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I am submitting herewith a thesis written by Jennapher Lynn Teunissen van Manen titled "Using Stable Isotopes to Assess Longitudinal Diet Patterns of Black Bears in Great Smoky Mountains National Park." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

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# USING STABLE ISOTOPES TO ASSESS LONGITUDINAL DIET PATTERNS OF BLACK BEARS (URSUS AMERICANUS) IN GREAT SMOKY MOUNTAINS NATIONAL PARK

# A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Jennapher L. Teunissen van Manen

May 2011

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# **DEDICATION**

This thesis is dedicated to Dr. Joan Brenchley-Jackson, my mentor and friend who I can never thank enough for igniting the fire in me 13 years ago to pursue my dreams and teaching me to view the natural world with the critical, curious and appreciative mind of a scientist.

To my husband, Frank, who makes me smile and laugh on a daily basis, and has shown me how to appreciate the subtle beauties of the natural world and life in general.

To my niece, Lily Cathleen LeBlanc, whose enthusiasm and love for her Auntie are the reason I want to preserve our natural ecosystems for future generations to enjoy.

To my girlfriends, Deniz Cline, Karen Fothergill, Marisa Parker, and Veronica Armijos, whose friendships are the backbone of my success and happiness.

To K'Ehleyr, Spiderman and Grenouille, you've kept me sane at the end of each day by showing me that all you really need in life is a string to chase, healthy food to eat, and a warm body to cuddle with to be happy.

And, to all the scientists who have challenged the dogma of their time and promoted the search for knowledge over belief, paving the road for a future society based on logic, truth and reason.

"It is far better to grasp the Universe as it really is than to persist in delusion, however satisfying and reassuring." — Carl Sagan

## **ACKNOWLEDGEMENTS**

I would like to express my sincere gratitude and appreciation to all the people who provided support, advice, and friendship throughout my graduate education. This project was made possible by funding from the University Of Tennessee Department Of Forestry, Wildlife and Fisheries, Great Smoky Mountains Conservation Association Carlos C. Campbell Fellowship. This project would not have been possible without the in kind support and use of equipment from the Stable Isotopes Lab at the University of Tennessee Department of Earth and Planetary Sciences. I would first like to thank my major professor, Dr. Lisa I. Muller, for giving me the opportunity to develop my own graduate project and the endless support necessary for me to complete my research, sending me to outside training to help better my career, and for our discussions on surviving being a woman in wildlife ecology. I will always appreciate that she is truly trying to help her students gain all the necessary skills for a career in science and not just trying to get a project done, I could not have asked for a better adviser. I would also like to thank my committee members; Dr. Arnold Saxton for his invaluable statistical advice and for understanding all my email questions even when I was not sure what I was asking; Dr. Zheng-Hua Li for his willingness to explain each time how to use the stable isotope machine, helping me calculate my isotope values and teaching me how to cut hundreds of hair samples into 1 mm segments; and of course Dr. Michael R. Pelton for having the foresight to collect these hair samples before stable isotope analysis was being used for wildlife nutrition studies.

I would like to thank the following people for donating hair and food samples to the project; Dr. Edward Ramsey from University of Tennessee School of Veterinary Medicine; Lisa Stewart from Appalachian Bear Rescue in Townsend, Tennessee; David Brandenburg of the Tennessee Wildlife Resources Agency; Kim Delozier, Bill Stiver and Joe Yarkovich of Great Smoky Mountains National Park Service, and Larry McKay, Department Head of the Earth and Planetary Sciences department at University of Tennessee.

I would especially like to thank Bill Stiver at Great Smoky Mountains National Park for always being so helpful with any question I had no matter how busy he was with work. I would also like to thank Dr. Jennifer Fortin-Noreus and Dr. Charlie Robbins at Washington State University for their advice to help me understand stable isotopes with wildlife nutrition.

I would like to thank my fellow graduate students, Carrie Lowe, Michael Drewery, and Mike Hooker, without them a statistics minor would have been impossible and defending and writing a thesis could never have been done without "all that popcorn". Carrie thanks for letting me turn you into a shoe connoisseur to help satisfy my need for boot shopping. I want to thank Jared Laufenberg for being the genius that he is and for a memorable time in Japan. Thanks to the rest of the bear lab graduate students, Jesse Troxler and Kaitlin O'Connell for good discussions about bear research and our Friday lunches. I'd also like to thank Dr. Joe Clark for giving me a space in the bear lab which made working a lot nicer having other graduate students around to discuss ideas

with and solve problems. And thank you to Terry White who always made me feel welcome and a part of things.

I want to thank my fellow foreigners, Niki Labbé and Nicolas Andre for keeping me sane while trying to adjust to the culture shock of East Tennessee. Our routine dinners talking about fresh fruits, vegetables, bread, sunshine, blue skies, mountains and the ocean have kept me going when it never seems to stop raining. Niki, thank you for being there to listen to me when I was homesick and about to lose my mind trying to adjust to leaving my friends behind and missing California sunshine, fruits, vegetables, and attitude. And thank you to my neighbors, Marc Houdeshel, Ann Fairhurst, Robbie Freestate, and Mary Moss for always accepting my outspoken California attitude with a smile and making me feel welcome.

I would like to thank my friends and family back home in northern California—3,000 miles away and they still support and encourage me in all my endeavors. They've answered the phone and listened to me, patiently offering words of understanding and motivation even when I've called way too early for California time. Thanks Mom and Dad for sending me California avocados and mandarins to help me cope with withdrawal symptoms from California fresh fruits and vegetables. I am thankful for the relationship we've been able to develop over the last few years and appreciate your encouragement and interest in my career. I can't say thank you enough to everyone back home who has at some point encouraged me to fulfill my dreams no matter how difficult; Harry Reynolds, Kathy Sorensen, Jeanie, Mark, Lily and Olivia LeBlanc, Oma, Nancy Reitz,

Lisa Levy, Thuy-By Bui (Vivian), Caitlin Kroeger, Abby Lawson, Jeff Brown, Faerthen Felix, and Rory Cornelison.

I want to say thank you to my friends and colleagues at California Fish and Game; Karen Fothergill, Joe Hobbs, Linda Sandoval, Craig Stowers, Russ Mohr, Mary Sommer, Jesse Garcia, Jim Powell, Sarah Edmonds, Mike and Nicole Carion, Margie Bowe, Jon Fisher, and Doug Updike. My supervisors in the deer program were always supportive and encouraging of me developing my career and learning everything I could while working, even lending me out to other programs so I could gain a wealth of experience. The friendships I developed while working in the Wildlife Branch mean the world to me and I will never forget the support and encouragement they gave me to get to graduate school even though they knew it meant I would leave my job. And, thank you to the professors at American River College and UC Davis for preparing me with an outstanding scientific education.

And, last but *most importantly*, I can't say thank you enough to my best friend, Frank. His unyielding support, patience, respect and love are more than I could have ever hoped for in a husband. I have been fortunate to have his advice and help as a scientist, and, he is an amazing husband who I know I can count on to keep me grounded yet let me be the crazy, outspoken, broccoli-loving, Californian that I am. I'm not sure how or why he puts up with me, but I'm glad he does. I could not have made it through this challenging time without him and now I look forward to the rest of our lives together.

# ABSTRACT

Long-term diet patterns based on stable isotope analysis may be helpful to understand changes in food selection of black bears (Ursus americanus) over time and guide management programs to reduce human-bear conflicts. An enriched stable carbon isotope signature indicates an anthropogenic food source in the diet and an enriched nitrogen signature indicates a higher tropic level for a species. I examined longitudinal feeding patterns from 117 hair samples of black bears live captured in Great Smoky Mountains National Park during 1980–2001 using stable carbon and nitrogen isotope analysis from hair samples. I developed a set of a priori models to examine if sex, age class, year, weight class, total hard mast index, white oak index (Quercus spp.), red oak index (*Quercus* spp.), nuisance status and hog harvest (*Sus scrofa*) affected stable isotope signatures. I used model averaging and an estimator of the unconditional variance was used to account for model uncertainty. The  $\delta[\text{delta}]^{13}$ C signatures differed by weight class with above average weight, ( $\beta[Beta] = 0.76\%$ ; 95% CI = 0.28 to 1.23) and average weight ( $\beta[\text{Beta}] = 0.42\%$ ; CI = 0.06 to 0.78) showing enriched values compared to below average bears. Bears had enriched δ[delta]<sup>15</sup>N signatures in years with low white oak mast production ( $\beta$ [beta] = -0.19, CI = -0.34 to -0.03) and depleted when white oak hard mast was abundant. Sub adult bears had enriched δ[delta]<sup>15</sup>N signatures compared to adult and older adult bears. Variation of nitrogen values was small during 1980–1991 ( $\bar{x}$ = 2.57, SD = 0.28) but increased substantially during 1992–2000 ( $\bar{x} = 2.29$ , SD = 0.71) when there was substantial variation in hard mast production. Bears in better physical condition appear more likely to access anthropogenic food sources. In years of low white

oak acorn production, the larger bears and sub adult bears are more likely to turn to alternative food sources. The long term variation detected in this study is important in identifying which bears are potentially more likely to seek out the anthropogenic food sources when changes occur in availability of natural foods.

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# **CHAPTER I**

# INTRODUCTION

# **HISTORY**

Bear management strategies have varied substantially since the establishment of the U.S. National Park System in 1872. In the early decades of the national parks, it was common to hold regular feedings and chain bears to posts to attract human visitors (Marsh 1972). Bears were intentionally fed garbage so they could be viewed by the public (Zardus and Parsons 1980, Stiver 1991). Great Smoky Mountain National Park (GSMNP) was established in 1934. Throughout the history of GSMNP, interactions between American black bears (Ursus americanus) and humans have occurred frequently. Human food and garbage were readily available to black bears and visitors routinely fed bears, leading to increased nuisance bear activity (Singer and Bratton 1980). As encounters with bears increased in many of the parks during the 1950s and 1960s, the National Park Service (NPS) implemented regulations prohibiting feeding of wildlife (Ise 1961, LaFollette 1974). Despite adoption of these regulations, incidents between bears and humans in national parks continue partially because of low visitor compliance, resulting in intentional feeding (Singer and Bratton 1980), improper food storage (National Park Service 2002), and an increase in black bear abundance and human visitation to the park (Singer and Bratton 1980).

Since the establishment of GSMNP, visitation has steadily increased. From 1953 to 1973, visitation increased on average 7 % per year (Singer and Bratton 1980). Approximately 10 million people per year visit GSMNP (National Park Service 2002). The black bear population also has steadily increased since the national park's establishment. During the late 1970s, the number of black bears in the national park was estimated around 500–700, but increased during the late 1980s, and peaked to >2,000 in the late 1990s (Coley 1995, Clark et al. 2005, F.T. van Manen, U.S. Geological Survey, personal communication). Black bears are mobile, curious, intelligent, and adaptable (Pelton 1982). Black bears are opportunistic omnivores and some bears may change their behavior to take advantage of easily obtained food sources. Those bears may become conditioned to anthropogenic foods and, consequently, habituated to humans (Herrero 1985). These behavioral traits, along with an increasing bear population and a large number of visitors, have lead to an increase of bear-human encounters. From 1964 to 1976, there were 1,028 reports of black bear incidents in GSMNP (86/year; Singer and Bratton 1980), whereas 1,414 nuisance bear incidents were reported from 1990 to 1998 (177/year; Clark et al. 2002).

Management practices can be directed to avoid human injuries from bear-human interactions. The mission of NPS is to preserve the historic wildlife, biological diversity and provide opportunities for the public to view the natural systems of the park (United States Congress 1916). Therefore, the goal of current nuisance bear management in GSMNP is to minimize bear-human conflicts while allowing wild bears to live naturally (National Park Service 2002). The 2002 Black Bear Management Guidelines emphasized

the importance of maintaining natural bear behavior (National Park Service 2002). Visitors must store food properly and dispose of trash in bear-proof dumpsters to help prevent bear habituation to anthropogenic food sources. The current Black Bear Management Guidelines require that the NPS wildlife biologist evaluate each situation on a case by case basis. Some options that can and are implemented include monitoring of bear activity, posting warning signs for visitors, closing areas to recreational use, aversive conditioning, relocation, and euthanasia (National Park Service 2002). Many factors can cause black bears to seek out anthropogenic food sources; these factors are known and actions have been taken to curtail nuisance activity. However, a better understanding of the biological variables that cause some bears to seek out anthropogenic food sources would be helpful to managers. Some of these factors may include physiological mechanisms, i.e., growth demand, competition, and environmental mechanisms, i.e., variability in food sources. With traditional wildlife nutrition studies, scat analysis has been used to determine the diet of a species (Beeman and Pelton 1980, Eagle and Pelton 1983, Seibert and Pelton 1994). Many dietary studies are conducted over a few years, which only provide a snapshot of the animals' foraging patterns and can miss variation that occurs within the environment. A long term-study of diet can examine the dietary trends of a population in connection with long-term environmental patterns and natural life history cycles.

# STABLE ISOTOPES IN WILDLIFE STUDIES

Stable isotope analysis can be used to differentiate the relative abundance of animal and plant matter and diets consisting of anthropogenic foods compared with natural food sources in individual animals (Robbins et al. 2004). Stable isotope analysis also allows quantitative analysis of trophic levels within a community (Crawford et al. 2008). The longitudinal dietary patterns of specific groups (e.g., sex, age, body mass) can be assessed (Greenleaf 2005). As part of an ongoing, long-term research project on black bears in GSMNP that started in 1969, hair samples have been collected from captured bears (Pelton and van Manen 1996). Those hair samples could provide valuable longitudinal information on assimilated diets and food habits of black bears (Robbins et al. 2004). Importantly, by using hairs collected from live captured animals, individual characteristics of each animal can be correlated with the sample analyzed.

## **OBJECTIVES**

I examined longitudinal feeding patterns of black bears live-captured in Great Smoky Mountains National Park (GSMNP) during 1980–2001 using stable carbon and nitrogen isotope analysis of hair samples. I used the ratio of stable carbon and nitrogen isotopes in bear hair to examine trophic levels and the use of anthropogenic foods by black bears. I wanted to investigate if anthropogenic food exploitation and increased protein intake were associated with age, sex, year, nuisance status, total hard mast index, white oak index, red oak index or hog kill. The use of long-term diet patterns based on stable isotope analysis may be helpful to understand changes in food selection of black bears over time and guide management programs to reduce human-bear conflicts. Specifically, I wanted to test the following hypotheses:

1) The availability of hard mast crops can affect the amount of anthropogenic food in the diet of black bears in the Southeast United States.

- 2) Black bears that are subsequently captured as nuisance bears will have enriched  $\delta^{13}C$  signatures (more anthropogenic food in diet) compared with bears not subsequently captured as nuisance bears.
- 3) Black bears in GSMNP will have enriched  $\delta^{13}C$  signatures in the later years of the study compared with depleted signatures in the earlier years of the study as human visitation in the park and black bear abundance increases.
- 4) Adult male black bears in better condition are more likely than other sex and age groups to have access to anthropogenic food sources because of their larger home ranges.
- 5) The number of hogs killed by park personnel as part of the wild hog management efforts is reflective of the amount of nitrogen in black bear diet, i.e., as the number of hogs killed increases;  $\delta^{15}N$  levels in the diet will also increase.

# **CHAPTER II**

# LITERATURE REVIEW

# ANTHROPOGENIC FOOD EXPLOITATION

A combination of factors can affect bear exploitation of anthropogenic food sources, such as high visitor density, high bear density, and poor natural food crops (Singer and Bratton 1980). Stiver (1991) found that mostly young females and subadult males accessed anthropogenic food sources. Food-conditioned bears were primarily a problem in campgrounds, picnic areas and other front-country sites (Stiver 1991). However, Hatch and van Manen (2007) suggested that older males captured in the backcountry may also access anthropogenic food sources. Adult male black bears may have regular access to anthropogenic food sources due to their larger home ranges compared with females and sub adults or there may be an increase in exploitation of anthropogenic food sources when bear densities increase and mast production fails. Clark et al. (2005) found that availability of hard mast was related to changes in population growth ( $\lambda$ ) of black bears in GSMNP. Therefore, changes in population density may be associated with hard mast availability, which, in turn, may influence incidence of nuisance activity by bears (Noyce and Garshelis 1997) and increase exploitation of human food sources.

## FEEDING ECOLOGY

Numerous black bear research projects have been conducted in GSMNP regarding mast production (Pelton 1989, Inman and Pelton 2002, Clark et al. 2005), population growth (Coley 1995, McLean and Pelton 1994), reproduction (Eiler et al. 1989, Pozzanghera 1990), visitor information and nuisance bear activity (Singer and Bratton 1980, Tate and Pelton 1983, Clark et al. 2003). Nutritional studies have mostly been based on scat collection (Beeman and Pelton 1980, Eagle and Pelton 1983, Seibert and Pelton 1994), which only provides information regarding nondigestible foods and not the assimilated diet of a species (Pritchard and Robbins 1990, Hewitt and Robbins 1996, Robbins et al. 2004). Thus, these earlier studies using scat analysis often underestimated the role of animal matter or anthropogenic foods in the diets of bears. The variation of digestive efficiency for bears ranges from 30% for plant matter to >90% for meat (Pritchard and Robbins 1990, Hewitt and Robbins 1996). An additional disadvantage of fecal analysis is the inability to correlate nutritional information from individuals with unique characteristics (e.g., body condition, reproductive status) unless the animal depositing the scat can be identified; this is often difficult because scat is usually collected after the animal has left the area (Greenleaf 2005).

# STABLE ISOTOPES

Stable isotopes are naturally occurring elements that contain an extra neutron and occur in parts per thousand (‰) relative to the abundant form of a particular element (Robbins et al. 2004). In the past, radioactive isotopes have been used as tracers in metabolic pathways because of their rapid decay rate. Stable isotopes are different from

radioactive isotopes because they do not decay and are incorporated into an organism's tissue (Crawford et al. 2008). Stable carbon and nitrogen isotopes exhibit natural variation in abundance (Karasov and del Rio 2007). The mass differences between the heavy element with the extra neutron and light element cause isotopes to react differently in physical and chemical processes called fractionation (Gannes et al. 1998). Isotopic variation within a species can be analyzed from tissue samples such as hair, plasma, or bone. The amount of tissue required for analysis is ≤2 mg (Robbins et al. 2004).

The common carbon isotope is  $^{12}$ C and the heavier isotope is  $^{13}$ C. Carbon fractionation in plants can differ because of 3 distinct photosynthetic pathways:  $C_3$ ,  $C_4$ , and Crassulacean acid metabolism (CAM).  $C_4$  and CAM plants evolved from C3 plants as an adaptation to declining atmospheric  $C0_2$  levels in the late Miocene. The photosynthetic pathways in  $C_4$  and CAM plants are adapted for concentrating  $C0_2$ . CAM plants concentrate the  $C0_2$  around Rubisco using a dual carboxyaltion pathway that is separated temporally in the same tissue compared with a spatial separation in C4 plants between the mesophyll cell and bundle sheath cell (Keeley and Rundel 2003). Because of this fractionation, the isotopic signature for  $C_3$  plants is unique compared with the isotopic signature of  $C_4$  and CAM plants (Gannes et al. 1998). The  $C_3$  plants are depleted in  $^{13}$ C (having a more negative delta ( $\delta$ ) value) in relation to an international standard of  $^{13}$ C: $^{12}$ C and  $C_4$  plants are enriched (having a more positive  $\delta$  value) with  $^{13}$ C (Karasov and del Rio 2007). The  $\delta$  notation refers to the difference in abundance of isotopes relative to common international standards (Crawford et al. 2008). The standard for

carbon is the Vienna Pee Dee Belemnite (VPDB) formation limestone with  $^{13}\text{C}$ : $^{12}\text{C}$  of 0.011237 (Jahren et al. 2006). The  $\delta^{13}\text{C}$  values for C<sub>3</sub> plants range from -34 to approximately -24  $^{0}$ / $_{00}$  with an average of approximately -27.1  $^{0}$ / $_{00}$  (Smith and Epstein 1971, Gannes et al. 1998, Jahren et al. 2006, Karasov and del Rio 2007, Jahren and Kraft 2008, Z. Li, University of Tennessee, personal communication).

Globally, most plants are C<sub>3</sub> plants (Jahren et al. 2006), which is the primitive photo-synthetic pathway and includes all native vegetation of GSMNP. Native C4 plants found in GSMNP include: Poaceae; Schizachyrium scoparium, Andropogon gerardii, Sorghastrum nutans, Andropogon virginicus, and Cistaceae; Hudsonia tomentosa (United States Department of Agriculture 2011). However; these perennial grasses are not known to be a part of the American black bear's natural diet and are found in the same habitat as the research bears in this study. The natural diet of the American black bear is almost exclusively comprised of C<sub>3</sub> plants (Hildebrand et al. 1996). An exotic C4 plant found in GSMNP is *Microstegium vimineum*, introduced from Japan and is an annual grass that is most abundant in the summer and early fall months when the flowers are produced (Plant Conservation Alliance 2008). With the abundant energy rich berries and acorns available during these months, this sprawling grass is an unlikely food choice of black bears in GSMNP. The natural food of black bears in GSMNP consists of berries, acorns, and grasses (Beeman and Pelton 1980, Eagle and Pelton 1983, Seibert and Pelton 1994) which are C<sub>3</sub> plants. Most anthropogenic foods contain high fructose corn syrup or other products that are derived from corn (Zea spp) or sugar cane (Saccharum spp), which are  $C_4$  plants (Smith and Epstein 1971, Jahren et al. 2006). The  $\delta^{13}$ C for  $C_4$  plants ranges

from -6 to -19  $^{0}/_{00}$  with an average of approximately -13.1  $^{0}/_{00}$  (Smith and Epstein 1971, DeNiro and Epstein 1978, O'Leary 1988, Jahren et al. 2006, Z. Li, University of Tennessee, personal communication). Stable isotopes of carbon can be used to differentiate natural diets, consisting of  $C_3$  plants, and diets from anthropogenic sources, consisting of  $C_4$  plants (Greenleaf 2005), because tissues of animals that consume food with  $C_4$  plant sources will have a distinctly high  $\delta^{13}$ C value (Jahren et al. 2006).

As with carbon, stable nitrogen isotopes (<sup>14</sup>N and <sup>15</sup>N) also undergo fractionation as the element is processed via different biochemical pathways. The fractionation occurs during deamination and transamination (DeNiro and Epstein 1981). Protein enters the body and is broken down to amino acids. These amino acids are taken up by cells in the liver and muscle tissue where they are converted to ammonia and proteins. The lighter stable nitrogen isotope, <sup>14</sup>N is partially removed from the digestive tract and the heavier <sup>15</sup>N stable isotope is assimilated preferentially into the body's tissues (biochemical fractionation; Greenleaf 2005). Because of the fractionation, animals at higher trophic level will have enriched <sup>15</sup>N value compared with animals at the lower trophic levels such as herbivores and omnivores (Gannes et al. 1998). Stable nitrogen isotope analysis provides a measure of the relative importance of meat in the diets of black bears. The standard for nitrogen is atmospheric nitrogen (AIR; DeNiro and Epstein 1981) because of its constant value (0.366%; Junk and Svec 1958). Used in combination with stable carbon isotope analysis, the importance of anthropogenic foods, such as human garbage, can be evaluated.

## USE OF STABLE ISOTOPES TO STUDY BEAR FOOD HABITS

Ratios of stable carbon and nitrogen isotopes have been used to measure feeding history in Asiatic black bears (*U. thibetanus*; Mizukami et al. 2005), American black bears (Greenleaf 2005, Hatch and van Manen 2007), brown bears (Hilderbrand et al. 1999a, 1999b; Felicetti et al. 2003, Fortin et al. 2007) and the extinct cave bear of Europe (U. speleaus; Hilderbrand et al. 1996). In Japan, Mizukami et al. (2005) found that hair from rural Asiatic black bears was enriched with  $\delta^{15}N$  and  $\delta^{13}C$  indicative of anthropogenic food sources in the diet compared with alpine bears. Rural bears with access to anthropogenic food and cornfields showed high variation in the isotope values during different seasons compared with the alpine bears, suggesting a diet of natural foods and anthropogenic foods for bears near human areas. Greenleaf (2005) found that stable nitrogen isotope ratios were significantly related to management status of bears in Yosemite Valley, California. Bears that were the most food-conditioned had enriched nitrogen values (Greenleaf 2005). Newsome et al. (2010) was able to detect differences in diets consumed by San Joaquin kit Foxes (Vulpes macrotis mutica) in urban and nonurban areas of Fresno, California. They found that foxes in urban areas exploited anthropogenic food sources by detection of an enriched  $\delta^{13}$ C signature. In a pilot study, Hatch and van Manen (2007) analyzed stable of carbon and nitrogen isotopes in 66 bears in the back country at GSMNP and found higher use of anthropogenic foods in larger, older male bears. However, this study was only for 1 year and could not examine effects of management changes over time. Therefore, a longitudinal study using stable carbon and nitrogen isotope analysis is needed to determine if access to anthropogenic food

sources has changed over time and what variables are associated with the changes. Better understanding of long term environmental and physiological factors affecting bear use of anthropogenic foods could help guide nuisance bear management. The purpose of this study is to examine if any trends exist over time for black bears in GSMNP with regard to selection of anthropogenic food sources over natural food sources and what the mechanisms are that drive those choices. Understanding these long term diet patterns can aide managers to understand what may cause black bears to seek out anthropogenic food sources.

# **CHAPTER III**

# **METHODS**

# STUDY AREA GENERAL DESCRIPTION

Great Smoky Mountain National Park is 2,072 km<sup>2</sup> and is located on the border of Tennessee and North Carolina between 35° 26′ and 35° 47′ N latitude and 83° 2′ and 84° 0′ W longitude. The park is bordered by the Cherokee National Forest, Tennessee to the Southwest, the Pisgah National Forest, North Carolina to the Northeast, and the Nantahala National Forest, North Carolina to the south. The Tennessee side of GSMNP includes the counties: Blount, Sevier, and Cocke. On the North Carolina side of GSMNP is Haywood and Swain counties. Land north of the park is privately owned and a single ridge is the geographical divide forming the political boundary between Tennessee and North Carolina (Stiver 1991). The Tennessee portion of GSMNP is bordered by private land which is developed for vacation homes and the tourism industry. Elevation within the park ranges from 270 to 2,024 m. Live-capture of black bears began in 1969 in the northwest quadrant (Figure 1) of the park (approximately 330 km<sup>2</sup>).

# **TOPOGRAPHY**

As part of the Unaka mountain range of the Blue Ridge Province, GSMNP is located within the southern division of the Appalachian Highlands. The park is characterized by rugged topography of ridges that extend outward from the main ridges separated by wide valleys (Fenneman 1938 in, Whittaker 1956). The range of elevations

within the park begins where Abrams Creek enters the Little Tennessee River at 270 m to Clingman's Dome at 2,024 m (Pivorun et al. 2009). The elevations within the northwest quadrant of the park for my study ranged from 318 m to 1,658 m (Laufenberg 2010).

#### CLIMATE

Because of the wide variation in elevation, aspect, and slope within GSMNP, there are several microclimates that exhibit substantial variation (Shanks, 1954).

Thornthwaite (1948) classified the area as mesothermal per-humid or warm-temperate rain forest. Average annual precipitation varies from 140 at lower elevations to 220 cm at higher elevations (Stephens 1969).

# FLORA AND FAUNA

Among the eastern forests in North America, Whittaker (1956) classified GSMNP with the greatest diversity. The variation in elevation yields a range of forest communities within the park. Low elevations are characterized by mixed hardwoods while the high elevations are characterized by spruce-fir (*Picea rubens*). Within the park there are over 1,300 flowering plants, 130 tree species, 2,000 fungi, 330 mosses, 230 lichens and 32 fern species that have been recorded (King and Stupka 1950, Stupka 1960). Within my study area, the primary vegetation of the hardwoods was composed of oaks (*Quercus* spp.), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), sweetgum (*Liquidambar sturaciflua*), yellow buckeye (*Aesculus flaca*) and dogwood (*Cornus florida*). The major understory vegetation within my study area was composed of rhododendron (*Rhododendron maxima*), mountain laurel (*Kalmia latifolia*), huckleberry (*Gaylussacia* spp.), blueberry (*Vaccinium* spp.) and wild grape (*Vitis* spp.)

(Laufenberg 2010). There are a recorded 71 mammal species within GSMNP. Four of those species were extirpated, 2 were reintroduced and 4 were non-native (Pivorun et al. 2009). King and Stupka (1950) recorded over 200 bird species, 30 reptile species, 39 amphibians, and 80 fish species.

# **BEAR TRAPPING**

During 1975–2007, field personnel collected 1,835 hair samples from black bears in GSMNP, along with information regarding nuisance status, body condition and mass, location of capture and release, and reproductive status (M. Pelton, University of Tennessee, unpublished data). Bears were captured using spring-activated, Aldrich foot snares; to reduce injuries during capture, a spring from an automobile hood was placed on the cable (Johnson and Pelton 1981). Various chemical immobilization drugs were used over the course of the study (Beeman and Pelton 1980, Wathen et al. 1986, van Manen 1994, Coley 1995, Clark et al. 2003, 2005, Stiver 1991). All bears captured were handled according to animal welfare protocols approved by the University Of Tennessee Institutional Animal Care and Use Committee (IACUC #1096).

# HAIR COLLECTION

Field personnel collected hair samples from captured bears in back-country of the northwestern quadrant of GSMNP. For this project, back-country refers to the locations of historic trap lines in GSMNP where bears are not normally associated with human visitation. From 1980 through 1988, samples were stored in glass vials or plastic bags. Starting in 1988, hair was stored in manila coin envelopes. All hair samples were stored away from light and at room temperature.

In the years 2007 and 2008, a reintroduction project of elk into the Cataloochee Valley of GSMNP, North Carolina, resulted in the removal of bears from the reintroduction site to prevent predation during the elk calving season (Yarkovich 2009); hair samples were collected from bears as part of the capture/relocation process along with weight, sex and age information. A total of 21 bear hair samples collected for the elk reintroduction project were donated for stable isotope analysis. Mean values of the hair samples from Cataloochee Valley in GSMNP, Knoxville Zoo bear, Appalachian Bear Rescue bears, Gatlinburg nuisance bears, and nuisance bears in the picnic and campground areas of GSMNP were combined and used as reference samples to verify that differences in diets could be detected with stable carbon and nitrogen isotope analysis.

# **SAMPLE SELECTION**

I used stratified random sampling to select hair samples for the period 1980–2001. This period coincided with the available mast survey data and covered sufficient temporal variation in feeding habits. Black bears molt once a year, usually starting in late spring/early summer and are variable depending on nutrition (C.T. Robbins Washington State University, personal communication). Hair collected in the late summer/early fall represents diet from the current season (Felicetti et al. 2003). Black bears have two types of hair, guard hairs and underfur. The guard hair in black bears is for protection of the skin and the underfur is mainly for insulation so these two different types of hair grow during different times of the year. The underfur primarily starts to grow in fall for thermoregulation during hibernation and the guard hair starts to grow depending on

nutrition in late summer (C.T. Robbins Washington State University, personal communication). I selected guard hair samples that were collected between May and August to represent the previous year of food eaten. I stratified the samples according to age, sex, and year. For each year, I selected 6 samples (3 for each sex; and 1 for each of the 3 age classes within sex; Table 1).

# SAMPLE PREPARATION

I transferred all hair samples to 15- x 45-mm glass vials and rinsed samples with deionized water to remove any large particles. Glass vials with rinsed hair were placed under a fume hood to dry. Enrichment of isotopes can differ for lipids synthesized from carbohydrates compared with lipids derived directly from fat (Gannes et al. 1998). To prevent erroneous results from fat deposited on the hair, I removed the lipids from hair samples by placing the hair in Soxhulet thimbles in a beaker with approximately 250 ml of a 2:1 chloroform methanol solution (Acros Organics, Morris Plains, New Jersey, USA; Fisher Chemical, Fairlawn, New Jersey, USA, respectively). The beakers were covered with aluminum foil and placed in a water bath for sonication using a Fisher Scientific Sonic Dismembrator Model 500 (Branson Ultrasonics, Danbury, Connecticut, USA). Hair samples were sonicated for 15 min at 30% amplitude. After sonication, I placed samples in clean, labeled glass vials and dried them in an oven at 40° C overnight (Z. Li University of Tennessee, personal communication).

I cut hair samples into 1-mm segments to obtain homogenous samples for analysis. To prevent any exogenous oils from contaminating the samples, I sterilized aluminum foil at 400°C for 3 hours. I placed hair samples into a square piece of foil

approximately 5 x 5 cm and rolled the hair in the foil. I cut the foil into 1-mm segments and removed the cut hair for storage until all samples were clipped and ready to weigh. Hair samples weighing from 1.3 to 1.5 mg were placed in a 5 x 9-mm pressed tin capsule (Costech Analytical Technologies, Valencia CA, USA) and folded for isotope analysis. I placed samples into a well tray until all samples were prepared and ready to be placed in the spectrometer (described under stable isotope analysis: Table 2).

Hair samples used for reference values were obtained as a courtesy from multiple sources (Table 3). All reference samples were already being collected for various projects; additional sample collection was approved by IACUC protocol #1930.

# FOOD ITEMS COLLECTION AND PREPARATION

I collected natural plant food items as available during 2009/2010 (oak acorns, huckleberry, blueberry, and wild grape). All food items were stored frozen in sealed plastic bags until ready for analysis. A variety of frozen acorn samples (Table 3) for analysis were donated by Appalachian Bear Rescue and mailed to the University of Tennessee where they were stored frozen until analyzed. Preparation of food items involved drying in glass vials at 40°C for 24-72 hours. After the samples were dried, I used a mortar and pestle to grind the food item to a fine powder and placed the pulverized food item back in a clean dry glass vial. Once all food items were ground into a fine powder, samples were weighed (1.4 – 1.5 mg) and placed in a 5 x 9-mm pressed tin capsule for isotope analysis. I placed samples into a well tray until all samples were prepared and ready to be placed in the mass spectrometer.

# STABLE ISOTOPE ANALYSIS

Stable isotopes of carbon and nitrogen were analyzed using a Thermo-Finnigan isotope ratio mass spectrometer Delta Plus XL, coupled with COSTECH Elemental Analyzer (ECS4010; Stable Isotope Laboratory at Department of Earth and Planetary Sciences, University of Tennessee, Knoxville, USA). The ratios were reported as parts per thousand of the isotope  $\binom{0}{00}$  relative to a standard  $x(R_{standard})$  as:

$$\delta^{13}$$
C or  $\delta^{15}$ N =  $\begin{bmatrix} R_{sample} / R_{standard} \end{bmatrix} - 1 \times 10^3$ ,

where, R is  $^{13}$ C/ $^{12}$ C or  $^{15}$ N/ $^{14}$ N (Smith and Epstein 1971; DeNiro and Epstein 1978, 1980; O'Leary 1981, 1988). The  $R_{standard}$  for  $\delta^{13}$ C is the Pee Dee Belemnite (PDB) standard for carbon (Craig 1957, Karasov and del Rio 2007). The  $R_{standard}$  for  $\delta^{15}$ N is atmospheric nitrogen (AIR; Mariotti 1983, Karasov and del Rio 2007). A total of 49 samples were analyzed per run. The 49 samples included: 2 bypass samples of Atropine  $\sim$ 1mg (Costech Analytical Technologies, Valencia CA, USA), 1 blank foil capsule (Costech Analytical Technologies, Valencia CA, USA), 4 standard samples (0.5, 1.0, 1.5, and 2.0mg) of Acetanilide to establish a calibration curve (Costech Analytical Technologies, Valencia CA, USA), and isotope reference samples ( $\sim$ 0.7-1.0mg) of USGS 40 and 41 L-Glutamic Acid (U.S. Geological Survey Reston, VA, USA). The first reference samples were run as the  $8^{th}$  and  $9^{th}$  sample after all bypass, blanks, and standard samples, thereafter I ran reference samples after every tenth hair sample (Table 2).

## **DISCRIMINATION FACTOR**

The assimilation of food items varies depending on composition of a species' diet but does not vary among bear species (Pritchard and Robbins 1990). When a consumer metabolizes organic matter, there is net difference (discrimination factor) between the food item and assimilated stable isotope value known as metabolic fractionation (Karasov and Martinez del Rio 2007). The value for metabolic fractionation depends on the type of diet consumed (Pritchard and Robbins 1990). The isotopic values of the consumer's diets are not always equal to the values of their organic diet because the proteins, carbohydrates, and lipids are routed to different tissues. This difference between the stable isotope signature of the consumer's diet and the stable isotope signature of a particular tissue i.e., serum, plasma, bone, hair, teeth, liver, etc. is termed the discrimination factor (Cerling and Harris 1999) or metabolic fractionation. There have been several studies examining the discrimination factor between consumer and diet and the results vary widely among studies. To determine an appropriate value to apply for the discrimination factor, results I compared the results from studies on appropriate values for discrimination factors. Ben-David (1996) and Ben-David et al. (1997a, 1997b, 2001, 2004), found that clotted blood cells had a 2% enrichment for  $\Delta\delta^{13}$ C when mammalian prey, avian prey and berries were consumed and 1% enrichment when salmon or invertebrates were consumed. For  $\Delta\delta^{15}$ N, Ben-David et al. (1997, 2001) used a 3% fractionation value. Hilderbrand et al. (1996) found that plasma  $\Delta\delta^{13}$ C was enriched by 0.4 to 4.5% when dietary values were -18.5 to -25.5% and  $\Delta\delta$  <sup>15</sup>N was enriched 4.1 ±0.5% in plasma in captive American black bears compared to their diet. DeNiro and

Epstein (1978) found that the whole body  $\Delta\delta^{13}$ C of animals were enriched 0.3±1.1‰. DeNiro and Epstein (1981) found that the whole body  $\Delta\delta^{15}$ N of animals were enriched 3.0±2.6‰ compared with their diets. Felicetti et al. (2003) found that the discrimination factor for  $\Delta\delta^{13}$ C diet to plasma of grizzly bear plasma (*U. arctos*) varied substantially and that the discrimination factor for  $\Delta\delta^{15}$ N to plasma was 3.0±5.0‰. Tieszen et al. (1983) reported fractionation values for hair using captive gerbils (*Meriones unguiculatus*) in captive feeding trials of 1‰ for  $\Delta\delta^{13}$ C over the diet. Lesage et al. (2002) used stable carbon and nitrogen isotope analysis to look at fractionation in Phocid seals and found a  $\Delta\delta^{13}$ C diet-hair fraction value of 2.3±0.1‰ and  $\Delta\delta^{15}$ N diet-hair fractionation value of 2.3±0.8‰. McCutchan et al. (2003) did a review of the literature regarding stable carbon and nitrogen isotope diet-tissue fractionation and found that for all animals within the various studies, the mean estimates of trophic shift for  $\Delta\delta^{13}$ C was + 0.4 ± 0.12‰ and + 2.0 ± 0.20‰ for  $\Delta\delta^{15}$ N.

To examine if the mean stable isotope signature of the population is different from mean stable isotope signature of the natural food items found in GSMNP, a fractionation correction value was applied to account for the known trophic shift between consumer and diet. I applied a value of 2‰ for  $\Delta\delta^{13}$ C and 3‰ for  $\Delta\delta^{15}$ N to account for the metabolic fractionation that occurs between diet and consumer based on average discrimination factor values (Table 4) from the reviewed literature (DeNiro and Epstein 1978, 1981; Ben-David 1996; Hilderbrand et al. 1996; Ben David et al. 1997a, 1997b, 2001, 2004; Tieszen et al. 1983; Lesage et al. 2002 Felicetti et al. 2003). Because there still is some question as to the correct fractionation value to apply depending on species,

diet, and tissue analyzed, I also compared the stable isotope signatures of natural food items in GSMNP with the hair samples using stable carbon and nitrogen isotope values without any discrimination factor. When examining changes in black bear diets over a long period of time, it was not necessary to use a discrimination factor because I was not directly comparing the stable isotope signatures in hair samples from bears with the stable isotope signatures of the natural food items consumed. However, for consistency I use the corrected values for the entire analysis.

#### STATISTICAL ANALYSIS

I used weight class because of the subjective assessments for body condition by different individuals over time. To account for differences in weight based on age and sex, I constructed the three weight categories (below average, average, and above average weight) using weight and standard deviation for the 6 combinations of age and sex from all recorded bears (Table 5). The categories were calculated as:

below average weight lower limit =  $\mu$  – (1.5 \* Std. dev), below average weight upper limit =  $\mu$  – (0.5 \* Std. dev), average weight lower limit =  $\mu$  – (0.5 \* Std. dev), average weight upper limit =  $\mu$  + (0.5 \* Std. dev), above average weight lower limit =  $\mu$  + (0.5 \* Std. dev) and, above average weight upper limit =  $\mu$  + (1.5 \* Std. dev).

GSMNP personnel conduct hard mast surveys each year and calculate a hard mast index using the methods developed by Greenberg and Warburton (2007). I used the values from this index as my hard mast variable. Acorn production varies by species

classified into two groups, red oak and white oak (Greenberg and Parresol 2000, Ober 2008). White oak species produce mast yearly and red oak species production occurs on a 2-year cycle. White oak acorn production is more variable than red oak acorn production (Greenberg 2000, McNutt 2002). Red oak acorns have higher protein and fat content and more calories but because of the higher tannin and fiber content they are not as easily digestible. Most wildlife species prefer white to red oak acorns because of the lower tannin content (Clark 2004, Ober 2008). Black bears will eat both red oak and white oak depending on the mast production for that year (Eagle and Pelton 1983). I used three hard mast variables in my model set, total hard mast index, white oak index and red oak index (Clark et al. 2005) to account for potential variation in acorn consumption by black bears depending on availability and preference. I offset the hard mast index by one year to correspond with the dietary time period of the hair sample (Table 5). Hair samples for analysis were collected from May to August representing the previous year's diet and the hard mast index is representative of the current year. Wild hogs were trapped or hunted during my study period for wildlife damage control. Hog carcasses were left in the park to be scavenged by native wildlife. I totaled the number of hogs killed in areas that corresponded to the historical trap lines as a measure of potential meat consumption by black bears. I compiled a list of all research bears that became nuisance bears at some future date and used this as a nuisance variable in my models. I used three age class categories for classification of bears; sub-adult (1.5 - 3yrs), adult (3.5-6.5yrs) and older adult ( $\leq 7$ yrs).

I developed an *a priori* suite of linear regression models examining if sex, age class, year, weight, total hard mast index (HMI), white oak mast index, red oak mast index, bear nuisance status, and hog kill were associated with stable carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) isotope signatures using an information-theoretic (IT) approach (Burnham and Anderson 2002, 2004; Anderson 2008). I constructed dummy variables for my categorical variables; weight class, sex, and nuisance status. Dummy variables were coded as:

if age\_class=1 then age\_class1=1; else age\_class1=0;

if age\_class=2 then age\_class2=1; else age\_class2=0;

if sex=1 then sex 1=1; else sex 1=0;

if nuisance=1 then nuisance1=1; else nuisance1=0;

if weight\_class=1 then weight\_class1=1; else weight\_class1=0;

if weight\_class=2 then weight\_class2=1; else weight\_class2=0;

I ran a Proc Reg (SAS Institute, 2009, Cary, North Carolina, USA) to calculate RSS values and used the equation from Anderson (2008) to calculate Akaike's second order information criterion (AIC<sub>c</sub>) and the relative weights of each model as:

$$AIC_c = n\log(\hat{\sigma}^2) + 2K\left(\frac{n}{n-K-1}\right),$$

Where, K is the number of parameters including the intercept and:

$$\hat{\sigma}^2 = \sum_{i} \hat{\varepsilon}_i^2 / n$$

To rank the models within the set, I used the AIC with a second-order correction criterion for small sample size values (AIC<sub>c</sub>) and Akaike weights to examine relative importance of each model. I ascertained a relative measure of empirical support by looking at the difference between the top model and other models within the candidate set. Models with  $\Delta$ AIC<sub>c</sub> values  $\leq$  2 were well supported and  $\Delta$ AIC<sub>c</sub> values  $\geq$ 10 were not considered to have much support. Akaike weights are a measure of the weight for the best model compared with all other models in the set. Model weights ( $w_i$ ) were calculated with the assumption that the best model is included as:

$$w_i = \frac{\exp\left(-\frac{1}{2}\Delta_i\right)}{\sum_{r=1}^{R} \exp\left(-\frac{1}{2}\Delta_i\right)}$$

where R is the number of models in the candidate set and r is the first model in the summation (Burnham and Anderson 2002, Anderson 2008). I used model averaging of the parameter estimates across my entire set of models to obtain a robust estimate for each of my parameters (Burnham and Anderson 2002, Anderson 2008).

$$\tilde{\overline{\beta}}_{j} = W_{+}(j)\hat{\overline{\beta}}_{j.}$$

where  $\beta_j$  is the linear regression coefficient associated with the predictor variable  $\left(x_j\right)$  and  $\hat{\beta}_j$  is the estimate of  $\beta_j$  averaged across all models where  $x_j$  appears, and (j) is the predictor variable.  $\tilde{\beta}$  is a second model-averaged estimator with  $w_+(j)$  being the sum of the Akaike weights over all models in the set where the predictor variable (j)

occurs and where  $x_j$  is not in a particular model,  $\beta_{j,i} \equiv 0$  is used (Burnham and Anderson 2002, 2004; Anderson 2008). I calculated unconditional variances for each parameter to take into account model selection uncertainty as:

$$\operatorname{var}\left(\hat{\overline{\theta}}\right) = \sum_{i=1}^{R} w_{i} \left\{ \operatorname{var}\left(\hat{\theta}_{i} \mid g_{i}\right) + \left(\hat{\theta}_{i} - \hat{\overline{\theta}}\right)^{2} \right\}$$

where  $\hat{\theta}$  is the model averaged estimate,  $w_i$  are the model probabilities and  $g_i$  is the *ith* model (Burnham and Anderson 2002, Anderson 2008). I calculated unconditional confidence intervals using the model averaged parameter estimates and standard error based on the unconditional variance (Burnham and Anderson 2002, Anderson 2008).

$$se(\hat{\overline{\theta}}) = \sqrt{var(\hat{\overline{\theta}})}$$

Using my model averaged estimates; I examined which parameters were associated with variations of stable carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) isotope signatures for black bears in GSMNP over a 20 year time period. All assumptions of normality for linear regression analysis were met.

I used the IT approach and AIC to estimate the size of the effect (Anderson 2008) on the stable carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) isotope signatures within the population of black bears in GSMNP. For general comparisons of food items and hair samples, estimating the effect size was not the goal. I wanted to examine if there was simply a difference in the mean signatures of the entire population of black bears in GSMNP compared to the natural food items of black bears in GSMNP. I used a confirmatory investigation (Anderson 2008), hypothesizing the mean stable carbon ( $\delta^{13}$ C) and nitrogen

 $(\delta^{15}N)$  isotope signatures of black bear natural food items in GSMNP would be depleted compared to mean stable carbon  $(\delta^{13}C)$  and nitrogen  $(\delta^{15}N)$  isotope signatures in hair samples from black bears in GSMNP. I used the Aspen-Welch unequal variance t-test (NCSS Software 2007, Kayscill, Utah, USA) to compare means between the sample population and natural food items. I compared means of the sample populations with both corrected and non-corrected values for fractionation differences between assimilated diets and food items.

To check for sample homogeneity, I randomly selected and analyzed one subsample from each year for stable carbon and nitrogen isotope values and looked at the standard deviation of the absolute value for the differences between sample sets.

#### **CHAPTER IV**

### **RESULTS**

#### STABLE CARBON AND NITROGEN ISOTOPES OF REFERENCE SAMPLES

Differences in diet were detected for both stable carbon and nitrogen isotope analysis from reference hairs (Figure 2A, 2B). The zoo bear, fed 4 whole fruits, 4 whole vegetables, 3 cups of chopped melon or grapes and a corn meal based kibble chow (Purina Proplan Weight Management Adult Chicken and Rice; Société des Products Nestlé S.A., Vevey, Switzerland) had enriched  $\delta^{13}$ C (-17.76%) and  $\delta^{15}$ N (4.47%) stable isotope signatures. Hair samples from nuisance bears (n = 3) in the Gatlinburg area collected from Tennessee Wildlife Resource Agency reflected a mixed diet of human foods and natural foods for  $\delta^{13}$ C ( $\bar{x} = -23.10\%$ , SD = 1.97) and  $\delta^{15}$ N ( $\bar{x} = 1.02\%$ , SD = 2.61). Bears from Appalachian Bear Rescue (n = 2) fed a mixed diet of natural food and vitamin supplements also had enriched  $\delta^{13}$ C ( $\bar{x} = -22.64\%$ , SD = 1.87) and  $\delta^{15}$ N ( $\bar{x} =$ 1.46%, SD = 2.02). Orphaned bear cubs also from Appalachian Bear Rescue (n = 7) fed a mixed diet of natural foods and vitamin supplements had slightly enriched  $\delta^{13}$ C ( $\bar{x}$  = 24.06‰, SD = 1.63) and  $\delta^{15}$ N ( $\bar{x} = 0.75$ ‰, SD = 1.06). Black bears (n = 7) that were captured in the campgrounds and picnic areas of GSMNP during the summer of 2010 had  $\delta^{13}$ C signatures similar to the research bears in this study ( $\bar{x} = -25.52\%$ , SD = 1.10) and  $\delta^{15}$ N signatures slightly enriched compared with the research bears ( $\bar{x} = 0.31\%$ , SD = 0.76). Bears (n = 21) from the Cataloochee Valley area in GSMNP that were a part of the relocation project (Yarcovich 2009), had  $\delta^{13}$ C ( $\bar{x}$  = -25.26‰, SD = 0.69) and  $\delta^{15}$ N ( $\bar{x}$  = 1.47‰, SD = 0.55) signatures similar to the research bears in this project. Reported results were all corrected for metabolic fractionation ( $\delta^{13}$ C = 2.0‰,  $\delta^{15}$ N = 3.0‰). Applying the correction factors to hair samples shifts uncorrected values (Figure 3A, 3B) of  $\delta^{13}$ C toward C<sub>3</sub> plant ranges and  $\delta^{15}$ N to ranges for lower trophic levels (Figure 4A, 4B).

#### STABLE CARBON ISOTOPE ANALYSIS

Top models for  $\delta^{13}$ C stable isotope data included weight class, white oak index, red oak index, total hard mast index, and nuisance status (Table 6). Five models (M17, M9, M10, M11, M19) had  $\triangle AIC_c$  values  $\leq 2.657$  and all included weight class. Cumulative weight of those five models was 0.772. The most parsimonious model (M17) included weight class and white oak index and had a weight of 0.370. The second top model (M9) included only weight class ( $w_i = 0.28$ ,  $\Delta AIC_c = 0.540$ ). In addition to weight class, the next three models included total hard mast index, nuisance, and red oak index, respectively (Table 6). All other models had  $\triangle AIC_c$  values  $\geq 7.280$  and weights  $\leq 0.010$ , with a cumulative weight of 0.029. The r-squared values for the top five models (M17, M9, M10, M11, M19) were: 0.15, 0.13, 0.13, 0.13, and 0.13 respectively. Based on model averaging, bears in the highest weight class had  $\delta^{13}$ C signatures that were 0.76‰ (95% CI = 0.28 to 1.23) greater compared with the low-weight class, and average-weight was 0.42% (95% CI = 0.06 to 0.78) greater compared with the low-weight class. There was a positive relationship with white oak index. In years with increased white oak acorn production,  $\delta^{13}C$  values were more enriched compared with years of lower production ( $\beta$ 

= 0.04, 95% CI = -0.08 to 0.16). All other variables (sex, age class, year, total hard mast index, red oak index, nuisance status and hog kill) had parameter estimates that were not greater than the analytical error of the mass spectrometer and 95% confidence intervals that included zero (Table 7).

### STABLE NITROGEN ISOTOPE ANALYSIS

Two models (M16, M14) accounted for a cumulative model weight of 0.893. The most parsimonious model (M16) included age class and white oak index ( $w_i = 0.732$ ). The next best model (M14) included white oak index ( $\Delta AIC_c = 3.030$ ,  $w_i = 0.161$ ). All other models had limited support ( $\Delta AIC_c \geq 5.746$ ,  $w_i \leq 0.041$ , cumulative  $w_i = 0.107$ ; Table 8). Based on model averaging, years with increased white oak mast production,  $\delta^{15}N$  values were depleted compared with years of lower white oak mast production ( $\beta = -0.19$ , 95% CI = -0.34 to -0.03). Older adult bears ( $\geq 7$  yrs) had depleted  $\delta^{15}N$  signatures ( $\beta = -0.36$ , 95% CI = -0.85 to 0.14) compared with subadult bears (1.5–3.0 yrs). Adult bears had slightly depleted  $\delta^{15}N$  signatures ( $\beta = -0.03$ , 95% CI = -0.35 to 0.30) compared to subadult bears. The r-squared value for the top model (M16) was 0.14 and for the next top model (M14) r-squared = 0.08. All other variables (sex, weight class, year, total hard mast index, red oak index, nuisance status and hog kill) had parameter estimates that were not greater than the analytical error of the mass spectrometer and 95% confidence intervals that included zero (Table 9).

#### **FOOD ITEMS**

Signatures of stable carbon isotopes of natural food items differed from those of black bear hair samples with or without the discrimination factor for metabolic

fractionation. Mean  $\delta^{13}$ C for black bear hair without the discrimination factor was - 22.74‰, whereas the mean  $\delta^{13}$ C value for natural food was -27.23‰ (t = 4.33, P = 0.001; Figure 5). After correcting for metabolic fractionation from prey to consumer, mean  $\delta^{13}$ C for black bear hair was -24.74‰, which also was different from food items (t = 2.40, P = 0.04; Figure 5).

The uncorrected mean  $\delta^{15}$ N for black bear hair (2.44‰) was greater than food items (-0.01‰; t = 2.89, P = 0.02; Figure 6). The corrected  $\delta^{15}$ N value for black bear hair was -0.56‰, which was not different from food items (t = 0.58, P = 0.08; Figure 6).

### SAMPLE HOMOGENEITY

The overall sample homogeneity for  $\delta^{13}C$  and  $\delta^{15}N$  were within analytical error (n = 22, SD = 0.17; SD = 0.13 respectively).

#### **CHAPTER V**

#### **DISCUSSION**

The metabolic fractionation between diet and consumer plays an important role in being able to determine if there is a difference between a species' diet and the natural food items that the species would consume under natural conditions. In order to apply a correction factor in stable isotope analysis of diet among wildlife, researchers must select an appropriate value from the literature; this value varies widely. I attempted to choose values that were closest to the diet of black bears (Ben-David et al. 1997a, 2001; Hilderbrand 1996) because the metabolic fractionation is dependent on the composition of a diet (Pritchard and Robbins 1990). However, the values I chose were appropriate to black bears in GSMNP. Different values may have changed the comparisons of food items and the diet, but would not change the conclusions for factors affecting  $\delta^{13}$ C and  $\delta^{15}$ N signatures.

#### **STABLE CARBON ISOTOPES**

Stable carbon isotope analysis often is used to examine differences in diets within a species resulting from consumption of  $C_4$  or  $C_3$  plants. Stable carbon isotopes ( $^{12}C$  and  $^{13}C$ ) are fractionated depending on the metabolic pathway of  $C_3$ ,  $C_4$ , and CAM vegetation. The  $C_3$  pathway (Calvin Cycle) is the most common and most primitive photosynthetic pathway used by plants. Smith and Epstein (1971) found that plants with enriched  $\delta^{13}C$  signatures are aquatic, desert, salt marsh and tropical grasses. Plants with

depleted  $\delta^{13}$ C signatures are found in the temperate regions and comprise the bulk of the plant kingdom. There is overlap in signatures between these two groups but the average  $\delta^{13}$ C signatures for each group is different which is the basis for using stable carbon isotope analysis in wildlife nutrition studies. I observed a distinct gradient of  $\delta^{13}$ C signatures ranging from zoo bears that were fed a corn-based chow diet to nuisance bears in the Gatlinburg area to research bears captured in backcountry areas (Figure 2). After applying a correction factor of 2‰ for metabolic fractionation between diet and consumer, a mean difference of 2.49‰ existed for  $\delta^{13}$ C between natural foods and assimilated diets of bears.

There was not support for sex and age class in association with changes in  $\delta^{13}C$  stable isotope values. Males were no more likely than females to be associated with changes in  $\delta^{13}C$  stable isotope values ( $\beta < 0.01$ , SE = 0.02, 95% CI = -0.04–0.05). Adults and old adults were no more likely than subadults to be associated with changes in  $\delta^{13}C$  stable isotope values ( $\beta < 0.01$ , SE < 0.01, 95% CI = -0.05–0.05;  $\beta \le 0.01$ , SE = 0.06, 95% CI = -0.11–0.12) respectively. The parameters sex and age class have been documented as being associated with the status of black bears in GSMNP i.e., panhandler or wild (McLean and Pelton 1990).

However, bears in better physical condition, adjusted for age and sex, were more likely to have assimilated diets that included  $C_4$  plants as a source based on  $\delta^{13}C$ . Previous studies also show that larger bears generally have access to higher quality foods than smaller bears. Dobey et al. (2005) found that larger body mass was associated with black bears that had greater access to  $C_4$  plant sources (e.g. corn). Black bear density

within GSMNP is high with 0.92 bears/km<sup>2</sup> (2003 estimate; Laufenberg 2010). Thus, competition for resources is likely high and bears in the high weight class, adjusted for age and sex, may be better competitors for food, including energy-rich, anthropogenic foods.

Because the natural diet of American black bears is C<sub>3</sub> plants (Hilderbrand 1996) and the C<sub>4</sub> plants that are native or exotic to GSMNP are not likely selected foods of black bears, the origin of the C<sub>4</sub> source must be anthropogenic, thus providing a useful indicator of anthropogenic food sources in the diet of black bears. The source of C<sub>4</sub> plants in bear diets is difficult to determine directly. Although nuisance behavior would seem to favor use of C<sub>4</sub> plants, nuisance status had little support. Out of 117 bear samples I analyzed, only 6 bears were subsequently captured for nuisance activity in the campgrounds or picnic areas. Tate and Pelton (1980) found that removal of nuisance bears within GSMNP was often random, and not always a result of nuisance behavior. Of the 6 bears, only 3 were actually observed exhibiting nuisance behavior. These observations are supported by Beeman and Pelton (1980), who estimated that 90–95% of bears rarely visited campgrounds or picnic areas. They speculated anthropogenic foods may comprise a substantial portion of the diet for a few bears but very little, if any, for most of the population. This is supported by the relatively low variation in  $\delta^{13}$ C signatures of bears over the 20-year period (Figure 7). Nuisance bears removed from the campgrounds and picnic areas in GSMNP during the summer of 2010 had  $\delta^{13}C$  signatures similar to those of the research bears.

The low numbers of bears that transition from research to nuisance bears suggests sources of  $C_4$  foods other than those available in campgrounds or picnic areas were responsible for the slightly enriched  $\delta^{13}C$  signature. A potential source of  $C_4$  plants could be corn bait used to trap wild hogs (*Sus scrofa*) in GSMNP. Since 1965, NPS personnel have removed wild hogs from park land, primarily by trapping. However, my analysis did not indicate support for the variable hog kill (Table 6). The amount of bait available to bears is small because trapping of hogs primarily occurs in winter, when bears are hibernating. A third potential source of  $C_4$  food items may originate with backpackers. An average of 83,675 (SD = 13,036) backpackers visit GSMNP annually (NPS 2011), particularly during months when bears are most active. Before bear-proof storage cables were installed in the latter part of the 1990s, bears frequently accessed foods at backcountry sites. Backcountry campsites likely remain a small but widely distributed source of human foods for bears.

#### STABLE NITROGEN ISOTOPES

Mean  $\delta^{15}N$  values of black bear hair showed substantial variation during 1990–2001 compared with 1980–1989 (Figure 8). The period of high variability in  $\delta^{15}N$  corresponded to several hard mast failures. Values of  $\delta^{15}N$  peaked during years when major hard mast failures occurred. Hard mast is crucial in fall to meet energetic requirement for the hyperphagic period prior to hibernation (Beeman and Pelton 1980, Eagle and Pelton 1983). Greenfell and Brody (1983) showed that the proportion of acorns in the diet of black bears was positively associated with acorn production. During years when hard mast was abundant,  $\delta^{15}N$  values were low. This pattern likely corresponded to

the low  $\delta^{15}N$  values I observed for acorns. Of the three mast indices I considered, white oak mast index showed the strongest association with  $\delta^{15}N$ . White oak acorns are preferred over red oak acorns (Clark 2004) but annual variation tends to be more extreme for white oaks. The enriched  $\delta^{15}N$  signature during years with poor or failing white oak crops may be a function of bears seeking alternative protein-rich foods. Those foods may include animal sources, such as colonial insects and carrion.

Basic energetic demand of protein requirement during the growth period may explain why subadult black bears had higher  $\delta^{15}N$  values than adult and older adult bears. Age class was an important variable likely because of the physiological mechanisms that drive protein consumption in animals. Growth of animals is related to the energy available from the different chemical constituents of the body i.e., minerals, proteins, and lipids. During growth, there is an accumulation of matter and energy into the developing organism known as the growth rate (Robbins 1993). The basic energetic demand required for sub adult black bears for growth is likely driving this class of bears to seek out more protein rich food.

Nitrogen values for this study were based on acorns and soft mast. Potential natural prey items i.e., white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), wild pig, yellow jackets (*Vespula maculifrons*), termites (*Reticulutermes spp.*), and other small mammals were not collected for this study. Therefore, it is difficult to draw any conclusions regarding the potential carnivorous food habits of black bears in GSMNP without  $\delta^{15}$ N values of the potential prey items. For future studies, it

would be beneficial to collect these prey items for stable isotope analysis to gain a better understanding of trophic relations of black bears in GSMNP.

#### **FUTURE RESEARCH NEEDS**

The models for both carbon and nitrogen had low r-squared values. This suggests much of the variation associated with changes in carbon and nitrogen levels were not explained. One potential source for this variation is the inability to measure how much meat or how much of a C<sub>4</sub> plant needs to be consumed to enrich a stable isotope signature. If the amount of food required to change a stable isotope signature could be measured, this could potentially explain the additional variation associated with carbon and nitrogen stable isotopes. An additional source of variation could be due to seasonal differences of diets within the population. Analysis of the entire hair provides an average signature for diet during the period of hair growth without capturing the variation that may exist within that period of different classes of bears i.e., sex, age, weight.

Additional research is needed to examine the source of the C<sub>4</sub> plants that is contributing to the diet of black bears in GSMNP. The use of mixing models may facilitate understanding of the relative contribution of food items to the diet of black bears. Mixing models analyze the relative proportion of different food sources that contribute to the diet of a species (Phillips 2001, Ben-David and Schell 2001, Phillips and Koch 2002, Moore and Semmens 2008). Mixing models have generally been used for more carnivorous species, particularly with marine and terrestrial diets to examine the relative contributions of particular stable isotopes from plant and meat sources either terrestrial or marine derived (Hilderbrand et al. 1999b, Felicetti et al. 2003, Robbins et al.

2004). I focused on main food items of black bears based on previous studies and did not collect meat sources. Black bears in GSMNP do not feed on a marine source. Thus, future work should collect all potential food items of black bears in GSMNP and use mixing models if the C:N ratios in the food items are substantially different. The mixing models would help examine the relative contributions of different food items (Robbins et al. 2002, 2004: Moore and Semmens 2008). By analyzing and incorporating the food stoichiometery and knowing the efficiency of assimilation for different food items, it would be possible to examine the proportional contributions of soft mast compared with hard mast for black bears.

#### **CHAPTER V**

#### MANAGEMENT IMPLICATIONS

Variation in  $\delta^{13}$ C and  $\delta^{15}$ N values over the 20-year period could not have been evaluated with a short-term study. A long-term study was needed to understand the overall feeding patterns of black bears in years of good or poor mast crops. The gradient of stable isotope signatures that I detected provides support that this technique is useful to examine potential anthropogenic food source exploitation by black bears and other wildlife species (Newsom et al. 2010). Bears in the best weight for sex and age class had higher use of anthropogenic foods. We do not know if their enhanced condition was due to their ability to exploit nutrient rich, anthropogenic foods or if the enhanced condition allowed them to outcompete for these human-based resources.

The lack of distinct groups exploiting anthropogenic foods within GSMNP suggests an abundant supply of primary and alternative food sources for black bears within the park. Moreover, the depleted  $\delta^{13}$ C signature in the nuisance bears captured in the campgrounds and picnic areas of GSMNP suggests they primarily fed on natural foods. It would be of interest to continue collecting hair on nuisance bears captured or removed from campground or picnic areas to examine if the depleted  $\delta^{13}$ C signatures persist over time. The  $\delta^{13}$ C signatures of live-trapped bears showed little variation over the 20-year period of this study (Table 9) and were similar to those of the nuisance bears captured or removed during the summer of 2010. Therefore, prompt management by

GSMNP personnel may be reducing the number of bears from accessing anthropogenic food sources.

Availability of alternate food sources and physiological requirements of animals affect their use of anthropogenic foods. The observations made by Beeman and Pelton (1980) found only a few bears accounted for the total exploitation of anthropogenic food sources within the population. I found sub-adult and larger bears were more likely to access anthropogenic food sources. In years of poor mast crops, managers should consider demographics and physiological requirements to identify which bears are potentially more likely to become nuisance bears.

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# **APPENDICES**

# **APPENDIX A: TABLES**

Table 1. Hair samples analyzed for stable carbon and nitrogen isotopes from black bears live-trapped in Great Smoky Mountain National Park, Tennessee, USA, <sup>a</sup>1980–2001.

Sample ID	Year	Age	Sex	Sample ID	Year	Age	Sex
1033701	1980	2.5	Male	1035501	1980	1.5	Female
1034601	1980	4.5	Male	1025303	1980	3.5	Female
1023702	1980	9.5	Male	1035401	1980	13.5	Female
1036302	1981	1.5	Male	1038801	1981	2.5	Female
1034502	1981	4.5	Male	1037901	1981	4.5	Female
1039401	1981	7.5	Male	1021302	1981	7.5	Female
1046501	1982	2.5	Male	1039801	1982	2.5	Female
1037302	1982	3.5	Male	1039901	1982	3.5	Female
1042101	1983	2.5	Male	1035204	1982	13.5	Female
1042201	1983	4.5	Male	1040104	1983	2.5	Female
1050501	1986	2.5	Male	1035903	1983	6.5	Female
1046603	1986	4.5	Male	1020112	1983	8.5	Female
1053901	1987	2.5	Male	1056101	1986	1.5	Female
1050902	1987	3.5	Male	1051701	1986	3.5	Female
1044102	1987	7.5	Male	1050201	1986	12.5	Female
1058101	1988	2.5	Male	1055201	1987	1.5	Female
1058701	1988	3.5	Male	1053101	1987	5.5	Female
1057901	1988	8.5	Male	1053501	1987	8.5	Female
1060501	1989	2.5	Male	1058501	1988	2.5	Female
1063601	1989	3.5	Male	1059101	1988	4.5	Female
1063001	1989	7.5	Male	1016608	1988	13.5	Female
1068201	1990	2.5	Male	1062601	1989	2.5	Female
1068801	1990	3.5	Male	1059801	1989	5.5	Female

Table 1 cont.

Sample ID	Year	Age	Sex	Sample ID	Year	Age	Sex
1067801	1990	9.5	Male	1064201	1989	8.5	Female
1070801	1991	3.5	Male	1066701	1990	2.5	Female
1070601	1991	4.5	Male	1057303	1990	5.5	Female
1069801	1991	8.5	Male	1060702	1990	7.5	Female
1074601	1992	2.5	Male	1071501	1991	3.5	Female
1073901	1992	3.5	Male	1059402	1991	5.5	Female
1060402	1992	8.5	Male	1055803	1991	9.5	Female
1074802	1993	1.5	Male	1075501	1992	2.5	Female
1076101	1993	3.5	Male	1073401	1992	4.5	Female
1071402	1993	7.5	Male	1075001	1992	7.5	Female
1079601	1994	2.5	Male	1077001	1993	3.5	Female
1081101	1994	4.5	Male	1076401	1993	5.5	Female
1063403	1994	8.5	Male	1070902	1993	14.5	Female
1083801	1995	1.5	Male	1079501	1994	3.5	Female
1082901	1995	3.5	Male	1079701	1994	4.5	Female
1062403	1995	9.5	Male	1069602	1994	8.5	Female
1092401	1996	1.5	Male	1084301	1995	1.5	Female
1080002	1996	5.5	Male	1076002	1995	3.5	Female
1077504	1996	7.5	Male	1082101	1995	8.5	Female
1136901	1997	1.5	Male	1089801	1996	1.5	Female
1138501	1997	3.5	Male	1091501	1996	3.5	Female
1066502	1997	8.5	Male	1073404	1996	8.5	Female
1151001	1998	1.5	Male	1089802	1997	2.5	Female
1146001	1998	3.5	Male	1084902	1997	6.5	Female
1078304	1998	7.5	Male	1060803	1997	10.5	Female
1151302	1999	2.5	Male	1144801	1998	2.5	Female

Table 1 cont.

Sample ID	Year	Age	Sex	Sample ID	Year	Age	Sex
1147302	1999	4.5	Male	1147001	1998	3.5	Female
1078602	1999	9.5	Male	1053802	1998	15.5	Female
1160201	2000	2.5	Male	1157001	1999	2.5	Female
1151502	2000	5.5	Male	1152601	1999	3.5	Female
1090702	2000	7.5	Male	1076910	1999	13.5	Female
1162301	2001	2.5	Male	1158601	2000	2.5	Female
1154202	2001	5.5	Male	1137702	2000	5.5	Female
1091902	2001	7.5	Male	1088302	2000	9.5	Female
				1165801	2001	2.5	Female
				1130006	2001	5.5	Female
				1142304	2001	7.5	Female

<sup>&</sup>lt;sup>a</sup> Samples from 1984 were omitted because of missing biological information. Samples from 1985 were omitted because hard mast data was not collected.

Table 2. Sample order for processing hair samples collected from black bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001 in mass spectrometer, starting with sample A1 and ending with E1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bypass	Bypass	Blank	Standard	Standard	Standard	Standard	USGS40	USGS41	S1 <sup>e</sup>	S2 <sup>e</sup>	S3 <sup>e</sup>
В	S4 <sup>e</sup>	S5 <sup>e</sup>	S6 <sup>e</sup>	S7 <sup>e</sup>	S8 <sup>e</sup>	S9 <sup>e</sup>	S10 <sup>e</sup>	USGS40	USGS41	S11 <sup>e</sup>	S12 <sup>e</sup>	S13 <sup>e</sup>
C	S14 <sup>e</sup>	S15 <sup>e</sup>	S16 <sup>e</sup>	S17 <sup>e</sup>	S18 <sup>e</sup>	S19 <sup>e</sup>	S20 <sup>e</sup>	USGS40	USGS41	S21 <sup>e</sup>	S22 <sup>e</sup>	S23 <sup>e</sup>
D	S24 <sup>e</sup>	S25 <sup>e</sup>	S26 <sup>e</sup>	S27 <sup>e</sup>	S28 <sup>e</sup>	S29 <sup>e</sup>	S30 <sup>e</sup>	USGS40	USGS41	S31 <sup>e</sup>	S32 <sup>e</sup>	S33 <sup>e</sup>
Е	S34 <sup>e</sup>	Empty <sup>f</sup>	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empt y	Empt y	Empt y
F	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empt y	Empt y	Empt y
G	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empt y	Empt y	Empt y

<sup>&</sup>lt;sup>a</sup> Atropine  $C_{17}H_{23}O_3$  (~1mg) used to condition furnace.
<sup>b</sup> An empty tin capsule is used as a blank reference.
<sup>c</sup> Acetanilide  $C_8H_9NO$  (0.5, 1.0, 1.5, 2.0 mg) respectively used for a calibration curve.
<sup>d</sup> Reference samples of USGS 40 and 41 L-Glutamic Acid (~0.7–1.0 mg).
<sup>e</sup> Bear hair or food samples
<sup>f</sup> A 96 well tray was used to store samples until ready for analysis in mass spectrometer, not all wells in tray were used.

Table 3. Black bear hair and food reference samples used to evaluate stable carbon and nitrogen isotopes from black bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA 1980–2001.

Sample Location	Source Type	Donator
Knoxville Zoo, TN, USA	Black bear hair, known food source	Dr. Ed Ramsey, University of Tennessee, Knoxville, School of Veterinary Medicine
Gatlinburg, TN, USA	Black bear hair, nuisance bears	Dave Brandenburg, Tennessee Wildlife Resources Agency
GSMNP Picnic and Campground Area, TN, USA	Black bear hair, nuisance bears	Bill Stiver, National Park Service, Great Smoky Mountains National Park
Cataloochee Valley, North Carolina, USA	Black bear hair, relocated bears for Elk reintroduction project	Joe Yarkovich, National Park Service, Great Smoky Mountains National Park
Townsend, TN, USA	Black bear hair	Lisa Stewart, Appalachian Bear Rescue Center
British Columbia, Canada	Black bear hair, hairs taken during legal harvest season	Larry McKay and sons, University of Tennessee, Knoxville, Department of Earth and Planetary Sciences
Townsend, TN, USA	Acorns (Quercus spp.)	Lisa Stewart, Appalachian Bear Rescue Center

Table 4. Discrimination factor values of metabolic fractionation between diet and consumer for  $\Delta\delta^{13}C$  (carbon) and  $\Delta\delta^{15}N$  (nitrogen) stable isotope analysis taken from wildlife nutrition and stable isotope literature.

Source	δ <sup>13</sup> C Metabolic fractionation Correction Value	δ <sup>15</sup> N Metabolic fractionation Correction Value	
Ben-David 1996; Ben-David et al. 1997a, 1997b, 2001, 2001	2.0‰	3.0‰	
Hilderbrand et al. 1996	0.4–4.5‰	$4.1\pm0.5\%$	
DeNiro and Epstein 1978, 1981	$0.3\pm1.1$ ‰	$3.0\pm2.6$ ‰	
Felicetti et al.	_	$3.0 \pm 5.0\%$	
Tieszen et al. 1983	1.0‰		
Lessage et al. 2002	$2.3\pm0.1$ ‰	$2.3\pm0.8~\% o$	
Average Value <sup>a</sup>	$1.75 \pm 1.6\%$	$3.08\pm0.65$	

 $<sup>^</sup>a$  Average values from literature were rounded and used for correction factors in this study; 2.0% for  $\Delta\delta^{13}C$  and 3.0% for  $\Delta\delta^{15}N.$ 

Table 5. Weight categories accounting for sex and age class based on a 95% confidence interval for all black bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.

Age Class	Sex	Average Weight (lbs) <sup>a</sup>	Standard Deviation	95% LCL <sup>b</sup>	95% UCL <sup>c</sup>	Weight Category
1.5–3.0yrs	F	69.44	24.90	32	57	Below Average
1.5–3.0yrs	M	93.46	42.39	30	72	Below Average
3.5–6.5yrs	F	94.57	21.92	62	84	Below Average
3.5–6.5yrs	M	148.49	58.41	61	119	Below Average
≥ 7yrs	F	110.26	21.54	78	99	Below Average
≥ 7yrs	M	236.78	49.70	162	212	Below Average
1.5–3.0yrs	F	69.44	24.90	58	82	Average
1.5–3.0yrs	M	93.46	42.39	73	115	Average
3.5–6.5yrs	F	94.57	21.92	85	106	Average
3.5–6.5yrs	M	148.49	58.41	120	178	Average
≥ 7yrs	F	110.26	21.54	100	121	Average
≥ 7yrs	M	236.78	49.70	213	262	Average
1.5–3.0yrs	F	69.44	24.90	83	107	Above Average
1.5–3.0yrs	M	93.46	42.39	116	157	Above Average
3.5–6.5yrs	F	94.57	21.92	107	127	Above Average
3.5–6.5yrs	M	148.49	58.41	179	236	Above Average
≥ 7yrs	F	110.26	21.54	122	143	Above Average
≥ 7yrs	M	236.78	49.70	263	311	Above Average

<sup>&</sup>lt;sup>a</sup> Mean weights for each combination of weight class and sex were calculated using weights collected in the field from all black bears live-trapped in Great Smoky Mountains National Park, USA, 1980–2001.

Below average weight lower 95% confidence limit calculated as:  $\mu - (1.5 * Std. dev)$ , average weight 95% lower confidence limit calculated as:  $\mu - (0.5 * Std. dev)$ , above average weight 95% lower confidence limit calculated as:  $\mu + (0.5 * Std. dev)$ .

<sup>&</sup>lt;sup>c</sup> Below average weight upper 95% confidence limit calculated as:  $\mu - (0.5 * Std. dev)$ , average weight 95% upper confidence limit calculated as:  $\mu + (0.5 * Std. dev)$ , above average weight 95% upper confidence limit calculated as:  $\mu + (1.5 * Std. dev)$ .

Table 6. Hard mast indices for Great Smoky Mountains National Park, 1979–2000 from Greenberg and Warburton (2007)<sup>a</sup>, with corresponding black bear hair collection year.

Year	White Oak Index	Red Oak Index	<b>Total Oak Index</b>	Hair Collection Year
1979	4.33 (59) <sup>b</sup>	3.19 (61)	3.91 (120)	1980
1980	0.78 (52)	4.00 (74)	2.87 (126)	1981
1981	3.86 (65)	2.32 (88)	3.11 (153)	1982
1982	0.67 (47)	2.23 (82)	1.79 (129)	1983
1983	_		_	1984
1984	_		_	1985
1985	2.60 (77)	1.90 (83)	2.34 (160)	1986
1986	1.60 (79)	3.04 (93)	2.53 (172)	1987
1987	2.94 (99)	2.62 (116)	2.91 (215)	1988
1988	2.96 (77)	3.21 (166)	3.33 (243)	1989
1989	0.66 (75)	3.08 (160)	2.49 (235)	1990
1990	1.25 (103)	1.61 (112)	1.53 (215)	1991
1991	1.35 (99)	1.05 (147)	1.24 (246)	1992
1992	0.50 (112)	0.85 (155)	0.76 (267)	1993
1993	0.45 (95)	2.67 (155)	1.98 (250)	1994
1994	0.79 (118)	2.20 (142)	1.68 (260)	1995
1995	1.97 (99)	5.04 (167)	4.16 (266)	1996
1996	3.94 (102)	1.87 (156)	2.81 (258)	1997
1997	0.66 (97)	2.76 (165)	2.14 (262)	1998
1998	1.73 (81)	3.77 (171)	3.33 (252)	1999
1999	1.23 (105)	1.29 (150)	1.35 (255)	2000
2000	0.78 (87)	1.61 (163)	1.42 (250)	2001

<sup>&</sup>lt;sup>a</sup> Visual surveys are used to determine the availability and distribution of mast (Whitehead 1969). Indices were calculated as:  $\left(PBA_{standard} = PBA_{year}/PBA_{max}\right) \times 100$  where PBA is the proportion of trees bearing acorns <sup>b</sup> Sample size of trees for given year.

Table 7. Model selection based on second-order Akaike's Information Criteria (AIC<sub>c</sub>) to evaluate stable carbon isotope delta ( $\delta^{13}$ C) values of hair collected from black bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.

Model No.	Models for δ <sup>13</sup> C Stable Isotope Values	RSS	Kª	AICc	ΔAICc <sup>b</sup>	w <sub>i</sub> <sup>c</sup>	Evidence Ratio
M17	$\gamma = \beta_0 + \beta_{1(WC)}^{d} + \beta_{2(WOI)}^{e} + \varepsilon$	67.359	4	-56.242	0.000	0.370	1.000
M9	$\gamma = \beta_0 + \beta_{1(WC)} + \varepsilon$	68.923	3	-55.703	0.540	0.283	1.310
M10	$\gamma = \beta_0 + \beta_{1(WC)} + \beta_{2(HMI)}^{\ \ f} + \epsilon$	68.679	4	-53.972	2.271	0.119	3.112
M11	$\gamma = \beta_0 + \beta_{1(WC)} + \beta_{2(NS)}^{g} + \epsilon$	68.873	4	-53.643	2.600	0.101	3.668
M19	$\gamma = \beta_0 + \beta_{1(WC)} + \beta_{2(ROI)}^{h} + \varepsilon$	68.907	4	-53.585	2.657	0.098	3.776
M16	$\gamma = \beta_0 + \beta_{1(AC)}^{i} + \beta_{2(WOI)} + \varepsilon$	71.684	4	-48.963	7.280	0.010	38.087
M14	$\gamma = \beta_0 + \beta_{1(WOI)} + \epsilon$	75.142	2	-47.702	8.540	0.005	71.536
M8	$\gamma = \beta_0 + \beta_{1(SEX)} + \beta_{2(AC)} + \beta_{3(YEAR)} + \varepsilon$	71.653	5	-46.829	9.413	0.003	110.668
M12	$\gamma = \beta_0 + \beta_{1(HK)}^{\ j} + \varepsilon$	76.694	2	-45.309	10.933	0.002	236.627
M6	$\gamma = \beta_0 + \beta_{1(SEX)} + \beta_{2(YEAR)} + \epsilon$	75.366	3	-45.247	10.996	0.002	244.175
M5	$\gamma = \beta_0 + \beta_{1(SEX)} + \beta_{2(AC)} + \epsilon$	74.052	4	-45.159	11.083	0.001	255.089
M2	$\gamma = \beta_0 + \beta_{1(YEAR)} + \varepsilon$	76.833	2	-45.099	11.144	0.001	262.927
M1	$\gamma = \beta_0 + \beta_{1(AC)} + \varepsilon$	75.598	3	-44.886	11.356	0.001	292.358
M7	$\gamma = \beta_0 + \beta_{1(AC)} + \beta_{2(HMI)} + \epsilon$	74.262	4	-44.828	11.415	0.001	301.043
M4	$\gamma = \beta_0 + \beta_{1(SEX)} + \epsilon$	77.509	2	-44.073	12.170	0.001	439.130
M3	$\gamma = \beta_0 + \beta_{1(HMI)} + \varepsilon$	77.577	2	-43.970	12.272	0.001	462.263
M18	$\gamma = \beta_0 + \beta_{1(AC)} + \beta_{2(ROI)} + \varepsilon$	75.563	4	-42.796	13.446	0.000	831.334
M15	$\gamma = \beta_0 + \beta_{1(ROI)} + \epsilon$	78.832	2	-42.093	14.150	0.000	1181.965
M13	$\gamma = \beta_0 + \beta_{1(NS)} + \epsilon$	78.859	2	-42.053	14.189	0.000	1205.408

<sup>&</sup>lt;sup>a</sup> Number of parameters plus 1 for intercept.

b Relative difference between AIC<sub>c</sub> of model and AIC<sub>c</sub> of model with lowest AIC<sub>c</sub>.

<sup>&</sup>lt;sup>c</sup> Model weight.

<sup>&</sup>lt;sup>d</sup> WC (weight class) Weight classes were calculated using weight recorded for all bears captured during the study period based on a normal distribution with 95% confidence intervals for each age and sex categories; low-weight, average-weight, and above- average weight.

<sup>&</sup>lt;sup>e</sup> WOI is the white oak index for GSMNP as calculated by Greenberg and Warburton (2007).

f HMI is the total hard mast index for GSMNP as calculated by Greenberg and Warburton (2007).

<sup>&</sup>lt;sup>g</sup> NS are research bears that subsequently were captured or removed from campgrounds or picnic areas of GSMNP.

<sup>&</sup>lt;sup>h</sup> ROI is the red oak index for GSMNP as calculate by Greenberg and Warburton (2007).

AC is the 3 age classes of black bears (sub-adult age class=1.5–3yrs, adult age-class = 3.5–6.5yrs, older adult age-class  $\geq 7$ yrs).

Hog kill (HK) is the number of hogs killed by park personnel for wild hog management.

Table 8. Parameter estimates of  $\delta^{13}C$  stable isotopes using model averaging for hair collected from black bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.

Variable	Model Averaged Parameter Estimate	Unconditional Standard Error	95% LCL	95%UCL
Intercept	-22.30	0.19	-2357	-22.83
Adult bears (3.5–6.5yrs)	0.0009	0.02	-0.05	0.05
Old Adult Bears (≥7yrs)	0.01	0.06	-0.11	0.12
Year	0.0006	0.01	-0.02	0.02
Total Hard Mast Index <sup>a</sup>	0.01	0.03	-0.06	0.07
Males	0.002	0.02	-0.04	0.05
Weight Class 1 <sup>b</sup>	0.42	0.18	0.06	0.78
Weight Class 2 <sup>b</sup>	0.76	0.24	0.28	1.23
Nuisance Status <sup>c</sup>	0.01	0.11	-0.21	0.23
Hog Kill <sup>d</sup>	0.000005	0.0001	-0.0003	0.0003
White Oak Index <sup>e</sup>	0.04	0.06	-0.08	0.16
Red Oak Index <sup>e</sup>	-0.01	0.05	-0.11	0.08

<sup>&</sup>lt;sup>a</sup> Total hard mast index for GSMNP as calculated by Greenberg and Warburton (2007).

b Weight classes were calculated using weight recorded for all bears captured during the study period based on a normal distribution with 95% confidence intervals for each age and sex categories; weight class 0=low-weight, weight class 1=average-weight, and weight class 2=above- average weight.

<sup>&</sup>lt;sup>c</sup> Nuisance status refers to research bears that subsequently were captured or removed from campgrounds or picnic areas of GSMNP.

d Hog kill is the number of hogs killed by park personnel for wild hog management.

<sup>&</sup>lt;sup>e</sup> White oak index and red oak index for GSMNP and as calculated by Greenberg and Warburton (2007).

Table 9. Model selection based on second-order Akaike's Information Criteria (AIC<sub>c</sub>) to evaluate stable nitrogen isotope delta ( $\delta^{15}$ N) values of hair collected from black bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.

Model No.	Models for $\delta^{15}N$ Stable Isotpe Values	RSS	K <sup>a</sup>	AICc	ΔAICcb	w <sub>i</sub> <sup>c</sup>	Evidence Ratio
M16	$\gamma = \beta_0 + \beta_{1(AC)}^{d} + \beta_{2(WOI)}^{e} + \epsilon$	76.498	4	-41.357	0.000	0.732	1
M14	$\gamma = \beta_0 + \beta_{1(WOI)} + \epsilon$	81.411	2	-38.327	3.030	0.161	4.549746
M17	$\gamma = \beta_0 + \beta_{1(WC)}^{f} + \beta_{2(WOI)} + \epsilon$	80.349	4	-35.611	5.746	0.041	17.68639
M7	$\gamma = \beta_0 + \beta_{1(AC)} + \beta_{2(HMI)}^{g} + \epsilon$	81.351	4	-34.161	7.196	0.020	36.5218
M1	$\gamma = \beta_0 + \beta_{1(AC)} + \varepsilon$	83.709	3	-32.962	8.395	0.011	66.50982
M3	$\gamma = \beta_0 + \beta_{1(HMI)} + \epsilon$	86.037	2	-31.860	9.497	0.006	115.3841
M11	$\gamma = \beta_0 + \beta_{1(AC)} + \beta_{2(HK)}^{h} + \varepsilon$	83.472	4	-31.150	10.207	0.004	164.5728
M18	$\gamma = \beta_0 + \beta_{1(AC)} + \beta_{2(ROI)}^{i} + \epsilon$	83.652	4	-30.898	10.459	0.004	186.700
M5	$\gamma = \beta_0 + \beta_{1(SEX)} + \beta_{2(AC)} + \varepsilon$	83.702	4	-30.828	10.529	0.004	193.3277
M9	$\gamma = \beta_0 + \beta_{1(WC)} + \epsilon$	85.778	3	-30.105	11.252	0.003	277.481
M10	$\gamma = \beta_0 + \beta_{1(WC)} + \beta_{2(HMI)} + \epsilon$	84.390	4	-29.869	11.487	0.002	312.2224
M2	$\gamma = \beta_0 + \beta_{1(\text{YEAR})} + \epsilon$	83.253	5	-29.503	11.853	0.002	374.9061
M8	$\gamma = \beta_0 + \beta_{1(SEX)} + \beta_{2(AC)} + \beta_{3(YEAR)} + \epsilon$	87.788	2	-29.273	12.084	0.002	420.6889
M12	$\gamma = \beta_0 + \beta_{1(HK)} + \epsilon$	87.990	2	-29.233	12.123	0.002	429.0707
M15	$\gamma = \beta_0 + \beta_{1(ROI)} + \varepsilon$	88.299	2	-28.824	12.532	0.001	526.4707
M13	$\gamma = \beta_0 + \beta_{1(NS)}^{j} + \varepsilon$	88.330	2	-28.783	12.574	0.001	537.4574
M4	$\gamma = \beta_0 + \beta_{1(SEX)} + \epsilon$	88.371	2	-28.729	12.628	0.001	552.1565
M19	$\gamma = \beta_0 + \beta_{1(WC)} + \beta_{2(ROI)} + \epsilon$	85.776	4	-27.963	13.393	0.001	809.677
M6	$\gamma = \beta_0 + \beta_{1(SEX)} + \beta_{2(YEAR)} + \epsilon$	87.787	3	-27.397	13.960	0.001	1074.974

<sup>&</sup>lt;sup>a</sup> Number of parameters plus 1 for intercept.

b Relative difference between AIC<sub>c</sub> of model and AIC<sub>c</sub> of model with lowest AIC<sub>c</sub>.

<sup>&</sup>lt;sup>c</sup> Model weight.

d AC is the 3 age classes of black bears (sub-adult age class=1.5–3yrs, adult age-class = 3.5–6.5yrs, older adult age-class ≥ 7yrs).

<sup>&</sup>lt;sup>e</sup> WOI is the white oak index for GSMNP as calculated by Greenberg and Warburton (2007).

WC (weight class) Weight classes were calculated using weight recorded for all bears captured during the study period based on a normal distribution with 95% confidence intervals for each age and sex categories; low-weight, average-weight, and above- average weight.

<sup>&</sup>lt;sup>g</sup> HMI is the total hard mast index for GSMNP as calculated by Greenberg and Warburton (2007).NS are research bears that subsequently were captured or removed from campgrounds or picnic areas of GSMNP.

h Hog kill (HK) is the number of hogs killed by park personnel for wild hog management.

i AC is the 3 age classes of black bears (sub-adult age class=1.5–3yrs, adult age-class = 3.5–6.5yrs, older adult age-class ≥ 7yrs).

j NS are research bears that subsequently were captured or removed from campgrounds or picnic areas of GSMNP.

Table 10. Parameter estimates of  $\delta^{15}N$  stable isotopes using model averaging for hair collected from black bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.

Variable	Model Averaged Parameter Estimate	Unconditional Standard Error	95% LCL	95%UCL
Intercept	2.91	0.20	2.52	3.30
Adult bears (3.5–6.5yrs)	-0.03	0.16	-0.35	0.30
Old Adult Bears (≥7yrs)	-0.36	0.25	-0.85	0.14
Year	-0.00005	0.0008	-0.002	0.001
Total Hard Mast Index <sup>a</sup>	-0.005	0.03	-0.05	0.05
Males	-0.00008	0.001	-0.002	0.002
Weight Class 1 <sup>b</sup>	-0.004	0.02	-0.04	0.03
Weight Class 2 <sup>b</sup>	-0.01	0.06	-0.13	0.10
Nuisance Status <sup>c</sup>	-0.0001	0.003	-0.01	0.01
Hog Kill <sup>d</sup>	0.00005	0.0007	-0.001	0.001
White Oak Index <sup>e</sup>	-0.19	0.08	-0.34	-0.03
Red Oak Index <sup>e</sup>	-0.0001	0.006	-0.01	0.01

<sup>&</sup>lt;sup>a</sup> Total hard mast index for GSMNP as calculated by Greenberg and Warburton (2007).

b Weight classes were calculated using weight recorded for all bears captured during the study period based on a normal distribution with 95% confidence intervals for each age and sex categories; weight class 0=low-weight, weight class 1=average-weight, and weight class 2=above- average weight.

<sup>&</sup>lt;sup>c</sup> Nuisance status refers to research bears that subsequently were captured or removed from campgrounds or picnic areas of GSMNP.

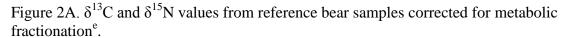
d Hog kill is the number of hogs killed by park personnel for wild hog management.

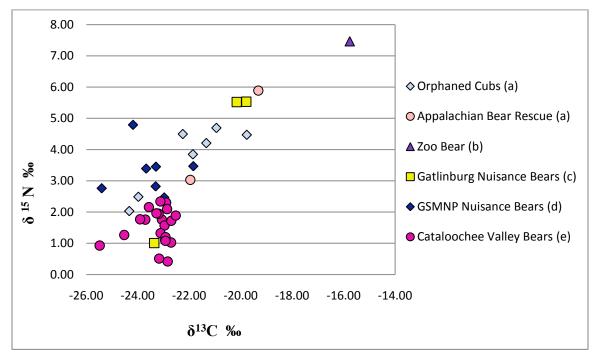
<sup>&</sup>lt;sup>e</sup> White oak index and red oak index for GSMNP and as calculated by Greenberg and Warburton (2007).

## **APPENDIX B: FIGURES**

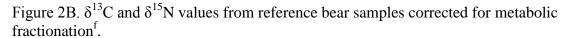
Tennessee North Carolina Live-trap site Sampling area County boundary National Park land 10 Kilometers 69

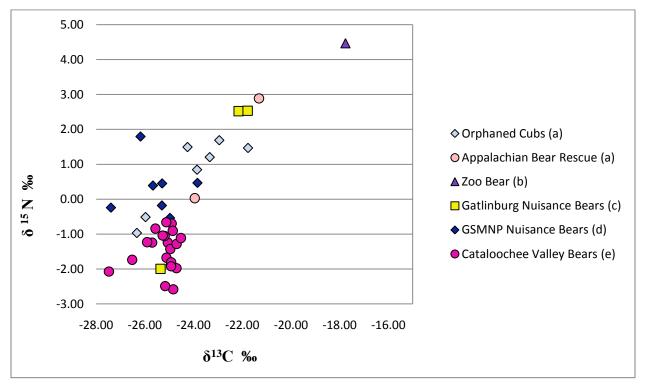
Figure 1. Study area map, Great Smoky Mountains National Park, Tennessee, USA.





- a Orphaned cub and Appalachian Bear Rescue hair samples were donated by Lisa Stewart from Appalachian Bear Rescue, Townsend, Tennessee, USA.
- b Knoxville zoo bear hair sample was donated by Dr. Ed Ramsey, University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee, USA.
- c Hair samples from Gatlinburg nuisance bears were donated by Dave Brandenburg from Tennessee Wildlife Resources Agency, Tennessee, USA.
- d GSMNP nuisance bear hair samples were donated by Bill Stiver of Great Smoky Mountains National Park, Tennessee, USA.
- e A correction factor value of 2‰ was applied for carbon and 3‰ for nitrogen.





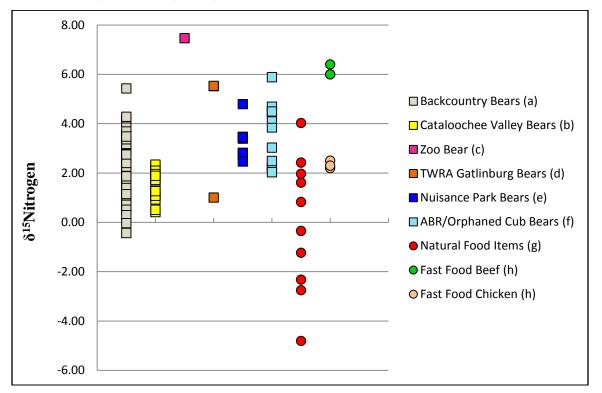
- a Orphaned cub and Appalachian Bear Rescue hair samples were donated by Lisa Stewart from Appalachian Bear Rescue, Townsend, Tennessee, USA.
- b The Knoxville zoo bear hair sample was donated by Dr. Ed Ramsey, University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee, USA.
- c Hair samples from Gatlinburg nuisance bears were donated by Dave Brandenburg from Tennessee Wildlife Resources Agency, Tennessee, USA.
- d GSMNP nuisance bear hair samples were donated by Bill Stiver of Great Smoky Mountains National Park, Tennessee, USA.
- e Hair samples were collected as part of project examining black bear predation on elk calves (Yarkovich 2009) and donated by Joe Yarkovich of Great Smoky Mountains National Park, North Carolina, USA
- $^{\rm f}\,$  A correction factor value of 2% was applied for carbon and 3% for nitrogen.

Figure 3A.  $\delta^{13}$ C values of all samples analyzed not corrected for metabolic fractionation to evaluate food habits of bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.



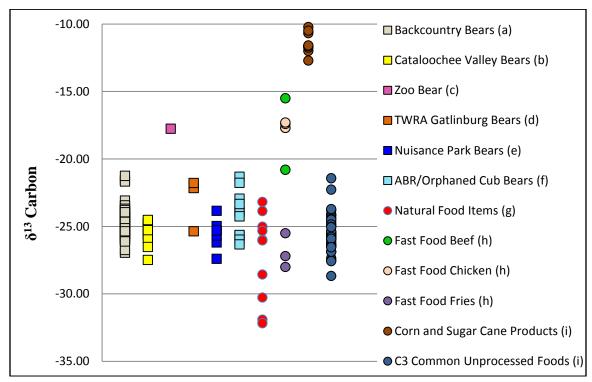
- a Research black bear hair samples for this project collected from Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.
- b Hair samples from elk relocation project Cataloochee Valley, Great Smoky Mountains National Park, North Carolina, USA, (Yarkovich 2009) donated by Joe Yarkovich, National Park Service.
- c Knoxville zoo bear hair sample was donated by Dr. Ed Ramsey, University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee, USA.
- d Hair samples from Gatlinburg nuisance bears were donated by Dave Brandenburg from Tennessee Wildlife Resources Agency, Tennessee, USA.
- e GSMNP nuisance bear hair samples were donated by Bill Stiver of Great Smoky Mountains National Park, Tennessee, USA.
- f Orphaned cub and Appalachian Bear Rescue hair samples were donated by Lisa Stewart from Appalachian Bear Rescue, Townsend, Tennessee, USA.
- g Natural food items included; red oak acorns (*Quercus* spp.), white oak acorns (*Quercus* spp.), blueberry (*Vaccinium* spp.), huckleberry (*Galussacia* sp.) and wild grape (*Vitis* spp.)
- h Values taken from Jahren and Kraft (2008).
- i Values taken from Jahren et al. (2006).

Figure 3B.  $\delta^{15}$ N stable isotope values of all samples analyzed not corrected for metabolic fractionation to evaluate food habits of bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.



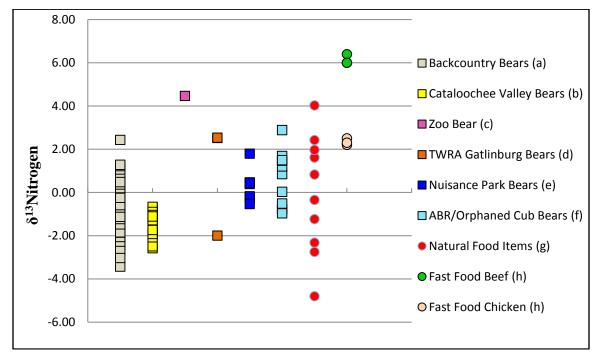
- a Research black bear hair samples for this project collected from Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.
- b Hair samples from elk relocation project Cataloochee Valley, Great Smoky Mountains National Park, North Carolina, USA, (Yarkovich 2009) donated by Joe Yarkovich, National Park Service.
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- e GSMNP nuisance bear hair samples were donated by Bill Stiver of Great Smoky Mountains National Park, Tennessee, USA.
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- h Values taken from Jahren and Kraft (2008).

Figure 4A.  $\delta^{13}$ C stable isotope values of all samples analyzed and corrected for metabolic fractionation<sup>j</sup> to evaluate food habits of bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.

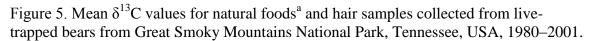


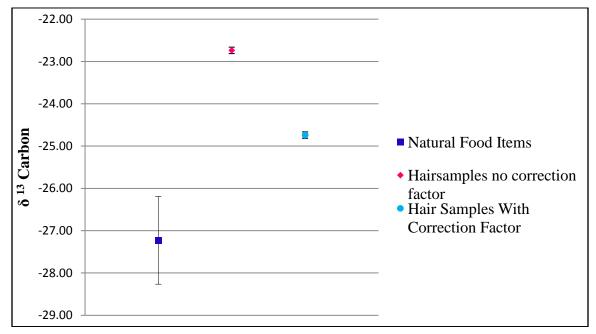
- a Research black bear hair samples for this project collected from Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.
- b Hair samples from elk relocation project Cataloochee Valley, Great Smoky Mountains National Park, North Carolina, USA, (Yarkovich 2009) donated by Joe Yarkovich, National Park Service.
- c Knoxville zoo bear hair sample was donated by Dr. Ed Ramsey, University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee, USA.
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- g Natural food items included; red oak acorns (*Quercus* spp.), white oak acorns (*Quercus* spp.), blueberry (*Vaccinium* spp.), huckleberry (*Galussacia* sp.) and wild grape (*Vitis* spp.)
- h Values taken from Jahren and Kraft (2008).
- i Values taken from Jahren et al. (2006).
- A correction factor value of 2‰ was applied for carbon and 3‰ for nitrogen.

Figure 4B.  $\delta^{15}N$  stable isotope values of all samples analyzed and corrected for metabolic fractionation<sup>i</sup> to evaluate food habits of bears live-trapped in Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.



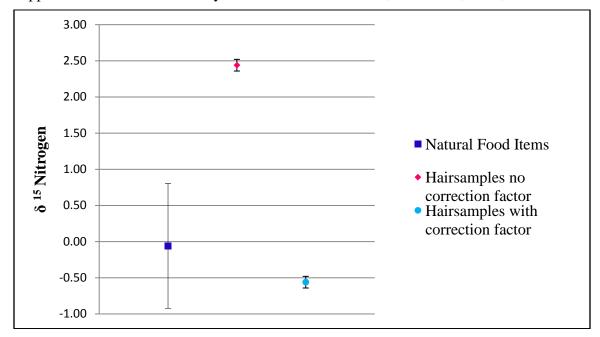
- a Research black bear hair samples for this project collected from Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.
- b Hair samples from elk relocation project Cataloochee Valley, Great Smoky Mountains National Park, North Carolina, USA, (Yarkovich 2009) donated by Joe Yarkovich, National Park Service.
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- h Values taken from Jahren and Kraft (2008).
- <sup>i</sup> A correction factor value of 2‰ was applied for carbon and 3‰ for nitrogen.





a Natural food items included; red oak acorns (*Quercus* spp.), white oak acorns (*Quercus* spp.), blueberry (*Vaccinium* spp.), huckleberry (*Galussacia* sp.) and wild grape (*Vitis* spp.).

Figure 6. Mean  $\delta^{15}$ N values for natural foods<sup>a</sup> and hair samples collected from live-trapped bears from Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.



a Natural food items included; red oak acorns (*Quercus* spp.), white oak acorns (*Quercus* spp.), blueberry (*Vaccinium* spp.), huckleberry (*Galussacia* sp.) and wild grape (*Vitis* spp.).

Figure 7. Mean  $\delta^{13}$ C values of research bears by year, Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.

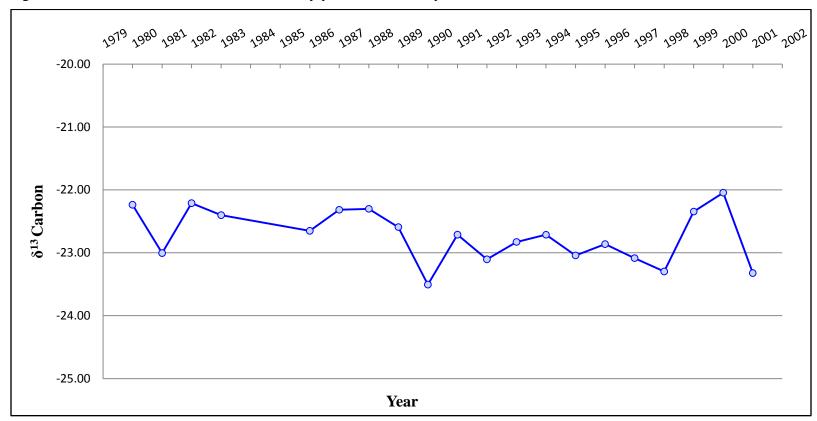
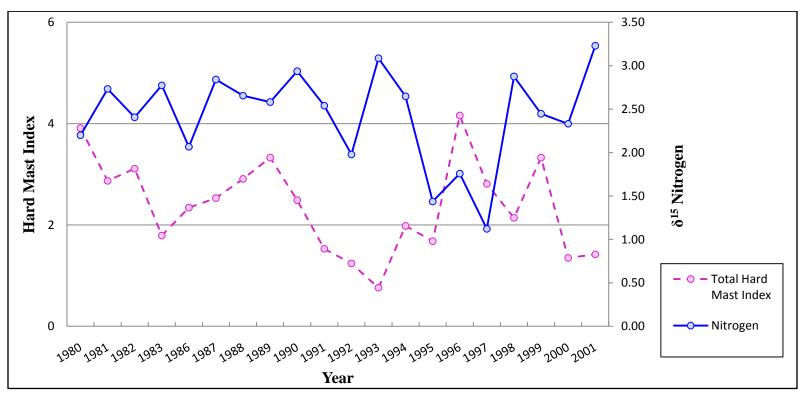


Figure 8. Total hard mast index values (Greenberg and Warburton 2007)<sup>a</sup> and mean  $\delta^{15}$ N values of research bears by year, Great Smoky Mountains National Park, Tennessee, USA, 1980–2001.



<sup>&</sup>lt;sup>a</sup> Visual surveys are used to determine the availability and distribution of mast (Whitehead 1969). The crown of each tree is surveyed estimating the percent of visible crown with mast. Indices were calculated as:

 $\left(PBA_{standard} = PBA_{year}/PBA_{max}\right) \times 100$  where PBA is the proportion of trees bearing acorns.

## Vita

Jennapher Teunissen van Manen was born in Salinas, California on 20 February 1970. She spent her childhood and teenage years camping with her family in the amazing landscapes of northern California. She graduated from Oakmont High School, Roseville, California in 1988 and did not begin her college career until 2000 when she decided to return to school for a degree in wildlife ecology. She worked at California Department of Fish and Game from 2000 to 2007 in the Wildlife Programs Branch and attended classes at American River College in the evenings until she was admitted to the University of California, Davis in 2004. In 2007, she graduated from UC Davis with a Bachelor of Science in Wildlife, Fish and Conservation Biology specializing in Physiological Ecology. In the fall of 2007, she married Frank Teunissen van Manen and moved to Maryville, Tennessee in December, 2007. She started a master's program at The University of Tennessee in the fall of 2008 under Dr. Lisa Muller and received her Master of Science degree in Wildlife and Fisheries Science and minor in Statistics under Dr. Arnold Saxton in May 2011.