ABSTRACT Dietary inferences using stable isotope analysis rely on comparing stable carbon and nitrogen isotope content of consumer to prey tissues by modeling discrimination between these tissues. Diet–tissue discrimination factors applied in these models for wild populations must be obtained from controlled feeding studies where consumer diets are known. Species-specific discrimination factors are lacking for wolves (Canis lupus), and most researchers assessing the diet of free-ranging wolves have used discrimination factors derived from red foxes (Vulpes vulpes) fed a commercial pellet diet. We calculated diet–tissue discrimination factors for various tissues from captive wolves fed a controlled diet of horse (Equus caballus) meat and also assessed the feasibility of seasonal delineation of diet through the partitioning of metabolically inactive tissues such as guard hairs and whiskers. Stable carbon isotopic discrimination in wolves was highest in whiskers (4.31‰), followed by guard hair (4.25‰), and lowest in serum (2.21‰) and red blood cells (2.16‰). Stable nitrogen isotopic discrimination was highest in serum (4.54‰), guard hair (3.09‰), and whiskers (3.05‰), and lowest in red blood cells (2.99‰). Using these values, we demonstrated the sensitivity of estimated wolf diet proportions to choice of discrimination factors. We also documented a decrease in growth rate of hair and whiskers from summer through autumn, which cautions estimating temporally explicit diet of mammals based on stable isotope analysis of discrete sections of hair and whiskers. We conclude that species-specific discrimination estimates should be used in dietary assessments based on stable isotope analyses to limit inaccuracies in diet interpretation of wild populations.

KEY WORDS blood, canid, δ-13-carbon, δ-15-nitrogen, hair, predator, prey, tissues, turnover, whisker.
of such samples to facilitate dietary inferences over several temporal scales.

A weakness of stable isotope analysis is the limited understanding of the change in isotope ratios as prey tissues are assimilated into consumer tissues (i.e., trophic discrimination). Discrimination factors for carbon and nitrogen of a given species can be determined from controlled feeding studies where the stable isotope content of both the consumer and its diet can be directly measured (e.g., Hobson et al. 1996, Sponheimer et al. 2003, Voigt et al. 2003, Miller et al. 2008, Parng et al. 2014). For wolves, species-specific discrimination factors for common tissues used in stable isotope analyses are lacking (but see Fox-Dobbs et al. 2007). Consequently, the best alternative for most studies assessing the diet of wolves is to use discrimination factors for stable carbon and nitrogen calculated by Roth and Hobson (2000) for captive red foxes (*Vulpes vulpes*) fed a commercial pellet diet (e.g., Urton and Hobson 2005, Adams et al. 2010, Kays and Feranec 2011, Milakovic and Parker 2011, Derbridge et al. 2012). However, researchers have challenged the appropriateness of using discrimination factors to infer diet broadly across different taxa (Caut et al. 2008; Crawford et al. 2008; DeMots et al. 2010; Milakovic and Parker 2011, 2013; Derbridge et al. 2012). Furthermore, application of discrimination factors to wild populations is most appropriate when such values are measured from a diet that mimics a natural diet (Fox-Dobbs et al. 2007, Parng et al. 2014). For free-ranging carnivores, such as wolves, discrimination factors obtained under natural conditions may differ from those measured using a commercial diet because of a difference in dietary sources of carbon and nitrogen expected between a high-protein meat-based diet versus a high-carbohydrate commercial diet (i.e., see Roth and Hobson 2000). Even slight changes to discrimination factors in diet models can alter inferences of prey composition in a consumer’s diet (Milakovic and Parker 2013), highlighting the importance of using appropriate discrimination factors. Advances in stable isotope analysis should, therefore, seek to determine species-specific discrimination factors and, perhaps more importantly, values inferred from controlled studies mimicking a natural diet of the consumer.

Further uncertainties in stable isotope analysis arise from the lack of knowledge about growth rates of animal tissues, such as hair or whiskers, which are sometimes used to infer temporal diet patterns of the consumer. For example, it is often assumed that guard hairs of wolves grow at a relatively constant rate during moult (e.g., summer to autumn) and that sectioning whole hairs for stable isotope analysis allows for seasonal dietary inferences by analyzing isotopic signatures of discrete sections along the hair length (Darimont and Reimchen 2002; Darimont et al. 2008; Milakovic and Parker 2011, 2013). However, neither guard hairs nor whiskers may grow continuously or synchronously, as has been shown for other species (e.g., gray seals [*Halichoerus grypus*]; Greaves et al. 2004). A greater understanding of the timing and growth rates of guard hairs and whiskers would improve our ability to use stable isotope analyses to infer diet at finer temporal scales than is presently feasible (Schwertl et al. 2003).

Our primary objective was to determine carbon and nitrogen discrimination factors of various tissues (red blood cells, serum, guard hairs, and whiskers) of captive wolves fed a simulated natural carnivore diet over a period of 4 months. Given previous suggestions that even modest changes in discrimination factors can dramatically affect estimated diet proportions, and that most previous wolf diet studies employing stable isotope analysis have used discrimination factors from captive red foxes fed a commercial pellet food (Roth and Hobson 2000), we next compared the estimated dietary proportions calculated from a published wolf data set using both our newly determined guard hair discrimination factor and that from Roth and Hobson (2000). Finally, we measured the rate of regrowth of both guard hairs and whiskers collected in our study to provide baseline data for future studies and to determine whether these tissues grow continuously throughout the summer and autumn.

**STUDY AREA**

We studied 3 wolves (1 M and 2 F) at the Toronto Zoo in Toronto, Ontario, Canada for 4 months between July and November 2013. All wolves were 2 years old and neither female was bred during the year of study. The male wolf came to the Toronto Zoo from Parc Safari in Québec, Canada, and the 2 females—sisters from the same litter—came from the Artis Royal Zoo in Amsterdam, the Netherlands. All 3 wolves arrived at the zoo approximately 2 months prior to the start of our study.

**METHODS**

We established 3 sampling periods to collect hair, whiskers, and blood from each wolf (0, 60, 120 days). During each sampling period, we chemically immobilized wolves with a combination of medetomidine (0.05 mg/kg)—midazolam (0.2 mg/kg)—ketamine (2.2 mg/kg) given intramuscularly by dart. The wolves weighed 41 kg (ad M), 35.6 kg, and 33.4 kg (ad F). During the first sampling period, we shaved a 5 × 5-cm patch of hair between the shoulder blades of each wolf as close to the skin as possible. Further, we clipped 5–6 whiskers at the skin’s surface on the muzzle of each wolf. We took digital photographs of each hair and whisker sample site immediately after sampling to facilitate subsequent sampling from the same locations on each wolf. We noticed, however, that sample sites were conspicuous during subsequent sampling periods because full regrowth was not achieved, thereby facilitating our ability to resample the exact locations on each wolf. We took a 5-mL blood sample using venipuncture into a 10-mL red top BD Vacutainer (Becton, Dickinson and Company, Mississauga, ON, Canada). We centrifuged blood samples at 9,000 revolutions/minute for 10 minutes to separate red blood cells from serum and kept both frozen at $-20^\circ$C until sample preparation. We collected successive samples at 60 days and 120 days after the initial sample period. During both subsequent sampling periods, we reshaved the patch of hair between the shoulder
blades and clipped the regrown whiskers on the muzzle down to the skin.

For the duration of the study, we fed the wolves an exclusive diet of horse (*Equus caballus*) meat balanced with vitamins, minerals, and fatty acids (<1% of the diet) according to the wolves’ nutritional requirements (referred to as the “study diet”; Toronto Zoo Canine Diet, Milliken Meat Products, Markham, ON, Canada). The horse meat, obtained from a Canadian Food Inspection Agency-approved slaughterhouse was provided in 2 forms: 2-kg bags of mixed horse meat pieces and 12-inch (30.5-cm) whole pieces of neck meat. We fed 3 2-kg bags to the wolves daily, while whole neck pieces were provided once per week. All 2-kg horse meat bags and each 12-inch (30.5-cm) neck piece was given a unique sample ID number, and we recorded the date each food item was given to the wolves. Water was available *ad libitum*. We collected samples from all bags of meat and neck pieces prior to feeding them to the wolves (*n* = 364) over the course of the study. Given the likelihood that each 2-kg bag of meat consisted of pieces from multiple individual horses, with possible varying carbon and nitrogen signatures per individual, we collected multiple samples per bag. Each bag was a square, so we picked approximately 1-cm² samples by hand (with nitrile gloves) from the center and the corners (like an “X”), then flipped the bag over and took samples from the center and sides (like a “T”). In total, we obtained 10 subsamples from each 2-kg bag (mean total wt of subsamples = 40 g) and placed all 10 subsamples into one sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI), sealed it, and labelled with the sample ID and date the food was given. We repeated this process for all 2-kg bags (*n* = 306). We only collected one sample (mean sample wt = 20 g) from each neck piece fed to the wolves (*n* = 58) because each piece was intact and, therefore, came from an individual horse. We collected neck meat pieces using a sterilized folding knife. We stored all food samples frozen at −80°C and homogenized them using a sterilized electric grinder. We subjected powdered meat samples to lipid extraction (see Pilote et al. 2012) through addition of a 2:1 chloroform:methanol solution to each sample tube, ensuring carbon ratios were not influenced by the presence of lipids in the samples (Rau et al. 1992). We agitated the samples and then left them undisturbed for 24 hours, after which they were centrifuged at 5,000 revolutions/minute for 15 minutes. We extracted the supernatant by pipette and discarded it. After lipid extraction, we freeze-dried meat samples overnight and homogenized them as described above.

We measured 1-mg samples of blood cells, serum, hair, whiskers, and meat from each respective sample type into 8 × 5-mm tin cups (Isomass Scientific, Inc., Calgary, AB, Canada) and analyzed for 13C and 15N composition using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, England, United Kingdom) at the Stable Isotope Facility at the University of California, Davis (USA). Signatures were expressed as delta (δ) notation using international standards V-PDB (Vienna PeeDee Belemnite) and Air for carbon and nitrogen, respectively. The difference in isotope signatures of replicates (*n* = 28) was 0.16 ± 0.11‰ for carbon and 0.10 ± 0.07‰ for nitrogen (mean ± SD).

**Statistical Analysis**

We tested for significant differences in δ13C and δ15N of wolf tissues collected at days 0, 60, and 120 using linear mixed-effects models with individual as the random effect. Sample type (i.e., hair, whisker, blood cells, serum) was included in the models as a main factor, but we expected, and were not interested in, difference in δ13C and δ15N by sample type. We performed *post hoc* analyses to determine which pairwise comparisons of sample periods showed significant differences in δ13C and δ15N. This allowed us to determine when tissues were in equilibrium with the diet. We then calculated discrimination factors for carbon and nitrogen for samples collected at days 60 and 120 using the absolute difference in mean δ13C and δ15N signatures between diet and biological samples. We were careful to only use diet samples reflective of the appropriate time period to represent assimilation of the study diet into each respective tissue type. Specifically, we used samples of horse meat fed 1 week prior to collection of wolf biological samples for discrimination...
factors of serum, using the suggested turnover of serum from Hilderbrand et al. (1996). We included diet samples collected from days 0 to 60 for calculation of red blood cell discrimination factors at day 60 and all diet samples collected during our 120-day study for red blood cell discrimination estimates at the end of our trial. Ideally, wolf discrimination factors of red blood cells should be calculated using diet samples from approximately the previous 4 months because of blood-cell turnover estimates (Christian et al. 1996). Given that we shaved and clipped hair and whiskers completely between sample periods, we averaged the discrimination estimates at the end of our trial. Ideally, wolf discrimination factors of hair and whiskers. We then used paired t-tests to test for significant differences in discrimination factors calculated for the day 60 and 120 sample periods.

To illustrate the potential importance of using species-appropriate discrimination factors in future wolf dietary studies, we reanalyzed the example wolf isotope data for 66 wolves in British Columbia provided with Program MixSIAR GUI (version 2.1, https://github.com/brianstock/MixSIAR; accessed 18 Sep 2014) and originally described by Darimont et al. (2009) and Semmens et al. (2009). Specifically, for each of the 3 study regions described by Darimont et al. (2009), we used MixSIAR to estimate diet proportions of deer (Odocoileus hemionus), marine mammals (Phoca vitulina, Lontra Canadensis, Neovison vison, and salmon (Oncorhynchus spp.) using the discrimination factor of hair estimated for captive red foxes by Roth and Hobson (2000) and commonly used for wolf diet studies across North America. We then repeated the analysis using all of the same specifications outlined by Stock and Semmens (2013), except that we substituted the experimentally derived discrimination factor of hair determined from our study. For each prey item, we compared the ratio of its estimated diet proportion obtained using our discrimination factor with its estimated proportion obtained using the fox discrimination factor to assess the differences in wolf diet results for each region.

To determine rates of regrowth for guard hairs shaved from each wolf at days 60 (Sep) and 120 (Nov), we began by measuring 10 hairs from each wolf collected from these 2 sample periods and then progressively increased our sample size in increments of 10 hairs until the mean of measured lengths stabilized. We found that the mean stabilized after measuring 30–40 hair samples (Supporting Information Appendix 1) and, therefore, we used the measured lengths of 40 randomly chosen individual hairs collected from each wolf at days 60 and 120 to determine mean hair growth rates (mm/day) between these periods. We measured all clipped whiskers collected at days 60 and 120 and obtained a mean growth rate (mm/day) for each wolf for each period. Using paired t-tests, we tested for significant differences in mean growth rates of regrown hair and whiskers between the days 60 and 120 sample periods to assess consistency of growth rates over our 4-month study.

All statistical analyses were performed in Program R (version 3.0.2, http://www.r-project.org/, accessed 17 Jun 2014). We used α = 0.05 to assess statistical significance in all analyses. Data are expressed as mean ± standard deviation, unless otherwise stated.

**RESULTS**

Carbon and nitrogen signatures of the diet were similar throughout our 4-month study ($\delta^{13}C = -25.67 \pm 0.94$, $\delta^{15}N = 5.61 \pm 0.53$; Fig. 1). Carbon signatures of wolf tissues differed significantly among sampling periods ($F_{2,28} = 10.49$, $P < 0.001$; Fig. 2a), whereas differences in nitrogen signatures approached significance ($F_{2,28} = 3.40$, $P = 0.047$; Fig. 2b). Tukey’s post hoc tests showed that $\delta^{13}C$ signatures of wolf tissues collected on day 0 were significantly different from those collected on days 60 ($P < 0.001$) and 120 ($P < 0.001$). There were no significant differences in $\delta^{13}C$ signatures of wolf tissues collected on days 60 and 120 ($P = 0.985$) and all pairwise comparisons for $\delta^{15}N$ showed no significance (all $P > 0.50$), confirming that wolf tissues were fully equilibrated with the horse meat diet before the end of the study.

Discrimination factors by sampling period for wolf hair, whiskers, blood cells, and serum are presented in Table 1. There were no significant differences between carbon (paired- $t = −0.34$, $df = 3$, $P = 0.753$) or nitrogen (paired- $t = −0.74$, $df = 3$, $P = 0.511$) discrimination factors calculated at days 60 and 120. Therefore, we pooled discrimination factors across the 2 sample periods to get an overall carbon and nitrogen discrimination factor for each tissue type (Table 1).

Our reanalysis of wolf diet based on stable isotope data originally presented by Darimont et al. (2009) and Semmens et al. (2009) showed discrepancies in diet estimates, particularly for Regions 2 and 3 (Fig. 3). Estimates of the proportion of salmon in the diet in Regions 2 and 3 increased considerably (995% and 326%, respectively) when substituting the discrimination factor of

**Figure 1.** Mean $\delta^{13}C$ (Φ) and $\delta^{15}N$ (□) isotope signatures in horse meat fed to captive wolves at the Toronto Zoo (Toronto, Canada) over a 4-month period in 2013. Bars represent ± standard deviation.
foxes with our experimentally derived discrimination factor, altering the ranking of prey items in the diet of wolves in these regions. Although we found increases (106%) in the proportion of deer in the diet of wolves in Region 1 when using our discrimination factor, deer remained the most common prey item of wolves in that region. Of the 3 prey types, the estimated proportion of marine mammals in the diet among the regions showed the least amount of change from the originally estimated proportions when using our discrimination factor (18–31%).

The rate of regrowth of hair for the first 60 days of our study was 1.35 ± 0.10 and 0.83 ± 0.10 mm/day, respectively, and of whiskers was 0.49 ± 0.10 and 0.37 ± 0.09 mm/day, respectively, over the course of the study (hair, paired t = 3.43, df = 2, P = 0.025; whiskers, paired t = 3.43, df = 2, P = 0.017).

Table 1. Diet–tissue discrimination factors of 13C and 15N (±SD) calculated at days 60, 120, and pooled for biological samples from wolves during a 4-month study in 2013 at the Toronto Zoo (Toronto, Canada).

<table>
<thead>
<tr>
<th>Wolf biological tissue</th>
<th>Day 60</th>
<th>Day 120</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ13C (%)</td>
<td>SD</td>
<td>Δ15N (%)</td>
</tr>
<tr>
<td>Hair</td>
<td>4.21</td>
<td>0.38</td>
<td>3.11</td>
</tr>
<tr>
<td>Whisker</td>
<td>4.13</td>
<td>0.28</td>
<td>3.02</td>
</tr>
<tr>
<td>Blood cells</td>
<td>2.29</td>
<td>0.13</td>
<td>3.04</td>
</tr>
<tr>
<td>Serum</td>
<td>2.30</td>
<td>0.30</td>
<td>4.34</td>
</tr>
</tbody>
</table>

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Figure 2. Comparison of (a) mean Δ13C (‰) and (b) mean Δ15N (‰) signatures of hair, whisker, blood cell, and serum samples collected from wolves at the Toronto Zoo (Toronto, Canada) during a 4-month study in 2013 at days 0 (start of study, ○), 60 (●), and 120 (●). Bars represent ± standard deviation.
Stable carbon and nitrogen discrimination factors for various tissues of captive wolves fed a simulated natural diet in our study differed from the widely referenced values obtained for captive red foxes (Roth and Hobson 2000). Our carbon discrimination factors were approximately 3 times higher than those obtained for foxes, while our nitrogen discrimination factors were only marginally higher (1.1 times) than the fox values. These differences led to our confirmation of the sensitivity of estimated diet proportions to the choice of discrimination factors used in mixing models. Although Derbridge et al. (2012) suggested that choice of discrimination factors could influence estimated diet proportions, they did not quantify this bias; and we suspect researchers may be alarmed that estimated diet proportions of individual prey items of wolves (from Darimont et al. 2009) could change considerably when our experimentally derived factor for guard hair was substituted for the often cited discrimination factor for hair from Roth and Hobson (2000). Such changes can alter the ranking of prey items in the diet and subsequent description of the diet of the consumer, as was the case of wolves in Regions 2 and 3 in our reanalysis. Certainly, future research into consumer diets using stable isotope analysis would benefit from obtaining taxon-, tissue-, and diet-appropriate discrimination factors for the study species in question.

Given the sensitivity of stable isotope mixing models to estimates of discrimination, a more accurate assessment of the diet of free-ranging wolves could be achieved using our wolf-specific discrimination factors, particularly because they were derived using an appropriate diet, an influential factor on such values (Caut et al. 2008, Florin et al. 2011, Parng et al. 2014). For example, carbon discrimination factors of consumer tissues, particularly keratinized tissues (e.g., hair; Fox-Dobbs et al. 2007 and whiskers; Newsome et al. 2010) decrease as the proportion of lipids in the diet increases. For wild wolves with low-lipid, protein-rich prey sources, using discrimination factors derived from diets that deviate from these conditions may lead to erroneous estimates of diet proportions. Further, diet composition, particularly the presence of grains in the diet (e.g., commercial domestic diets), determines the source of amino acids available for consumer tissue synthesis and subsequently affects carbon and nitrogen discrimination factors (Martínez del Río et al. 2009, Parng et al. 2014). We consider the horse meat diet of wolves in our study a suitable natural prey substitute because our wolves had carbon signatures that closely mimicked the highly negative carbon signature of wild canids more so than that of domestic canids (Kays and Feranec 2011). Isotope signatures are a direct reflection of a consumer’s diet and the similarities to wild wolves supports the appropriateness of our controlled study diet from which we derived our discrimination factors. We suspect that differences in the proportion of lipids in our wolf diet and that of red foxes and the incorporation of grains in the fox diet (which would substitute for moisture content in our study diet) can explain the observed differences in our carbon and nitrogen discrimination factors more so than protein quality and quantity. Slight variation in the protein composition of our study diet compared with that used by Roth and Hobson (2000) could have contributed to small differences in

![Figure 3](image-url)

**Figure 3.** Differences in estimated proportions of prey in diets of wolves from 3 regions in British Columbia, Canada (original data from Darimont et al. 2009), based on choice of discrimination factor used in stable isotope mixing models. Bars represent ± standard deviation.

**DISCUSSION**

Stable carbon and nitrogen discrimination factors for various tissues of captive wolves fed a simulated natural diet in our study differed from the widely referenced values obtained for
discrimination factors. Given our use of a 100% animal-source diet, our factors should more closely reflect natural discrimination processes of wild wolves and are, therefore, recommended for use in mixing models to make dietary inferences for free-ranging wolves feeding primarily on animal protein. We note the specificity of our factors to wolves because our values differed from those calculated for captive felids also fed animal-source diets (Parng et al. 2014); this indicates that diet alone may not explain observed differences in discrimination factors. Therefore, our discrimination factors would be most useful for wolves consuming prey with stable isotope signatures similar to those from our study diet.

Although we only had a few wolves to work with, we observed very little variation in $\delta^{13}$C and $\delta^{15}$N among our study wolves and their tissues used to calculate discrimination with the diet. Had we observed large variation in any of our samples, our confidence in our results would be reduced. All wolves used in our study were adults, so any potential effects due to growth (e.g., Roth and Hobson 2000) would also have been negligible. Further, the wolves were brought to the zoo from different geographic regions (Canada and the Netherlands) and, therefore, the different origins of the wolves combined with the low variance in their stable isotope signatures indicate the robustness of our results. At the very least, our research speaks to the need for appropriate discrimination factors for wolves and cautions against interpretation of diet information based on previously published discrimination factors.

We could not detect significant differences in $\delta^{13}$C and $\delta^{15}$N signatures of metabolically active wolf tissues (i.e., red blood cells in serum) and tissues stimulated to grow (i.e., hair and whiskers) sampled at days 60 and 120 after the initiation of our feeding trial. However, $\delta^{13}$C signatures of samples collected during these 2 sampling periods differed from those of samples collected at the start of the study. Zoo wolves were fed a similar horse meat diet for approximately 2 months prior to our study initiation and $\delta^{13}$C signatures of red blood cells continued to change until day 60 of our study, representing an overall turnover period of approximately 4 months. Although determining tissue-specific turnover rates was not a primary objective of our study, these results suggest that turnover of metabolically active tissues such as red blood cells of wolves is similar to that of domestic dogs (110 days; Christian et al. 1996). Therefore, stable isotope analysis of blood samples collected from wild wolves likely represents the diet over the previous 3–4 months, rather than 1–2 months, as commonly referenced from turnover rates of red blood cells in American black bears (Ursus americanus; Hilderbrand et al. 1996).

Our finding of a decrease in hair and whisker growth between September and November validated our concerns regarding assumptions of continuous growth of these tissues. We are not aware of any reasons that repeated shaving and clipping of hair and whiskers would decrease growth of these tissues, so we believe that our results are reflective of biological processes rather than methodological bias. This result is an important consideration for research using stable isotope analysis to obtain diets at fine temporal scales by sectioning metabolically inert tissues such as guard hair and whiskers into equal sections and assuming each section reflects the diet during a consistent and predictable length of time. For example, dividing guard hairs in half as a proxy for spring and summer (distal end of hair) and autumn (proximal end of hair) diets may in fact lead to overrepresentation of the summer diet in stable isotope results from the proximal end of the hair, particularly if hair shows a slowed growth during this period. For wolves, such a crude approach to partitioning seasons may work, however, when there is a pronounced change in diet between the start and end of the moulting season (see Darimont et al. 2008). Relating isotopic content of discrete sections of whiskers to assess changes in diet over specific intervals of time should also be cautioned against until more specific data on rates and timing of whisker regrowth throughout the year are available. In the meantime, supplementing analysis of sectioned hair and whiskers with analysis of metabolically active tissues, such as blood cells, can help to increase precision of seasonal diet inferences.

The goal of our study was to address some uncertainties inherent in the use of stable isotope analysis to infer diets of wild wolves and provide a better understanding of potential sources of error in dietary studies based on stable isotope analysis. Our research demonstrates that the use of appropriate discrimination factors for diet inferences of wild wolves is crucial. The values we calculated for stable isotopic carbon and nitrogen discrimination will be useful for future studies on wolves because they are species-appropriate and they were obtained from a diet-appropriate controlled feeding study. Our results also suggest that published diet assessments of wolves based on stable isotope data should be reassessed using the discrimination factors presented here to evaluate any significant changes in conclusions. This is particularly important in cases where the role of wolves as predators of specific prey species, as inferred from results of stable isotope analysis, is used to guide wolf management. Furthermore, the precise growing season of guard hairs remains largely unknown for most mammal species, and likely varies geographically; both topics warrant further study. Finally, we recommend that future research focuses on obtaining additional species-specific discrimination factors using feeding studies mimicking wild diets. Such research will help minimize the uncertainties of stable isotope analysis and will improve the reliability of this methodology in complementing current research on the feeding ecology of free-ranging consumers.

ACKNOWLEDGMENTS

Primary funding for this research was provided by the Ontario Ministry of Natural Resources and Forestry (OMNRF), Wildlife Research and Monitoring Section. From the Toronto Zoo, we thank J. Wensvoort for advice and providing the diets for the study and E. Di Nuzzo for her thorough collection and storage of diet samples. We further thank all veterinarian technicians and keepers for facilitating wolf sample collections. We are indebted to A. Silver and S. Taylor of the OMNRF for their help with laboratory sample...
preparation. We also thank 2 anonymous reviewers and the Associate Editor for their comments on an earlier draft of this manuscript.

LITERATURE CITED


**Associate Editor: Berkman.**

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article at the publisher’s website (Appendix 1). A figure is used to display the mean length of wolf guard hairs, measured in sets of 10, with stabilization in the mean length being achieved between 30 and 40 hair samples for days 60 and 120.