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Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears

Laura A. Felicetti, Charles C. Schwartz, Robert O. Rye, Mark A. Haroldson, Kerry A. Gunther, Donald L. Phillips, and Charles T. Robbins

Abstract: Whitebark pine (*Pinus albicaulis*) is a masting species that produces relatively large, fat- and protein-rich nuts that are consumed by grizzly bears (*Ursus arctos horribilis*). Trees produce abundant nut crops in some years and poor crops in other years. Grizzly bear survival in the Greater Yellowstone Ecosystem is strongly linked to variation in pine-nut availability. Because whitebark pine trees are infected with blister rust (*Cronartium ribicola*), an exotic fungus that has killed the species throughout much of its range in the northern Rocky Mountains, we used stable isotopes to quantify the importance of this food resource to Yellowstone grizzly bears while healthy populations of the trees still exist. Whitebark pine nuts have a sulfur-isotope signature (9.2 ± 1.3‰ (mean ± 1 SD)) that is distinctly different from those of all other grizzly bear foods (ranging from 1.9 ± 1.7‰ for all other plants to 3.1 ± 2.6‰ for ungulates). Feeding trials with captive grizzly bears were used to develop relationships between dietary sulfur-, carbon-, and nitrogen-isotope signatures and those of bear plasma. The sulfur and nitrogen relationships were used to estimate the importance of pine nuts to free-ranging grizzly bears from blood and hair samples collected between 1994 and 2001. During years of poor pine-nut availability, 72% of the bears made minimal use of pine nuts. During years of abundant cone availability, 8 ± 10% of the bears made minimal use of pine nuts, while 67 ± 19% derived over 51% of their assimilated sulfur and nitrogen (i.e., protein) from pine nuts. Pine nuts and meat are two critically important food resources for Yellowstone grizzly bears.

Résumé : Le pin albicaule (*Pinus albicaulis*) est un arbre à glandée qui produit des noix relativement grosses et riches en graisses et en protéines, dont se nourrissent les grizzlis (*Ursus arctos horribilis*). Les arbres donnent des récoltes abondantes certaines années, pauvres d'autres années. La survie des grizzlis de l'écosystème du Grand Yellowstone est étroitement liée aux variations de la disponibilité des noix de pin. Le pin albicaule est sujet aux infections de rouille vésiculaire du pin (*Cronartium ribicola*), un champignon exotique qui a décimé les populations sur une grande partie de la répartition de l'espèce, dans le nord des Rocheuses; c'est pourquoi nous avons utilisé des isotopes stables pour quantifier l'importance de cette ressource alimentaire pour les grizzlis tandis qu'il existe encore des populations de pins en bonne santé. Les noix du pin albicaule ont une signature d'isotopes du soufre de 9,2 ± 1,3 ‰ (moyenne ± 1 écart type) qui diffère de celles que l'on retrouve dans les autres aliments du grizzli dont les signatures de soufre se situent entre 1,9 ± 1,7 ‰ pour les autres plantes et 3,1 ± 2,6 ‰ pour les ongulés. Des expériences alimentaires sur des grizzlis en captivité ont servi à identifier les relations entre les signatures d'isotopes du soufre, du carbone et de l'azote dans la nourriture et celles du plasma des ours. Les relations du soufre et de l'azote ont servi à estimer l'importance des noix de pin chez des grizzlis libres dans leur milieu à partir d'échantillons de sang recueillis entre 1994 et 2001. Les années de production réduite de noix de pin, 72 % des grizzlis maintenaient minimale leur consommation de noix de pin. Les années d'abondance, 8 ± 10 % des ours utilisaient peu les noix de pin, alors que 67 ± 19 % des ours assimilaient plus de 51 % de leur soufre et de leur azote (i.e. protéines) à partir de noix de pin. Les noix de pin et la viande sont des ressources alimentaires essentielles aux grizzlis de Yellowstone.

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Introduction

Whitebark pine (*Pinus albicaulis*), a masting species with a fat- and protein-rich nut, produces significant cone and nut crops at irregular intervals in the northern Rocky Mountains (Lanner and Gilbert 1994). Annual cone production varies from as many as 303 to no cones per tree (Haroldson 2000). Consumption of pine nuts by grizzly bears (*Ursus arctos horribilis*) in the Greater Yellowstone Ecosystem (GYE) is considerable in years of abundant crops (Mattson et al. 1991). Most of the nuts consumed by bears are from cones excavated from the food middens of red squirrels (*Tamiasciurus hudsonicus*) (Kendall 1983; Mattson and Reinhart 1994). As an indication of the importance of whitebark pines to grizzly bears in the GYE, grizzly bear mortality is 1.8–3.3 times greater in years of poor nut production (Mattson 1998). During years when they feed heavily on pine nuts, grizzly bears range in high mountainous areas distant from roads and human facilities. During years when pine nuts are unavailable, bears forage in lower elevation habitats and search for alternative foods near humans, with resulting conflicts and elevated mortality (Mattson et al. 1992).

Whitebark pine in the GYE is infected with an exotic fungus, white pine blister rust (*Cronartium ribicola*) (Kendall and Keane 2001). In many ecosystems in the western United States and Canada, 50–100% of the extant whitebark pine are either dead or dying. Recent surveys suggest that the rust is spreading in the GYE (Kendall and Keane 2001). Loss of whitebark pine has the potential to impose significant nutritional stress on the threatened Yellowstone grizzly bear.

Quantifying the nutritional importance of pine nuts to individuals and thus the potential ecological effects of their decline to this population of grizzly bears has been difficult. Uncorrected fecal prevalence has been used previously as an indicator of food habits and the nutritional importance of pine nuts to Yellowstone grizzly bears (Mattson et al. 1991), but nondigested fecal residues can be a good indicator of either nutritional importance or its antithesis. Similarly, fecal prevalence gives only a broad population average and does not indicate what percentage or component of the population is making significant use of the resource. Stable isotopes, particularly carbon and nitrogen, have become important tools in estimating assimilated diets of numerous wild animals, including bears (e.g., Hobson and Welch 1992; Hilderbrand et al. 1996). In this study, we used both sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to grizzly bears in the GYE. Preliminary studies (Rye et al. 2002; Chaffee et al. 2003) indicated that sulfur-isotope ratios might be particularly useful tracers of pine-nut consumption.

Methods

Study area

The GYE includes Yellowstone and Grand Teton national parks and adjacent federal, state, and private lands in portions of Montana, Wyoming, and Idaho. The GYE contains the headwaters of three major continental-scale river systems: the Missouri and Mississippi, Snake and Columbia, and Green and Colorado. Long cold winters and short summers characterize the climate of the GYE. Grizzly bears use habitats that range from 1500 to 3600 m. At low elevations,

foothill grasslands or shrub steppes occur. With increasing moisture, open stands of Rocky Mountain juniper (*Juniperus scopulorum*), limber pine (*Pinus flexilis*), and Douglas-fir (*Pseudotsuga menziesii*) occur. Lodgepole pine (*Pinus contorta*) dominates at mid-elevations where poor soils formed from rhyolite predominate. With increasing elevation, spruce–fir or subalpine forests dominate. Engelmann spruce (*Picea engelmannii*) and whitebark pine form the upper tree line. Alpine tundra occurs at the highest reaches of all major mountain ranges (Patten 1963; Waddington and Wright 1974; Despain 1990).

Field collections of bear foods

Major plant and animal foods consumed by grizzly bears (Mattson et al. 1991) were collected throughout the GYE to determine if whitebark pine nuts have a unique isotopic signature relative to other foods. Plant samples were collected at sites used by radio-collared grizzly bears and included whitebark pine nuts; the foliage of clover (*Trifolium* spp.), horsetails (*Equisetum arvense*), elk thistle (*Cirsium scariosum*), cow parsnip (*Heracleum lanatum*), dandelion (*Taraxacum* spp.), spring beauty (*Claytonia lanceolata*), sedges (*Carex raynoldsii* and *C. praticola*), and grasses (*Bromus anomalous*, *Phleum alpinum*, *Agropyron caninum*, *Poa* spp., and *Festuca idahoensis*); and the bulbs or roots of oniongrass (*Melica spectabilis*), biscuitroot (*Lomatium* spp.), and yampa (*Perideridia gairdneri*). Fleshy fruits or berries are not significant grizzly bear foods in the GYE and therefore were not collected (Mealey 1975; Kendall 1983; Mattson et al. 1991). Collected animal matter included army cutworm moths (*Euxoa auxiliaris*) at alpine aggregation sites, cutthroat trout (*Oncorhynchus clarki*) in spawning streams around Yellowstone Lake, and bison (*Bison bison*), elk (*Cervus elaphus*), and mule deer (*Odocoileus hemionus*) from throughout the park where they had been killed in collisions with cars. All foods were stored frozen at –20°C.

Feeding trials using captive grizzly bears

Feeding trials were used to determine the fractionation occurring between the carbon-, nitrogen-, and sulfur-isotope signatures of the diet and that of bear plasma. Plasma samples were used rather than hair or other tissues, because plasma isotopic signatures equilibrate (i.e., become asymptotic) with the diet within 10–14 days and have the same isotopic signatures as hair (Hilderbrand et al. 1996). Six (three male and three female siblings) 16- to 21-month-old grizzly bears housed at the Washington State University Bear Research, Education, and Conservation Facility in Pullman, Washington, were used. Two were born in captivity, two were wild-caught from the GYE, and two were wild-caught from the Northern Continental Divide Ecosystem. Bears ranged in mass from 40 kg in the spring to 100 kg in the fall. Each bear was fed each of 5 diets (Table 1) for 21 days. Blood-plasma samples were collected at the end of each trial and frozen. Diet samples were collected daily and pooled and homogenized at the end of the trial for isotopic analyses.

Field collection of bear tissues

Grizzly bear hair and blood samples were collected from the GYE between 1994 and 2001, as part of ongoing natural-history, population-monitoring, and habitat studies conducted

Table 1. Isotopic signatures (‰) of diets fed to captive grizzly bears (*Ursus arctos horribilis*) during 21-day feeding trials to determine the isotope enrichments occurring between diet and consumer plasma.

Diet	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Chinook salmon (*Oncorhynchus tshawytscha*)	−20.1	11.2	19.5
Apples (*Malus* sp.)	−26.7	0.7	7.7
Commercial bear chow	−19.0	3.8	−0.7
Pelleted chow			
15.4% protein	−22.2	2.3	2.2
3.3% protein	−22.8	3.6	3.0

Note: Diet composition for the two pelleted diets can be found in Felicetti et al. (2003).

by the Interagency Grizzly Bear Study Team (Schwartz and Haroldson 2002). Hair samples were collected from live-captured bears and mortalities (e.g., bears killed by humans) and stored at room temperature in paper envelopes (Haroldson and Anderson 1997). Blood samples, collected from live-captured bears, were centrifuged, separated into plasma and red blood cells, and stored frozen.

Although hair and blood samples were available from May through November of each year, our initial step in relating the isotopic signatures of the bears to the foods available in a specific year, particularly pine nuts, was to determine when the tissue was produced. While plasma samples reflect the isotopic signature of the foods consumed during the preceding 10–14 days and red blood cells that of the foods consumed during the past 3 months (Hilderbrand et al. 1996), hair samples are much more ambiguous (Jacoby et al. 1999). Bears have one hair molt per year. That molt generally starts during very late spring and summer and continues into the fall when significant sources of dietary protein are available. Thus, mature full-length hair that was collected from early May to mid-June was produced during the preceding year and represented that diet. Hair collected in September, October, and November was produced during the current year, as the previous year's hair coat would have been completely replaced by new hair. Because we could not be certain which year was represented by hair collected in late June, July, and August, these samples were not used in this study. Ultimately, hair or blood samples from 77 different bears met the necessary criteria and were used in this study.

Whitebark pine cone counts

Yearly production of cones (number of cones/tree) in the GYE was determined along 19 transects each fall before cone maturation in mid-July (Haroldson and Podruzny 2002). We used these cone counts from years corresponding to blood and hair sampling to develop relationships between cone production and bear isotopic signatures. Typically, pine nuts are available only in the fall if the crop is small, but are also available the following spring and summer if the crop is large (Kendall 1983; Mattson and Jonkel 1990; Mattson et al. 1991; Haroldson and Podruzny 2001).

Sample preparation and analysis

All blood and food samples were freeze-dried and ground prior to isotope analyses. Hair samples were treated with a 2:1 chloroform:methanol solution to remove oils (Hilder-

brand et al. 1996). Sulfur-isotope content and composition were determined either after Eschka extraction (e.g., plant samples containing low levels of sulfur) or directly without prior extraction (e.g., animal flesh or hair). All samples were weighed into tin boats and analyzed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ by continuous flow methods using a Carlo Erba NC2500 elemental analyzer coupled to either a Micromass Optima mass spectrometer or a Finnigan Delta Plus XL mass spectrometer (Fry et al. 1992; Giesemann et al. 1994; Kester et al. 2001). Results are reported as per mil (parts per thousand (‰)) ratios relative to PDB ($\delta^{13}C$) and atmospheric N ($\delta^{15}N$), with internal laboratory standards calibrated against ANU sucrose ($\delta^{13}C$ = −10.4‰), NBS 22 ($\delta^{13}C$ = −29.6‰), USGS 25 ($\delta^{15}N$ = −30.4‰), and USGS 26 ($\delta^{15}N$ = 53.7‰). Isotopic compositions of $\delta^{34}S$ are reported relative to CDT, using internal laboratory standards calibrated against NBS 127 ($\delta^{34}S$ = 21.1‰) and IAEA-S-1 ($\delta^{34}S$ = −0.3‰). Internal reproducibility based on hundreds of standards run over the last 5 years is ±0.2‰ for C, N, and S analyses. [PDB, Pee Dee Belemnite; ANU, Australian National University; NBS, National Bureau of Standards; USGS, U.S. Geological Survey; CDT, Canyon Diablo Troilite; IAEA, International Atomic Energy Agency.]

Model estimates of assimilated diet

Because the carbon-isotope signatures in bear plasma from the captive feeding trials did not track dietary carbon signatures as well as nitrogen and sulfur plasma signatures tracked their respective dietary signatures (see Results and Discussion), only nitrogen and sulfur isotopes were used to estimate the dietary contribution of pine nuts for Yellowstone grizzly bears. With two isotopic ratios ($\delta^{15}N$, $\delta^{34}S$), unique solutions only exist for contributions of three or fewer sources when standard mixing models are used (Phillips and Gregg 2003). However, there were five major food sources for the Yellowstone bears (Table 2). Consequently, the IsoSource model described by Phillips and Gregg (2003) was used to find the range of feasible dietary contributions of each of these five food sources. First, the $\delta^{15}N$ and $\delta^{34}S$ isotopic signatures of each food source were adjusted to reflect their corresponding bear plasma isotopic signatures, as shown in Fig. 1. In the IsoSource model, all combinations of food-source contributions summing to 100% were examined in increments of 1%. For each combination, the resultant predicted bear $\delta^{15}N$ and $\delta^{34}S$ signatures were compared with the observed signatures. If they matched within 0.1‰ for both elements, that combination of the five food sources represented a feasible solution. The range of all such feasible solutions indicated the minimum and maximum contributions for each food source that were consistent with isotope mass balance. While this model approach could be extended to include concentration-dependent effects, as outlined in Phillips and Koch (2002), we did not incorporate concentration-dependent effects, as (*i*) we were interested in quantifying the relative contribution of pine nuts to the bears' total sulfur and nitrogen pools rather than estimating food habits or concentration-weighted assimilated biomass and, (*ii*) many of the intake and metabolic variables necessary to use a concentration-dependent model could not be quantified (Robbins et al. 2002).

These analyses were performed on two different data sets of isotopic signatures. Yearly mean bear $\delta^{15}N$ and $\delta^{34}S$ signatures

Table 2. Mean stable isotope signatures (‰; mean ± SD) for major foods consumed by grizzly bears in the Greater Yellowstone Ecosystem.

Sample	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Whitebark pine nuts	−24.0 ± 1.2 a (18)	−1.0 ± 1.1 a (9)	9.2 ± 1.3 a (6)
All other plant matter	−27.9 ± 1.4 b (15)	−1.0 ± 2.6 a (13)	1.9 ± 1.7 b,c (15)
Army cutworm moths	−26.1 ± 0.5 c (21)	6.4 ± 2.0 b (20)	1.3 ± 2.2 b (3)
Ungulates	−23.6 ± 0.6 a (17)	4.4 ± 0.6 c (17)	3.1 ± 2.6 c (12)
Cutthroat trout	−22.4 ± 2.3 d (13)	8.5 ± 0.8 d (13)	2.0 ± 0.9 b,c (10)

Note: Values in parentheses are sample size. Values in each column followed by different letters are significantly different ($P < 0.01$), while those followed by the same letter are not.

Fig. 1. The relationships between diet and plasma stable-isotope signatures for grizzly bears (*Ursus arctos horribilis*; current study), American black bears (*Ursus americanus*; Hilderbrand et al. 1996), and polar bears (*Ursus maritimus*; Hobson and Welch 1992). The carbon regression is for single-species diets only, whereas the nitrogen and sulfur relationships are for both single-species and mixed diets. Carbon and nitrogen regressions are for all three species of bears. Sulfur data are available only for grizzly bears in the current study.
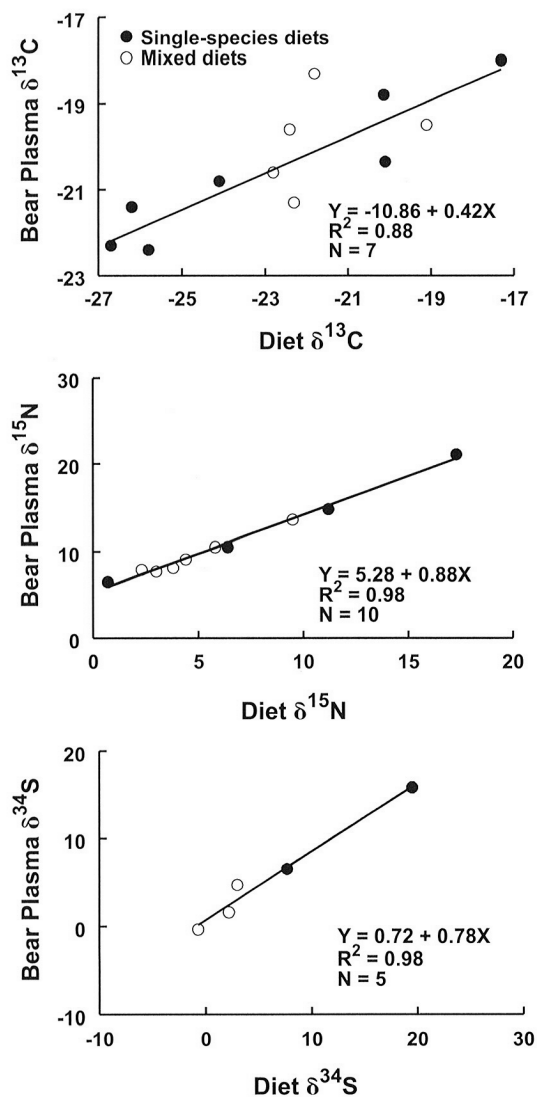
were analyzed, to characterize overall population differences in pine-nut utilization between years. Isotopic signatures for individual bears within each year were also analyzed, to characterize individual variation in pine-nut utilization. For individual bears, the mean solution for pine-nut dietary contribution was categorized by quartiles (i.e., 0–25, 26–50, 51–75, or 76–100% dietary content), and the distributions of these quartiles were compared among years of good and poor pine-nut availability.

Statistical analyses

Linear least-squares regression (PROC REG; SAS Institute Inc. 1998) was used to model the isotope relationships. We used ANOVA and least squares means to test for differences between the carbon-, nitrogen-, and sulfur-isotope signatures of the diets and dietary components of Yellowstone grizzly bears (PROC GLM and LS Means; SAS Institute Inc. 1998).

Results

Isotopic signatures of Yellowstone bear foods

The sulfur-isotope signature of whitebark pine nuts is unique, with values ranging from 6.1 to 7.9‰ higher than those of all other food groups analyzed in the ecosystem ($F = 59.1$, $P < 0.0001$; Table 2) and 4.7‰ higher than those of any other individual plant isotopic signature. The total sulfur content of pine nuts (0.035 ± 0.006% of the dry matter) is similar to that of other plants (0.11 ± 0.17%, ranging from 0.02 to 0.58%) and army cutworm moths (0.07%), but is 28 times less than that of cutthroat trout and ungulates (~1%). Pine nuts have a nitrogen-isotope signature that is identical with the mean nitrogen-isotope signature for other plants in the ecosystem but significantly lower than the nitrogen-isotope signatures of the various meat resources ($F = 116.6$, $P < 0.0001$). The carbon-isotope signature of pine nuts, while lower than the carbon-isotope signatures of most other plants ($F = 48.8$, $P < 0.0001$), is similar to the carbon-isotope signatures of the various meat resources ($F = 48.8$, $P = 0.9933$).

Captive-bear feeding trials

Nitrogen- and sulfur-isotope signatures of bear plasma closely tracked the isotopic signature of the diet, even when mixed diets were fed (Fig. 1). There was much more variation in the diet to plasma carbon isotope relationship (Fig. 1). Plasma $\delta^{15}N$ signatures were enriched from 3.2 to 5.0‰ across the range of diets fed, whereas plasma $\delta^{34}S$ signatures ranged from slightly enriched (0.9‰) for the lowest

Fig. 2. The relationship between mean whitebark pine cone production in specific years and mean annual free-ranging grizzly bear hair or plasma isotopic signatures produced in the same years for the Greater Yellowstone Ecosystem. Data for 2000 were omitted because of the extensive use of pine nuts that overwintered from the bumper crop produced in 1999.
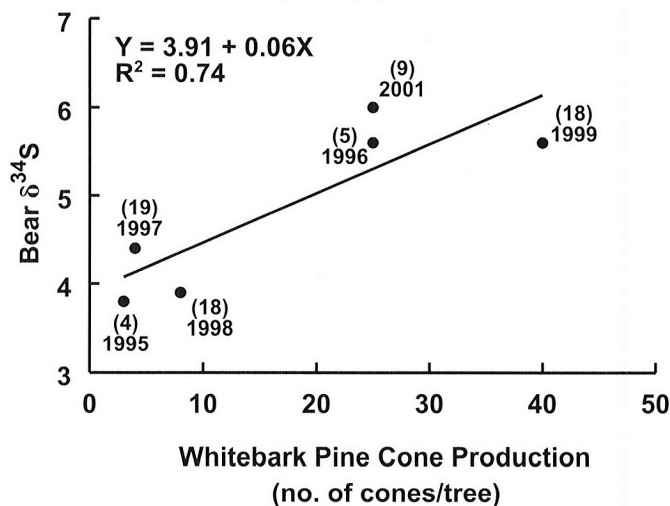
dietary sulfur isotope signatures to depleted (−3.6‰) for the highest dietary sulfur isotope signatures.

Yellowstone pine-nut production and bear isotopic signatures

Mean cone production ranged from 3 cones per tree in 1995 to 40 cones per tree in 1999. Cone production was very poor in 1995, 1997, 1998, and 2000 and of intermediate size in 1996 and 2001 but, in 1999, the second best crop in 20 years was produced.

With one exception, the mean yearly $\delta^{34}S$ signature for grizzly bears increased with an increase in cone production (Fig. 2). The one exception to this relationship between cone production and grizzly bear $\delta^{34}S$ occurred in 2000, when mean cone production was 6 cones per tree and the mean yearly $\delta^{34}S$ signature for grizzly bears was the highest measured (7.8‰; Fig. 3). Pine nuts were estimated to have provided over 76% of the assimilated sulfur and nitrogen for 80% of the bears (Fig. 4). Because the grizzly bear samples for the year 2000 constituted hair that was collected in September 2000 and May 2001, the above signature represents the diet for virtually the entire year.

To determine if the high sulfur-isotope signature in 2000 was real and caused by elevated pine-nut intake, we hypothesized that the $\delta^{15}N$ signature of the same bears would be relatively low and reflective of a primarily herbivorous diet. Mean yearly $\delta^{34}S$ and $\delta^{15}N$ signatures for Yellowstone grizzly bears were inversely related, with bears in 2000 having the lowest signature measured (Fig. 3). The mean grizzly bear $\delta^{15}N$ signature (4.5‰) in 2000 was similar to the mean Yellowstone ungulate signature (4.4 ± 0.6‰) and to the estimated bear signature (4.0‰) that would have occurred for bears consuming a 100% plant-based diet (Table 2, Fig. 3).

Estimates of assimilated dietary pine nut content by Yellowstone grizzly bears

Estimated mean assimilated dietary content of pine nuts

Fig. 3. Mixing diagram for the major grizzly bear foods and the mean annual grizzly bear $\delta^{34}S$ and $\delta^{15}N$ isotopic signatures in the Greater Yellowstone Ecosystem. The isotopic signatures of the five food sources are labeled with letters. The prime letters at the ends of the broken lines indicate the shift in these food signatures to the hypothetical grizzly bear signatures that would occur if each food were consumed as the sole diet (calculated using the regression equations in Fig. 1).
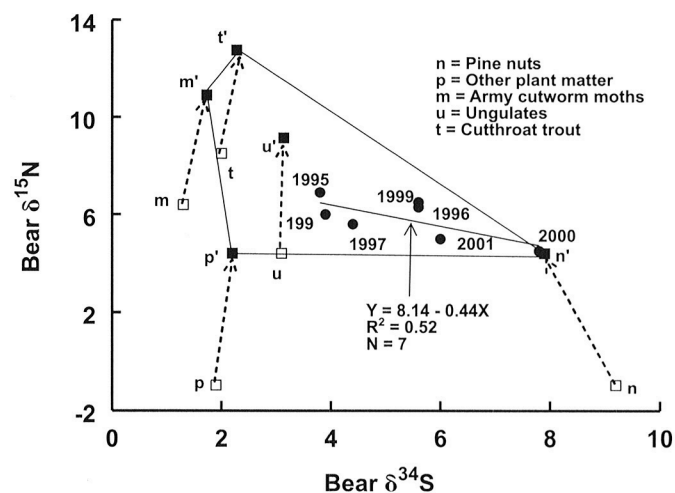

for the population ranged from 19% in 1995 (range of model estimates 11–26%) to 97% in 2000 (range of model estimates 96–100%). During years of poor cone production with minimal carryover of cones from the preceding year, 72% of the bears made minimal use of pine nuts (Fig. 4). During years of good cone production (1996, 1999, and 2001) and the single year with annually abundant cones (2000) produced during the preceding year, 8 ± 10% of the bears made minimal use of pine nuts while 67 ± 19% of the bears derived over 51% of their assimilated sulfur and nitrogen (i.e., protein) from pine nuts.

Discussion

Variation in the diet to plasma carbon isotope enrichments and the low slope of the regression observed in this study when single-species diets were fed (Fig. 1) warns of a significant problem in the use of this isotope to estimate assimilated diets (e.g., marine versus terrestrial or C_3 versus C_4 dietary divisions). The variation could be due to (i) the different chemical constituents of a particular food having different concentrations, carbon signatures, and digestibilities, so that the overall diet signature does not accurately represent the signature of the digested carbon compounds (Phillips and Koch 2002) or (ii) physiological processes within the animal that vary with age, sex, season, or a wide range of other variables that affect the routing or metabolism of individual carbon compounds (Ben-David and Schell 2001; Robbins et al. 2002). The latter source of variation may be most important, as the variation in diet to plasma enrichment occurring when the same highly digestible diet (e.g., salmon) was fed at different times was similar to the variation occurring within all other diets along the regression.

Nitrogen and sulfur isotopes may be much more useful for estimating assimilated diets, as their diet to consumer fractionation relationships have minimal variation and the slopes

Fig. 4. Assimilated dietary content of the sulfur and nitrogen from pine nuts in the diet of Yellowstone grizzly bears calculated using the mixing model of Phillips and Gregg (2003). Because the isotopic distribution of the individuals varied on the basis of the current and preceding year's crop of pine nuts, three different scenarios are shown. Minimal pine-nut availability occurred during the 2nd year of 2 successive years of poor cone production (1994 and 1995, 2 and 3 cones/tree, respectively; 1997 and 1998, 4 and 8 cones/tree, respectively). Intermediate or seasonal pine-nut availability occurred when either the preceding year's cone crop was adequate to provide some carryover into the following year and the current year's crop was poor (1996 and 1997, 25 and 4 cones/tree, respectively) or when the preceding year's crop was poor but the current year's crop was good or very good (1995 and 1996, 3 and 25 cones/tree, respectively; 1998 and 1999, 8 and 40 cones/tree, respectively; 2000 and 2001, 6 and 25 cones/tree, respectively). Annually abundant pine-nut availability occurred when the preceding year's crop was the second best in 20 years and cones were available throughout the entire following year, even though the current year's crop was poor (1999 and 2000, 40 and 6 cones/tree, respectively).
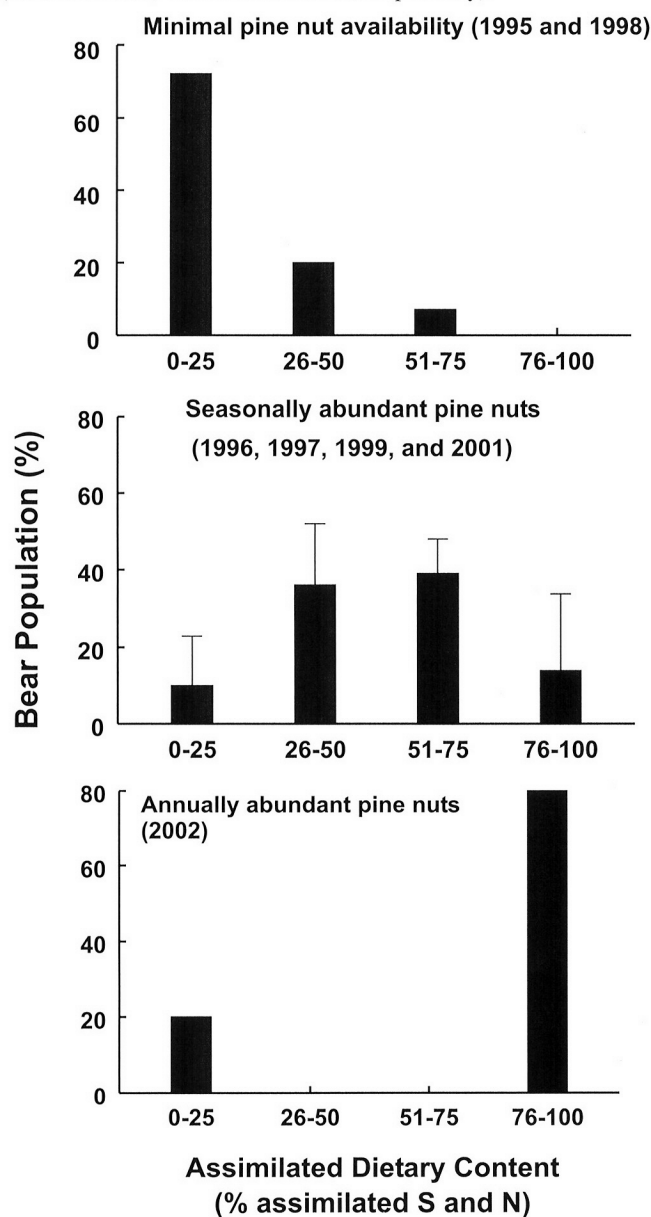
of their relationships are generally twice as large as that for carbon (Fig. 1). Sulfur may be particularly useful in making the marine versus terrestrial diet division for which carbon has been used previously. Plants and animals in terrestrial ecosystems (excluding immediate coastal areas that can be isotopically enriched) generally have $\delta^{34}S$ signatures that range from 2 to 6‰, whereas marine systems range from 17 to 21‰ (Peterson and Fry 1987; Kester et al. 2001).

The lack of variation in the nitrogen and sulfur fractionation relationships presumably occurs because the bulk of both nitrogen and sulfur in plants and animals occurs in amino acids and, therefore, protein (Izhaki 1993; Van Soest 1994; Yeoh and Wee 1994). Because protein is highly digestible in the low-tannin foods consumed by bears (Pritchard and Robbins 1990), the overall dietary $\delta^{34}S$ and $\delta^{15}N$ signatures are the same as the combined signatures of the absorbed amino acids. Additionally, in contrast with carbon, which can move repeatedly between all organic compounds in the animal, nitrogen and sulfur are largely restricted to proteins and are excreted when no longer serving that role. Thus, there may be little chance for either dietary or physiological processes to alter nitrogen and sulfur signatures beyond the initial fractionation.

Our quantification of the importance of pine nuts to Yellowstone grizzly bears supports the earlier conclusions of Mattson et al. (1991) and Mattson and Reinhart (1994) that were based on the frequency of pine-nut residues in bear feces. However, the value of pine nuts to the energy budgets of Yellowstone grizzly bears is much greater than is indicated by the change in $\delta^{34}S$ in the bears. On a dry-matter basis, 28 times more pine nuts (0.035 ± 0.006% sulfur) than cutthroat trout or ungulates (~1% sulfur) must be consumed to provide the same amount of isotopically labeled sulfur. When such large amounts of pine nuts replace meat, as is indicated by the inverse relationship between $\delta^{34}S$ and $\delta^{15}N$ in Fig. 3, the amount of pine nuts provides 14 times more energy than meat, as pine nuts have approximately the same gross energy content as meat on a dry-mass basis (27 kJ/g for pine nuts relative to 22–31 kJ/g for cutthroat trout and ungulates) but are half as digestible as meat (50% for pine nuts relative to 94% for cutthroat trout and ungulates) (Pritchard and Robbins 1990; Lanner and Gilbert 1994). These and earlier dietary estimates and the increased mortality of bears when pine nuts are not available indicate that pine nuts and meat are two critically important foods for Yellowstone grizzly bears (Mattson et al. 1991; Mattson and Reinhart 1994; Mattson 1998; Jacoby et al. 1999).

Although the results from 2000 did not fit the simple pattern observed in other years, cone production in 1999 was the second highest observed between 1980 and 2000. Cones produced in 1999 were still plentiful in 2000, and grizzly bear scats were dominated by pine-nut residues throughout the spring and summer of 2000 (Haroldson and Podruzny 2001). A very similar pattern was reported by Mattson et al. (1991) in their 11-year study, when Yellowstone bears made the greatest use of pine nuts during 1979. Cone production in 1978 was the highest observed up to that time, and crop size was "moderate" in 1979 (Kendall 1983; Mattson et al. 1991). Because many pine nuts overwintered in the cones stored in red squirrel middens between 1978 and 1979, residues from pine nuts "composed most of the fecal matter in

May through October" of 1979 and "... pine nuts were used to the near exclusion of other foods" (Mattson et al. 1991). Thus, the levels of pine-nut consumption reported by Mattson et al. (1991) and Haroldson and Podruzny (2001) in the year following a bumper crop would produce the hair isotopic signatures we observed in 2000.

In summary, pine nuts and other critically important foods for Yellowstone grizzly bears are threatened by humans or introduced diseases and organisms (Kendall 1983; Mattson et al. 1991; Jacoby et al. 1999; current study). For example, introduced lake trout (*Salvelinus namaycush*), which spawn in deep lake waters and are not available to bears, threaten stream-spawning Yellowstone cutthroat trout that are available; an expanding wolf population within the GYE, sport-hunting outside the Park, and agricultural-based control programs for brucellosis (*Brucella abortus*) may reduce bison and elk herds; agricultural practices in the Great Plains may threaten the numbers of army cutworm moths that migrate to Yellowstone National Park each summer; and white pine blister rust threatens whitebark pine nut production. Although recent trends indicate that the GYE grizzly bear population has increased (Haroldson and Schwartz 2002) and expanded in distribution (Schwartz et al. 2002), one has to be concerned about the future of the various food resources used by Yellowstone grizzly bears (Reinhart et al. 2001; Mattson and Merrill 2002). The stable-isotope technology demonstrated in this study provides the first opportunity to link the dynamics of reproduction and survival of individual grizzly bears and the overall dynamics of the GYE grizzly bear population to the use of these changing food resources.

Acknowledgements

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