FRUIT, NUTS, AND FISH: THE IMPORTANCE OF THREE FOODS TO GRIZZLY BEARS

By

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Lastly, and most importantly, I thank my family. Thanks to my mom and dad for their continual mental, emotional and let's not forget financial support. Thanks for suffering through graduate school with me. Finally, we are finished. Ok, maybe not.

FRUIT, NUTS, AND FISH: THE IMPORTANCE OF THREE FOODS TO GRIZZLY BEARS

Abstract

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December 2003

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The following dissertation contains three manuscripts. The first chapter investigated how dietary protein content alters energy expenditure and composition of mass gain in grizzly bears. Bears fed low-protein diets ad libitum gained up to 3 times the fat of bears consuming the higher protein diet and gaining mass at the same rate. Thus, bears eating fruit can either consume other foods to increase dietary protein content and reduce energy expenditure, intake, and potentially foraging time, or overeat high fruit diets and use dietary-induced thermogenesis and fat synthesis to deal with their skewed energy to protein ratio.

The second chapter is an application of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. 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 from pine nuts.

The third chapter used naturally occurring mercury in Yellowstone Lake to quantify the importance of cutthroat trout to grizzly bears in the Yellowstone Ecosystem. Intake of cutthroat trout per year by the grizzly bear population was < 1% of the spawning population, and a fraction of that estimated by previous investigators. Males consumed 92% of all trout ingested by grizzly bears, which suggests that males dominate feeding sites on streams and preclude females from obtaining large quantities of fish.

The technology demonstrated in the whitebark pine and cutthroat trout studies 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 changing food resources. The study assessing the effects of low dietary protein levels on fat metabolism suggests that berries may be a valuable commodity for young bears trying to accumulate fat. These three papers combined provide managers with detailed information regarding dietary habits of bears in the wild that will hopefully help in the management of the species.

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GENERAL INTRODUCTION

The following dissertation contains three manuscripts. The first chapter investigated how dietary protein content alters energy expenditure and composition of the mass gain in grizzly bears. The second chapter is an application of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. The third chapter used naturally-occurring mercury in Yellowstone Lake, and therefore cutthroat trout, to quantify the importance of these fish to grizzly bears in the Yellowstone Ecosystem.

Each of these manuscripts has been or will be submitted for publication in peer-reviewed journals. Therefore, the format of each manuscript is in accordance with the requirements specified by the appropriate journal. Chapter one has been published in Physiological and Biochemical Zoology and is co-authored by my committee members C. Robbins, and L. Shipley. Chapter two has been published in Canadian Journal of Zoology and is co-authored by Charles C. Schwartz, Robert O. Rye, Mark A. Haroldson, Kerry A. Gunther, Donald L. Phillips, and Charles T. Robbins. C. Schwartz, M. A. Haroldson, and K. A. Gunther provided field samples from the Greater Yellowstone Ecosystem; R. Rye provided funding and laboratory expertise; D.Phillips provided a model to analyze portions of data. The third chapter will be submitted to the Canadian Journal of Zoology and is co-authored by Charles C. Schwartz, Robert O. Rye, Kerry A. Gunther, James G. Crock, Mark A. Haroldson, Lisette Waits, and Charles T. Robbins. C. Schwartz, M. A. Haroldson, and K. A. Gunther provided field samples from the Greater Yellowstone Ecosystem; R. Rye provided funding and laboratory expertise; and J. G. Crock and L. Waits conducted laboratory analysis.

Dietary protein content alters energy expenditure and composition of the mass gain in grizzly bears (Ursus arctos horribilis)

Abstract

Many fruits contain high levels of available energy but very low levels of protein and other nutrients. The discrepancy between available energy and protein creates a physiological paradox for many animals consuming high fruit diets as they will be protein deficient if they eat to meet their minimum energy requirement. We fed young grizzly bears both high-energy pelleted and fruit diets containing from 1.6 to 15.4 % protein to examine the role of dietary-induced thermogenesis and fat synthesis in dealing with high energy-low protein diets. Digestible energy intake at mass maintenance, where bears neither lose nor gain mass, increased 2.1 times and composition of the gain changed from primarily lean mass to entirely fat when the protein content of the diet decreased from 15.4% to 1.6%. Daily fat gain was up to 3 times higher in bears fed low-protein diets ad libitum as compared to bears consuming the higher protein diet and gaining mass at the same rate. Thus, bears eating fruit can either consume other foods to increase dietary protein content and reduce energy expenditure, intake, and potentially foraging time, or overeat high fruit diets and use dietary-induced thermogenesis and fat synthesis to deal with their skewed energy to protein ratio. These are not discrete options but a continuum that creates numerous solutions for balancing energy expenditure, intake, foraging time, fat accumulation, and ultimately fitness depending on food availability, foraging efficiency, and bear size and body condition.

Introduction

Many fruits are excellent energy sources because of their high concentration of soluble carbohydrates or fats, but the same fruits are frequently low in protein, specific amino acids, and many important vitamins and minerals (Izhaki and Safriel 1989; Rogers et al. 1990; Leighton 1993; Welch et al. 1997; Rode and Robbins 2000; Felicetti et al. 2000). This discrepancy in fruit between available energy and other nutrients, particularly protein, may require animals that eat large amounts of fruit to develop adaptive foraging or metabolic strategies (Raubenheimer and Simpson 1997). These strategies can range from 1) consuming mixed diets in which other dietary components are used to meet the protein, mineral, or vitamin needs not met by fruit, 2) evolving efficient systems for nutrient conservation that reduce requirements to the level that can be supplied by fruit when intake is regulated to meet minimum energy requirements, and/or 3) overeating in excess of minimum energy requirements and developing metabolic systems that shunt a significant amount of the energy into fat storage or heat production such that protein or other nutrient requirements can be met (Izhaki and Safriel 1989; Delorme and Thomas 1996; Korine et al. 1996; Raubenheimer and Simpson 1997; Rode and Robbins 2000; Witmer 2001).

North American grizzly bears are omnivores that feed heavily on seasonally available fruits during the late summer and fall in many interior areas where salmon or other more nutrient-rich foods are not available (McLellan and Hovey 1995; Welch et al. 1997). Thus, they must have developed one or more foraging or metabolic strategies for handling the high energy to protein ratio in fruits. Because grizzly bears do not have exceptionally low maintenance protein requirements (Pritchard and Robbins 1990;

Robbins 1993), Rode and Robbins (2000) hypothesized that grizzly bears could use either the mixed diet strategy or overeat energy, dissipate much of the excess energy in fruit as heat, and, thereby, meet minimum protein requirements. Bears apparently use both strategies. Energy intake for mass maintenance was up to 3.3 times higher when captive bears were fed fruit-only diets in comparison to high-protein (35%) pelleted diets (Rode and Robbins 2000). At the population level, free-ranging grizzly bears and black bears (U. americanus) with ad libitum access to fruit consumed mixed diets containing 13 ± 5% dietary protein to presumably minimize energy expenditure, intake, and foraging time (Rode and Robbins 2000).

Although captive bears can gain weight on fruit-only diets, Rode and Robbins (2000) did not measure the composition of the gain to determine if fat accumulation was a significant component of the bears' adaptive strategy. For small to medium-sized bears that are not limited by the rate of fruit harvesting (Welch et al. 1997), the ability to store excess energy as fat rather than dissipating that energy as heat would be beneficial in preparing for hibernation. In this study, we extended the work of Rode and Robbins (2000) by investigating the composition of the mass gain and the partitioning of excess energy intake between heat production and fat accumulation when captive grizzly bears consume high energy-low protein fruit and pelleted diets relative to higher protein pelleted diets. We also tested if diet form, water content, or bulk (fresh fruit, dried fruit, and pelleted diets) alter energy expenditure or composition of the gain.

Methods

Feeding trials

Six (3 male and 3 female siblings), 16– to 21–month-old grizzly bears housed at the Washington State University Bear Research, Education, and Conservation Facility in Pullman, WA were used in this study. Young bears were chosen to maximize the opportunity to observe dietary-induced fat accumulation caused by the consumption of high energy-low protein diets. Whereas older, mature bears have completed skeletal and lean mass growth and thus have a yearly cycle dominated by fat gain and loss, mass gain in young bears is predominantly lean mass when consuming protein-sufficient diets (Farley and Robbins 1994; Barboza et al. 1997; Hilderbrand et al. 1999). Bears ranged in mass from 40 kg in the spring to 100 kg in the fall, well below the mass of adult females (180 kg) and males (360 kg) in this facility. When bears were not being used in the study, they were fed a maintenance diet of salmon, apples, and commercial chow (21% crude protein, Command Chunk, Land O' Lakes Feeds, Seattle, WA 98119). This study was approved by the Washington State University Institutional Animal Care and Use Committee (Protocol No. 2731).

From May through October 2001, each bear was fed each of 4 diets (a high energy-low protein (3.3%) pelleted diet, a high energy-higher protein (15.4%) pelleted diet, fresh apples (Malus pumila, 3.5 % protein), and dried apples (1.6 % protein)) during separate 21-day trials (Table 1). No bear was fed two successive low-protein diets because of concern for the health consequences of a long-term protein deficiency. After each bear finished a low-protein diet trial, they were either switched to a higher protein diet trial or to the high-protein maintenance diet. The two pelleted diets were used to directly test the isolated effect of protein content. Fresh and dried apples were used to determine if the high water content or bulk of most wild fruits would increase energy metabolism relative to the dried, pelleted diets used in this and other studies as hypothesized by Rode and Robbins (2000). When fed the apple diets, bears were supplemented with the same vitamin and mineral mix and at the same level (3.6% of the diet on a dry weight basis) used in the pelleted diets in order to not confound the energy-protein questions with potential mineral or vitamin deficiencies (Table 1). All feeding levels were at or above mass maintenance because of our desire to understand the composition of the gain, although some bears voluntarily restricted intake to below maintenance. Each bear was housed individually in a 3.3 x 9.1 m pen containing an interior air-conditioned den and exterior run, fed a weighed amount of the test diet each day, and all rejected food was collected and weighed the following day before they received additional food. Dry matter content of each food was determined by drying sub-samples of fresh and rejected food at 100°C.

Body mass was determined using electronic scales weighing to the nearest 0.1 kg, and body composition was estimated using dual energy x-ray absorptiometry (DEXA) (Hologic Inc., Bedford, MA, Model QDR 4500A, software QDR version 11.1) at the beginning and end of each trial. We used DEXA analysis because it can be used repeatedly on the living animal and more accurately determines body composition than does water dilution or bioelectrical impedance (SE of DEXA body composition analyses is < 2%; Toll et al. 1994; Burkholder 2001). DEXA also gives very precise measurements across time and can detect diet-induced changes in body composition (Toll et al. 1994; Burkholder 2001).

Bears were fasted for 16 hrs prior to all measurements to ensure gastrointestinal emptying (Pritchard and Robbins 1990). The DEXA algorithm for the adult human whole body composition was used rather than that for the dog, cat or others because of the more similar morphology and tissue distribution between bears and humans (i.e., plantigrade feet with distally muscled limbs). Bear weights estimated from DEXA imaging and the adult human alogrithm averaged 98.5 ± 2.6% of that measured by the electronic scale.

To determine the nutritional content and digestibility of all diets, we conducted a series of 7-day total collection digestion trials using four bears per diet. Samples of fresh apples and dried apples were frozen, freeze-dried and ground. Pelleted rations were ground without further drying. All feces were oven-dried at 100°C and subsampled for grinding and analyses. All feeds and feces were analyzed for protein using Kjeldahl analysis (N X 6.25) and gross energy content using bomb calorimetry. All analyses were run in duplicate and corrected to a 100% dry matter basis (100°C). Although urine was not collected during the digestion trials, metabolizable energy coefficients as a percent of digestible energy have ranged from 93.4 to 98.3% when bears consume fruit or high protein vegetation (Pritchard and Robbins 1990).

Statistical analyses

Linear least-squares regression (PROC REG; SAS Institute Inc. 1998) was used to model the relationships between digestible dry matter intake, rate of gain, and composition of the gain. We used an analysis of covariance and contrasts (PROC GLM; SAS Institute Inc. 1998) to test for significant difference between regression slopes and y-intercepts.

We used 95% confidence intervals (CI) to test for differences between regression x-intercepts (Neter et al. 1990).

Results

Slopes (all t's < 1.06; all P's > 0.14) and intercepts (all t's < -1.05; all P's > 0.30) of regressions between intake and rate of gain by bears consuming the three high energy-low protein diets (pellets, fresh apples, and dried apples) did not differ. Thus, because neither dietary form, water content, nor bulk were determinants of maintenance energy intake or efficiency of gain, we combined the data for these diets into one regression (Fig. 1). Rate of gain by grizzly bears consuming high and low-protein diets increased linearly as digestible dry matter intake increased (Fig. 1). Maintenance intake increased 2.1-fold from 32 ± 12g (95% CI) dry matter intake/kg $^{0.75}$/day (144 kcal digestible energy/ kg$^{0.75}$/day) on the 15.4% pelleted-protein diet to 69 ± 3g (95% CI) dry matter intake/kg $^{0.75}$/day (305 kcal digestible energy/ kg$^{0.75}$/day) on all low-protein diets. The reduction in maintenance energy intake caused by increasing dietary protein content in high-energy diets was curvilinear with the relationship becoming asymptotic above approximately 15% dietary protein (Fig 2). The efficiency of gain (i.e., slope of the relationship between intake and gain) decreased 2.7-fold from 0.66 g gain/g digestible dry matter intake when consuming the 15.4% pelleted-protein diet to 0.24 g gain/g digestible dry matter intake on the low-protein diets (P < 0.0001).

Many of the bears gained mass on the low-protein diets (Fig. 1). That gain was entirely fat, as all animals lost lean mass, although lean mass stasis was approached at the highest levels of intake (Fig. 3a). In contrast, bears eating the pelleted diet containing

15.4% protein gained either purely lean mass at lower total mass gains or both lean mass and fat at higher total mass gains (Fig. 3b). At the highest intake of the 15.4% protein diet, the gain was 72% lean mass and 28% fat.

The rate at which bears gained lean mass was directly proportional to protein intake (Fig. 4). The rate at which bears gained fat was directly proportional to digestible energy intake and did not vary with dietary protein content (all t's < 1.31; all P's > 0.13; Fig. 5). Thus, lean mass and fat gains in young bears consuming high-energy diets with low to moderate levels of protein are controlled by two largely separate dietary cues, and the increased energy intake at mass maintenance in bears consuming low-protein diets equaled the energy content and inefficiency in the synthesis of the lean mass gained in bears consuming the 15.4% protein diet. Bears achieved the highest digestible energy intakes and therefore highest gains of fat on the low-protein diets (Fig. 5).

Discussion

The increased maintenance energy intake, decreased rate of gain per unit of feed intake, and increased fat accumulation by young grizzly bears that consumed high energy-low protein diets relative to higher protein diets are consistent with parts or all of earlier results on vertebrates (e.g., bears, bats, and domestic rats) and invertebrates (e.g., locusts) (White et al. 1994; Delorme and Thomas 1996; Zanotto et al. 1997; White et al 1998; Stock 1999; Du et al. 2000; Rode and Robbins 2000). The reduced efficiency of gain (g gain/g digestible dry matter intake) when bears consumed low-protein diets relative to the 15.4% protein diet is largely attributable to the much higher energy content of the gain on the low-protein diets (primarily fat at 9.1 kcal/g) relative to the mixture of fat and lean mass gain on the 15.4% protein diet (3.4 kcal/g) (Robbins 1993; Farley and Robbins 1994).

Rode and Robbins (2000) assumed that the higher maintenance intake of bears consuming high energy-low protein diets was entirely due to increased heat production. That assumption was based partially on the observation that bears consuming fruit ad libitum panted continuously, even when lying in shaded, air-conditioned dens; while bears consuming higher protein diets did not pant under similar conditions. However, that assumption is not correct as the composition of the bear changed at mass maintenance as lean mass was lost and replaced with an equal amount of fat (i.e., energy was accumulated at mass maintenance) (Fig. 3a). By multiplying the slope of Fig. 5 (0.06 g fat accumulated per kcal of digestible energy intake) by the energy equivalent of fat (9.1 kcal/g) and correcting digestible energy intake to metabolizable energy intake (metabolizable energy coefficient of fruit consumed by bears = 97.5% of the digestible energy), we estimate that 56% of the excess metabolizable energy intake at mass maintenance or above was stored as fat and 44% was lost as heat (Pritchard and Robbins 1990, Farley and Robbins 1994). Thus, very similar processes and efficiencies of fat synthesis and heat production are occurring at and above mass maintenance.

Free-ranging bears with ad libitum access to fruit can use either a mixed diet strategy to meet their protein requirement and reduce the energy cost of maintenance or consume a high fruit diet and effectively dump the excess energy into increased heat production and body fat accumulation. Whereas the earlier analyses of Rode and Robbins (2000) indicated that populations of bears use the mixed diet strategy to increase dietary protein content to the optimum level that reduces maintenance energy intake, we hypothesize that bears within a population may use different strategies. For example, small to medium-sized bears that are not limited by the rate of fruit intake (i.e., young bears of either sex and smaller adult females) could purposefully manipulate their body fat content by consuming a greater proportion of fruit than large bears whose daily intake is limited by harvesting rate (Welch et al. 1997). The increased dietary fruit content consumed by smaller, leaner bears would maximize fat accumulation and potentially survival during hibernation when fat is the major energy source (Barboza et al. 1997). This strategy would necessarily be very temporary during fall fruit abundance as young bears must have a yearly net increase in lean mass size. However, in contrast to the failure of bears in this study to maintain lean mass when consuming diets with 1.6 to 3.5% protein, ≥ 4% dietary protein would be adequate for maintaining lean mass at the maximum intakes observed in this study. That level of protein occurs in many wild fruits which would minimize the need for smaller bears to consume a mixed diet in order to maintain lean mass (Welch et al. 1997). Similarly, because water content of the fresh apples used in this study (82.7%) did not limit intake relative to the dried apples (Fig. 1) and is similar to the water content in many important wild berries (e.g., huckleberry (Vaccinium membranaceum) (85.4% water), soapberry (Shepherdia canadensis) (82.0%), or serviceberry (Amelanchier alnifolia) (70.8%)), maximum daily intake of wild fruits should be similar to the values measured in this study if harvest rate is not limiting (Welch et al. 1997).

Finally, nutritionists and nutritional ecologists have long studied energy and protein requirements in isolation (Robbins 1993). This and other similar studies indicate that for some species, energy and protein intake and requirements are more intimately connected than previously appreciated. The ability to over-consume energy to meet other nutrient requirements when consuming foods with unbalanced energy-to-nutrient ratios appears widespread in the animal kingdom (Raubenheimer and Simpson 1997; Zanotto et al. 1997; Stock 1999; current study). This recognition opens the opportunity for many exciting studies of energy and nutrient metabolism of captive animals and diet selection by free-ranging animals as they attempt to optimize energy and nutrient intake when minimum energy and nutrient requirements can not be met simultaneously (Raubenheimer and Simpson 1997).

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Table 1. Composition and apparent digestibility (100% dry matter basis) of four diets fed to captive grizzly bears (Rode and Robbins 2000; current study). All items are in percent except gross energy.

	Pelleted diets		Fruit diets	
Item	High protein	Low protein	Fresh apples	Dried apples
Cellulose	—	12.0	—	—
Soybean meal	19.4	—	—	—
Tapioca	45.5	53.4	—	—
Sawdust	6.0	6.0	—	—
Dry molasses	10.0	10.0	—	—
Corn	9.0	9.0	—	—
Tallow	5.0	5.0	—	—
Fish meal	1.5	1.0	—	—
Fresh apples	—	—	96.4	—
Dried apples	—	—	—	96.4
Limestone	0.4	0.4	0.4	0.4
TM salt w/ selenium	0.7	0.7	0.7	0.7
Dicalcium phosphate	1.0	1.0	1.0	1.0
Vitamin/mineral premix	0.3	0.3	0.3	0.3
Monosodium phosphate	0.9	0.9	0.9	0.9
Magnesium oxide	0.3	0.3	0.3	0.3
Dry matter (as fed)	91.0	91.0	17.3	95.0
Dry matter digestibility	78.2 ± 1.3	70.6 ± 1.5	67.7 ± 2.8	76.3 ± 0.2
Crude protein content	15.4	3.3	3.5	1.6
Protein digestibility	79.8 ± 1.5	50.4 ± 0.7	13.5	3.0 ± 5.0
Gross energy (kcal/g)	4.5	4.4	4.3	4.1
Energy digestibility	78.1 ± 1.6	70.6 ± 1.4	65.7 ± 3.2	74.8 ± 0.5

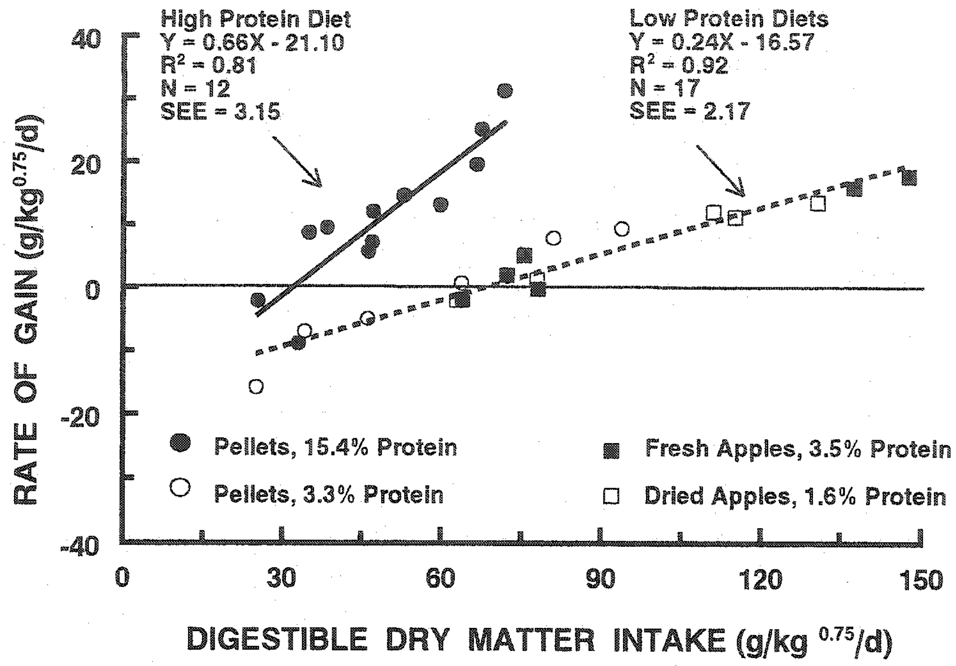


Figure 1. The relationships between daily mass gain in captive, 16– to 21–month-old grizzly bears and digestible dry matter intake of two pelleted diets and two fruit diets.

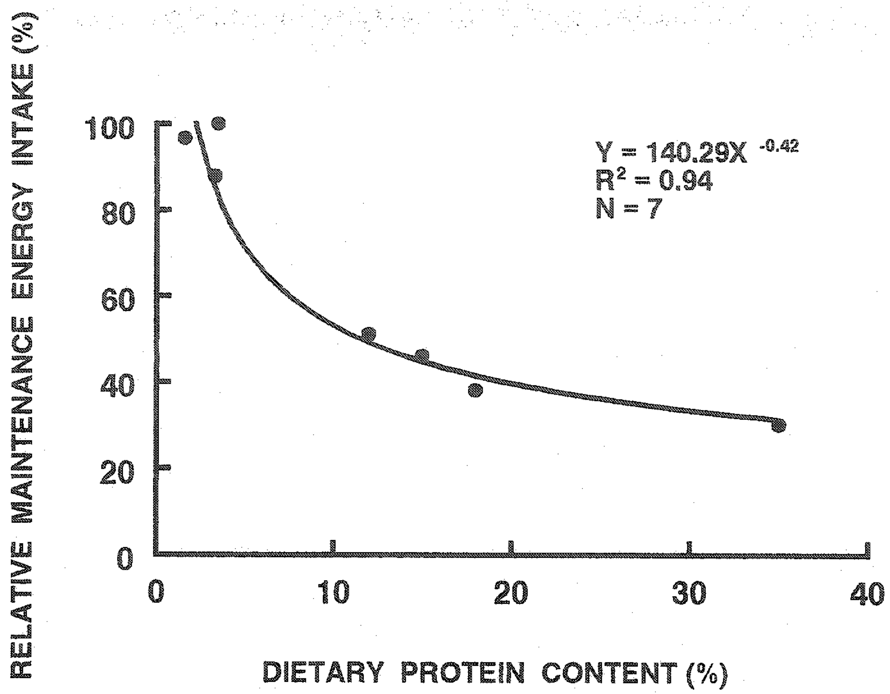
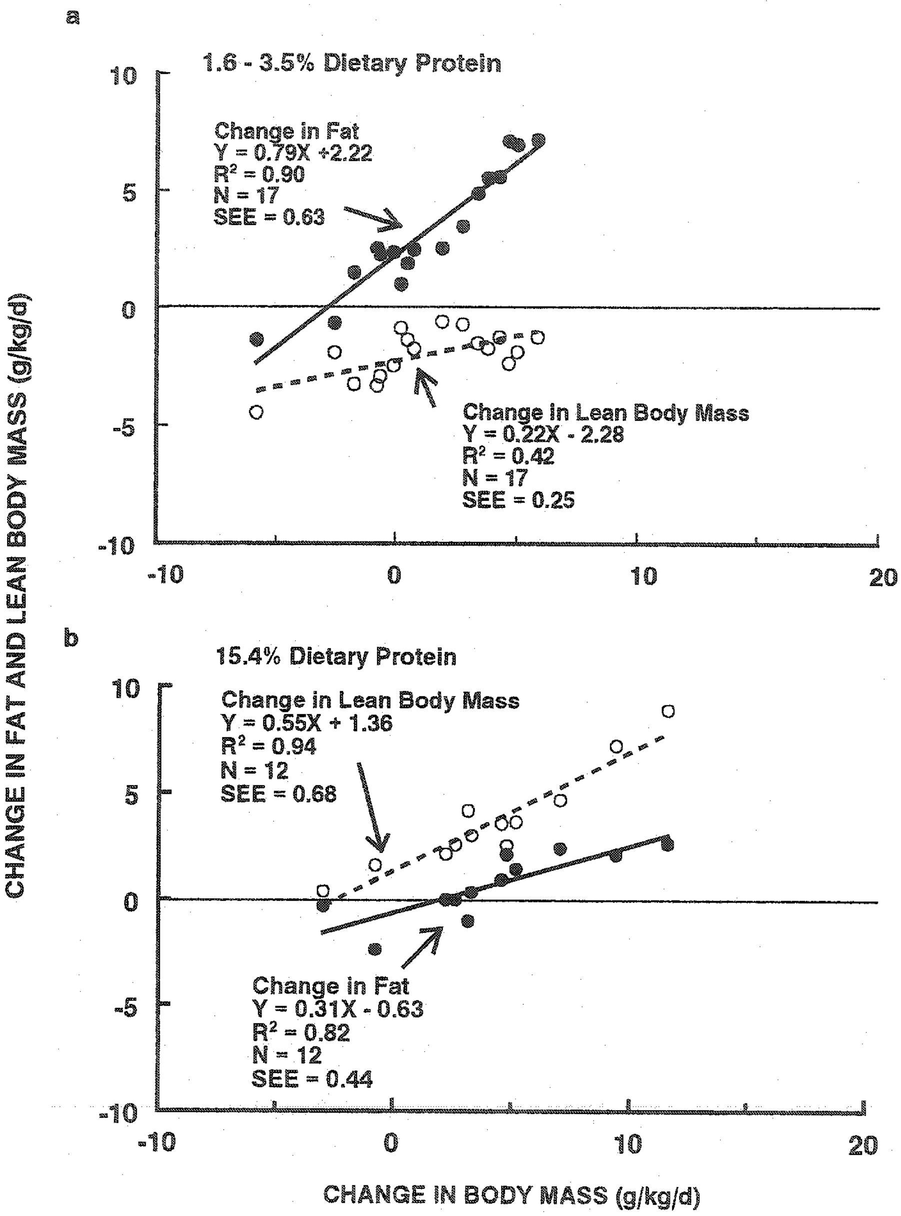


Figure 2. The relationship between dietary protein content and energy intake at mass maintenance for bears consuming a range of plant-based diets as a percent of the maintenance intake for bears eating a fruit diet (fresh apples) (Rode and Robbins 2000; current study). For example, the maintenance energy cost in the current study for bears consuming the 15.4% pelleted, protein diet (144 kcal digestible energy/ $kg^{0.75}$/day) is expressed as a percent of the maintenance energy cost when consuming fresh apples (310 kcal digestible energy/ $kg^{0.75}$/day) (i.e.., 46%). Because both studies (Rode and Robbins 2000; current study) used bears of different ages and therefore report slightly different maintenance energy intakes when consuming fresh apples, the maintenance cost specific to each study was used in the calculations. Diets included pelleted diets containing 3.3%, 12.0%, 15.4%, 18.0%, and 35.0% protein, fresh apples (3.5% protein), and dried apples (1.6% protein).



Figures 3a and b. The relationship between the gain or loss of lean mass and fat when captive, 16– to 21–month-old grizzly bears were fed low-protein fruit and pelleted diets and a higher protein pelleted diet.

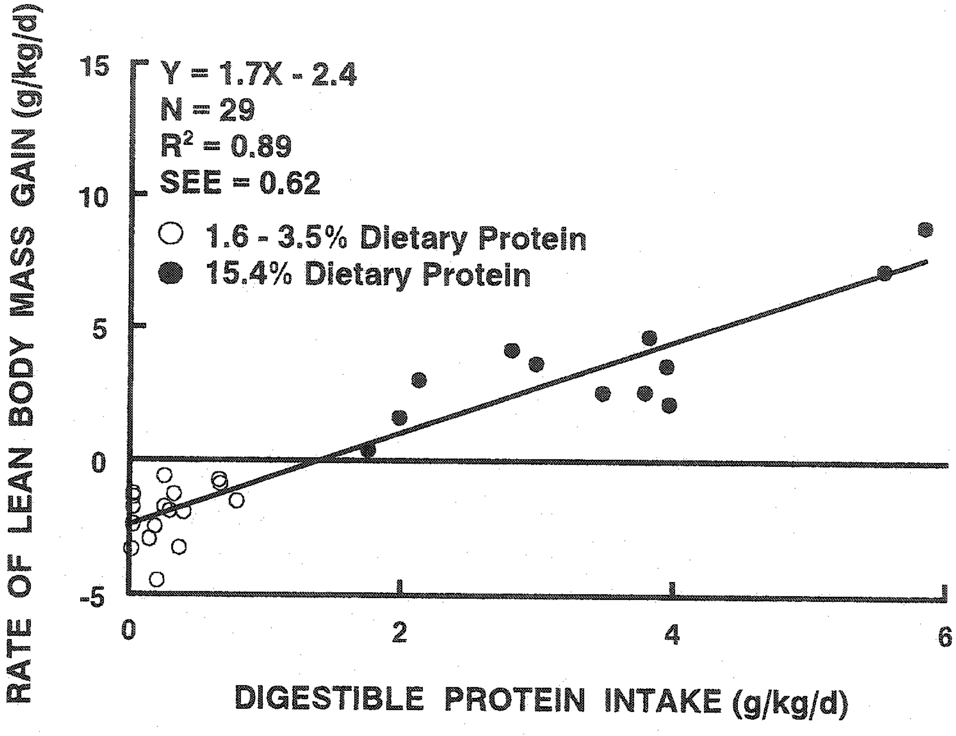


Figure 4. The relationship between the rate of lean mass gain and digestible protein intake when captive, 16– to 21–month-old grizzly bears were fed low-protein fruit and pelleted diets and a higher protein pelleted diet. Data distribution is similar and linear when both axes are expressed on a per kg$^{0.75}$ basis ($Y = 2.0X - 6.8$, $N = 29$, $R^2 = 0.90$, $SEE = 1.68$).

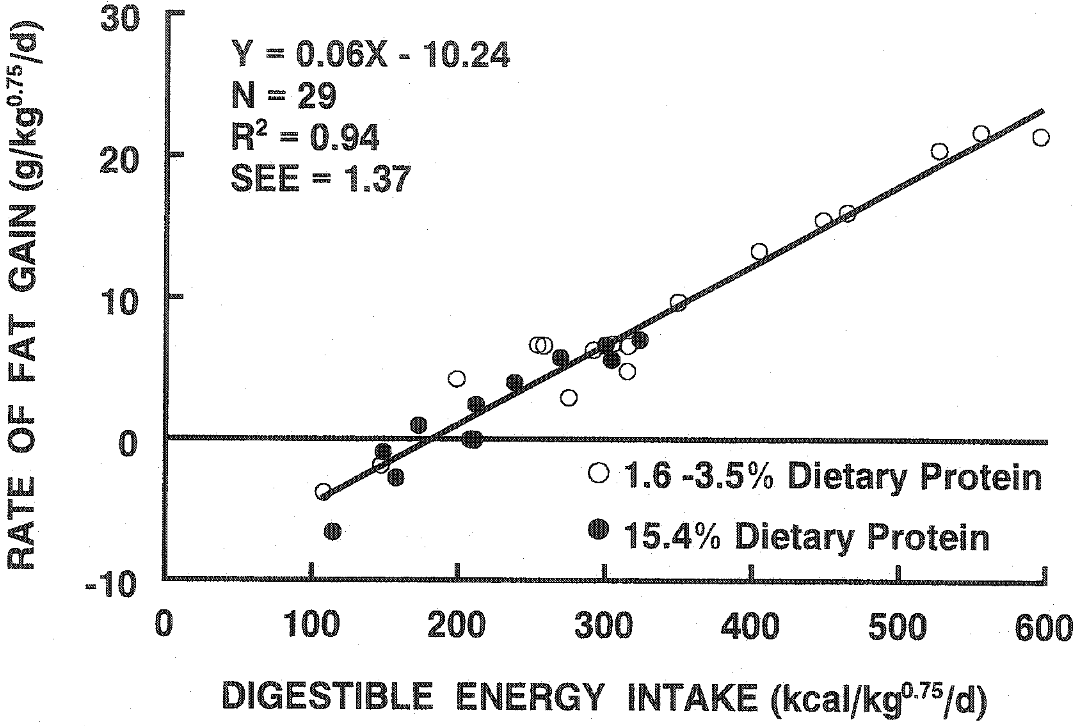


Figure 5. The relationship between rate of fat gained and digestible energy intake when captive 16– to 21–month-old grizzly bears were fed low-protein fruit and pelleted diets and a higher protein pelleted diet.

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

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 ± 1SD) that is distinctly different from those of all other grizzly bear foods that range 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 whereas 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.

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 cones per tree 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 to 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 alternate 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 extant whitebark pine trees are either dead or dying. Recent surveys suggest that 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. While 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), non-digested fecal residues can either be a good indicator of nutritional importance or its antithesis. Similarly, fecal prevalence gives only a broad population average and does not indicate what percent or component of the population is making significant use of the resource. Stable isotopes, particularly carbon and nitrogen, have become important tools to estimate 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 Greater Yellowstone Ecosystem (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 m 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 the 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 onion-grass (*Melica spectabilis*), biscuitroot (*Lomatium* spp.), and yampa (*Perideridia gairdneri*). Fleshy fruits or berries are not significant grizzly bear foods in the GYE and were therefore 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 isotopic signatures of the diet and that of bear plasma. Plasma samples were used rather than hair or other tissues because plasma isotope signatures equilibrate (i.e., become asymptotic) with the diet within 10 to 14 days and have the same isotope signatures as hair (Hilderbrand et al. 1996). Six (3 male and 3 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, 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 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 May through November of each year, our initial step in relating the isotope 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 isotope signature of the foods consumed during the preceding 10 to 14 days and red blood cells 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 had 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, those 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 (no. 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 with 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 (Hilderbrand et al. 1996). Sulfur isotope content and composition were determined either after Eschka extraction (e.g., low sulfur-containing plant samples) 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; Giessemann et al. 1994; Kester et al. 2001).. Results are reported as per mil 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.

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 did nitrogen and sulfur signatures (see Results and Discussion), only nitrogen and sulfur isotopes were used to estimate the dietary contribution of pine nuts to 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 which 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), the requisite information on food source digestibility and many other metabolic variables was not available (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 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 an 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

Isotope Signatures of Yellowstone Bear Foods

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

Captive Bear Feeding Trials

Nitrogen and sulfur isotope signatures of bear plasma closely tracked the isotope signature of the diet, even when mixed diets were fed (Fig 1). Much more variation occurred 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‰) at the lowest dietary sulfur isotope signatures to depleted (-3.6‰) at the highest dietary sulfur isotope signatures.

Yellowstone Pine Nut Production and Bear Isotope Signatures

Mean cone production ranged from 3 cones per tree in 1995 to 40 cones in 1999. Very poor cone production occurred in 1995, 1997, 1998, and 2000, intermediate cone production occurred in 1996 and 2001, whereas the second best crop in 20 years occurred in 1999. 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 the above relationship between cone production and grizzly bear $\delta^{34}S$ was in 2000 when mean cone production was 6 cones per tree, but the mean yearly $\delta^{34}S$ signature for grizzly bears was the highest measured (7.8‰, Fig. 3) and pine nuts were estimated to have provided over 76% of the assimilated sulfur and nitrogen for 80% of the bears (Fig. 4). Because the 2000 grizzly bear samples were 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 the estimated bear signature (4.0‰) that would occur for bears consuming a 100% plant-based diet (Table 2 and Fig. 3).

Estimates of Assimilated Dietary Pine Nut Content by Yellowstone Grizzly Bears

Estimated mean assimilated dietary content of pine nuts for the population ranged from 19% in 1995 (range of model estimates: 11 to 26%) to 97% in 2000 (range of model estimates: 96 to 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) warn 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 1) the different chemical constituents of a particular food having different concentrations, carbon signatures, and digestibilities such that the overall diet signature does not accurately represent the signature of the digested carbon compounds (Phillips and Koch 2002) or 2) 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 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 ranging 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; Yeoh and Wee 1994; Van Soest 1994). Because protein is highly digestible in the low-tannin foods consumed by bears (Pritchard and Robbins 1990), the overall dietary δ^{34}S and δ^{15}N signatures are the same as the combined signatures of the absorbed amino acids. Additionally, in contrast to 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 what is indicated by the change in δ^{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 δ^{34}S and δ^{15}N in Fig. 3, that 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 weight basis (27 kJ/g for pine nuts relative to 22 to 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 highest 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 over-wintered 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 isotope 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.

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Table 1. Isotope signatures (‰) of diets fed to captive grizzly bears during 21-day feeding trials to determine the isotope enrichments occurring between diet and consumer plasma. Diet composition for the two pelleted diets can be found in Felicetti et al. (2003).

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
Pelleted chow (3.3% protein)	-22.8	3.6	3.0

Table 2. Mean stable isotope signatures (‰, mean ± SD) for major foods consumed by grizzly bears in the Greater Yellowstone Ecosystem. Values in parenthesis are sample size. Values in each isotope column that are followed by different letters are significantly different (*P*<0.01) while those with the same letter are not.

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)

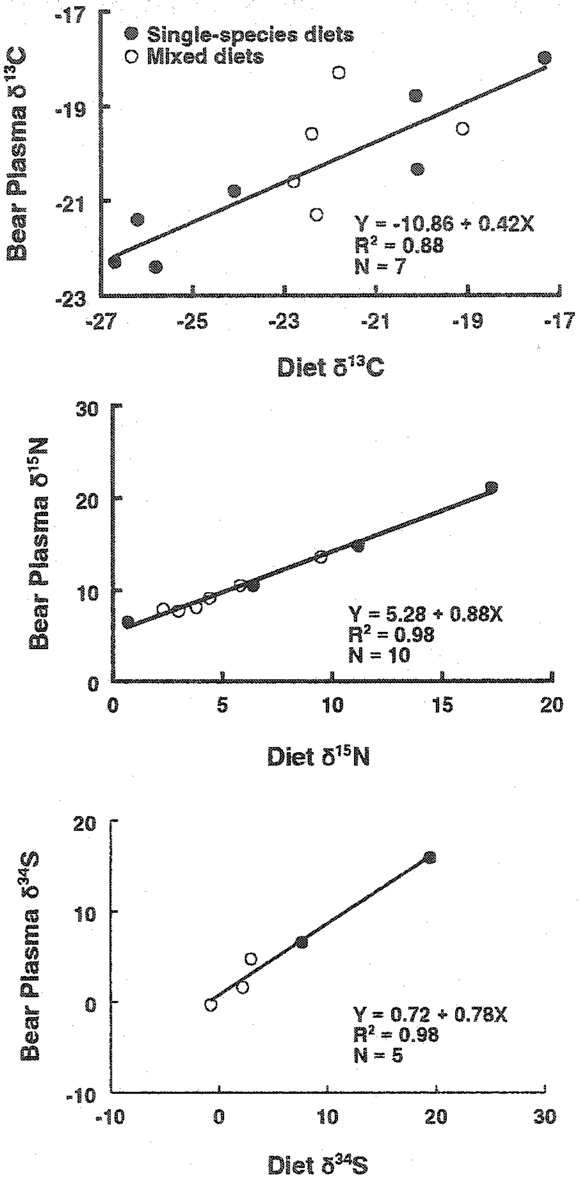

Figure 1. The relationships between diet and plasma stable isotope signatures for grizzly bears (current study), American black bears (Hilderbrand et al. 1996), and polar bears (*U. maritimus*) (Hobson and Welch 1992). The carbon regression is for single-species diets only, whereas the nitrogen and sulfur 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.

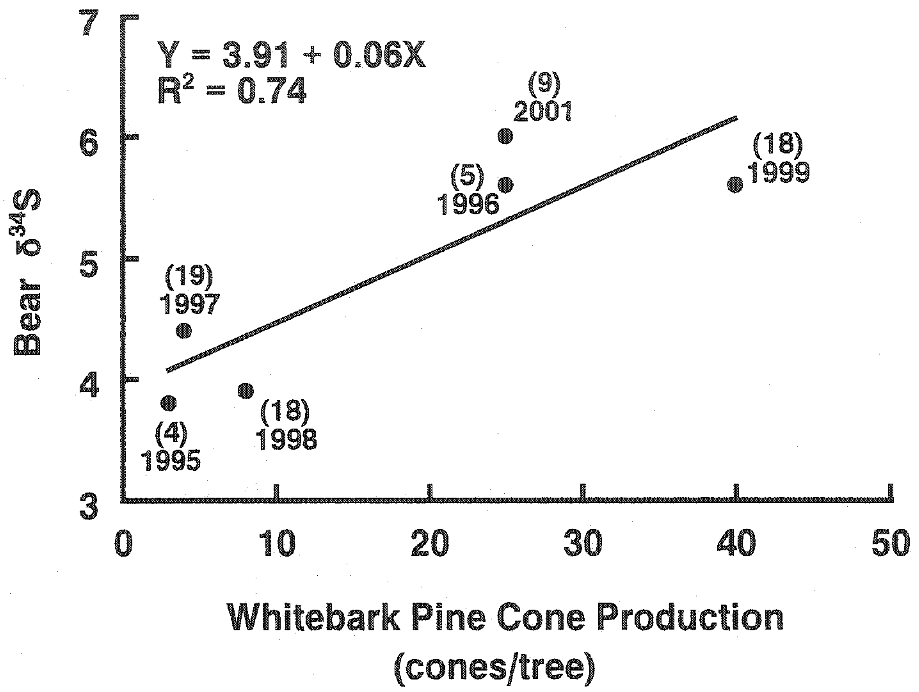

Figure 2. The relationship between the mean whitebark pine cone production in specific years and the mean, annual, free-ranging grizzly bear hair or plasma signatures produced in the same years for the Greater Yellowstone Ecosystem. Data for 2000 were omitted because of the extensive use of pine nuts that over-wintered from the bumper crop produced in 1999.

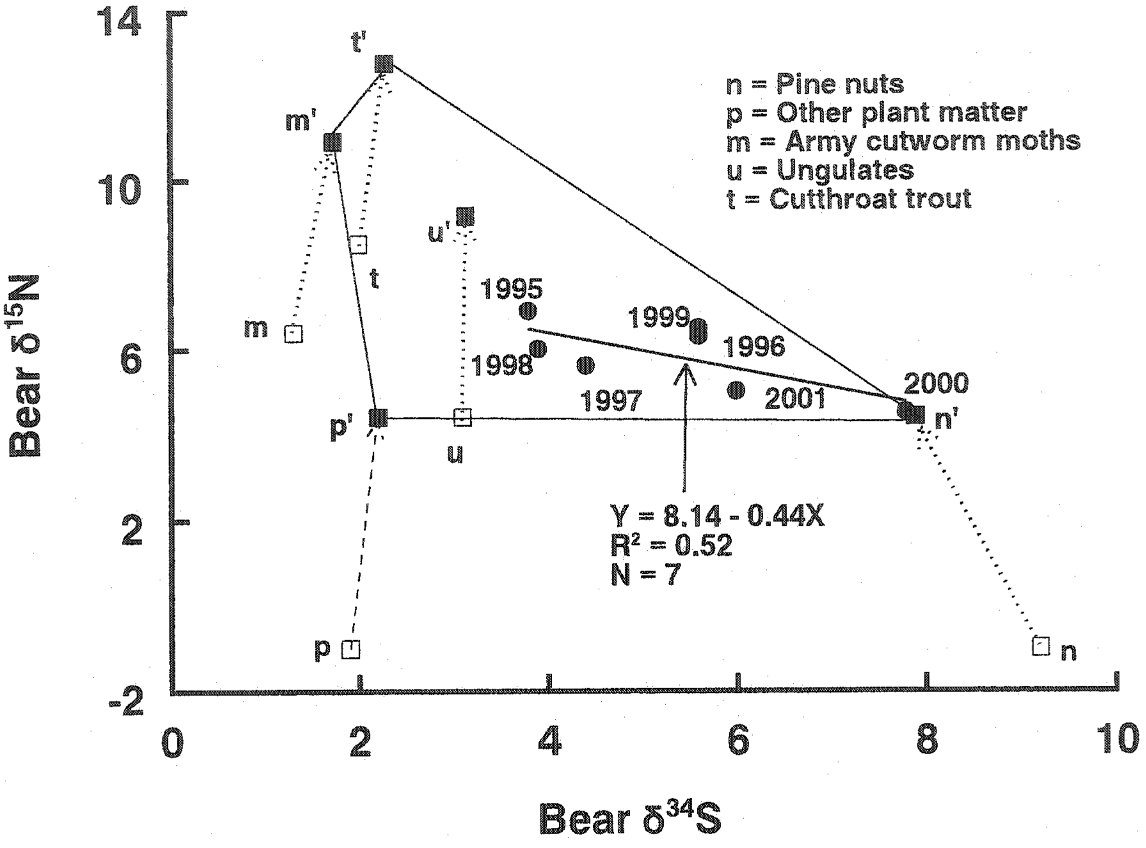

Figure 3. Mixing diagram for the major grizzly bear foods and the mean annual grizzly bear $\delta^{34}S$ and $\delta^{15}N$ isotope signatures in the Greater Yellowstone Ecosystem. The isotopic signatures of the five food sources are labeled with letters. The prime letters at the end of the dashed lines 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).

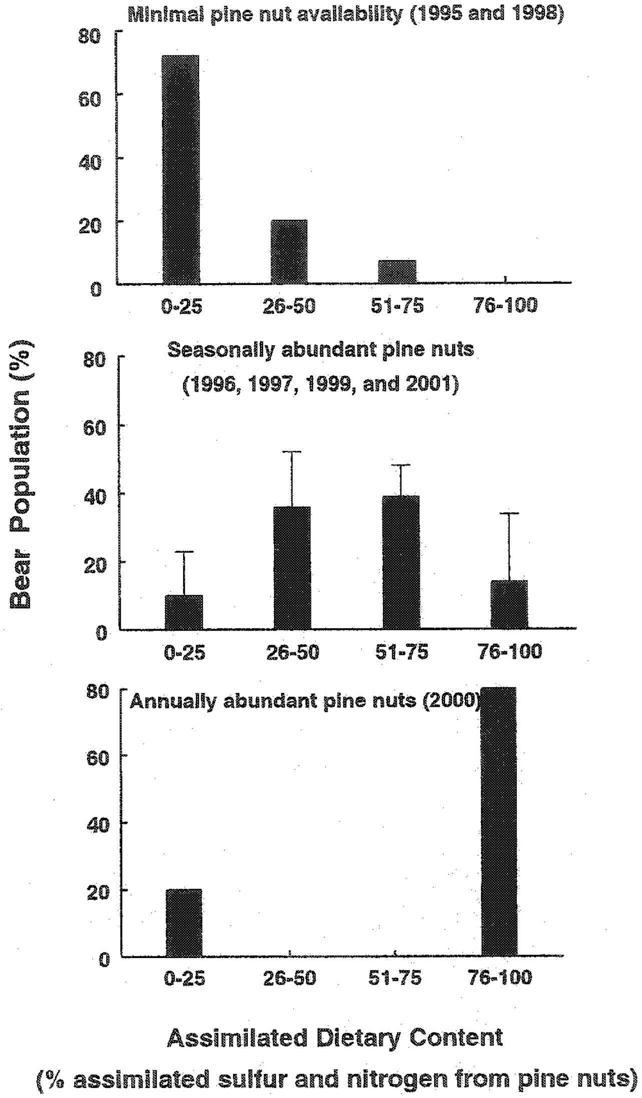

Figure 4. Assimilated dietary content of the sulfur and nitrogen from pine nuts in the diet of Yellowstone grizzly bears calculated using the mixing model of Phillips and Gregg (2003). Because the isotopic distribution of the individuals varied based on the current and preceding year's crop of pine nuts, three different scenarios are shown. Minimal pine nut availability occurred during the second year of two successive years of poor cone

production (1994–1995, 2 and 3 cones/tree; and 1997–1998, 4 and 8 cones/tree). 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 (1996–1997, 25 and 4 cones/tree) or when the preceding year's crop was poor but the current year's crop was good or very good (1995– 1996, 3 and 25 cones/tree; 1998–1999, 8 and 40 cones/tree; and 2000–2001, 6 and 25 cones/tree). 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 (1999–2000, 40 and 6 cones/tree).

Use of naturally-occurring mercury to determine the importance of cutthroat trout to Yellowstone grizzly bears

Abstract: Spawning cutthroat trout (*Oncorhynchus clarki*) are a potentially important food resource for grizzly bears (*Ursus arctos horribilis*) in the Greater Yellowstone Ecosystem. We developed a method to measure the amount of cutthroat trout ingested by individual grizzly bears living in the Yellowstone Lake area. The method used 1) the relatively high, naturally-occurring concentration of mercury in Yellowstone Lake cutthroat trout (508 ± 93 ppb) and its virtual absence in all other bear foods in the park (≤ 6 ppb), 2) hair snares to remotely collect hair samples from bears visiting spawning cutthroat trout streams between 1997 and 2000, 3) DNA analyses to identify the individual and gender of grizzly bears leaving a hair sample, 4) feeding trials with captive bears to develop relationships between fish and mercury intake and hair mercury concentrations, and 5) mercury analyses of hair samples collected from wild bears to determine the amount of trout consumed by each bear. Of the 74 grizzly bears identified by DNA analysis of hair collected on snares near cutthroat trout spawning streams, 42 bears (19 males, 14 females and 9 of unknown sex) left enough hair for mercury analyses. Male grizzly bears consumed an average of 5 times more trout/kg bear than did female grizzly bears. Ninety-two percent of the grizzly bears of known gender with ≥ 200 ppb mercury in their hair were males. Seventy-five percent of the bears of known gender that had < 100 ppb mercury in their hair were females. Cutthroat trout intake per year by the grizzly bear population was only a small fraction (2266 cutthroat trout, or < 1% of the spawning population) of that estimated by previous investigators, and males consumed 92% of all trout ingested by grizzly bears. Disproportionate consumption of

cutthroat trout by males suggests that they are dominating feeding sites on streams and precluding females from obtaining large amounts of fish.

INTRODUCTION

Spawning cutthroat trout (*Oncorhynchus clarki*) are a highly digestible, energy and protein-rich food resource that is readily accessible to grizzly bears (*Ursus arctos horribilis*) in the Greater Yellowstone Ecosystem (GYE) (Pritchard and Robbins 1990; Mattson and Reinhart 1995). Until recently, Yellowstone Lake was the last pristine habitat for native Yellowstone cutthroat trout (Kaeding et al. 1996). However, non-native lake trout (*Salvelinsu namaycush*) were discovered in 1994 and found in substantial numbers in the lake in 1995. Because lake trout are efficient predators of cutthroat trout, lake trout have the potential to reduce the cutthroat trout population by 80-90% (McIntyre 1995). A decline of this magnitude may negatively impact 28 wildlife species that feed on cutthroat trout, including the threatened grizzly bear. Lake trout, unlike cutthroat trout that spawn in small streams in late spring and summer, are not accessible to bears and other wildlife because they spawn in the deeper water of the lake (Schullery and Varley 1996).

Previous studies of grizzly bear use of spawning cutthroat trout in the tributaries of Yellowstone Lake found or suggested that 1) 59 of the 124 tributaries to the Lake contained spawning cutthroat trout and 36 of those streams showed evidence of fishing by bears, 2) a minimum of 44 individual bears fished those streams in 1987, 3) female grizzly bears used the vicinity of streams more consistently and made greater use of the spawning cutthroat trout than did males, and 4) 90% of the bears' diet during the spawning season was cutthroat trout (Reinhart and Mattson 1990; Mattson and Reinhart 1995). A more recent study conducted from 1997 to 2000 that used hair snares and DNA analyses identified 74 individual grizzly bears (64% male:36% female) and estimated that 60 bears per year (12 to 18% of the GYE grizzly bear population) visited the spawning streams and immediate area around Yellowstone Lake during the cutthroat trout spawning season (Haroldson et al. 2003). Because of the large number of grizzly bears using the Yellowstone Lake area, determining the nutritional importance of trout to both individual grizzly bears and the population is critical for evaluating the ecological impact of the loss of this food resource.

Mattson and Reinhart (1995), in the most extensive study of grizzly bear use of spawning cutthroat trout, made several critical assumptions regarding cutthroat trout use by grizzly bears that, if incorrect, could lead to an overestimate of their nutritional importance. Those assumptions included: 1) bears that had ≥ 1 radiotelemetry relocation within 500 m of a known spawning stream during the spawning season ate cutthroat trout and 2) food habits estimated from feces collected adjacent to spawning streams represented the diet of all bears that had ≥ 1 relocation within 500 m of a spawning stream.

To avoid making these assumptions while determining the nutritional importance of cutthroat trout, we sought a predictor of trout consumption that could be measured in grizzly bear hair. Mercury, a biological contaminant that accumulates in many aquatic ecosystems, is readily absorbed and deposited in hair in proportion to its intake (Huckabee et al. 1973; Ben-David et al. 2001). Recently, Yellowstone Lake cutthroat trout were found to contain relatively high levels of naturally-occurring mercury (W. C.

Shanks, letter to Yellowstone National Park Superintendent Finley dated September 9, 1999). Thus, we hypothesized that the mercury content of the hair of Yellowstone grizzly bears could be a direct measure of cutthroat trout intake if cutthroat trout were the only significant dietary source of that element.

METHODS

Study Area

The Greater Yellowstone Ecosystem (GYE) includes Yellowstone (YNP) 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 (Marston and Anderson 1991). Grizzly bears use habitats that range from 1500 m to 3600 m (Schwartz et al. 2003). 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 (*Pinus albicaulis*) 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).

Yellowstone Lake is a high elevation (2,358 m), oligotrophic lake that covers 35,391 ha, has a mean depth of 42 m, and a basin capacity of 14 x 10^9 m^3 (Benson 1961).

The lake is usually frozen from December until May or June (Reinhart and Mattson 1990). The Yellowstone Lake watershed area is estimated to be 261,590 ha. The west and north drainages of the Yellowstone Lake basin contain small streams draining from low relief plateaus with lodgepole pine forests and alluvial meadows, whereas higher relief mountain topography, closed canopy mixed forests, and subalpine slopes characterize the east and southeast drainages (Reinhart and Mattson 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 their mercury content and, thereby, if cutthroat trout had a unique mercury signature relative to all 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.), fireweed (*Epilobium angustifolium*), sticky geranium (*Geranium viscosissimum*), horsetails (*Equisetum arvense*), elk thistle (*Cirsium scariosum*), strawberry (*Frageria sp.*), cow parsnip (*Heracleum lanatum*), dandelion (*Taraxacum* spp.), spring beauty (*Claytonia lanceolata*), bluebells (*Mertensia ciliata*), sedges (*Carex raynoldsii* and *C. praticola*), and grasses (*Bromus anomalous, Phleum alpinum, Phleum alpinum, Agropyron caninum, Poa* spp., *Danthonia spp., and Festuca idahoensis*); the bulbs or roots of onion-grass (*Melica spectabilis*), biscuitroot (*Lomatium triternatum*.), false truffles (*Rhizopogon* spp.), and yampa (*Perideridia gairdneri*): and fleshy fruits or berries from huckleberry (*Vaccinium globulare*), currant (*Ribes sp.*), strawberry (*Frageria sp.*), serviceberry (*Amalancier alnifolia*), roses (*Rosa woodsii*) and soapberry (*Shepherdia canadensis*) (Mealey 1975; Kendall 1983; Mattson et al. 1991). Collected animal matter included cutthroat trout from 11 spawning tributaries of

Yellowstone Lake and one tributary of Trout Lake in the northeast corner of YNP, lake trout, bison (*Bison bison*), elk (*Cervus elephus*), moose (*Alces alces*), and mule deer (*Odocoileus hemionus*). All foods were stored frozen at –20°C.

Field Collection of Bear Tissues and Their Analyses

Grizzly bear hair samples were collected from May to mid-August of 1997 through 2000 using hair snares set along cutthroat trout spawning streams surrounding Yellowstone Lake (Haroldson et al. 2003). MtDNA amplification was used to identify hair samples to species (Murphy et al. 2000), microsatellite loci for individual identification (Wood et al. 1999), and co-amplification of X and Y-chromosomes for sex determination (Ennis and Gallagher 1994). Field and laboratory methods are described in detail in Haroldson et al (2003).

All plant and animal tissue samples were freeze-dried and ground prior to mercury analysis. Hair samples collected from the same bear in different years were analyzed separately to determine interannual variation in mercury content. Samples were analyzed by the U.S. Geological Survey laboratories in Denver, CO. Samples were digested in a nitric acid – sodium dichromate solution, diluted with 12 ml of water and preserved with a 1 percent sodium dichromate/nitric acid solution, and analyzed for total mercury using continuous-flow cold vapor-atomic absorption spectrometry using a Perkin-Elmer 3030B Spectrophotometer (Kennedy and Crock 1987).

Feeding Trials Using Captive Grizzly Bears

Six, three year-old, captive grizzly bears (3 male and 3 female siblings) were used in a year-long feeding trial to determine the relationships between consumption rates of mercury-contaminated trout and bioaccumulation of mercury in hair, plasma and whole blood. Bears were housed at the Washington State University Bear Research, Education, and Conservation Facility in Pullman, Washington. Two bears 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 69 kg in the spring to 136 kg in the fall.

In the summer of 2001, 2800 kg of lake trout and 360 kg of cutthroat trout were collected from Yellowstone Lake. These fish were gill-netted as part of the park's annual effort to control the lake trout population. Freshly netted fish were stored frozen in waxed cardboard boxes and shipped to Washington State University where they were stored (–20°C) until fed. In the feeding trials, we wanted to simulate the normal time course of cutthroat trout consumption occurring in GYE grizzly bears. Although spawning trout are available from as early as May 4 to as late as August 17, the average duration of trout availability in 22 streams is 33 ± 14 days with peak spawning numbers occurring from June 4 to June 21 (Haroldson et al. 1999; 2000; 2001)

Thus, we began the feeding trials on May 30, 2002 and fed fish for 33 days. One male and one female bear were not fed fish and served as controls. The remaining bears were fed either ad libitum fish (one male and one female) or 50% of ad libitum (one male and one female). The two ad libitum bears were housed in concrete-floored pens and fed only trout during the 33-day experimental period. The two bears receiving 50% ad libitum began eating fish one day later than the ad libitum bears as their daily fish allotment was determined from the preceding day's ad libitum intake. Bears receiving fish were fed 89% lake trout and 11% cutthroat trout because not enough cutthroat trout were available for the entire trial. Before and after the 33-days of fish-feeding (ad libitum bears) or throughout the study for the 2 control bears and the 50% ad libitum bears, all bears were fed limited amounts of low-mercury commercial bear chow (21% crude protein, Command Chunk, Land O' Lakes Feeds, Seattle, WA 98119) and apples and grazed low-mercury white clover (*Trifolium repens*) 12 hrs/day (Table 1).

Blood and hair samples were collected at the start of the feeding trial, at the end of the 33 days of fish-feeding, and once a month thereafter until the bears hibernated in early November. Before the feeding trial began, we shaved several patches along the middle of the back of each captive bear where hair would be most likely be sampled when wild bears moved under hair snares. The shaved areas allowed us to measure the length of newly growing hair during each sampling. After all hair measurements were taken for each month we collected hair samples by shaving the newly grown hair, thus enabling us to measure the new growth every month. The study complied with the principles and guidelines of the Canadian Council on Animal Care and was approved by the Washington State University Institutional Animal Care and Use Committee (protocol ASAF # 3181).

Statistical analyses

Linear least-squares regression (PROC REG; SAS Institute Inc. 1998) was used to model the relationship between total content of mercury in fish eaten by a bear and the content of mercury accumulated in bear hair, and the relationship between the amount of fish consumed by a bear and the amount of mercury in the bear hair. We used an ANOVA and least squares means to test for differences between the mercury content of foods consumed by Yellowstone grizzly bears and to test for differences between the mercury content of male and female grizzly bears (PROC GLM and LS Means, SAS Institute Inc. 1998).

RESULTS

Mercury in Yellowstone Bear Foods

The only significant source of mercury in foods consumed by grizzly bears in the GYE was cutthroat trout (Table 1), which contained a minimum of 88 times more mercury than either plants or ungulates ($F = 118.55$, $P < 0.0001$). Gill-netted cutthroat trout (530 ± 90 ppb) had slightly higher mercury concentrations than did cutthroat trout caught in spawning streams (508 ± 93 ppb) and gill-netted lake trout (430 ± 60 ppb), although none were significantly different ($F = 1.45$, $P = 0.2972$). Spawning cutthroat trout caught in the single spawning stream for Trout Lake also had elevated mercury levels (Table 1).

Mercury Content of Yellowstone Grizzly Bear Hair

Of the 74 bears identified from hair snares set on cutthroat trout spawning streams flowing into Yellowstone Lake, 42 (19 males, 14 females and 9 unknowns) left enough hair for mercury analyses (Fig. 1). Hair mercury contents ranged from 17 to 2600 ppb. Males had higher mean mercury concentrations in their hair (526 ± 639 ppb) than did females (134 ± 282 ppb) ($F = 4.93$, $P = 0.0336$). Five bears (3 males, 1 female, and 1 unknown) were sampled in two successive years. All five maintained qualitatively similar hair mercury concentrations between years. Of the bears of known gender and $\geq$ 200 ppb mercury in their hair, 92% were males. For bears of known gender and $<$ 100 ppb mercury in their hair, 75% were female.

Captive Bear Feeding Trials

Blood mercury levels peaked at the end of trout-feeding before declining toward background levels prior to hibernation (Fig. 2). Bear hair began growing in early May and continued growing into October at approximately 1.5 cm/month. The mercury content of the hair grown by each bear during a particular month tracked that bear's average, monthly, plasma mercury content with a considerable enrichment in hair (Fig. 3). Mercury content of the fully-grown hair collected in October increased curvilinearly with increasing fish and mercury intake (Fig. 4). However, the curvilinearity was minimal and there was virtually no variation caused by gender or any other variable except total fish and mercury intake.

Estimates of Cutthroat Trout Intake by Yellowstone Grizzly Bears

We estimated intake of cutthroat trout based on mercury concentrations in the hair of wild bears (Fig. 1), the mercury content (Table 1) and dry matter content (27.8 ± 2.0%) of spawning cutthroat trout, and trout intake required to produce the level of mercury observed in the fully-grown hair of captive bears (Fig. 4). Intake ranged from 2.4 g cutthroat trout/kg bear/year to 1090 g cutthroat trout/kg bear/year. Mean fish intake per kilogram by male grizzly bears was over 5 times greater (135 g/kg bear/year) than the mean fish intake by females (26 g/kg bear/year).

DISCUSSION

All grizzly bears identified by hair samples as having been near spawning cutthroat trout streams had consumed trout, as indicated by elevated mercury signatures. Grizzly bear hair collected adjacent to spawning streams in May through July was very likely fully-grown hair from the preceding year. Bears have one molt/year that is initiated in late spring and summer as abundant, high-protein foods are consumed, and old hair is not lost until new hair is well along in its growth cycle. Newly growing hair accumulates the trout-mercury signature very quickly such that any new hair snagged late in the spawning season would carry a mercury signature similar to or higher than that of fully-grown hair. Thus, it is unlikely that the hair caught in the Yellowstone hair snares grew prior to the bears' feeding on spawning cutthroat trout and therefore carried an unrepresentative or abnormally low mercury signature.

Our data indicate that male grizzly bears were the primary consumers of cutthroat trout, which differs from Reinhart and Mattson's (1990) and Mattson and Reinhart's (1995) conclusions that females fed more heavily on trout. Whereas we cannot exclude the possibility that cutthroat trout declined significantly between 1975–1989 (Reinhart and Mattson 1990; Mattson and Reinhart 1995) and 1997–2000 (current study) with a resultant change in bear behavior, the contradictory findings are likely explained by differing sampling methods. Reinhart and Mattson (1990) estimated gender and age composition of bears near streams based on track analyses, whereas Mattson and Reinhart (1995) used telemetry locations to determine proximity of collared bears to trout streams. Both authors inferred equality between time spent by bears in proximity to spawning streams and trout ingestion, although they lacked any support for that claim. Our results indicated that proximity does not equal level of consumption.

Whereas others have compared the cutthroat trout spawning streams of Yellowstone Lake with the salmon spawning streams of Alaska (Mattson and Reinhart 1995; Craighead et al. 1995), the two can be quite different both temporally and spatially. Cutthroat trout spawning occurs primarily in the spring and early summer, whereas salmon spawning occurs primarily in the summer and fall. Because bears have very different levels of food requirements during spring and summer versus summer and fall, consumption levels of fish can change dramatically between these spawning seasons (Hilderbrand et al. 1999). Also, cutthroat trout spawning streams are typically smaller than salmon spawning streams and, thereby, contain a potentially valuable food resource that is spatially defendable. Adult male grizzly bears would be expected to dominate such a food resource (Stonorov and Stokes 1972; Jacoby et al. 1999), and our results support such a conclusion. Our results also help clarify anomalies in Mattson and Reinhart's (1995) observations—females living in the vicinity of cutthroat trout spawning streams first reproduced at a later age and had smaller litters than females elsewhere in GYE. These authors had difficulty rationalizing these observations relative to their conclusion that female grizzly bears consuming trout should have been in better condition and, therefore, more productive than those not eating fish. Our data suggest that trout are not consumed in large quantities by most female grizzly bears; thus, females living in the Yellowstone Lake area are likely to have a poorer quality diet than suggested by Mattson and Reinhart (1995).

Using data from Reinhart and Mattson (1990), Mattson and Reinhart (1995), and Mattson (1997), Stapp and Hayward (2002) estimated that Yellowstone grizzly bears annually consume 20,910 spawning cutthroat trout, or approximately 5% of the spawning population. The mercury-based estimates of trout intake from our study for an average adult male (195 kg) and female (135 kg) in GYE (Blanchard 1987) are 26 kg and 4 kg of fish/bear/year, respectively. Based on an average cutthroat size of 468 g (Stapp and

Hayward 2002), adult male grizzly bears consumed an average of 55 trout/year and females 8 trout/year. The maximum trout intake for a male with the highest hair mercury level was 180 kg of trout (385 fish) and for the highest female was 44 kg of trout (94 fish). Based on an annual visitation of the streams by approximately 60 grizzly bears, 38 males and 22 females (Haroldson et al. 2003), 2266 cutthroat trout would be consumed, or 2090 trout by male grizzly bears and 176 trout by female grizzly bears. This level of grizzly bear trout consumption is only 11% of that estimated by Stapp and Hayward (2002) and < 2% of the cutthroat trout being consumed by lake trout (Ruzycki et al. 2003).

Because females do not use cutthroat trout to the same extent as males, a significant decline in the Yellowstone Lake cutthroat trout population may have little to no impact on female reproductive success. Available meat from large ungulates, combined with whitebark pine nuts (Felicetti et al. 2003), cutworm moths (*Euxoa auxilaris*), and plants may provide adequate amounts of protein and energy to maintain the current reproductive rates observed for female grizzly bears in the GYE. However, loss of cutthroat trout could increase competition between the sexes and/or age classes of bears for the remaining foods, in which case adult females and subadults of both sexes could be displaced by adult males.

Because bioaccumulation of mercury is relatively high in aquatic food systems, using mercury for estimating fish intake by grizzly bears may extend beyond the GYE and Yellowstone Lake (Clarkson 1992; Duffy et al. 1998; Ben-David et al 2001, Bowles 2001). Because spawning salmon (*Oncorhynchus* spp.) may be the only significant contributor of mercury to some salmon-feeding brown bear populations, hair mercury levels may be useful in quantifying the amount of salmon consumed by bear populations in Alaska and Canada (Ben-David et al. 2001, current study). Previous estimates of salmon intake by bears required multiple captures of the same bear (Hilderbrand et al. 1999). Estimates based on hair mercury levels and appropriately timed captive bear feeding trials would require only one capture or could be done remotely with hair snares.

Finally, significant mercury consumption and accumulation could have negative health consequences to YNP grizzly bears (Lippmann 2000). Because no controlled studies have examined the interaction of mercury intake and reproduction by grizzly bears, we cannot exclude the possibility that the reduced reproductive success reported by Mattson and Reinhart (1995) for adult females living in the Yellowstone Lake area relative to the rest of the ecosystem is caused by the negative consequences of mercury ingestion. However, captive adult grizzly bears (n = 6) that were fed large amounts of salmon (240 ppb) over multiple years had hair mercury concentrations of 4778 ± 631 ppb. These bears appear completely healthy, are now 18 years old, and have produced numerous sets of healthy twin cubs (personal observation). The hair mercury levels in the captive bears are from 9 to 35 times higher than the mean hair mercury concentrations in male and female grizzly bears in YNP. Thus, we do not expect detrimental health consequences associated with the levels of mercury consumption currently occurring in the grizzly bears of the GYE.

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Table 1. Mercury concentration in parts per billion (mean ± SD, 100% dry matter basis) of foods fed to captive bears and consumed by grizzly bears in the Greater Yellowstone Ecosystem. Numbers in parentheses are sample size.

Sample	Mercury content
Yellowstone Animal Matter	
Cutthroat trout	
Gill-netted in Yellowstone Lake	530 ± 90 (6)
Caught in Yellowstone Lake spawning streams	508 ± 93 (16)
Caught in Trout Lake spawning stream	485 (2)
Lake trout	
Gill-netted in Yellowstone Lake	430 ± 60 (6)
Ungulates (bison, elk, moose and mule deer)	all ≤ 6 (10)
Yellowstone Plant matter	
Foliage, roots and bulbs, fruits and berries in GYE	all ≤ 6 (47)
Washington State University Bear Foods	
White clover	6 (1)
Apples (*Malus* spp.)	≤ 6 (2)
Commercial bear chow	≤ 6 (2)

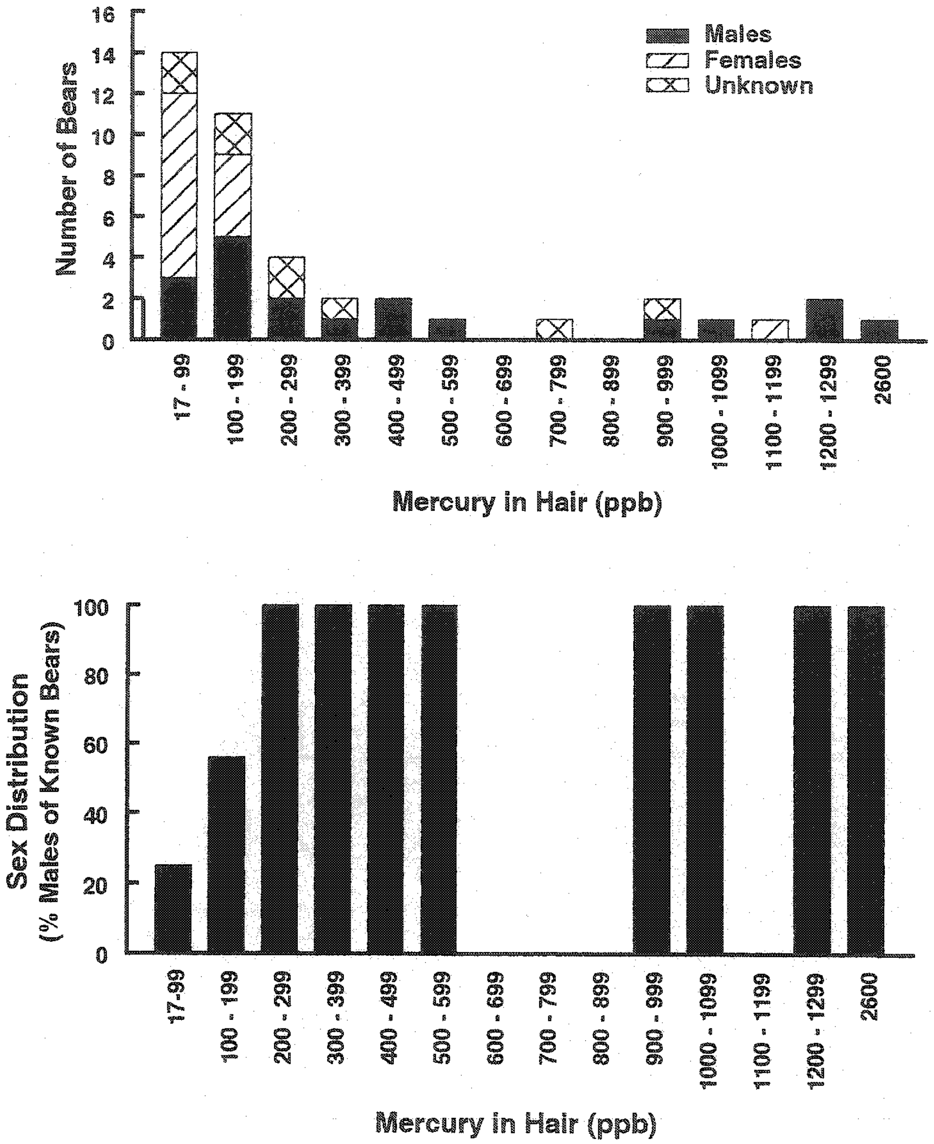

Figure 1. The amount of mercury (100% dry matter basis) in hair collected from 42 grizzly bears that encountered hair snares adjacent to cutthroat trout spawning streams flowing into Yellowstone Lake between 1997 and 2000. The dark line at the far left of the top graph is the background level of 6 ppb.

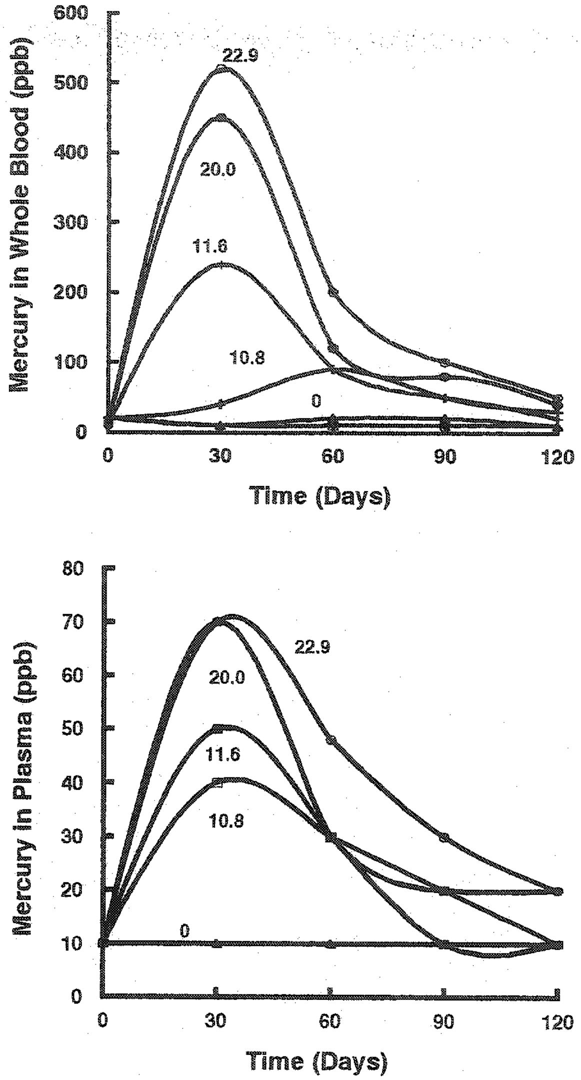

Figure 2. The relationships between Yellowstone Lake trout consumption and captive grizzly bear plasma and whole blood mercury concentrations. Trout were fed in the ratio of 89% lake trout and 11% cutthroat trout and had a dietary mercury concentration of 441 ppb. Trout were fed at ad libitum (2 bears) and 50% ad libitum (2 bears) levels. Trout feeding occurred between May 30 and July 1, 2002, before and after which low-mercury foods were fed. Two bears (controls) received no trout and were fed low-mercury foods throughout the study. The numbers accompanying each line are the amounts of mercury consumed (mg) in trout per bear during the entire 33-day feeding trial.

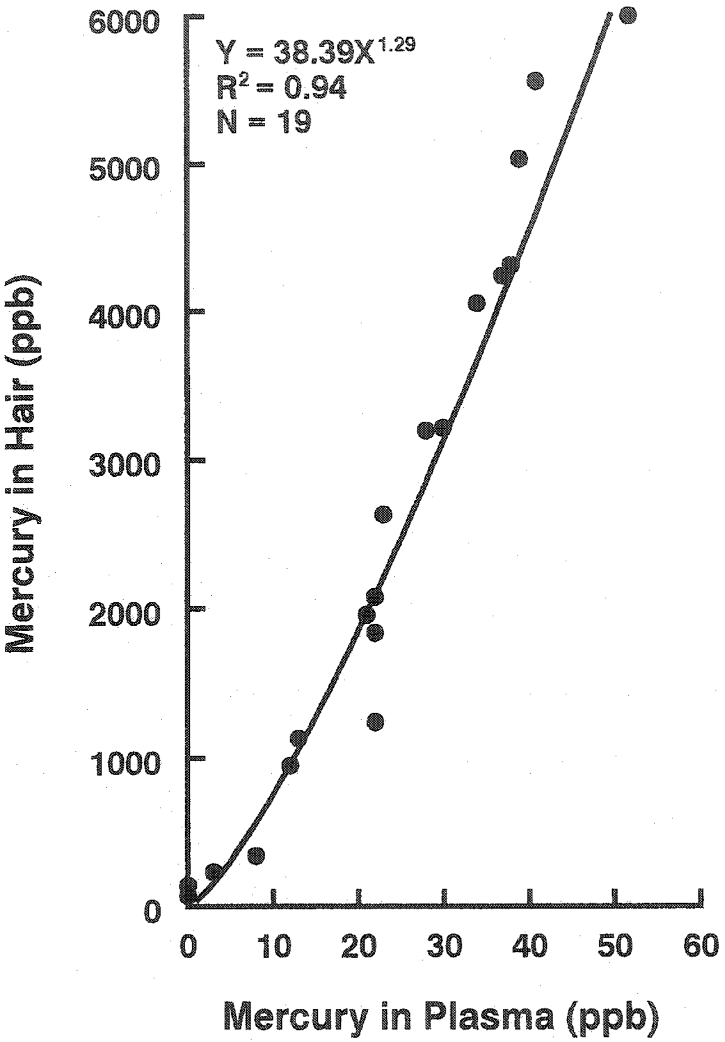

Figure 3. The average, monthly mercury content of grizzly bear plasma and the mercury content of the hair grown during that month in six captive grizzly bears fed varying levels of lake and cutthroat trout.

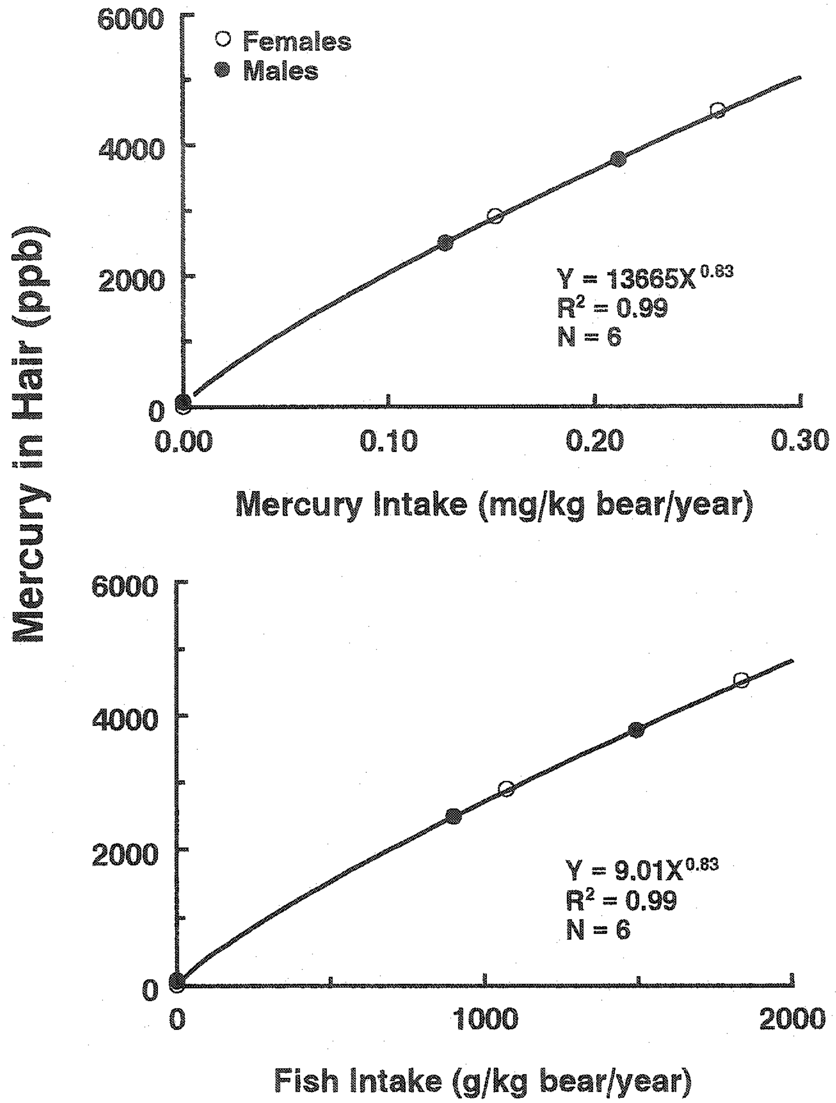

Figure 4. The relationships between mercury consumption, hair mercury concentrations, and Yellowstone Lake cutthroat trout intake by six captive grizzly bears. The hair was collected in early October.

GENERAL DISCUSSION

Grizzly bears have been extirpated from much of the their original range in the lower 48 states. In 1975 the grizzly bear was listed as a threatened species in the lower 48 states under the U. S. Endangered Species Act. Because grizzly bears are listed as a threatened species, current management goals focus on the conservation of existing populations and their habitats. Our understanding of habitat requirements for grizzly bears and our ability to manage for the conservation of grizzly bears in the lower 48 states improves with an increased understanding of the importance of specific dietary items and how these foods physiologically affect grizzly bears. This dissertation is a small step in understanding the importance of three food items to grizzly bears in the lower 48 states.

The first manuscript, Dietary protein content alters energy expenditure and composition of the mass gain in grizzly bears (Ursus arctos horribilis), applies to bears in or near Glacier National Park where berries can be abundant during the fall. We explored the physiological benefits of berries to bears and determined that berries can be a very important source of energy. Free-ranging bears with ad libitum access to fruit can use either a mixed diet strategy to meet their protein requirement and reduce the energy cost of maintenance, or consume a high fruit diet and effectively "dump"excess energy into increased heat production and body fat accumulation. We hypothesized that bears within a population may use different strategies. For example, small to medium-sized bears that are not limited by the rate of fruit intake (i.e., young bears of either sex and smaller adult females) could purposefully manipulate their body fat content by consuming a greater proportion of fruit than large bears whose daily intake is limited by harvesting rate (Welch et al. 1997). The increased dietary fruit content consumed by smaller, leaner bears would maximize fat accumulation and, potentially, survival during hibernation when fat is the major energy source (Barboza et al. 1997).

In the second manuscript, we used sulfur and nitrogen isotopes to study the importance of whitebark pine (*Pinus albicaulis*) nuts, a significant fall food rich in fat (Lanner and Gilbert, 1994), to grizzly bears in the Greater Yellowstone Ecosystem (GYE). Whitebark pine in the Yellowstone Ecosystem is infected with an exotic fungus, white pine blister rust (*Cronartium ribicola*) (Kendall and Keane 2000). Loss of whitebark pine has the potential to impose significant dietary stress on the threatened Yellowstone grizzly bear. We determined that during years of abundant cone availability, 8 ± 10% of the bears made minimal use of pine nuts whereas 67 ± 19% derived over 51% of their assimilated sulfur and nitrogen (i.e., protein) from pine nuts. During years of poor pine nut availability, 72% of the bears made minimal use of pine nuts and meat became a critically important food resource for Yellowstone grizzly bears.

In the third manuscript, we used naturally-occurring mercury to determine the importance of cutthroat trout to Yellowstone grizzly bears. During 1994, non-native lake trout were discovered in Yellowstone Lake. Lake trout are efficient predators and in the absence of management, have the potential to reduce the native cutthroat trout population by 80-90% (McIntyre 1996). A decline of this magnitude could negatively impact 28 wildlife species, including the threatened grizzly bear (Schullery and Varley 1996). We found that cutthroat trout intake per year by the grizzly bear population was only a small fraction (2266 cutthroat trout, or < 1% of the spawning population) of that estimated by previous investigators, and that males consumed 92% of all trout ingested by grizzly bears. This disproportionate consumption of cutthroat trout by males suggests that they dominate feeding sites on streams and preclude females and subadults from obtaining large amounts of fish.

The three manuscripts within this dissertation contribute important information about three major food resources for grizzly bears: berries, whitebark pine nuts, and cutthroat trout. We do not yet fully understand the cost/benefit tradeoff of feeding on berries to bears. Berries may benefit certain size and age classes of bears more than others in their quest to accumulate fat stores for hibernation. However, the "real life" tradeoffs between berries and higher protein foods, such as salmon, need to be evaluated. Whitebark pine nuts and cutthroat trout, two food resources for grizzly bears in Yellowstone, are threatened. The loss of whitebark pine nuts could increase the number of bear-human interactions, which threatens the safety of both humans and bears within the GYE. Although the decline of cutthroat trout may be negatively impacting other predatory species, at this time, a decline in the cutthroat trout population does not seem to be negatively impacting the grizzly bear population. However, it is crucial from management, safety, and conservation standpoints that we understand the value of these three foods, and others, to grizzly bears.

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Title: Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears
Authors: Laura Felicetti, Charles Schwartz, Robert Rye, Mark Haroldson, Kerry Gunther, Donald Phillips, and Charles Robbins
Citation: Canadian Journal of Zoology 81: 763-770 (2003).

Manuscript #2 - being reviewed by CJZ.

Title: Use of naturally-occurring mercury to determine the importance of cutthroat trout to Yellowstone grizzly bears
Authors: Laura Felicetti, Charles Schwartz, Robert Rye, Mark Haroldson, James Crock, Kerry Gunther, Lisette Waits, and Charles Robbins

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