lipid-extracted diet) provided by Derbridge et al[38] for guard hairs sampled in a similar age class. However, the $\Delta^{13}C$ values in adult wolves of our study were half of those (4.25 ± 0.36‰, n = 3) provided by McLaren et al[27] for the same tissue and age group. Wolf $\Delta^{15}N$ values (3.68 ± 0.28‰) were higher in our study than those in Derbridge et al[38] (3.04‰) and McLaren et al[27] (3.09 ± 0.2‰). We suggest that differences in $\Delta^{13}C$ values across studies are explained by differences in the isotopic ratios of the diet fed to wolves. In our study and that of Derbridge et al,[38] wolves were fed items encompassing a wide range of isotopic values: dry commercial dog food in our study versus deer, beaver, and goose in Derbridge et al,[38] while McLaren et al[27] used horse meat exclusively. Horse meat is characterized by low and rather uniform $\delta^{13}C$ values (see Table S3, supporting information). Differences in $\Delta^{15}N$ values among the three studies were rather low given the similar trophic level of the food provided to wolves, yet

the slightly higher $\Delta^{15}N$ value reported in our study could be associated with the dry mixture provided. Interestingly, the mean $\Delta^{13}C$ value (2.60 ± 0.8‰) in juvenile wolves was higher than in adult wolves (2.03 ± 0.7‰) suggesting that age could be an important source of variation for this particular discrimination factor. Although our small sample size (adult wolves) may undermine our capacity to validate such an effect, we could nonetheless confirm that the range of variation and average $\Delta^{13}C$ value of juvenile wolves from our study were different than the ones reported for adult wolves by Derbridge et al[38] (with $n = 10$). Very few experimental studies have detected age effects on discrimination factors (but see[9,41]), and most of them showed age effect on $\Delta^{15}N$ only. However, Lecomte et al[4] documented important variation in $\Delta^{13}C$ among age groups in captive Arctic foxes *Vulpes lagopus* fed a mixed diet. Contrary to our study, their results showed lower $\Delta^{13}C$ values in juveniles (M: 1.98 ± 0.16‰, $n = 10$; F: 1.89 ± 0.13‰, $n = 10$) than in adults (M: 2.16 ± 0.32‰, $n = 10$; F: 2.65 ± 0.22‰, $n = 10$) with the hypothesis that such an age effect could be related to different metabolic pathways or syntheses in yearlings from those in adults.

To our knowledge, only two studies[15,37] have experimentally determined discrimination factors in bears. Hilderbrand et al[37] did not document any diet-plasma and diet-red blood cells isotopic discrimination in black bears *Ursus americanus*. Rode et al[15] addressed the effect of isotopic composition in diet on $\Delta^{13}C$ and $\Delta^{15}N$ values in plasma tissue in brown bears and polar bears. Diet-hair $\Delta^{13}C$ values measured on captive adult grizzly bears in our study (2.60 ± 0.65‰, $n = 2$) were higher than the diet-plasma $\Delta^{13}C$ values (0.6 ± 0.1‰, $n = 4$) measured on juvenile captive brown bears fed with a diet with similar proportions of lipids (~11%). On the other hand, our grizzly bear's diet-hair $\Delta^{15}N$ value (2.10 ± 0.30‰) was lower than their diet-plasma values (3.4 ± 0.1‰). The scale and the sign of the difference between diet-hair and diet-plasma $\Delta^{13}C$ and $\Delta^{15}N$ values were nonetheless similar to those reported in other experimental studies.[4] It is noteworthy that age and lipid extraction (not performed in Rode et al[15]) are potential confounding effects in this comparison. The diet-hair $\Delta^{13}C$ and $\Delta^{15}N$ values of our adult polar bear (2.46 ± 0.4‰ and 1.58 ± 0.17‰, respectively) were similar to ($\Delta^{13}C$) and different from ($\Delta^{15}N$) the diet-plasma results obtained in adult polar bears fed with a lipid-rich diet (2.0 ± 0.6‰ and 2.5 ± 0.2‰, $n = 4$, respectively[15]). Such differences could be associated with tissue types and differences in lipid content. The $\Delta^{15}N$ value in polar bear cubs was higher (M: 2.74 ± 0.21‰; F: 2.90 ± 0.21‰) than that reported in Rode et al[15] and in our adult female. Mother-offspring ^{15}N enrichment is typical in both capital[42] and income[43,44] mammalian breeders during lactation, with a fading trend during the weaning phase. The female polar bear that we studied was still providing milk to her two cubs, yet not in its prime lactating period (cubs of 1.5 years old are mostly weaned in captivity). Hence, despite our low sample size, the ^{15}N enrichment observed in the polar bear cubs could probably be attributed to lactation. Apart from this, the observed variation in $\Delta^{15}N$ value between mother and cubs could be associated with differences in the isotopic composition of the diet (the proportion of herring with elevated $\delta^{15}N$ values being less in the diet of cubs than in the diet of the mother). Overall, the effect of age on isotopic discrimination in polar bears still requires more attention given our limited ability to draw conclusions on this matter.

Finally, the wolverine discrimination factors were determined from the most consistent diet fed in our experiment (CAZA meat for terrestrial carnivores) and thus they contain less uncertainty. These results are the first published for this species.

4.2 | Diet-dependent discrimination

Several studies have insisted that estimated discrimination factors depend on the diet's isotopic composition.[4,6-8] They also warn against using discrimination factors determined in controlled conditions for free-ranging individuals, especially when derived from a single diet source, as this can blur results of diet reconstruction models. Caut et al[6] showed an error of 2‰ in ca 35% of the studies reviewed. These authors recommend in particular the use of their diet-dependent discrimination equations (derived from linear models between diet ratio and discrimination values) for species without discrimination factors determined experimentally. Here, the predicted estimates (0.86 ± 0.41‰ and 3.29 ± 0.17 for $\Delta^{13}C$ and $\Delta^{15}N$, respectively) from the hair models of Caut et al[6] were still lower than our experimental results for $\Delta^{13}C$ (2.45 ± 0.52‰), but comparable for $\Delta\delta^{15}N$ (3.03 ± 0.22‰). Because our discrimination factors were calculated on an average diet made of items encompassing a wide range of isotopic ratios, we suggest that our results represent a more realistic approximation of discrimination factors for free-ranging carnivores than the equations for corrections, particularly for $\Delta^{13}C$. Henceforth, we recommend that the equations of Caut et al[6] should be used with care, particularly in studies dealing with large carnivores with opportunistic foraging behaviours.

4.3 | Effect of lipid normalization on isotopic discrimination

The presence of lipids in tissues depletes the ^{13}C amount (thus decreasing the $\delta^{13}C$ value) with a potential bias in the calculation of $\Delta^{13}C$. However, some meta-analyses[6] and experimental studies[14] did not detect any differences in $\Delta^{13}C$ values between lipid-extracted and bulk diets. However, the topic is still vigorously discussed and most recent studies still consistently address the effect of lipid extraction on their results.[13,15] To cut the costs of isotopic laboratory analyses, we used mathematical lipid corrections (normalization) developed by Post et al[32] for animal muscle and vegetal tissue, a reliable alternative to chemical extraction.[31] The lipid corrections ($\Delta\delta^{13}C$) applied to the Total Diet Average $\delta^{13}C_{bulk}$ values were higher (in all species) than the uncertainty term (±SD) (Table 2). This was especially true in polar bears (fed with lipid-rich diet items) where the Total Diet Average $\Delta\delta^{13}C$ (0.81‰ for adult female was twice the error term (0.46‰). In our study, the use of $\delta^{13}C_{bulk}$ values in the calculation of $\Delta^{13}C$ values would inflate results in all species, from 0.51‰ in wolverines to 0.85‰ in polar bear cubs. The lack of experiments on discrimination factors in large Arctic carnivores makes comparisons difficult and it is a challenge to determine which of the $\delta^{13}C_{bulk}$ or $\delta^{13}C_{LN}$ values should be applied. In wolves, both Derbridge et al[38] and McLaren et al[27] determined diet-hair $\Delta^{13}C$ values based

on lipid-extracted diet. Hence, no $\delta^{13}C_{bulk}$ diet-based carbon discrimination factors are available for further comparisons. Nevertheless, both our study and that of Derbridge et al[38] applied diet lipid correction upon the logic that in situations of heterogeneous lipid contents among several diet items, lipid correction is typically worthwhile in order to standardize the contribution of these items to the total diet $\delta^{13}C$ value of a consumer.[32] However, Newsome et al[45] recommend the use of $\delta^{13}C_{bulk}$ values in determining discrimination factors of keratinous tissue such as animal hairs, based upon the argument that keratin structural carbon can originate from lipids.

5 | CONCLUSIONS

The development of non-invasive research approaches is warranted to efficiently monitor and conserve large Arctic carnivores. The increasing use of inactive and easy to collect tissues such as hairs is promising despite the logistical challenges associated with low animal density.[24] Using stable isotopes ratios, it is now possible to reconstruct the diet of wild animals, although the accuracy of models is sensitive to diet-consumer isotopic discrimination. The large Arctic carnivore diet-hair discrimination factors provided in this study are directly applicable to wild animals but with caution. Although we characterized discrimination factors for several species, the conditions of our experiment did not allow extensive replications nor comparisons among age classes, sex classes, or diet types, except for wolves. However, to document average values and variation on such elusive species was still an important first step to guide future field studies of free-ranging large carnivores. When using our discrimination factors, we recommend considering the range of variation provided (and to run sensitivity analysis) rather than the average value solely. This would account for sampling size limitations in this study and better capture the inherent intra-population variation.[4]

Nevertheless, by documenting potential age effects on carbon isotope ratio discrimination in wolves (so far, only reported in Lecomte et al[4]), our study suggests that population structure can alter isotopic discrimination and thus the accuracy of diet reconstruction models. This may bear implications for the management of the species, as wolf packs are typically made of genetically related individuals of different age classes.[46] Experimental designs emphasizing population structure and discrimination factors are needed for this species. Such an endeavour could help our understanding of the metabolic mechanisms involved in the partitioning of carbon (and nitrogen) isotopes among age and sex classes. Future work should also address the effect of different diets (with distinct isotopic compositions) on isotopic discrimination of large carnivores.[7]

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