Carnivore-specific stable isotope variables and variation in the foraging ecology of modern and ancient wolf populations: case studies from Isle Royale, Minnesota, and La Brea

K. Fox-Dobbs, J.K. Bump, R.O. Peterson, D.L. Fox, and P.L. Koch

Abstract: We use carbon and nitrogen isotope data collected from two North American gray wolf (*Canis lupus* L., 1758) populations (Isle Royale and northern Minnesota) to both calculate carnivore-specific isotopic variables and investigate wolf foraging ecology. The isotopic enrichments of ^{13}C and ^{15}N that occur between mammalian carnivores and their prey have not been well defined in modern populations. We use bone collagen from the Isle Royale National Park wolf, moose (*Alces alces* (L., 1758)), and beaver (*Castor canadensis* Kuhl, 1820) populations to determine trophic enrichment factors of 1.3‰ ± 0.6‰ for $\delta^{13}C$ and 4.6‰ ± 0.7‰ for $\delta^{15}N$. We apply these carnivore-specific fractionation factors to a case study from the fossil record, and reconstruct the diets of late-Pleistocene dire wolves (*Canis dirus* (Leidy, 1858)) from the La Brea tar pits. We use the Minnesota wolf tissue (collagen, hair, muscle) isotopic data to estimate carnivore population subsample sizes needed to replicate the mean values of the whole population within one standard deviation. Finally, we compare the Isle Royale and Minnesota collagen and hair isotopic data to published $\delta^{13}C$ and $\delta^{15}N$ values for North American gray wolf populations. We find that interpopulation differences in isotope variances provide insight into wolf foraging ecology.

Résumé : Nous utilisons des données sur les isotopes de carbone et d'azote récoltées dans deux populations (Isle Royale et nord du Minnesota) de loups gris (*Canis lupus* L., 1758) pour calculer les variables isotopiques spécifiques aux carnivores et aussi pour étudier l'écologie alimentaire des loups. Les enrichissements isotopiques de ^{13}C et de ^{15}N qui se produisent entre les mammifères carnivores et leurs proies n'ont pas été bien définis dans des populations actuelles. Nous utilisons du collagène des os provenant de populations de loups, d'orignaux (*Alces alces* (L., 1758)) et de castors (*Castor canadensis* Kuhl, 1820) du parc national de l'Isle Royale pour déterminer les facteurs d'enrichissement trophique, soit de 1,3 ‰ ± 0,6 ‰ pour $\delta^{13}C$ et de 4,6 ‰ ± 0,7 ‰ pour $\delta^{15}N$. Nous appliquons ces facteurs de fractionation spécifiques aux carnivores dans une étude de données fossiles provenant du puits de goudron de La Brea pour reconstituer le régime alimentaire du loup noir (*Canis dirus* (Leidy, 1858)) de la fin du pléistocène. Nous utilisons les données isotopiques du tissu (collagène, cheveu, muscle) de loups du Minnesota pour déterminer la taille des sous-échantillons de carnivores nécessaire pour pouvoir retrouver les valeurs moyennes de la population totale dans un intervalle de moins d'un écart type. Finalement, nous comparons les données isotopiques du collagène et des cheveux obtenus à l'Isle Royale et au Minnesota avec les valeurs de $\delta^{13}C$ et de $\delta^{15}N$ publiées pour les populations nord-américaines de loups gris. Nous trouvons que les différences de variance isotopique entre les populations ouvrent des perspectives sur l'écologie alimentaire des loups.

[Traduit par la Rédaction]

Introduction

Terrestrial carnivores generally exist at low densities, move frequently within large home ranges, and feed opportunistically (Mech and Boitani 2003; Peterson and Ciucci 2003). These factors make traditional measures of foraging ecology, such as observation and fecal analysis, difficult to obtain for wild carnivores and highlight the importance of stable isotope analyses as an alternative method for estimating diet. Stable isotope values are routinely measured in animal tissues as a quantitative method for investigating trophic relationships and interspecies connectivity within modern and ancient food webs. Paradoxically, interpretations of isotopic results and patterns are often not quantitative, and may even be subjective, without taxon- and diet-specific information on how isotopes are sorted (or fractionated) between diet and different body tissues in consumers.

A growing body of experimental research is available to facilitate dietary interpretations of isotopic data measured in tissues from a range of animals, including ungulates, small mammals, and birds (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson and Clark 1992*a*, 1992*b*; Sponheimer et al. 2003*a*, 2003*b*; Jim et al. 2004; Passey et al. 2005), and

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these experiments are concordant with results from field studies. Our current understanding of how carbon and nitrogen stable isotopes are fractionated between terrestrial carnivore body tissues and their diet is primarily drawn from a few feeding studies done with captive carnivores fed omnivorous and carnivorous diets (Hilderbrand et al. 1996; Roth and Hobson 2000; Ben-David and Schell 2001). In addition, a few studies of wild carnivore populations have reported diet–tissue fractionations, but in each case diet could not be completely constrained because of food-web complexity (Lee-Thorp et al. 1989; review in Bocherens and Drucker 2003). To our knowledge, diet–tissue trophic fractionations have not been measured for a large, terrestrial carnivore species with a fully characterized hypercarnivorous (pure animal tissue) diet.

We present $\delta^{13}C$ and $\delta^{15}N$ data for two North American gray wolf (*Canis lupus* L., 1758) populations, and use these data to calculate carnivore-specific trophic fractionations and to investigate the foraging ecology of these populations. Both populations live in boreal ecosystems in the northern United States (Isle Royale National Park, Michigan, and northern Minnesota) (Fig. 1), but their ecological settings are quite different. The wolves of Isle Royale are part of a geographically isolated and simple food web, with available prey limited to moose (*Alces alces* (L., 1758)), beavers (*Castor canadensis* Kuhl, 1820), and snowshoe hares (*Lepus americanus* Erxleben, 1777). The island is protected as a national park and the wolves are not affected by humans. We analyzed wolf, moose, and beaver bone collagen from animals that died on Isle Royale from 1965 to 2004 and interpret our results within the ecologic context defined by the large body of published data on the Isle Royale food web. Thus, we reduce the number of assumptions required to determine wolf–diet isotopic spacings from this food web and can identify ecologically relevant patterns within the stable isotope data sets. In contrast to the Isle Royale wolves, the wolves from northern Minnesota exist in a mosaic of protected, uninhabited, and agricultural lands. These wolves feed within a more complex food web that includes two ungulate species (white-tailed deer, *Odocoileus virginianus* (Zimmermann, 1780), and moose) and multiple small-mammal species (Fritts and Mech 1981; Fuller 1989). Additional food sources may include human-derived waste (e.g., garbage) and domesticated animals (e.g., livestock and pets). We analyzed bone collagen, hair, and muscle from Minnesota wolves that died in the summer of 2004.

We explore several ecologic and isotopic questions with these wolf data sets. First, we investigate temporal and spatial patterns of isotopic variability in the Isle Royale food web. Previous work identified significant geographic differences in the isotope values of modern vegetation across the island (Tischler 2004), but we do not know how long these differences have persisted or how they propagate through the food web. Second, we account for spatial and temporal variations within the Isle Royale wolf and prey data sets, and calculate robust $\delta^{13}C$ and $\delta^{15}N$ trophic fractionations between wolf collagen and diet (moose and beaver) collagen values (referred to as $\varepsilon^*_{wolf-diet}$). These are the first collagen trophic fractionations to be reported for a terrestrial hypercarnivore with a fully characterized diet. We emphasize that collagen–collagen fractionations between carnivores and their prey are not equivalent to collagen–diet fractionations derived from direct measurement of diet (prey flesh) isotope values. Skeletal remains are often all that we have for study, and therefore we depend upon collagen–collagen trophic fractionations when reconstructing the diets of past and present carnivore populations. Third, we present a case study from the fossil record to illustrate how varying $\delta^{13}C$ and $\delta^{15}N$ trophic fractionations can affect dietary interpretations drawn from carnivore and prey stable isotope data sets. We reconstruct the diets of late-Pleistocene dire wolves (*Canis dirus* (Leidy, 1858)) from the La Brea tar pits (Fig. 1), using both conventional trophic fractionations and trophic fractionations calculated from the Isle Royale wolves. Fourth, we use the Minnesota wolf tissue data sets to estimate carnivore population subsample sizes needed to replicate the mean values of the whole population within one standard deviation (1 SD). Since the stable isotope values of each tissue represent a different dietary "time window", we compare the subsample sizes required for different tissue types from the same population. Although subsample sizes should ultimately be calculated for each specific population under investigation, conservative estimates derived from the Minnesota wolf population provide a guideline for the development of future isotopic studies of carnivores. Fifth, we compare the Isle Royale and Minnesota wolf collagen and hair $\delta^{13}C$ and $\delta^{15}N$ data sets with the collagen and hair stable isotope data sets from other North American wolf populations. Genetically, all modern North American gray wolf populations are closely related and are remnants of a formerly abundant and continent-wide gray wolf population (discussion in Leonard et al. 2005). We quantify the range of dietary specialization, derived from intrapopulation isotopic variability, found in these remnant gray wolf populations.

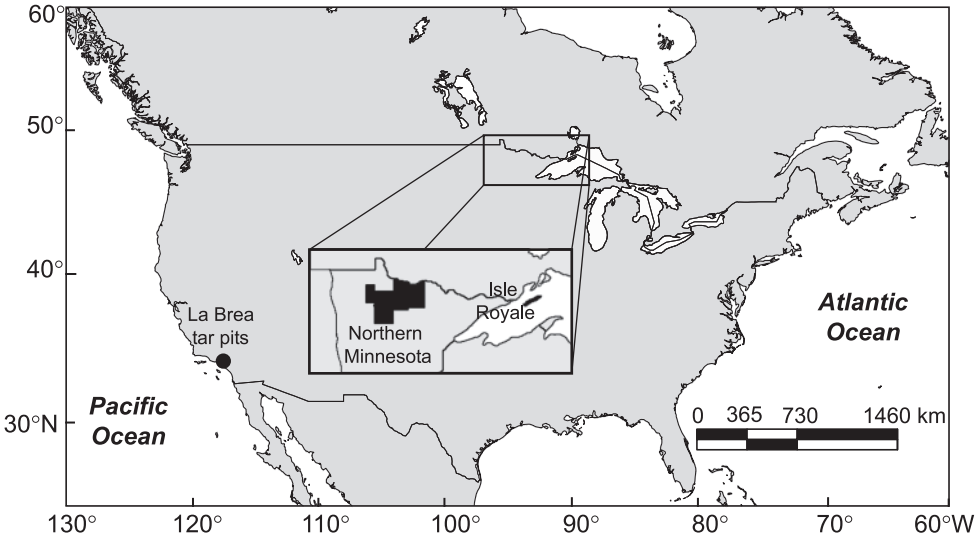
Methods

Study areas

Isle Royale National Park

Isle Royale National Park, USA, is an archipelago (544 km^2) in northwestern Lake Superior, 24 km southeast of the Canadian mainland. Variable glacial retreat resulted in significant deposits of glacial debris at the western end of the island, but little material at the eastern end. Consequently, soils on the eastern two-thirds are generally thin and azonal, with exposed bedrock on sloping ridges. Soils on the western third are comparably deeper and more developed, with less exposed bedrock (Wolf and Huber 1973). The island bedrock comprised Precambrian volcanics, conglomerates, and sandstones (Huber 1975). The variable geology coupled with fire history and browsing creates a forest mosaic of boreal and northern hardwood tree species. Balsam fir (*Abies balsamea* (L.) P. Mill.), white spruce (*Picea glauca* (Moench) Voss), paper birch (*Betula papyrifera* Marsh.), and quaking aspen (*Populus tremuloides* Michx.) are found near the Lake Superior shoreline. Sugar maple (*Acer saccharum* Marsh.) and yellow birch (*Betula alleghaniensis* Britt.) dominate higher elevations. Cedar (*Thuja occidentalis* L.) is also common in lowland areas (Peterson 1977). The aforementioned soil differences result in more

Fig. 1. Map showing locations of Isle Royale and northern Minnesota (Beltrami and Koochiching counties) gray wolf (*Canis lupus*) populations, as well as the La Brea tar pits fossil site in southern California.



forest disturbance by wind at the east end of the island, yielding higher forest floor light levels (MacLaren and Janke 1996). In contrast, soils at the west end of the island support older and taller deciduous forests that heavily shade the forest floor. These east–west distinctions yield differential regeneration of balsam fir, an ecologically important browse species, with high regeneration at the east end but not at the west end (Peterson et al. 2003). This vegetative trend presumably explains the typical spatial pattern in moose density: the highest moose densities (~5.4/km^2) are at the east end, low densities mid-island (~0.8/km^2), and moderate densities (~1.8 to 3.4/km^2) at the west end (Vucetich and Peterson 2004).

Moose likely colonized Isle Royale sometime between 1905 and 1913 (Murie 1934), followed by wolves in the late 1940s (Mech 1966). Island moose and wolf populations have fluctuated dramatically during the past half-century in what appears to be two decade intervals with significant predation, food, and weather effects on moose (Peterson et al. 2003; Vucetich and Peterson 2004). The wolf population size has varied from 12 to 50 individuals. Introduced disease (canine parvovirus) significantly reduced wolves at one point (Peterson 1999) and invertebrate parasites likely affect moose levels (R.O. Peterson, personal communication).

Northern Minnesota

Northern Minnesota is characterized by dense boreal forests and abundant lakes, with a low density of agricultural development. The wolves in our study are from Beltrami and Koochiching counties, which are located in the north-central part of the state (Fig. 1). Both the geographic range and size of the northern Minnesota wolf population have been increasing since the 1970s (Fuller et al. 1992). During the winter of 1997–1998, there were approximately 2500 wolves in northern Minnesota, at an estimated density of 2.8–3.3 wolves/100 km^2 (Berg and Benson 1999; Fuller et al. 1992). Wolves in northern Minnesota have spatially and seasonally variable diets, but white-tailed deer is the primary prey for most individuals (Fritts and Mech 1981; Fuller 1989). Other prey include moose, beavers, and small mammals (Fritts and Mech 1981; Fuller 1989).

La Brea tar pits

For our case study, we reconstructed trophic relationship between a hypercarnivorous predator (dire wolves) and a suite of potential herbivorous prey (bison, *Bison antiquus* (Leidy, 1852); horse, *Equus occidentalis* (Leidy, 1865); camel, *Camelus hesternus* (Leidy, 1854); ground sloth, *Paramylodon harlani* (Owen, 1840); mastodon, *Mammut americanum* (Kerr, 1791)), using late-Pleistocene specimens from the La Brea tar pits (Los Angeles Basin, California). In general, coastal California is considered to have been an ''ice age refugium'' for biota during the Last Glacial Maximum; the climate was slightly wetter and cooler than present, but was not strongly affected by the continental ice sheets (Johnson 1977). Much like today, the vegetation varied along the moisture gradient from the Pacific coast into the interior desert and mountain regions (Mock and Bartlein 1995).

Sample collection

Bone samples of Isle Royale wolves (n = 42), moose (n = 58), and beavers (n = 10) were taken from specimens housed in the Isle Royale National Park collection at Michigan Technological University (MTU), Houghton, Michigan. The wolf and moose specimens were collected from individuals that died naturally from 1965 to 2004. Life-history information such as year of death (YOD), location of death (LOD), age, and sex were recorded for some wolves and most of the moose. Limited life-history data were available for beavers, although most died between 1990 and 2002. Researchers opportunistically collected wolf specimens, whereas the moose specimens were systematically collected from carcasses that were surveyed by MTU researchers each winter and summer. We sampled all available wolf specimens and indiscriminately selected moose specimens from the extensive collection, with emphasis on temporal and age class representations.

Bone samples of La Brea dire wolves (n = 24) were collected from fossil specimens housed at the George C. Page Museum, Los Angeles, California. We sampled dire wolf

specimens from tar pits (pits 61 and 67) that are dated to ~12 000 years BP (Marcus and Berger 1984).

Bone, hair, and muscle samples were collected by personnel from the Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture (USDA), responding to cases of wolf predation on domestic livestock in northern Minnesota from June to August 2004. All 19 wolves weighed >14 kg at death, including the 3 pups that were sampled.

Sample preparation and analysis

Bone chunks (Isle Royale and Minnesota samples) were drilled from the specimens using a handheld Dremel® microdrill. Approximately 25 mg samples were decalcified in 0.5 mol/L HCl for 1–2 days at 4 °C. Lipids were extracted with five rinses of a 2:1 chloroform and methanol solution, with sonication of each rinse for 0.5 h. Guard hairs (Minnesota) were rinsed in methanol to remove surface contaminants. Several hairs (5–10) were then homogenized in a CryoMill® grinder. Muscle samples (Minnesota) were lipid extracted as described above and then homogenized in a CryoMill® grinder.

Small bone chunks were drilled from La Brea dire wolf specimens. Samples were prepared following the methods in Fox-Dobbs et al. (2006). In brief, ~120 mg samples were crushed to a coarse powder, continuously rinsed with solvents (petroleum ether and acetone, 24 h each) in a Soxhlet extractor to remove tar, and then decalcified as above. The collagenous residue was gelatinized in 0.01 mol/L HCl at 57 °C for 12 h and then passed across a 1.5 μm glass-fiber filter, with retention and lyophilization of the filtrate.

For stable isotope analyses, collagen, hair, and muscle samples (1.0 mg) were weighed into precombusted tin capsules. Stable isotope ratios were measured using an elemental analyzer coupled with a mass spectrometer (Europa Hydra 20/20) at the University of California Davis Stable Isotope Facility. Stable isotope compositions are reported using the standard δ notation, and are referenced to Vienna PeeDee Belemnite and air for carbon and nitrogen, respectively. The standard deviation for replicates of a gelatin standard was <0.2‰ for carbon and nitrogen.

Data analysis

We include 40-year $\delta^{13}C$ and $\delta^{15}N$ records for Isle Royale wolf, moose, and beaver, but only apply statistical analyses to the beaver data set and the temporal subsets of wolf (1975–1995) and moose (1970–2000) values that we used to calculate wolf–diet trophic enrichment factors. We selected subset time frames that contained the highest frequency of wolf and moose individuals, and were long enough to reduce any short-term variability owing to anomalous environmental conditions. The moose subset extends 5 years before and after the wolf subset to include moose that were in the diet of the oldest and youngest wolves, respectively. We follow the logic outlined in Passey et al. (2005), and calculate the isotopic spacings between wolf collagen and diet collagen as ε^* values (* is the nonequilibrium fractionation factor): $\varepsilon^*_{wolf-diet} = (\alpha_{wolf-diet} - 1) \times 1000$, where $\alpha_{wolf-diet} = (\delta_{wolf} + 1000)/(\delta_{diet} + 1000)$.

Prior to statistical analysis of the Isle Royale $\delta^{13}C$ records, we removed the effects of anthropogenically driven changes in the $\delta^{13}C$ value of the atmosphere that have occurred over the past ~150 years (Long et al. 2005). Specifically, we used an atmosphere-derived rate of $\delta^{13}C$ change of 0.032‰ per year to correct the values to modern (2004) (Francey et al. 1999; Keeling et al. 2005). Quantitative analyses were preformed with JMP® version 5.0.1a (SAS Institute Inc. 2002). In cases where data did not meet the assumptions of normality or homogeneity of variances, we used nonparametric tests.

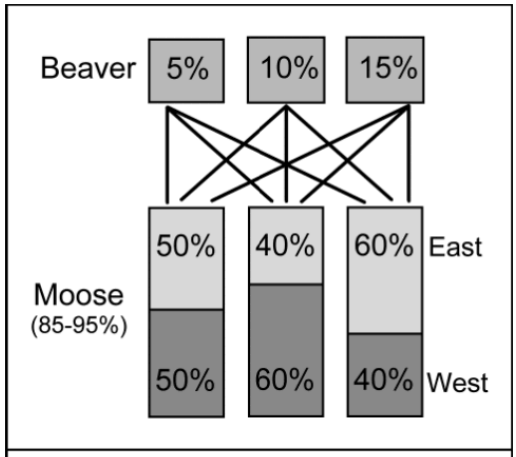
Isle Royale model diet construction

To calculate collagen $\varepsilon^*_{wolf-diet}$ values from Isle Royale data, we modeled nine wolf diets that account for known variation in wolf feeding preferences, as well as intrapopulational isotopic variability in moose (Fig. 2). Each modeled diet included different proportions of the three dietary inputs: moose that died on the eastern end of the island (east moose), moose that died on the western end (west moose), and beavers. We combined the weighted means of these dietary inputs and additively propagated the variances associated with each dietary input. We then calculated nine collagen $\varepsilon^*_{wolf-diet}$ values from the model diet and wolf $\delta^{13}C$ and $\delta^{15}N$ values. To determine uncertainties for each of the nine wolf–diet trophic enrichment values, we summed the model diet and wolf variances, and then calculated the standard deviation from this variance. This method of error propagation allowed us to assign conservative estimates of uncertainty to the $\varepsilon^*_{wolf-diet}$ values that we report here. We relied upon extensive observational records to make the following assumptions when constructing the modeled diets: (*i*) moose compose 90% ± 5% of wolf diet by biomass, (*ii*) wolves consume equal moose biomass (50% ± 10%) from the east and the west ends of the island, (*iii*) adult and young (<1 year old) moose account for 85% and 15%, respectively, of moose biomass consumed by wolves, (*iv*) beavers compose 10% ± 5% of wolf diet by biomass, and (*v*) hares are a negligible fraction of wolf diet (Peterson 1977; Thurber and Peterson 1993).

Minnesota wolf subsample size calculations

To examine the minimum sample size necessary to represent with a stated degree of confidence the mean isotope composition of a population, we modified the method of Clementz and Koch (2001) and used a standard bootstrapping approach to determine the minimum sample sizes necessary to represent the mean of the $\delta^{13}C$ and $\delta^{15}N$ values for each tissue in the Minnesota wolf data set (bone: $n = 18$; muscle and hair: $n = 19$ each). For each set of measurements ($\delta^{15}N$, $\delta^{13}C$) of each tissue (bone, muscle, hair), the bootstrapping method determines the proportion of 1000 subsamples of n_{sub} values chosen at random without replacement that have a mean value within 1 SD of the mean of the whole data set for that measurement of that tissue. The standard deviation of the whole data set is calculated assuming that the whole data set represents the total population rather than a sample of a larger population. Thus, the term in the denominator of the formula for standard deviation is n instead of $(n - 1)$, which is used for calculating the standard deviation of a sample of a population. Bootstrapping is replicated for all values of n_{sub} between 2 and $n - 1$. For a given value of n_{sub}, the number of subsamples within 1 SD

Fig. 2. Diagram detailing construction of the nine Isle Royale model wolf diets. Dietary inputs for the models include east (E) moose (*Alces alces*), west (W) moose, and beavers (*Castor canadensis*). The model diets account for observed variation in Isle Royale wolf diet.



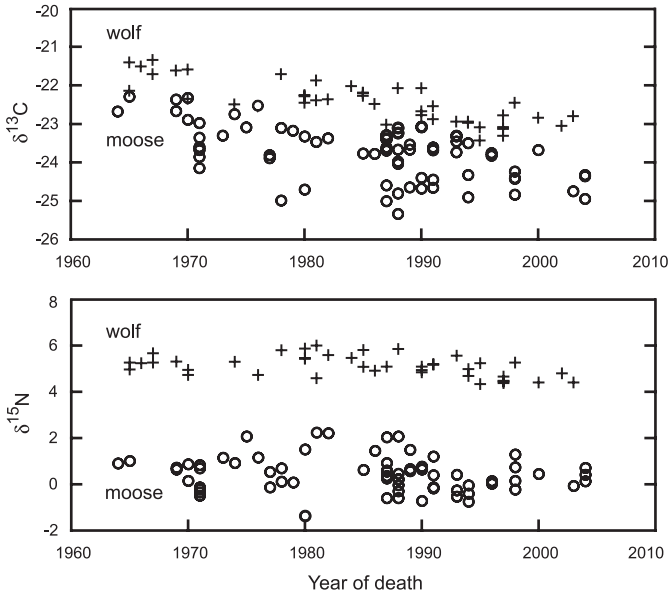
of the mean of the whole data set provides a bootstrapped estimate of the probability that a subsample of s values is within 1 SD of the mean for the whole sample. We have 95% confidence that subsamples of n_{sub} are a good estimator of the population mean if ≥950 subsamples out of 1000 have mean values within 1 SD of the mean of the whole data set. This method is sensitive to the distribution of the values in the whole data set, so relying on results from our Minnesota wolf sample to design other empirical studies must assume that the empirical population has a variance structure like that of the Minnesota wolf population used here. A limitation of this method is that it does not provide a straightforward means to compare the variance in subsamples of n_{sub} values with that in the whole data set.

Results

Isle Royale $\delta^{13}C$ and $\delta^{15}N$ records

The full $\delta^{13}C$ and $\delta^{15}N$ records of the Isle Royale wolf and moose from 1965 to 2004 are shown in Fig. 3. All Isle Royale wolf, moose, and beaver specimen numbers and isotopic values are provided in Table S1.[2] In Fig. 3, the $\delta^{13}C$ data have not been detrended to account for temporal changes in atmospheric $\delta^{13}C$ values (see Methods above). In all subsequent figures and tables the wolf and moose $\delta^{13}C$ values are detrended, and the detrended values are included in Table S1.[2]

Fig. 3. Plots of the full Isle Royale wolf and moose $\delta^{13}C$ and $\delta^{15}N$ records (1965–2004). The decrease in wolf and moose $\delta^{13}C$ values with time is primarily attributed to anthropogenically mediated changes in the carbon isotope composition of the atmosphere.



The means, SD, and ranges of the 1975–1995 wolf $\delta^{13}C$ (detrended) and $\delta^{15}N$ values are given in Table 1. Within the wolf population, we investigated the effects of YOD and LOD on variance in $\delta^{13}C$ and $\delta^{15}N$ data. The wolves were divided into three LOD groups (east, west, and middle). There were no significant differences (two-way ANOVA; whole model, $p > 0.05$) among individuals based on these factors. We only analyzed adult (>9 month old) wolves, so we did not investigate differences among age classes.

The means, SD, and ranges of the 1970–2000 moose $\delta^{13}C$ (detrended) and $\delta^{15}N$ values are provided in Table 1. For LOD, moose were only divided into two groups (east and west) because of the lack of moose specimens located in the island's middle. There was a significant difference in moose $\delta^{15}N$ values owing to the combined effects of age (adult vs. young (<1 year of age)), LOD (east vs. west), and YOD (three-way ANOVA; whole model, $F_{[7,34]} = 4.0$, $p = 0.003$). Yet, the only significant effect within the whole model was LOD ($F_{[1,40]} = 13.3$, $p = 0.0009$); east moose had $\delta^{15}N$ values approximately 1‰ lower than those of west moose. There was a significant difference in moose $\delta^{13}C$ values owing to the combined effects listed above (three-way ANOVA; whole model, $F_{[7,34]} = 3.8$, $p = 0.004$), but the only significant effect within the whole model was age ($F_{[1,40]} = 19.2$, $p = 0.0001$). Young moose had $\delta^{13}C$ values approximately 1‰ lower than those of adult moose.

Without life-history data for the beavers, we could not determine demographic sources of variance within the beaver isotope data. Based on the size of the skeletal elements analyzed, we assumed that all of the specimens were adults or young adults. The means and SD of the beaver $\delta^{13}C$ and $\delta^{15}N$ values were –24.1‰ ± 0.6‰ and 2.4‰ ± 0.9‰, re-

[2] Supplementary data for this article are available on the Journal Web site (cjz.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5152. For more information on obtaining material refer to cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

Table 1. Summary of Isle Royale wolf (*Canis lupus*), moose (*Alces alces*), and beaver (*Castor canadensis*) collagen $\delta^{13}C$ and $\delta^{15}N$ values (‰) used in trophic enrichment calculations.

	Wolf (n = 25)	East moose (n = 21)	West moose (n = 21)	Beaver (n = 20)
$\delta^{13}C$				
Mean	–23.2	–24.5	–24.5	–24.1
1 SD	0.3	0.5	0.6	0.6
Range	–23.8 to –22.6	–25.5 to –23.9	–26.0 to –23.6	–25.0 to –23.1
$\delta^{15}N$				
Mean	5.2	0.0	0.8	2.4
1 SD	0.4	0.4	0.8	0.9
Range	4.3 to 6.0	–0.6 to 0.8	–0.6 to 2.2	1.2 to 4.0

spectively (Table 1). The beaver and moose $\delta^{13}C$ values were not significantly different (one-way ANOVA; $p > 0.05$). Beaver $\delta^{15}N$ values were significantly different than both the east (one-way ANOVA; $F_{[1,29]} = 114.0$, $p < 0.0001$) and the west (one-way ANOVA; $F_{[1,29]} = 26.1$, $p < 0.0001$) moose $\delta^{15}N$ values.

Isle Royale trophic fractionations

The $\delta^{13}C$ and $\delta^{15}N$ values of Isle Royale wolves, east moose, west moose, and beavers used to calculate collagen $\varepsilon^*_{wolf-diet}$ values are presented in Fig. 4. Based upon the significant effect of LOD in the $\delta^{15}N$ moose data, we treated the moose populations on the east and west sides of the island as separate inputs for the model wolf diets. Although there was a significant difference between adult and young moose $\delta^{13}C$ values, we included a constant proportion (~15%) of young moose (Peterson 1977; Thurber and Peterson 1993) in the east and west moose dietary inputs. We found that varying the proportion of young by as much as 10% had a negligible effect on $\varepsilon^*_{wolf-diet}$ values.

The nine model diet $\delta^{13}C$ values were essentially invariant, from –24.5‰ to –24.4‰, whereas model diet $\delta^{15}N$ values ranged more, from 0.4‰ to 0.8‰. The mean wolf $\delta^{13}C$ and $\delta^{15}N$ values were –23.2‰ and 5.2‰, respectively. The nine collagen $\delta^{13}C$ $\varepsilon^*_{wolf-diet}$ ranged from 1.29‰ to 1.35‰, while the $\delta^{15}N$ $\varepsilon^*_{wolf-diet}$ ranged from 4.47‰ to 4.81‰ (Table 2). The means and SD of the nine $\delta^{13}C$ and $\delta^{15}N$ $\varepsilon^*_{wolf-diet}$ values were 1.3‰ ± 0.6‰ and 4.6‰ ± 0.7‰, respectively.

Minnesota wolf tissues

The means and SD of $\delta^{13}C$ and $\delta^{15}N$ values for each of the Minnesota wolf tissue types are presented in Fig. 5. Specimen numbers and isotope values are provided in Table S2.[2] We treated the $\delta^{13}C$ and $\delta^{15}N$ data sets differently for quantitative analyses, since the tissue $\delta^{13}C$ values were not normally distributed (e.g., not appropriate for parametric statistics), whereas the $\delta^{15}N$ values were normally distributed. Mean $\delta^{13}C$ and $\delta^{15}N$ values were significantly different among tissue types ($\delta^{13}C$: Kruskal–Wallis test, $\chi^2 = 27.6$, $p < 0.0001$; $\delta^{15}N$: one-way ANOVA, $F_{[2,53]} = 5.2$, $p = 0.009$). We tested for equal variances in $\delta^{13}C$ and $\delta^{15}N$ values across tissue types using Levene's test, which is robust to departures from normality ($\delta^{13}C$: Levene's test, $F_{[2,53]} = 1.1$, $p = 0.3$; $\delta^{15}N$: Levene's test, $F_{[2,53]} = 0.1$, $p = 0.9$) (Schultz 1985). For both $\delta^{13}C$ and $\delta^{15}N$ values, the SDs in all tissue types were within 0.2‰ of each other.

Fig. 4. Plot of Isle Royale wolf (1975–1995), east and west moose (1970–2000), and beaver $\delta^{13}C$ and $\delta^{15}N$ values, and mean values. $\delta^{13}C$ values have been detrended to account for changes in the $\delta^{13}C$ value of the atmosphere through time.

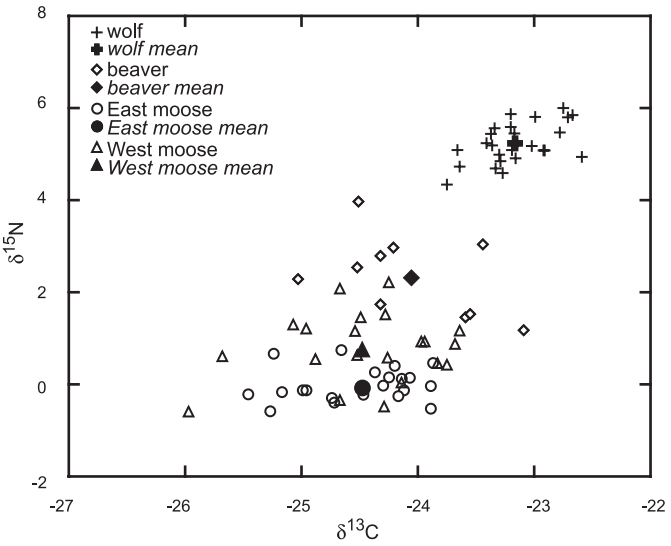


Table 2. Collagen $\delta^{13}C$ and $\delta^{15}N$ trophic enrichments (‰) between Isle Royale wolves and model diets.

	$\varepsilon^*_{wolf-diet}$ ± 1 SD	
Model diet	$\delta^{13}C$	$\delta^{15}N$
A	1.34±0.64	4.74±0.72
B	1.33±0.65	4.66±0.75
C	1.35±0.63	4.81±0.69
D	1.32±0.64	4.64±0.73
E	1.31±0.65	4.57±0.76
F	1.32±0.64	4.71±0.71
G	1.29±0.65	4.54±0.75
H	1.29±0.66	4.47±0.77
I	1.30±0.64	4.61±0.72
Average	1.32±0.64	4.64±0.74

Note: The SD was calculated from the summation of variances associated with the wolf and each model diet $\delta^{13}C$ and $\delta^{15}N$ values.

Minnesota wolf subsample sizes

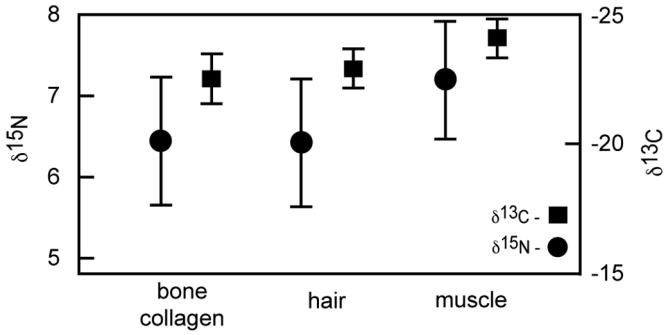
The bootstrapping results for the $\delta^{15}N$ and $\delta^{13}C$ values of bone, hair, and muscle of Minnesota wolves are similar to the results of Clementz and Koch (2001) despite the differ-

Table 3. Results of bootstrap analysis of $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen, hair, and muscle of Minnesota wolves (bone: n = 18; hair and muscle: n = 19 each).

	$\delta^{13}C$			$\delta^{15}N$		
Tissue	SD (‰)	n_{sub}	Subsamples within 1 SD*	SD (‰)	n_{sub}	Subsamples within 1 SD*
Bone	0.93	2	874	0.72	2	851
		3	961		3	951
		4	979		4	970
		5	994		5	998
		6–17	1000		6–17	1000
Hair	0.68	2	863	0.75	2	849
		3	926		3	925
		4	980		4	977
		5	996		5	998
		6	999		6	999
		7–18	1000		7–18	1000
Muscle	0.71	2	887	0.69	2	860
		3	937		3	943
		4	974		4	978
		5	994		5	994
		6–18	1000		6–18	1000

*Number of subsamples out of 1000 replicates of size n_{sub} within 1 SD of the mean of all 18 or 19 measurements for each measurement of each tissue.

Fig. 5. Plot of northern Minnesota wolf mean (±1 SD) $\delta^{13}C$ and $\delta^{15}N$ values for bone collagen, hair, and muscle.



ences in resampling methodologies. For $\delta^{13}C$ and $\delta^{15}N$ values of bone of the Minnesota wolves (n = 18), more than 95% of subsamples of $n_{sub} \geq 3$ are within 1 SD of the mean of the whole data sets. For both measurements of the other two tissues, more than 95% of subsamples of $n_{sub} \geq 4$ had mean values within 1 SD of the mean of the whole data set (Table 3). Moreover, for both measurements of all three tissues, all 1000 subsamples were within 1 SD of the mean of the whole data set for subsamples of n_{sub} = 6 or 7, depending on the tissue. Thus, for muscle $\delta^{15}N$ and $\delta^{13}C$ values, we can be more than 95% confident that samples of four randomly chosen individuals from a population with the variance structure of the Minnesota wolf sample will be within 0.69‰ and 0.71‰ of the means of a much larger sample, respectively. For these same measures, if the sample size increases to only six, respectively, we are effectively guaranteed to be within 0.69‰ and 0.71‰ of the means of a much larger sample, respectively. For the other tissues, more than 95% of samples of either three (bone) or four (hair) randomly chosen individuals would be within 0.93‰–0.68‰ of the population mean.

Discussion

Stable isotope ecology of the Isle Royale food web

The Isle Royale food web shows significant isotopic heterogeneity, both today and in the past. The two main prey species of Isle Royale wolves, moose and beaver, are isotopically distinct. Furthermore, there is a significant difference in the $\delta^{15}N$ values between moose from the east and the west ends of the island that has persisted for at least the past ~35 years. Spatial differences in the $\delta^{15}N$ values of Isle Royale moose have been observed in other tissues or wastes (hoof: Tischler 2004; tooth dentine: Bada et al. 1990; urine: R.O. Peterson, unpublished data), but our results put this difference into historical perspective. In contrast to the moose results, we found that the Isle Royale wolf isotope values and variances are remarkably constant in both time and space. Below we briefly discuss the patterns and potential causes of spatial and temporal isotopic variability at Isle Royale.

The moose and beaver mean $\delta^{13}C$ values are within the range expected for herbivores feeding within a pure C_3 environment (Cerling et al. 1997). The beaver $\delta^{15}N$ values are, on average, 1.6‰ higher than those of west moose and 2.4‰ higher than those of east moose. This difference may reflect a greater proportion of aquatic plants in the diet of beavers. Observations suggest that beavers may feed almost entirely on aquatic plants in summer, when they take in most of their annual intake (R.O. Peterson, personal communication). Aquatic plants are a high-protein (nitrogen-rich) forage relative to terrestrial summer vegetation (McCracken et al. 1993; Tischler 2004). Isotopic research at Isle Royale and in other boreal ecosystems has shown that aquatic plants have higher $\delta^{15}N$ values than local terrestrial plants (Ben-David et al. 2001; Tischler 2004). The elevated beaver $\delta^{15}N$ values verify that their diet includes a large amount of aquatic vegetation.

The significant difference in east and west moose $\delta^{15}N$ values may be due to differences in diet composition, body condition, or the stable isotope values of forage (plants) at either end of the island. There is a well-documented variation in the distribution of available summer forage across the island, primarily related to soil development and the distribution of lakes on the island. Specifically, in the west there are more deciduous trees, whereas in the east there are more lakes and habitat for aquatic plants (Huber 1973). The moose of Isle Royale consume a wide range of terrestrial and aquatic plants during the summer (Belovsky and Jordan 1978; Tischler 2004). The higher relative abundance of aquatic plant habitat on the east end, and the elevated $\delta^{15}N$ values of aquatic plants compared with terrestrial plants, should translate to higher $\delta^{15}N$ values in east versus west moose. Yet, we found the opposite pattern in moose $\delta^{15}N$ values (i.e., east moose have lower values than west moose), so consumption of aquatic plants does not seem to explain the observed pattern in moose $\delta^{15}N$ values. This suggests that there is either a difference in moose body condition or a difference in terrestrial plant values. Based on research by Tischler (2004), we rule out differences in body condition between moose subpopulations; instead, we suggest that the difference in moose $\delta^{15}N$ values is correlated to winter forage $\delta^{15}N$ values. During the winter, diets of moose across Isle Royale converge on a limited number of woody plant species and arboreal lichens (Tischler 2004). Balsam fir is the most common winter forage for moose (~60% of diet), and Tischler (2004) found the $\delta^{15}N$ values of balsam fir from the west end were significantly higher than those from the east end. A thorough understanding of what factors (e.g., soil type and nitrogen cycling) drive the difference in balsam fir $\delta^{15}N$ values is a topic for future research, but we suggest that the ecosystems at both ends of the island have been in nitrogen cycling equilibrium since 1970.

We found that $\delta^{15}N$ values of young and adult moose were indistinguishable, whereas the young moose $\delta^{13}C$ values were, on average, 0.8‰ lower than adult moose values. The young moose $\delta^{13}C$ values may reflect an average diet that is a mixture of vegetation and their mother's milk, since moose do not wean until ~5 month of age (Wilson and Ruff 1999). Milk is a lipid-rich food source, and therefore is ^{13}C depleted relative to plant food sources. The contribution of milk to the diet of young animals is generally also evident in their $\delta^{15}N$ values, since while nursing the young are essentially feeding at a trophic level higher than that of adults (Jenkins et al. 2001). If consumption of lipid-rich milk explains the low $\delta^{13}C$ values of young moose, it is unclear why the $\delta^{15}N$ values of young moose are not higher than those of adults.

The Isle Royale wolf $\delta^{13}C$ and $\delta^{15}N$ records reveal that the population has had a constant diet for the past 30 years. This is in agreement with observational records that span this time period (Mech 1966; Peterson 1977; Peterson and Page 1988; Peterson et al. 1998). By analyzing many individuals from multiple generations, we found a sustained level of intrapopulation variability for Isle Royale wolves through time. Changes in diet over time as a result of documented changes in wolf pack dynamics (e.g., geographic range, duration, membership) and wolf population size are not reflected at the population level in bone collagen stable isotope values (a review of wolf population dynamics from 1959 to 2005 is presented in Peterson and Vucetich 2005). We therefore treat the 1975–1995 wolves as a single population and assume that isotopic variability among individuals is due to individual physiological or dietary differences. As predators within a single-prey (>90% moose) food web, the wolves of Isle Royale provide a unique opportunity to characterize the baseline isotopic variance that we can expect within a large carnivore population. This variance can be attributed to fundamental physiological differences among individuals owing to age, sex, health, and reproductive status, and to sustained individual dietary differences that are due to social status and feeding behaviors.

Carnivore-specific collagen $\delta^{13}C$ and $\delta^{15}N$ trophic fractionations

Among ecologists using stable isotopes techniques, there has been a renewed call for the measurement and application of tissue- and consumer-specific trophic fractionations. Ultimately, tissue–diet trophic fractionations are most rigorously calculated from well-designed, controlled feeding experiments. Several recent controlled feeding studies of quickly replaced (blood, muscle), or continuously grown (hair, nail), tissues have yielded robust tissue–diet fractionations for a range of taxa (Hobson and Clark 1992*a*, 1992*b*; Roth and Hobson 2000; Ben-David and Schell 2001; Sponheimer et al. 2003*a*, 2003*b*; Passey et al. 2005). Yet, experimental measurements of collagen trophic fractionations for a large-bodied carnivore are lacking. In addition to ethical considerations regarding experimental duration and extraction of bone collagen for analysis, the replication of a "natural" hypercarnivorous diet in captivity is potentially costly and difficult. The ungulate prey of most wild large carnivores are a very lean (fat-poor, protein-rich) food source for carnivores (Robbins et al. 1974). Preliminary work with captive wolves fed a diet of domestic cow hearts (~25% fat by wet mass) revealed that wolf hair $\delta^{13}C$ values strongly recorded the fat fraction of the diet (K. Fox-Dobbs, unpublished data). These results suggest that carnivore trophic fractionations must be measured from a diet that nutritionally and biochemically "mimics" a natural carnivore diet in order for the fractionations to be applicable to studies of wild populations.

The Isle Royale wolf–moose system is a close approximation of a controlled feeding study for a large hypercarnivore in a nonexperimental setting. The collagen $\varepsilon^*_{\text{wolf–diet}}$ values that we report account for all likely variations in wolf diet (model diets A–I). The fractionations vary by only 0.03‰ for $\delta^{13}C$ values and 0.34‰ for $\delta^{15}N$ values. The average $\delta^{13}C$ $\varepsilon^*_{\text{wolf–diet}}$ value (1.3‰) for Isle Royale wolf collagen is similar to values reported in other studies of wild terrestrial carnivores and their prey (Bocherens and Drucker 2003), as well as values calculated from numerical dietary models (Hedges 2003). Consumer collagen $\delta^{13}C$ values are strongly correlated to the $\delta^{13}C$ values of dietary protein (Ambrose and Norr 1993; Jim et al. 2004), indicating that carbon from dietary protein is routed to consumer body proteins, including collagen. In a high-protein consumer such as a wild carnivore, protein is the primary source (~90% by mass) of dietary carbon, with lipids as a secondary dietary

Table 4. La Brea dire wolf prey (diet source) $\delta^{13}C$ and $\delta^{15}N$ values (‰), calculated with general and carnivore-specific trophic enrichment values.

		Mean		General $\varepsilon^*_{tissue-diet}$		Carnivore $\varepsilon^*_{tissue-diet}$	
Taxon	*n*	$\delta^{13}C$	$\delta^{15}N$	Mean $\delta^{13}C$ + ε = 1.0‰	Mean $\delta^{15}N$ + ε = 3.0‰	Mean $\delta^{13}C$ + ε = 1.3‰	Mean $\delta^{15}N$ mean + ε = 4.6‰
Camel, *Camelus hesternus**	10	–20.6	9.1	–19.6	12.1	–19.3	13.7
Horse, *Equus occidentalis**	7	–21.4	6.3	–20.4	9.3	–20.1	10.9
Ground sloth, *Paramylodon harlani**	3	–21.4	8.9	–20.4	11.9	–20.1	13.5
Bison, *Bison antiquus**	9	–20.6	9.4	–19.6	12.4	–19.3	14.0
Mastodon, *Mammut americanum**	4	–20.6	4.2	–19.6	7.2	–19.3	8.8
Dire wolf, *Canis dirus*	24	–19.9	11.6	–18.9	14.6	–18.6	16.2

*Data from Coltrain et al. 2004.

component (discussion based on Hedges 2003). The relatively large, positive metabolic fractionation associated with assimilation of carbon from dietary protein (herbivore body proteins) into consumer body proteins is what drives the $\delta^{13}C$ trophic enrichment between high-protein consumer (carnivore) and diet (herbivore) collagen. This assumes that the ^{13}C enrichment of collagen compared with other body proteins (owing to the high content of ^{13}C enriched glycine in collagen) is the same for both herbivores and carnivores.

The average $\delta^{15}N$ $\varepsilon^*_{wolf-diet}$ value (4.6‰) for Isle Royale wolf collagen is higher than fractionations that were estimated for other wild terrestrial carnivore populations (Bocherens and Drucker 2003). Dietary characterization can be difficult in more complex food webs and it is possible that the diets of the other carnivore populations were not fully constrained. The first-order pattern of increasing $\delta^{15}N$ values (regardless of tissue type) with trophic level is broadly attributed to the preferential excretion of ^{14}N in urea, which is the main efflux of nitrogen in most animals. This results in a body pool that is enriched in ^{15}N relative to diet. Variation in the magnitude of the $\delta^{15}N$ trophic enrichment observed in mammalian feeding studies appears to correlate well with variation in dietary protein content (Robinson et al. 2001; Sponheimer et al. 2003*b*; Robbins et al. 2005). Yet, there is no consensus on the nature of this relationship; it has been reported as both an inverse relationship (Robbins et al. 2005) and a direct relationship (Robinson et al. 2001; Sponheimer et al. 2003*b*). Our carnivore $\delta^{15}N$ $\varepsilon^*_{wolf-diet}$ value is higher than many trophic enrichment values estimated for animals with omnivorous or herbivorous diets (e.g., DeNiro and Epstein 1981; Roth and Hobson 2000; Sponheimer et al. 2003*b*), a pattern that supports the direct relationship between trophic enrichment value and dietary protein content. Sponheimer et al. (2003*b*) postulated that animals on a high protein diet lose proportionally more nitrogen as urea than feces compared with animals on a low protein diet. In essence, animals lose a relatively constant amount of nitrogen in feces regardless of dietary protein content, but urinary nitrogen loss will vary with dietary protein content. Increased ^{14}N loss as urea results in a body pool that is relatively more enriched in ^{15}N for animals with high versus low protein diets.

Trophic fractionation case study: dietary reconstruction for dire wolves from La Brea

We provide an example from the late Pleistocene to illustrate how varying fractionation factors can change the interpretations that are drawn from predator and prey $\delta^{13}C$ and $\delta^{15}N$ records. To do this, we use the isotope values of dire wolves and their potential megafaunal prey from the La Brea tar pits (southern California). Dire wolves are an extinct species of canid and were abundant in North America until the end of the Pleistocene (~10 000 years ago), when they disappeared rapidly, along with much of the Pleistocene mammalian and avian megafauna (Koch and Barnosky 2006). The demise of large-bodied Pleistocene carnivores (predators and scavengers), such as dire wolves, has been linked to the extinction of megafaunal prey species (Van Valkenburgh and Hertel 1993; Binder et al. 2002; Fox-Dobbs et al. 2006).

We compared the dire wolf isotope values (Table S3[2]) to published isotope values for a suite of herbivores (horse, bison, mastodon, ground sloth, and camel) from the same tar pits as the dire wolves (Coltrain et al. 2004). To reconstruct the diet of dire wolves, we calculated the mean $\delta^{13}C$ and $\delta^{15}N$ values for each potential prey, and input those values as the dietary endmembers in a multisource isotope mixing model (Isosource version 1.3.1; Phillips and Gregg 2003). Isosource is an appropriate model to use when there are a large number of potential diet sources (e.g., greater than n + 1 diet sources, where n is the number of stable isotopes used in the model) for a given consumer. We treated each of the megafaunal herbivores as a potential diet source, along with dire wolves (Table 4). Carnivores are the most abundant animals in the La Brea tar pits (Stock and Harris 1992), thus it is feasible that carnivores were prey of, or carrion consumed by, other carnivores. To test the effects of variation in trophic fractionation factors, we ran the Isosource model twice: first, with general nonspecific trophic enrichments of 1‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ (DeNiro and Epstein 1978; DeNiro and Epstein 1981) (Fig. 6A), and second with the carnivore-specific fractionations presented here (1.3‰ for $\delta^{13}C$ and 4.6‰ for $\delta^{15}N$) (Fig. 6B). We report the contribution of each prey source to dire wolf diet as a range (1 SD) of percentages.

The dietary inferences that we can make from the mixing model results are different, depending upon which trophic fractionations are used. The model with nonspecific fractionations predicts a larger contribution of dire wolf, bison, and camel to the wolf diets. The model with carnivore-specific fractionations suggests that horses were important prey for wolves, and that sloth, mastodon, and grazers (bison and camel) contributed equally but were less common prey. The

Fig. 6. Isosource dietary mixing polygons for late Pleistocene La Brea dire wolves (*Canis dirus*). The mean dire wolf $\delta^{13}C$ and $\delta^{15}N$ values (+) are plotted with potential diet sources (prey). Contributions of each diet source are presented as a range of possible percentages (defined by 16%–84% percentiles of food-source distributions). (A) General trophic enrichment values of 1.0‰ for $\delta^{13}C$ and 3.0‰ for $\delta^{15}N$ were added to the mean $\delta^{13}C$ and $\delta^{15}N$ values of potential diet sources. (B) Carnivore-specific trophic enrichment values of 1.3‰ for $\delta^{13}C$ and 4.6‰ for $\delta^{15}N$ were used.

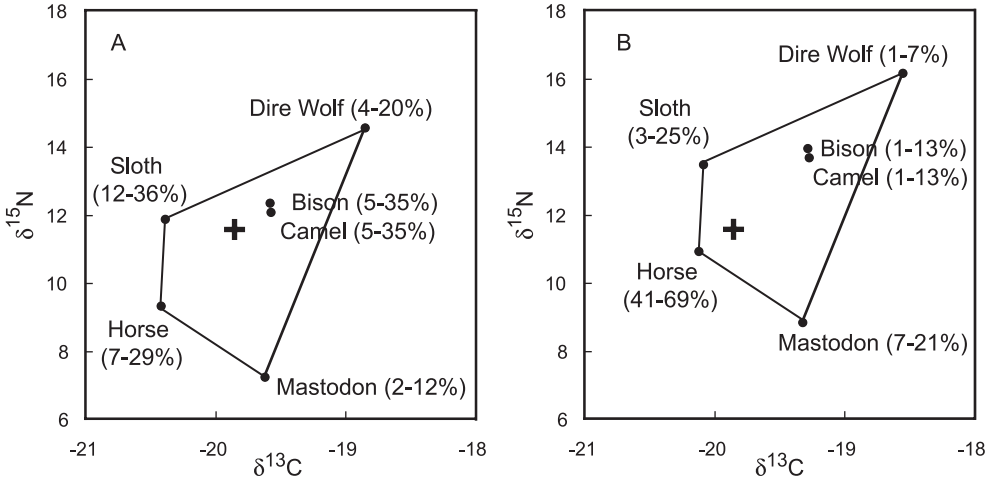


Table 5. Compilation of published $\delta^{13}C$ and $\delta^{15}N$ values for North American gray wolf populations (collagen and hair).

Wolf population	*n*	$\delta^{13}C$ (mean ± 1 SD)	$\delta^{15}N$ (mean ± 1 SD)	Inferred or observed diet	Reference
Bone collagen					
USA					
Northern Minnesota	18	–22.5±0.9	6.7±0.7	Terrestrial, range of prey	This study
Isle Royale National Park	25	–23.2±0.3	5.2±0.4	Terrestrial; moose and beaver only	This study
Coastal island Alaska	101	–22.7±1.0	7.5±1.0	Terrestrial or marine; range of prey	Szepanski et al. 1999
Coastal mainland Alaska	62	–21.4±2.4	7.6±2.4	Terrestrial or marine; range of prey	Szepanski et al. 1999
Interior Alaska	50	–19.6±0.7	6.4±0.7	Terrestrial; caribou, moose	Szepanski et al. 1999
Canada					
Central Ontario	10	na	5.9±0.5	Terrestrial; range of prey	Schwarcz 1991
Hair keratin					
USA					
Northern Minnesota	19	–22.9±0.7	6.4±0.8	See above	This study
Canada					
Coastal British Columbia	17	–22.2±1.0	6.6±1.0	Terrestrial or marine; salmon and deer	Darimont and Reimchen 2002
PANP, Saskatchewan	16	–22.9±0.3	6.5±0.6	Terrestrial; limited prey	Urton and Hobson 2005
Outside PANP, Saskatchewan	14	–22.5±1.2	7.4±1.0	Terrestrial; range of prey	Urton and Hobson 2005
La Ronge, Saskatchewan	17	–21.7±1.3	7.9±1.5	Terrestrial; range of prey	Urton and Hobson 2005

Note: PANP, Prince Albert National Park.

fraction of wolf in the wolf diets dropped between models, and the carnivore-specific fractionations appeared to provide a more plausible estimate of dietary composition. Morphologic work on La Brea dire wolves suggested that these extinct hypercarnivores experienced relatively high levels of tooth breakage and that their skulls were "robust" (e.g., wider palate) compared with those of modern gray wolves (Binder et al. 2002). Based upon cranial morphology and tooth breakage levels, dire wolves appear to have been adapted for hunting large prey and (or) scavenging upon the carcasses of large prey. The isotope mixing model results support previous paleoecologic interpretations for dire wolves and suggest thta they were not specialized predators. At the population level, late-Pleistocene dire wolves were hunting or scavenging all off the most abundant megafaunal herbivores present in the La Brea region.

Minnesota wolf tissue $\delta^{13}C$ and $\delta^{15}N$ values and subsample sizes

Different metabolically active body tissues record diet differently, depending upon the rate at which the tissue is replaced or turned over. For example, bone collagen, hair, and muscle tissue samples taken from the same individual represent isotopically averaged dietary "time windows" of years, months, and weeks, respectively (Hilderbrand et al. 1996; Roth and Hobson 2000). The population-level variances in the Minnesota wolf muscle and hair $\delta^{13}C$ and $\delta^{15}N$ values reflect individual differences in summer and spring foraging behaviors. These differences are likely driven by short-term opportunistic foraging trajectories. In contrast, population-level variance in the Minnesota wolf bone collagen values reflects both long-term (annual or multi-annual) trends in individual foraging behaviors (e.g., prey presence

or absence, migration) and any baseline changes to the food web or ecosystem (e.g., landscape modification) $\delta^{13}C$ and $\delta^{15}N$ values.

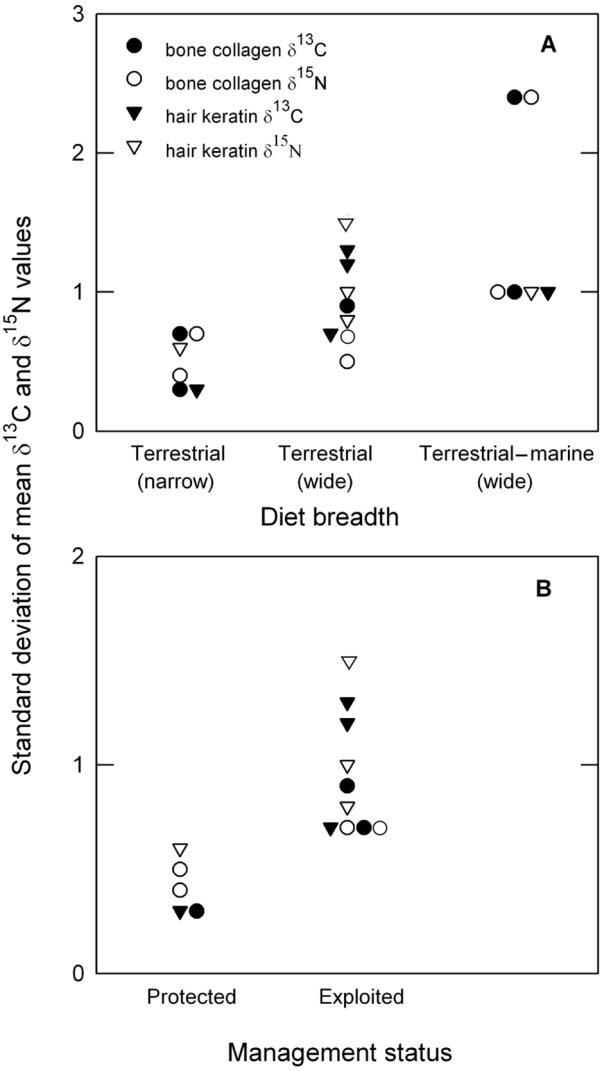
In any study that involves destructive sampling of zoological or paleontological specimens, the experimental design must balance the need to minimize the impact on individual specimens and collections against the need for sufficiently large sample sizes to address ecological and paleoecological questions adequately (e.g., statistically). Clementz and Koch (2001) used a resampling method to address this question using carbon and oxygen isotope measurements on tooth enamel from 42 individuals of black-tailed deer (*Odocoileus hemionus hemionus* (Rafinesque, 1817)) from a population around Monterey, California. The method used suggested that 5–10 samples from a population with the variance structure of the Monterey deer were sufficient to get a decent estimate of the population mean and standard error, but the method did not have a quantitative measure of "decency" of the estimate in terms of statistical confidence. To examine the minimum sample size necessary to represent with a stated degree of confidence the mean isotope composition of a population, we modified the method of Clementz and Koch (2001) and used a standard bootstrapping approach to determine the minimum sample sizes necessary to represent the mean of the $\delta^{13}C$ and $\delta^{15}N$ values for each tissue in the Minnesota wolf data set.

Clementz and Koch (2001) found that the standard error on the normalized estimate of the mean $\delta^{13}C$ value of the whole data set of black-tailed deer dropped to 0.01‰ for $n_{sub} = 5$ and the standard error on the estimate of the mean deer $\delta^{18}O$ value dropped to 0.01‰ for $n_{sub} = 4$. Using our bootstrapping routine on the black-tailed deer data set of Clementz and Koch (2001) confirms their results and corroborates ours for the Minnesota wolf data. Despite the larger size of the deer data set, more than 95% of subsamples with $n_{sub} = 4$ were within 1 SD of the mean $\delta^{13}C$ and $\delta^{18}O$ values of the whole data set, and for subsamples with $n_{sub} = 10$ ($\delta^{13}C$) and 11 ($\delta^{18}O$), all 1000 subsamples were within 1 SD of the mean value. Thus, we concur with the recommendation of Clementz and Koch (2001) that minimum sample size of wild populations of large-bodied, terrestrial mammals for stable isotope analysis be set at five and increased as materials and curators allow.

North American wolf populations: comparisons of collagen and hair $\delta^{13}C$ and $\delta^{15}N$ values

Intrapopulation isotopic variability may provide a good approximation of dietary breadth in wolves (Urton and Hobson 2005). When we compare the Isle Royale and Minnesota wolf data to those collected from other North American wolf populations, we uncover predicable differences in $\delta^{13}C$ and $\delta^{15}N$ variability based on inferred or observed dietary patterns (Table 5, Fig. 7). The wolves with the lowest variances, Isle Royale and Prince Albert National Park (PANP), are geographically isolated and protected populations; at the population level, these wolves are specialized predators of locally abundant prey. In contrast, coastal wolf populations exhibit high variances owing to feeding on a range of isotopically distinct food sources across the marine–terrestrial interface (Szepanski et al. 1999; Darimont and Reimchen 2002).

Fig. 7. Plot of published standard deviations for collagen and hair $\delta^{13}C$ and $\delta^{15}N$ values for North American gray wolf populations (see Table 5). (A) Values categorized by diet breadth. (B) Values categorized by management status. Note different *y*-axis scales between panels.



Wolf populations feeding in complex terrestrial food webs (Minnesota, Saskatchewan, Ontario, interior Alaska) have levels of variance that are intermediate between the two endmembers described above. At the individual level, carnivores feeding in complex food webs are either opportunistic predators of several types of prey or specialists on a particular prey type. It is possible for this difference in individual foraging ecology to be reflected in the population-level variance of stable isotope values measured in tissues that form over long time intervals (e.g., collagen and long sections of hair). A population of opportunistic predators (type-A generalist population) should have lower variances in collagen and hair isotope values than a population of predators consisting of individuals specialized on different prey (type-B generalist population) (Bearhop et al. 2004). Determining if a wolf population is a type-A or type-B generalist population can be important for wildlife management decisions and conservation efforts. Urton and Hobson (2005) proposed that exploitation of wolves and their prey facilitate the

breakdown of wolf social structure, which may lead to the more individualistic foraging behaviors of a type-B generalist population. Habitat loss or fragmentation is expected to have a similar effect on wolf foraging ecology (Darimont et al. 2004). Among the North American wolf populations feeding in complex terrestrial food webs, the Minnesota wolves have more variable collagen isotope values than the interior Alaska and central Ontario populations. This suggests the Minnesota wolves are relatively more like type-B generalists, and interior Alaska and Ontario wolves are relatively more like type-A generalists. The exploited wolf populations of Saskatchewan (outside PANP and La Ronge) have the highest hair variances of the noncoastal populations, indicating that they are type-B generalist populations. Although a number of complicating factors can arise when interpopulation comparisons are made across broad spatial and environmental scales, we find that relative differences in the magnitude of isotopic variance among wolf populations appear to reflect long-term differences in foraging ecology. Interpopulation (both spatial and temporal) comparisons of stable isotope variances may be a useful tool for monitoring the direct and indirect impacts of exploitation and habitat destruction upon carnivore foraging ecology.

Conclusions

1. The collagen $\delta^{13}C$ (detrended) and $\delta^{15}N$ values of Isle Royale wolves have not varied spatially or temporally for the past 30 years, indicating that their diet and dietary breadth have remained constant. The difference in collagen $\delta^{15}N$ values of moose from the east and the west ends of Isle Royale has persisted for the past 35 years. This pattern is likely driven by variation in soil type and development, and not the relative contribution of aquatic versus terrestrial plants to the summer diet of the moose. The beavers of Isle Royale have variable $\delta^{13}C$ and $\delta^{15}N$ values and are isotopically distinct from moose.
2. Carnivore-specific collagen $\varepsilon^*_{\text{wolf–diet}}$ values calculated from the Isle Royale wolves, moose, and beaver data are 1.3‰ ± 0.6‰ for $\delta^{13}C$ and 4.6‰ ± 0.7‰ for $\delta^{15}N$. These are the first trophic fractionations to be reported for a wild carnivore with a fully constrained diet.
3. When the diets of late-Pleistocene dire wolves from the La Brea tar pits are reconstructed using carnivore-specific trophic enrichment values, we find that they were consuming a range of megafauna prey. The same reconstruction done with general trophic enrichment values underestimates the contribution of horse to dire wolf diet and overestimates the contribution of grazers.
4. For $\delta^{13}C$ and $\delta^{15}N$ measurements of all three tissues (bone, muscle, hair) of the Minnesota wolves, more than 95% of subsamples of $n_{\text{sub}} \geq 4$ had mean values within 1 SD of the mean of the whole data set.
5. Our comparison of published collagen and hair $\delta^{13}C$ and $\delta^{15}N$ values for North American gray wolf populations shows that interpopulation differences in isotope variances provide insight into wolf foraging ecology. Wolves feeding in both marine and terrestrial food webs have the highest variances. Among the wolf populations feeding purely in terrestrial food webs, the protected populations are specialized predators of a preferred prey type(s) and have the lowest variances, whereas exploited populations are generalist predators and have the highest variances.

Stable isotope analyses will continue to be an important and practical tool for studying modern and ancient carnivores, such as gray wolves and dire wolves. Establishing carnivore-specific baseline isotopic parameters and patterns will also continue to be a challenge, but can be accomplished through future studies of well-researched wild populations or captive colonies of carnivores.

Note added in proof

While the bone collagen $\delta^{13}C$ and $\delta^{15}N$ values of northern Minnesota wolf WJP-887 (Table S2[2]) are analytically sound, the $\delta^{15}N$ value of this individual affects the population-level variation in bone collagen values. To present these data in a more conservative manner, we removed this wolf from the subsampling and statistical analyses, resulting in slight modifications to the Results section compared with that of the original manuscript. This removal changed neither the significance of the results nor the conclusions drawn from the northern Minnesota wolf bone collagen data set.

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