Foraging ecology and aging of black bears in human-modified landscapes

By

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A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Wildlife Ecology)

at the

UNIVERSITY OF WISCONSIN-MADISON

2016

Date of final oral examination: 08/17/2016

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Acknowledgements

I am grateful to many people that have contributed to this dissertation along the way. First, I would like to thank my advisor, Jon Pauli, for offering his generous support, time, expertise, and helping me to become an independent researcher. I would also like to thank my collaborators, Mat Alldredge and Dave MacFarland for their guidance and support of this research. Further, thank you to my committee members Bill Karasov, Lisa Naughton, and Zach Peery for their advice and encouragement throughout my studies.

I would also like to acknowledge the organizations that supported my research: Colorado Parks and Wildlife, Wisconsin Department of Natural Resources, American Society of Mammalogists, and the Department of Forest and Wildlife Ecology at University of Wisconsin-Madison. I also thank the Department of Zoology for helping to support me with Teaching Assistantships, allowing me to foster my love of teaching.

I am indebted to the numerous hunters and wildlife managers that have aided in sample collection and kindly offered their stories and insights over the last few years. I am particularly appreciative of many field and lab assistants, without whom this work would not have been achieved, especially Sam Paddock, Sonia Petty, Carissa Freeh, Cristina Vaughan, Rachel Larson, Jenna Carlson, and Garret Johnson. I am also thankful to Heather Johnson for the most recent collaboration leading to the last chapter of this dissertation.

I must again thank Jon Pauli for putting together a fantastic group of graduate students with whom I have shared the lab. There are too many folks to mention by name, but the countless hours of talking science and everything else with the Pauli lab, FWE department, and wider UW community has been invaluable. Finally, I am truly grateful to my family for their unwavering love and support.

Dissertation abstract

As human-modified landscapes now predominate globally, understanding the role of novel habitats in modifying animal behavior is increasingly important. Such new conditions can change animal foraging behavior, with important implications for individual condition, and biological aging. American black bears (*Ursus americanus*) are long-lived opportunistic omnivores that exhibit highly plastic foraging strategies, utilize seasonal hibernation, and are increasingly found in developing landscapes. As such, bears may alter their behavior to take advantage of differing availabilities of food subsidies, with important consequences at the individual- and population-level. My dissertation investigates the interactions of habitat and human activity on the foraging and aging ecology of black bears. Using stable isotopes to analyze diet, and telomeres as a molecular marker for biological aging, I examined bears at multiple scales in Colorado and Wisconsin.

Each chapter of this dissertation is written (and formatted) as a manuscript for publication in a scholarly journal. **Chapter 1** (published in *Biological Conservation*) investigated bear foraging throughout the Colorado landscape, and found that levels of human activity drove bear diet, and foraging on subsidies increased risk of conflict. **Chapter 2** (in review at *Evolutionary Ecology*) explored the relative importance of such environmental factors and individual characteristics on biological aging in these Colorado black bears, and discovered a latitudinal pattern in telomere length that is likely driven by habitat differences. **Chapter 3** (in review at *Journal of Applied Ecology*) characterized the high reliance of northern Wisconsin black bears on intentionally provisioned subsidies in the form of bear bait – over 40% of their lifetime diet was derived from bait sources. Finally, **Chapter 4** (prepared for submission to *Functional Ecology*) inspected a small population of longitudinally sampled black bears in southwestern Colorado to consider how foraging and hibernation influence stress and biological aging, and found that hibernation length slowed biological aging, but that foraging on subsidies could shorten hibernation. Overall, the work here demonstrates the remarkable habituation of black bears to food subsidies at multiple scales, and how accompanying changes in behavior and hibernation characteristics may have associated effects at the population and molecular levels, and suggests strategies for managing such populations.

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The diet of black bears tracks the human footprint across a rapidly developing landscape

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Abstract

Food subsidies have become a widely available and predictable resource in human-modified landscapes for many vertebrate species. Such resources can alter individual foraging behavior of animals, and induce population-wide changes. Yet, little consensus exists about the relative influence of the availabilities of native and human food subsidies to wildlife foraging throughout altered landscapes. We explored this unresolved question by analyzing the effects of landscape factors on American black bear (*Ursus americanus*) diet across the state of Colorado, USA. We estimated assimilated diet using stable isotope analysis of harvested black bear tissues to determine the contribution of human-derived foods to bear diets throughout Colorado, as well as how increasing reliance on human-derived food subsidies increases the risk of conflict. We found that bears (n = 296) showed strong regional diet variability, but substantial use of human-derived food subsidies in eastern Colorado (>30% assimilated diet). The age-sex class of the bear and housing density of its harvest location were the most influential predictors of ^{13}C enrichment (a tracer of human food subsidies). Furthermore, foraging on subsidies increased risk of conflict; the odds of being a nuisance bear increased by 60% for each ~ 1‰ increase in $\delta^{13}C$. Our study confirms the efficacy of $\delta^{13}C$ as a proxy for human activity, and indicates that while demographic differences play a clear role in the foraging ecology of bears, availability of subsidies coincident with varying levels of human activity appears to be a major driver in predicting black bear diet throughout the western United States.

Highlights

- We used stable isotopes to analyze black bear diet across a western U.S. landscape.
- Increased food subsidy consumption was primarily related to increased human development.

- Black bear use of food subsidies predicted risk of conflict with humans.
- Isotopic signatures of bears tracked development across a regional landscape.

Keywords: foraging, human-wildlife conflict, resource subsidies, stable isotopes, *Ursus americanus*

Introduction

Human-modified landscapes now prevail globally (Ellis et al., 2010), and urban landscapes show particularly extreme changes in productivity and resource availability (Shochat et al., 2006). Human-derived foods, especially in the form of food waste (Parfitt et al., 2010) or agricultural crops (Oro et al., 2013), are often widely available (Fedriani et al., 2001; Newsome et al., 2014a) and are a temporally and spatially predictable supplemental resource for many species of wildlife (Oro et al., 2013; Yirga et al., 2012). While supplemental food can enhance individual nutritional status and reproduction (Marzluff and Neatherlin, 2006), it also can have substantial ecological costs (Parker and Nilon, 2008). For example, it can shift phenological timing (Beckmann and Berger, 2003a; Robb et al., 2008) or modify prey use (Newsome et al., 2014a), altering well-established interspecific interactions (Rodewald et al., 2011) and potentially restructuring trophic cascades (Newsome et al., 2014b). Across mammalian species, increasing reliance on food subsidies can increase population sizes and decrease home ranges and activity levels (Newsome et al., 2014b; Parker and Nilon, 2012). Furthermore, wildlife habituation to supplemental food can have societal costs through increased conflict with humans (Beckmann and Lackey, 2008). Understanding then, how anthropogenic inputs to the environment are utilized has important ecological and conservation implications.

As opportunistic omnivores, American black bears (*Ursus americanus*) exhibit highly plastic foraging strategies and are increasingly found in modified landscapes (Beckmann and Berger, 2003a). Diet preferences and food intake vary by sex, reproductive status, and season (Jacoby et al., 1999; Robbins et al., 2004). In the spring, bears consume primarily herbaceous plants and graminoids (Raine and Kansas, 1990), incorporating a wide variety of soft and hard mast during summer and fall as they enter hyperphagia (Hellgren et al., 2005; Ryan et al., 2007).

Ungulates, small mammals, and insects, especially ants, can also be significant in the diet of some populations (Noyce et al., 1997; Zager and Beecham, 2006), as well as human-derived foods (Breck et al., 2009; Hopkins et al., 2014; Merkle et al., 2013). Despite high variability among populations, food availability is generally the primary predictor for habitat use, reproduction, denning chronology, and population density (Baldwin and Bender, 2010; Costello et al., 2003; Hilderbrand et al., 1999; Noyce and Garshelis, 1994; Rogers, 1987). Further, foraging preferences may vary by age-sex class to avoid risky competition or take advantage of differing prey availabilities (Ben-David et al., 2004; Edwards et al., 2011). Regardless of food availability in a particular year, adult survival in most black bear populations tends to be high (Noyce and Garshelis, 1994), but cub production declines in years with limited food availability (Bridges et al., 2011; Elowe and Dodge, 1989). Urbanized areas can supplement diets during these food-limited years, stabilizing cub production, though in some cases also increasing adult mortality due to lethal conflict with humans (Baruch-Mordo et al., 2014; Beckmann and Lackey, 2008). Increasing bear-human conflicts have been attributed to a combination of growing bear populations (Garshelis and Hristienko, 2006; Spencer et al., 2007), natural food failures (Hristienko and McDonald, 2007), and the availability of and attraction to human-derived foods (Beckmann et al., 2008; Can et al., 2014; Greenleaf et al., 2009; Hopkins et al., 2014).

The western U.S., in particular, is experiencing both increasing urbanization, and the expansion of anthropogenic impacts into exurban and rural areas through infrastructure such as roads and power lines (Hansen et al., 2005; Leu et al., 2008). The Colorado Front Range is one of the largest wildland-urban interfaces (Radeloff et al., 2005), exposing black bears to variable levels of human activity and habitat quality, and increasing bear-human conflicts (Baruch-Mordo et al., 2008). These conflicts appear to be highly variable across space and time, and among age-

sex class (Baruch-Mordo et al., 2008, 2014; Beston, 2011), generating uncertainty about accurately predicting which bears might become problematic. Further, the relative influence of native and human-derived foods on such conflicts remains in question. Some studies suggest that habituated bears will return to previously encountered food subsidies (Beckmann et al., 2004; Hopkins and Kalinowski, 2013; McCarthy and Seavoy, 1994; Merkle et al., 2013), while others indicate that use of urban environments is more flexible (Johnson et al., 2015), and increases primarily in poor food years (Baruch-Mordo et al., 2014; Kavčič et al., 2015). Due to high local variation in diet, few generalizations about bears across regions exist (Bojarska and Selva, 2012).

Traditional methods for diet reconstruction (e.g., scat, stomach content analyses) tend to underestimate highly digestible resources, including human-derived foods (e.g. Newsome et al., 2010). However, isotopic analysis examines assimilated diet, avoiding this bias, and has been successfully used to detect human-derived food consumption in omnivorous mammals including foxes (Lavin et al., 2003; Newsome et al., 2010), coyotes (Garwood et al., 2015; Newsome et al., 2015), and bears (Hobson et al., 2000; Hopkins et al., 2012, 2014; Mizukami et al., 2005). For example, Hopkins et al. (2014) demonstrated that consumption of human-derived foods by black bears in Yosemite National Park varied temporally with management policies of trash. Additionally, in British Columbia (Hobson et al., 2000) and central Japan (Mizukami et al., 2005) bears identified as nuisances or caught near human development were enriched in ^{13}C relative to their conspecifics. Because corn- and cane sugar-dominated human foods and their derivatives are enriched in ^{13}C relative to a C_3 native plant base (Jahren et al., 2006; Jahren and Kraft, 2008; Chesson et al., 2008), such bears could be consuming either human food waste, agricultural crops, or even livestock that are enriched in ^{13}C due to their feed (Jahren and Kraft, 2008). Most recently, black bears residing in an agricultural landscape in Minnesota were found

to regularly consume corn crops, based on GPS movements and ^{13}C enrichment (Ditmer et al., 2015b). Further, Hopkins et al. (2012) and Mizukami et al. (2005) found management bears also were enriched in ^{15}N, suggesting foraging at a higher trophic position, which they could obtain from consuming either natural or human foods that were rich in animal matter (meat, insects). Although there have been a number of site-specific studies, there have been few landscape-level studies on wildlife diet (e.g. Mowat and Heard, 2006) because diet reconstruction with stable isotopes depends on adequately characterizing the prey base.

Herein, we used stable isotopes to examine black bear diets across the state of Colorado. We analyzed individual bear hair and blood samples, and potential prey across the state, to determine the extent to which bears relied on human-derived food subsidies, and considered how landscape factors representing human activity and primary productivity related to diet. We anticipated that bears in poorer quality habitat and exposed to more human subsidies would consume a greater proportion of anthropogenic foods, which would vary as a function of age and sex class (Johnson et al., 2015). We further compared nuisance bears (lethal removal) to the hunter-harvested population to determine if eating anthropogenic foods increased the risk of being a conflict bear, as has been shown in other populations (Hopkins et al., 2014). Our findings provide insight to bear foraging at the landscape-level, and how food subsidies associated with the expanding human footprint influence black bear foraging ecology and management.

Materials and methods

Study area

We examined black bear diet throughout their range in Colorado, USA. Black bears occupy the western two-thirds of the state, in the Southern Rocky Mountains, which is a complex patchwork of land use types consisting of forests, agricultural lands, and urban developments. Black bears are hunted throughout their range from September – January, with the majority of harvest in September and October. Colorado does not allow baiting, and manages the bear population at the level of Game Management Unit (GMU), which are on average ~1900 km^2. Housing density within GMUs ranges from 0.04 to 121 housing units/km^2, and tends to be denser in eastern Colorado ($t = 5.98$, $P < 0.001$) along the Front Range, a rapidly expanding wildland-urban interface. The vegetation community varies widely throughout the state, transitioning from ponderosa pine and piñon-juniper (*Pinus edulis/Juniperus* spp.) woodlands at lower elevations into lodgepole pine (*Pinus contorta*), aspen (*Populus tremuloides*), Douglas-fir (*Pseudotsuga menziesii*), and spruce (*Picea* spp.)-subapline fir (*Abies lasiocarpa*) forests at higher elevations. Areas of Gambel oak (*Quercus gambelli*), considered especially high-quality bear habitat, are distributed throughout the Southern Rockies (Beck 1991).

Sample collection and preparation

We sampled hair and blood from hunter-harvested black bears across Colorado during the fall hunting season in 2011 ($n = 296$ hair; $n = 113$ blood). Because we opportunistically sampled bear carcasses during registration with hunters' permission, sample locations are not evenly distributed, and not all carcasses contained sufficient blood for sampling. For each harvested

bear, GPS locations (or minimally the management unit) are provided by the hunter (Fig. 1). Our samples represent ~27% of Colorado bear harvest in 2011.

Hair growth in black bears typically occurs late spring into the fall, and its isotopic signature is representative of assimilated diet throughout its growth (Hilderbrand et al., 1996, Jacoby et al., 1999). Whole blood represents more recent diet than hair, approximately the last month or two in bears (Hilderbrand et al., 1996). We examined seasonal changes in diet within individuals by analyzing hair and blood isotopic signatures, comparing spring-summer diet (hair) to late summer-fall diet (blood). Though we recognize the uncertainty in the exact time period each tissue represents, as well as some temporal overlap, we hereafter refer to "spring-summer" or "late summer-fall" for simplicity. Due to the wide geographic spread of bear samples, we aimed to first characterize the isotopic signatures of the native forage base in five general regions: Northeastern Front Range, Southeastern Front Range, Southwestern San Juan Mountains, Uncompaghre Plateau, and Northwestern Colorado. We collected known bear foods that grouped broadly into native vegetation (n = 288) (acorns, berries, and herbaceous plants) or animal matter (n = 116) (mule deer, rabbit, ants and other insects) (see Appendix A, Table A1).

We rinsed hair samples three times with 2:1 chloroform:methanol solution to remove surface oils, homogenized them with surgical scissors, and dried samples for 72 hours at 56°C (Pauli et al., 2009). Whole blood samples were dried for 72 hours at 60°C, and homogenized with a spatula. Vegetation samples were dried at 56°C for a minimum of 72 hours and homogenized in a ball mill (Mixer Mill MM200, Restch Inc. Newton, PA, USA). We weighed samples (>50% in duplicate) into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis at the University of Wyoming's Stable Isotope Facility using a Costech 4010 and Carlo Erba 1110 Elemental Analyzer (Costech, Valencia, CA) attached to a Thermo Finnigan Delta Plus XP Continuous

Flow Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). We provide results as per mil (‰) ratios relative to the international standards of Vienna Peedee Belemnite for C and atmospheric N_2 for N, with calibrated internal laboratory standards.

Stable isotope analyses

Choice of discrimination factors can strongly influence mixing models, and be particularly challenging in omnivores where sources can differ greatly in isotopic signature (Caut et al., 2009). We applied tissue-specific mean discrimination factors recently developed for omnivorous mammals to each sampled bear food group for hair and blood samples separately (hair: animal matter, $\Delta^{13}C$: 2.1 ± 0.1 and $\Delta^{15}N$: 3.9 ± 0.3, native vegetation, $\Delta^{13}C$: 3.4 ± 0.2 and $\Delta^{15}N$: 2.4 ± 0.2; blood: animal matter, $\Delta^{13}C$: 0.6 ± 0.1 and $\Delta^{15}N$: 3.0 ± 0.3, native vegetation, $\Delta^{13}C$: 1.3 ± 0.2 and $\Delta^{15}N$: 1.9 ± 0.2; Kurle et al., 2014). Though we analyzed whole blood, we assumed the appropriate discrimination factors would be most similar to those of red blood cells (e.g. Caut et al., 2009).

To define isotopically distinct diet groups, we used a K nearest-neighbor randomization test (KNN; Rosing et al., 1998), comparing forage items first within each region, and subsequently across regions. Uncompaghre Plateau samples were indistinguishable from Southwestern Colorado, so we combined these areas, and proceeded with 4 geographical regions of Colorado. To define a human-derived foods signature, we used human hair samples from across the U.S. ($\delta^{13}C$ = -16.9 ± 0.8; $\delta^{15}N$ = 8.8 ± 0.5; Bowen et al., 2009), which has been applied in previous work on omnivorous carnivores (Hopkins et al., 2012; Newsome et al., 2010). We make the assumption then that black bears would discriminate an all human foods diet similarly to humans, and thus apply no trophic correction for hair samples (Hopkins and

Ferguson, 2012) (Fig. 2). For blood sample analysis, it was necessary to correct the human hair samples to blood samples, so we applied a trophic correction to the human-derived foods diet group by calculating the difference between discrimination factors developed for red blood cells and those developed for hair in an omnivore consuming a mixed diet ($\Delta^{13}C$: -1.7 ± 0.1 and $\Delta^{15}N$: -0.7 ± 0.2; Kurle et al., 2014).

We estimated proportional importance of each forage group to regional bear populations with Bayesian-based mixing models in the package Stable Isotope Analysis in R (SIAR; Parnell et al., 2010). Models were parameterized with uniform priors, allowing the data to drive the model, as well as trophic discrimination and concentration dependence (mean digestible elemental concentrations for each diet group; Hopkins and Ferguson, 2012). Data are expressed as medians of the probability density functions with 95% credible intervals, which represent each diet group's likely level of contribution to bear diet (Parnell et al., 2010). We also estimated dietary proportions by age-sex class. To ensure that minor differences in the isotopic signatures of regional diet sources (i.e., regional mixing spaces) did not bias results, we also re-ran models parameterized with mean statewide diet sources, and found no significant differences in group estimates. Therefore, we were confident in using raw isotopic values as proxies for diet throughout the state, with increased enrichment in ^{13}C as indicative of increased anthropogenic food consumption and increased enrichment in ^{15}N as indicative of either increased anthropogenic food or animal matter consumption (i.e. trophic position).

Variable predictors of diet and model selection

We examined how potential variables, specifically demographic class, habitat productivity, and human activity, were correlated with isotopic signature, and therefore diet. For these analyses we

limited our dataset to bear samples with GPS harvest locations (hair $n = 273$; blood $n = 104$). Although black bears can vary widely in their home range size, collared bears in two Colorado studies generally ranged up to 50 km^2 (Baldwin, 2008; Baruch-Mordo et al., 2014). Thus, we buffered each bear harvest location by a radius of 4 km (~50 km^2) to analyze variables representing both habitat productivity and human activity. To examine whether our analysis was sensitive to scale, we also considered initially buffers of 10, 250, and 1000 km^2. As we found the same patterns regardless of scale, we present results from 50 km^2 as the most representative of a black bear home range. To estimate habitat productivity in 2011, we examined the Normalized Difference Vegetation Index (NDVI) (Wiegand et al., 2008), with higher NDVI values representing greater primary productivity. We took global monthly composites (0.1 degrees) for 2011 collected by Terra/MODIS (NASA Earth Observations) and averaged them across the growing season (April – October) in ArcGIS (ESRI, v.10). Mean growing season NDVI of each buffered bear location was extracted using Geospatial Modelling Environment (GME, v. 0.7.2.1), and then used in subsequent comparisons. We considered three possible measures of human activity that could be related to bear diet: road density from TIGER 2013 primary and secondary Colorado road layer (US Census Bureau), housing density from 2010 Block Level Housing Density (Radeloff et al., 2010) and percent crop cover from National Landcover Database (NLCD, 2011). Because road density and housing density were highly correlated ($r = 0.60$, $t = 11.86$, $P < 0.001$), likely capturing similar measures of human development, we selected housing density to represent human development and crop cover for agricultural development. Bears were not harvested in areas of high crop cover – 87% of their locations contained no croplands. We accordingly categorized bear location for presence or absence of any amount of croplands. As Colorado bear habitat can vary with elevation (e.g., high elevations

have greater precipitation and colder temperatures), we also calculated average elevation of each location from National Elevation Dataset (USGS, 2009).

We first examined variables individually with Pearson's correlations or ANOVAs. We then compared linear models using $\delta^{13}C$ and $\delta^{15}N$ as response variables, and used Akaike's Information Criterion to select the best models to predict $\delta^{13}C$ and $\delta^{15}N$ separately. Covariates examined include age-sex class (adult female, adult male, subadult female, subadult male), elevation, housing density, crop cover, and growing season NDVI. Housing density was log transformed to meet assumptions of normality. We also initially considered environmental covariates including precipitation, snow, and temperature, but these were highly correlated with elevation and growing season NDVI, therefore we did not include them in the models. We conducted these analyses for both hair and blood samples. We tested for spatial autocorrelation in the model residuals by running a spline correlogram with 1,000 permutations (Bjørnstad and Falck, 2001), and found no significant autocorrelation that would warrant additional spatial analyses (Appendix A, Fig. A1).

Because hunter-harvested bears are more likely to be killed along transportation corridors, our samples of hunter-harvested bears may underrepresent remote wildland bears that have little access to human-derived food subsidies. However, as hunting is not permitted within urban areas, we are likely also missing bears that have the greatest access to subsidies. Regardless, analyses up to this point did not include known roadkill or nuisance bears. We considered conflict bear diet independently by analyzing samples from bears killed by vehicle collision (n = 14) and lethal nuisance removal by CPW (n = 14), representing ~16% and 11% of each mortality type in 2011, respectively, and were killed during the same time period as the hunted bears and in similar locations. Nuisance bears may have been lethally removed due to

conflicts around housing developments (e.g. property damage) or agricultural operations (e.g. crop depredation). Roadkill bears are also classified broadly as conflict bears by Colorado Parks and Wildlife due to injury and damage to humans and property (e.g. Baruch-Mordo et al., 2008). To determine whether isotopic signature is predictive of being a conflict bear, we compared nuisance bears and roadkill bears to a subset of harvested bears (n = 62) from within the same areas (GMUs) as the conflict bears, thus removing potential geographical bias. We compared dietary estimates among bears grouped by mortality type, and used logistic regression to estimate the odds ratios for mortality types based on isotopic signature.

Results

Regional bear diet

Comparisons between spring-summer and late summer-fall diet yielded similar patterns of forage within regions. Bear hair isotopic values exhibited large variation between individuals in both $\delta^{13}C$ ($\bar{x}$ = -21.78; range: -24.23 to -17.15), and $\delta^{15}N$ ($\bar{x}$ = 5.17; range: 2.12 to 10.19). Because we found significant, albeit slight, isotopic differences by region – vegetation in southwest Colorado was enriched relative to the northeast (KNN, $P = 0.01$) and animal matter in the southeast was enriched relative to the northeast (KNN, $P < 0.001$) – we estimated proportional diet contributions separately for each region. As expected, native vegetation made up the primary spring-summer diet group for Colorado bears in all regions, ranging from a low of 66% in the northeast to a high of 80% in the southwest (Table 1). However, there were strong longitudinal differences in estimates of human food contributions, with eastern bears consuming > 30% human-derived foods, while western bears consumed ≤ 21%. Because we used region-specific diet samples to parameterize the model, these differences are not based on an isotopic difference in prey base.

Bear blood samples were less enriched in ^{13}C than hair samples, but slightly enriched in ^{15}N, showing a tissue-specific (i.e., season-specific) effect (RM-MANOVA, Pillai's trace, $F_{2,111} = 0.80$, $P < 0.001$), suggesting that any seasonal change in diet was consistent across individuals. Blood samples indicated similar consumption of food subsidies during the fall compared to the summer, with a low of 22% in the west and a high of 36% in the northeast (Table 1). The 95% credible intervals for diet estimates from blood samples overlap with estimates derived from hair samples, but were larger, likely due to smaller sample sizes with greater variation. Regardless of

minor differences in model estimates, both seasons show a consistent longitudinal pattern, with higher human-derived food consumption in the eastern region.

Covariates influencing bear diet

For spring-summer diet, we found that presence of crop cover was unrelated to ^{13}C enrichment (t = -1.53, P = 0.13); however, it was related to housing density as bear locations with some crop cover tended to have greater housing densities than locations without any crop cover (t = -2.66, P = 0.01). Because of the very limited crop cover in our study area, we could not adequately tease it apart from the broader measure of human activity at this scale. Thus, we did not include crop cover in regression analyses.

Age-sex class was an influential predictor of the hair isotopic signature of bears, with all four age-sex groups exhibiting significant differences. Adults were enriched in both ^{13}C and ^{15}N over subadults, though adult females were the most enriched in ^{13}C, while adult males were the most enriched in ^{15}N (MANOVA, Wilk's $\lambda = 0.84$, $P < 0.001$). Stable isotope mixing model estimates suggest that adults consumed more human-derived foods than subadults (Table 2).

The top linear model for hair $\delta^{13}C$ also included age-sex class, NDVI, and housing density (Table 3). NDVI tended to have a slight negative relationship with $\delta^{13}C$ (β = -0.001, P = 0.01), suggesting that bears in areas of higher productivity consumed more native vegetation. Housing density was positively related to ^{13}C enrichment (β = 0.650, $P < 0.001$), and thus, to bear reliance on human-derived foods, regardless of age-sex class (Fig. 3). The top model for hair $\delta^{15}N$ included NDVI as an important covariate (β = -0.001, P = 0.005) (Table 3).

For late summer-fall diet, age-sex class was not significantly related to blood $\delta^{13}C$ and $\delta^{15}N$ (MANOVA, Wilk's $\lambda = 0.97$, $P = 0.74$) suggesting diet later in the season was less variable

across age-sex classes than earlier (Table 2). Similar to hair samples, however, housing density and NDVI were influential covariates for blood $\delta^{13}C$ and $\delta^{15}N$ (Table 3), though the relationships were not as strong as with hair samples. Housing density was positively related to $\delta^{13}C$ (β = 0.622, P = 0.090) and NDVI was negatively related to both $\delta^{13}C$ (β = -0.003, P = 0.002) and $\delta^{15}N$ (β = -0.001 P = 0.068). Though there are clearly some seasonal differences, hair and blood samples corroborate the relationships of housing density and NDVI with bear diet.

Conflict bears

Conflict bears and the subset of hunter-harvested bear samples were similarly distributed among ages (t = -1.48, P = 0.15), and split evenly between males and females. Hair samples from either type of conflict bears (nuisance removals or vehicle collisions) were typically enriched in isotopic signature compared to hunter-harvested bears (MANOVA, Wilk's λ = 0.93, P = 0.04), with nuisance bears being the most enriched (Fig. 4a). Enrichment in ^{13}C is related to an increased probability of being a nuisance bear, as opposed to a hunter-harvested bear. Because $\delta^{13}C$ and $\delta^{15}N$ are correlated in this system (r = 0.44, t = 4.63, P < 0.001), we report only $\delta^{13}C$, as it is a tracer of human foods. The odds of being a nuisance bear increased by 60% for each ~ 1 ‰ increase in $\delta^{13}C$ (odds-ratio: 1.6, 95% CI: 1.1-2.51, P = 0.02). This pattern is also still significant for the more general conflict bear, which includes vehicle collisions in addition to nuisance removals ($\delta^{13}C$ odds-ratio: 1.4, 95% CI: 1.03-2.0, P = 0.04). This corresponds to an increased estimated dietary contribution from human-derived foods - nuisance bears consumed on average 12% more human-derived foods than hunter-harvested bears (Fig. 4b).

Discussion

We found that black bears across Colorado exhibited regional variability in diet. As has been demonstrated in previous populations (e.g. Hellgren et al., 2005), vegetation is the most important dietary group regardless of location. However, bears in the eastern regions along the Front Range consumed a high amount of human-derived foods (over 30% of assimilated diet), while in western Colorado, bears relied more on native vegetation. Native animal matter contributed little to total bear diet. At the landscape scale, human density and activity (as indexed by housing density) appeared to be the strongest predictor of human-derived food consumption (Table 3, Fig. 3). This relationship held regardless of age-sex class, tissue type, or native vegetative productivity. Further, use of food subsidies was predictive of conflict, confirming that lethally removed nuisance bears consumed more human-derived foods than hunter-harvested bears (Fig. 4b).

Whether bears turn to food subsidies only in food-limited years (Baruch-Mordo et al., 2014) or utilize subsidies regardless of natural food availability (Beckmann et al., 2008) has been studied with conflicting results. Most recently, an analysis of bears in three systems in the western U.S. indicated that individual bear use of development was a dynamic interaction between their physiological state and environmental conditions – bears tended to use developed areas more during poor food years, later in the season, and as they aged, with males using development more overall (Johnson et al., 2015). Our study scale allowed for examination of broad dietary patterns across a range of variable black bear habitat. Though these bears were only sampled in a single year, Colorado Parks and Wildlife estimated 2011 as an average year for bear fall forage (Apker, unpublished data), suggesting that in a mast failure year, human-derived food consumption could be even higher. Previous work has found black bears consuming

crops, livestock, trash, and other human-derived foods can lead to conflict in Colorado (Baruch-Mordo et al., 2008). Our results indicate that crop cover is not substantial in areas of bear harvests. Crop cover is also unrelated to ^{13}C enrichment, suggesting, at the landscape scale, human-derived food consumption is not being strongly driven by agricultural crops. Rather, we suggest that subsidies associated with human development and habitation are primarily driving bear diet. Such subsidies may include trash, planted fruit trees, and bird feeders (e.g. Baruch-Mordo et al., 2008; Beckmann and Berger, 2003a; Merkle et al., 2008). Our study is further confirmation that managing human behavior to reduce availability of subsidies is paramount to reducing bear reliance on human-derived foods. Future work examining different types of human development at a finer scale (e.g. housing developments as opposed to campgrounds) could also be valuable to ascertain differences in subsidy availability. It is worth noting that although our study area had little crop cover, the few areas with non-negligible crop cover also possessed high housing densities. So, while crops are not an important factor driving bear food subsidy use throughout the Colorado landscape, they may still contribute to ^{13}C enrichment, at least in some locations.

We also found a slight negative relationship between NDVI, as a measure of vegetative productivity, and ^{13}C enrichment. This suggests that the quality of the available habitat could play a role in food subsidy use that we have not adequately captured with NDVI, which may be limited when averaged over a period of months. It is possible that we have underestimated natural forage availability as NDVI can miss some important bear foods, such as berries or insects (Wiegand et al., 2008), or over-represent conifers, which offer little forage for Colorado bears. We further found adults were more likely to be consuming food subsidies than subadults (Table 2). Males, however, were enriched in nitrogen-15, indicating higher trophic level foraging

(and theoretically better forage), while females were more enriched in carbon-13. As adult male bears typically access the best food resources, our study suggests that human-derived foods may be considered a preferred resource, which could increase the potential for conflicts. Complicating this situation, female consumption of high caloric food subsidies can enhance reproduction and overall increase population size (Beckmann and Lackey, 2008). Further, females that forage with their cubs in developed areas are more likely to rear cubs that prefer developed areas as adults (Mazur and Seher 2008), creating a population that is ever more reliant on food subsidies.

The large amount of anthropogenic foods consumed by eastern Colorado bears is comparable to that estimated for bears inhabiting Yosemite National Park during years of poor trash management (Hopkins et al., 2014) and when food subsidies were abundant. Human-derived food subsidies are likely abundant throughout eastern Colorado, which features more human development compared to western Colorado. Whether individuals are flexible in their use of subsidies between years or become habituated to them remains in question. Previous behavioral studies on black bears in Nevada found that dependency on food subsidies was irreversible (Beckmann and Berger, 2003a), but another study in northern Colorado showed interannual flexibility in subsidy use (Baruch-Mordo et al., 2014). Locations featuring more human development may constrain dietary options for bears, leading to a greater reliance on human foods. As urbanization is predicted to continue to increase throughout bear range (Bierwagen et al., 2010), the increasing dependence on food subsidies seems likely, and research such as this into patterns of consumption will be critical to mitigating human-bear conflicts.

Bears most frequently involved in conflicts throughout their range tend to be subadults, particularly males (Hristienko and McDonald, 2007), or females with cubs (Rode et al., 2006). Though we found adult males in the hunter-harvested Colorado population consumed the

greatest amount of food subsidies, females with cubs were likely underrepresented in our sample, as hunters are prohibited from harvesting them. Thus, we may be underestimating the amount of human foods consumed by adult females, in general. On the other hand, we also recognize that our sample of hunter-harvested bears are a subset of the population that could potentially miss the most wildland bears with less access to subsidies. We did, however, find that even within our sample pool, $\delta^{13}C$ in Colorado bear hair is predictive of risk of conflict regardless of age-sex class. As we considered only bears in GMUs that had both non-conflict and conflict mortalities, simply residing in an area of high human density did not necessarily lead to conflict, but increased foraging on food subsidies did. Some GMUs with high human development also feature high quality habitat for bears. Thus, regardless of whether bears were selecting for or against this habitat as measured at the GMU level (e.g. due to intraspecific competition; Elfström et al., 2014), some bears may have still avoided human-derived foods and, thus, decreased their risk. Feeding trials suggest that bears may have innate food preferences and sex-specific differences in use of novel foods, with males more likely to try unfamiliar diet items (e.g. crops), compared to females. However, after exposure to novel foods, females will eventually preferentially seek out those food sources (Ditmer et al., 2015a). Our results also suggest that such individual differences in foraging preferences could predict the likelihood of conflict. Regardless, roadkills were enriched over hunter-harvested bears, and nuisance bears were even enriched over roadkills (Fig. 4a), indicating $\delta^{13}C$ could be indicative of conflict type. This corroborates previous work indicating that nuisance bears tend to be enriched in ^{13}C (Hobson et al., 2000; Mizukami et al., 2005). Our results suggest some individuals are certainly consuming more human-derived foods than others. Because some bears reside in developed habitats and avoid use of subsidies, a general strategy of lethal removals of urban bears (Hopkins et al., 2014;

Lewis et al., 2014) may not be sustainable long-term as they will likely be replaced with other individuals.

More generally, our findings corroborate the utility of $\delta^{13}C$ as a tracer of human-derived food in temperate North American systems (Hopkins et al., 2014; Lavin et al., 2003; Newsome et al., 2010, 2015). Carbon-13 enrichment is primarily attributed to high use of corn in human foods (Jahren et al., 2006; Jahren and Kraft, 2008); the similar enrichment of ^{15}N in human hair is likely due to high meat consumption in North America (Schoeller et al., 1986). Traditional diet studies tend to underestimate highly digestible food such as human-derived food, and few studies have reconstructed diet using stable isotopes on such a large regional scale, because changes in forage base will affect mixing model estimates (e.g. Phillips et al., 2005). We are confident in our characterization of the potential forage mixing space, however, because surveying native plants and animals throughout the regions did not yield important regional differences (Appendix A, Table A1). In particular, vegetation in eastern Colorado was not enriched in ^{13}C, confirming that enrichment found in those bears was not inflated due to a difference in carbon-13 signature of vegetation. Native animals, however, were slightly enriched in ^{13}C in southeastern Colorado, signifying a general increased use of food subsidies throughout the area. Regardless of slight isotopic differences in forage base, $\delta^{13}C$ appears to track human food consumption well in animals throughout this altered landscape.

Because of its relationship to human foods, we have primarily restricted our discussion to $\delta^{13}C$; however, $\delta^{15}N$ is correlated with $\delta^{13}C$ in this system and we found it to be similarly predictive of conflict bears as well as human-derived food consumption, as has been shown in other bear populations (Hopkins et al., 2012; Mizukami et al., 2005). The primary landscape variable associated with nitrogen-15 enrichment was a negative relationship with NDVI,

suggesting that bears forage at a slightly lower trophic level in areas of higher productivity. Whether this relationship is driven by the correlation between $\delta^{15}N$ and $\delta^{13}C$ and their incorporation into tissues (Hobson et al., 2000) or by true differences in foraging is unknown. Future research could benefit from combining individual level landscape selection with isotopic diet analysis to disentangle some of these remaining questions.

Synanthropic species tend to show pronounced plasticity in their diet as part of their association with anthropogenic features (Luniak, 2004), but whether individuals overlap in their resource use or some specialize on human-derived foods remains unknown. We found less variability within individuals than between tissue types, suggesting that some specialize more on food subsidies. We also found similar levels of consumption throughout summer and fall, which is in contrast to increased bear use of development during the fall found by Johnson et al. (2015). This discrepancy may be due to differences in the study scales, as well as the overlap in foraging periods represented by hair and blood samples. Though we used tissue-specific discrimination factors, segmenting hair samples in future work could provide a finer timescale of diet changes to compare with seasonal foraging movements.

Clearly, black bears can use human food subsidies extensively, and such bears tend to have higher reproduction, shorter activity and denning, and larger body sizes (Beckmann and Berger, 2003b; Graber, 1982), which are all indicative of better forage availability. While there may be some individual specialization occurring, those consuming the most human-derived foods could be marginalized to such habitat (Elfström et al., 2014) or driven by physiological necessity (Johnson et al., 2015). Those consuming more human-derived foods within such altered habitats though are further at an increased risk of conflict. Though high-caloric food subsidies may enhance reproduction, the costs due to conflict mortality (Beckmann and Lackey,

2008), increased stress (Malcolm et al., 2014; Rode et al., 2006), and unknown effects of human "junk food" (Heiss et al., 2009), suggest that urban areas may in effect be an ecological trap, and use of food subsidies in particular may not be an adaptive strategy. As urbanization and human activity expands, this is further evidence that proper trash management to minimize human food subsidies will be essential in mitigating conflict. Evaluating the underlying causes of and degree to which such species as black bears are exploiting human food subsidies is a crucial component when considering human impacts on ecosystems.

Acknowledgements

Funding was provided by Colorado Parks and Wildlife, Wisconsin Department of Natural Resources, and the American Society of Mammalogists. We thank Christina Vaughan, Garrett Johnson, and Samantha Paddock for laboratory assistance and Benjamin Zuckerberg for consultation on spatial analyses. We especially thank the many wildlife managers and bear hunters for collecting and providing samples.

Appendix A. Supplementary material

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Table 1. Assimilated dietary estimates from Bayesian mixing models (SIAR) for hunter-harvested black bears in the spring-summer and late summer-fall seasons in 2011, obtained from the isotopic signatures of hair and blood, respectively. Estimates provided by region of Colorado.

	Median Proportion (95% credible intervals)			
Diet Groups	NE CO	SE CO	NW CO	SW CO
Hair (n)	*29*	*71*	*104*	*92*
Native vegetation	0.66 (0.58-0.72)	0.67 (0.63-0.72)	0.78 (0.76-0.80)	0.80 (0.78-0.83)
Animal matter	0.01 (0.00-0.05)	0.00 (0.00-0.02)	0.01 (0.00-0.03)	0.01 (0.00-0.03)
Human-derived foods	0.33 (0.26-0.40)	0.32 (0.28-0.37)	0.21 (0.18-0.23)	0.19 (0.16-0.21)
Blood (n)	*9*	*29*	*37*	*38*
Native vegetation	0.45 (0.20-0.63)	0.61 (0.54-0.69)	0.68 (0.60-0.73)	0.73 (0.69-0.77)
Animal matter	0.19 (0.00-0.49)	0.02 (0.00-0.07)	0.10 (0.00-0.22)	0.04 (0.00-0.10)
Human-derived foods	0.36 (0.17-0.52)	0.36 (0.28-0.45)	0.22 (0.14-0.29)	0.22 (0.17-0.28)

Table 2. Assimilated dietary estimates from Bayesian mixing models (SIAR) for age-sex class of black bears in the spring-summer and late summer-fall seasons in 2011, obtained from the isotopic signatures of hair and blood, respectively.

	Median Proportion (95% credible intervals)			
	Adult male	Adult female	Subadult male	Subadult female
Hair (n)	*93*	*71*	*93*	*39*
Native vegetation	0.68 (0.66-0.71)	0.75 (0.72-0.78)	0.75 (0.73-0.78)	0.77 (0.73-0.81)
Animal matter	0.01 (0.00-0.02)	0.01 (0.00-0.02)	0.01 (0.00-0.02)	0.02 (0.00-0.06)
Human-derived foods	0.31 (0.28-0.33)	0.24 (0.21-0.27)	0.24 (0.21-0.26)	0.21 (0.16-0.25)
Blood (n)	*38*	*30*	*35*	*10*
Native vegetation	0.63 (0.58-0.68)	0.65 (0.60-0.69)	0.66 (0.60-0.72)	0.64 (0.43-0.77)
Animal matter	0.06 (0.00-0.14)	0.02 (0.00-0.08)	0.04 (0.00-0.14)	0.10 (0.00-0.39)
Human-derived foods	0.31 (0.22-0.39)	0.32 (0.27-0.37)	0.29 (0.21-0.36)	0.25 (0.07-0.39)

Table 3. Top linear models to predict $\delta^{13}C$ and $\delta^{15}N$ signatures in hair and blood, representing spring-summer diet and late summer-fall diet, respectively. Covariates tested were age-sex class, mean housing density (log transformed), growing season productivity (NDVI), and mean elevation (all calculated within a 50 km^2 buffer of harvest location). Models were ranked using AIC (only < 2 ΔAIC are shown).

	AIC	Δ AIC	wt.	Adj. R^2
Hair				
$\delta^{13}C$				
Age-Sex Class + Housing density + NDVI	25.56	0.00	0.59	0.16
Age-Sex Class + Housing density + NDVI + Elevation	26.64	1.08	0.34	0.16
$\delta^{15}N$				
Age-Sex Class + NDVI	109.39	0.00	0.42	0.10
Age-Sex Class + NDVI + Elevation	110.69	1.31	0.22	0.10
Age-Sex Class + NDVI + Housing density	110.97	1.59	0.19	0.10
Blood				
$\delta^{13}C$				
Housing density + NDVI + Elevation	59.41	0.00	0.44	0.19
NDVI + Elevation	60.42	1.01	0.27	0.17
Housing density + NDVI	60.92	1.51	0.21	0.17
$\delta^{15}N$				
NDVI	40.74	0.00	0.24	0.02
NDVI + Elevation	41.50	0.76	0.16	0.02
Intercept	42.16	1.42	0.12	0.02
NDVI + Elevation + Housing density	42.41	1.67	0.10	0.02
NDVI + Housing density	42.49	1.76	0.10	0.02

Figure Legends

Figure 1. Sample locations of hunter-harvested bears (*n* = 273) in 2011, shown with Colorado black bear range (as estimated by Colorado Parks and Wildlife) and management regions.

Figure 2. Isotopic signatures of potential diet items and black bear hair samples, 2011. Generalized diet groups for Colorado shown with means and standard deviations, corrected for trophic discrimination: native vegetation, animal matter, human-derived foods. Geographic origin of hunter-harvested bear samples indicated by symbols: eastern CO (*dark gray circles*), western CO (*light gray circles*).

Figure 3. Linear regression of $\delta^{13}C$ on housing density within 50 km^2 of bear harvest location (log transformed), showing a positive relationship between increased housing density and ^{13}C enrichment of hunter-harvested bear hair. Though age-sex class was also a significant predictor variable, slopes were similar across classes, so only a single regression is shown, with points representing age-sex classes: adult male (*filled black circles*), adult female (*filled gray circles*), subadult male (*open black circles*), subadult female (*open gray circles*).

Figure 4. (a) Isotopic signatures of bears separated by mortality type: hunter-harvested (*light gray*), roadkill (*medium gray*), and nuisance bears (*black*); and (b) Assimilated dietary estimates shown as proportional density distributions for each group of bears.

Figure 1.

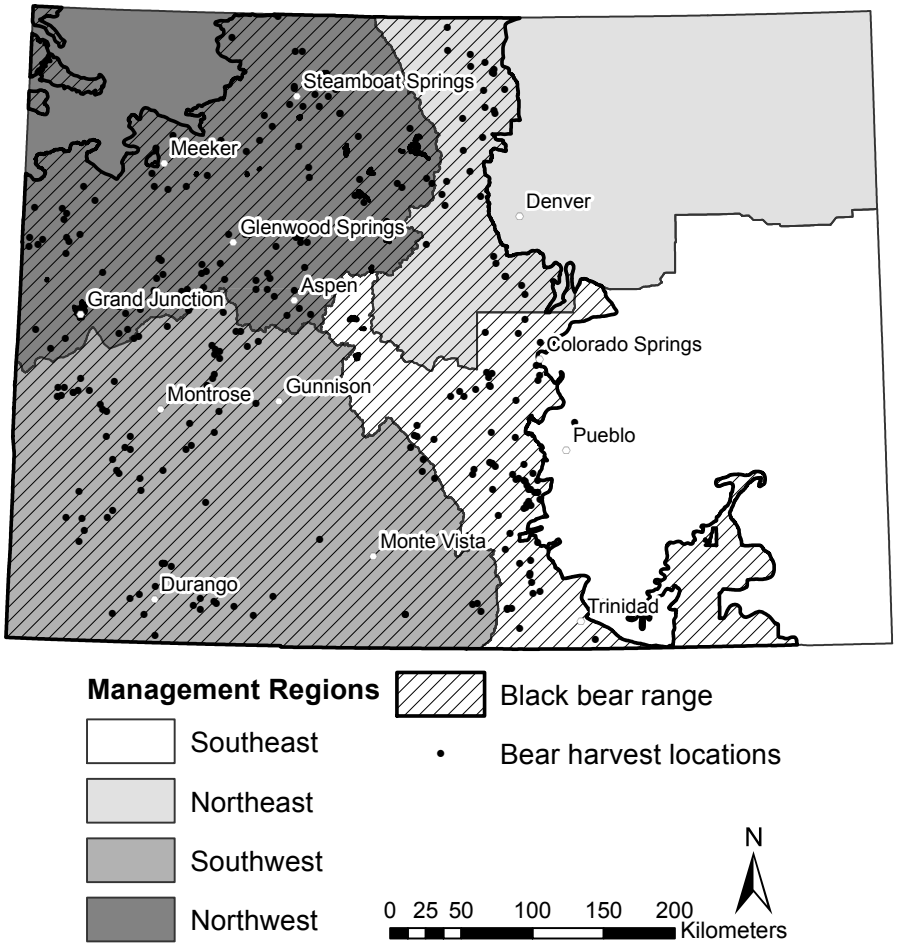

Steamboat Springs
Meeker
Denver
Glenwood Springs
Aspen
Grand Junction
Colorado Springs
Montrose
Gunnison
Pueblo
Monte Vista
Durango
Trinidad
Management Regions
Southeast
Northeast
Southwest
Northwest
Black bear range
Bear harvest locations
N
0 25 50 100 150 200
Kilometers

Figure 2.

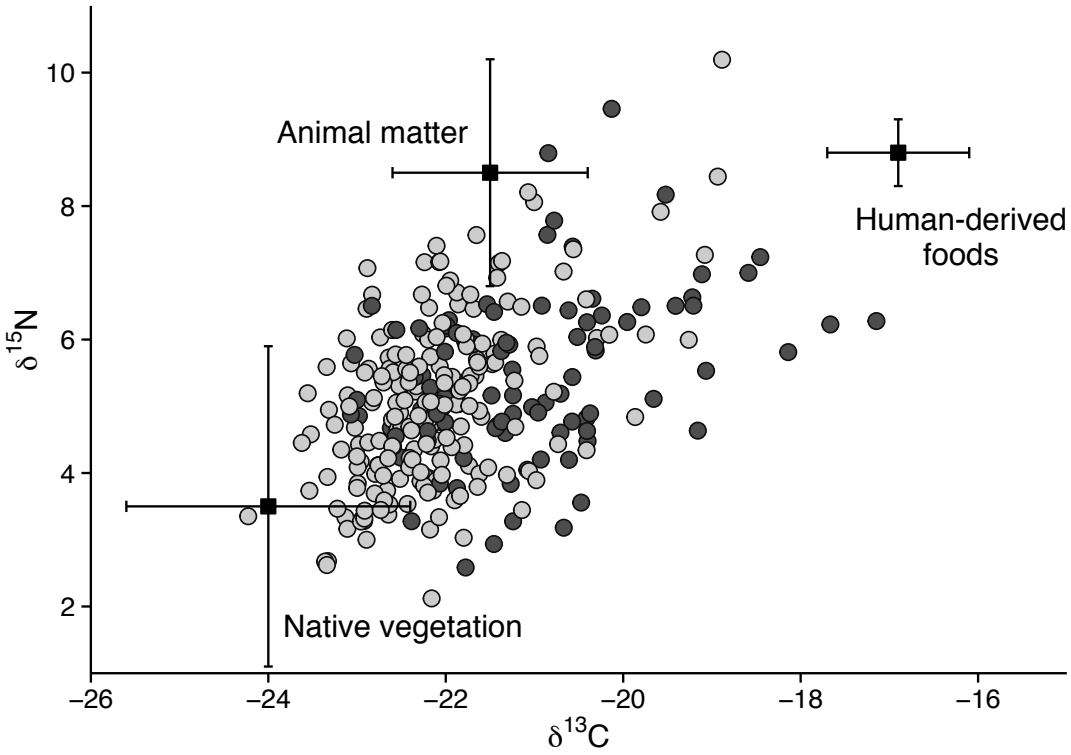

Animal matter
Human-derived foods
Native vegetation
δ15N
δ13C
10
8
6
4
2
–26
–24
–22
–20
–18
–16

Figure 3.

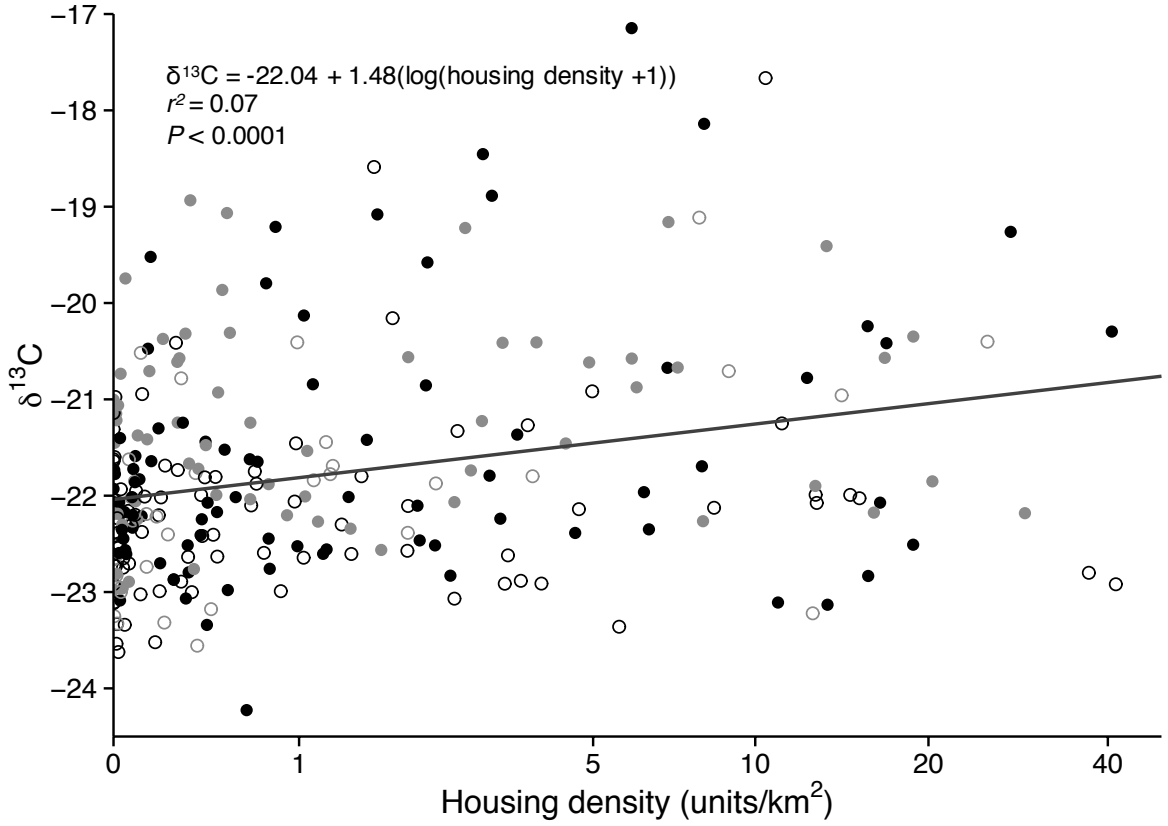

δ13C = -22.04 + 1.48(log(housing density +1))
r2 = 0.07
P < 0.0001
−17
−18
−19
−20
−21
−22
−23
−24
δ13C
0
1
5
10
20
40
Housing density (units/km2)

Figure 4.

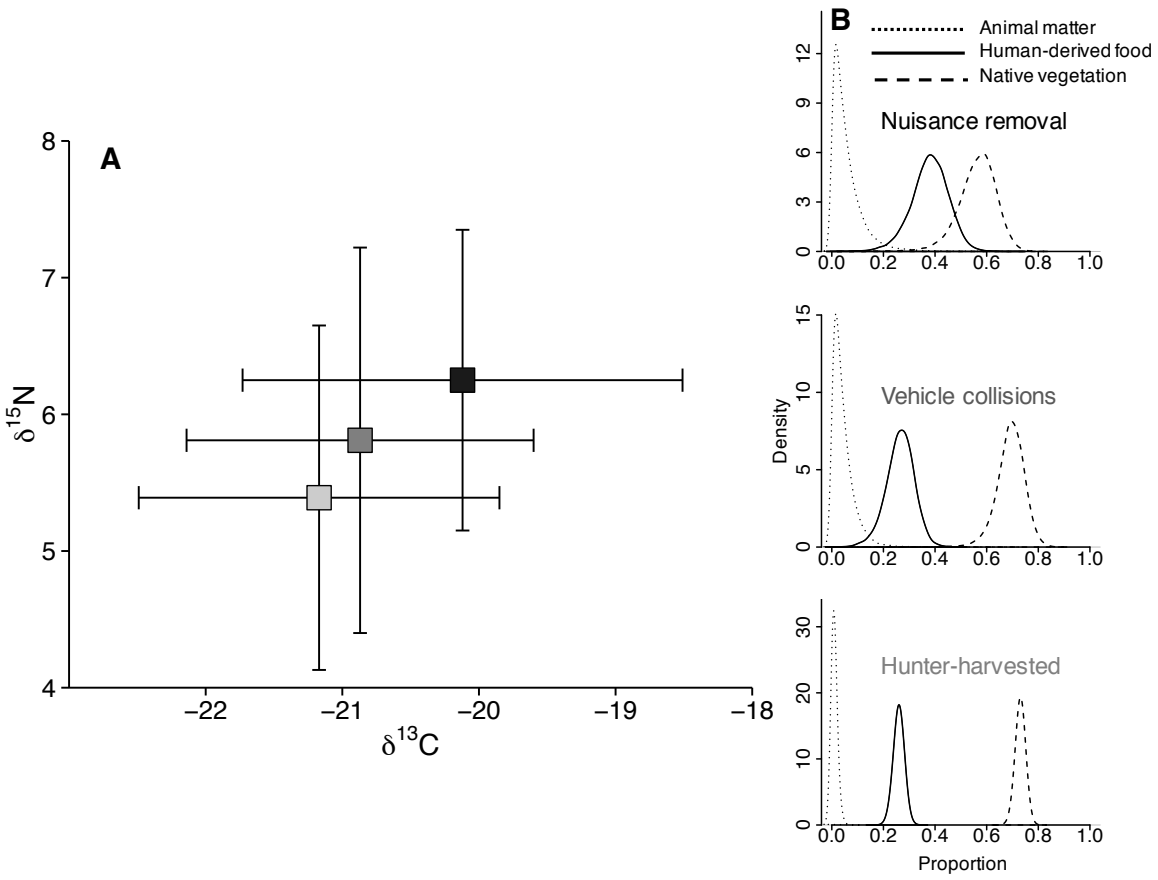
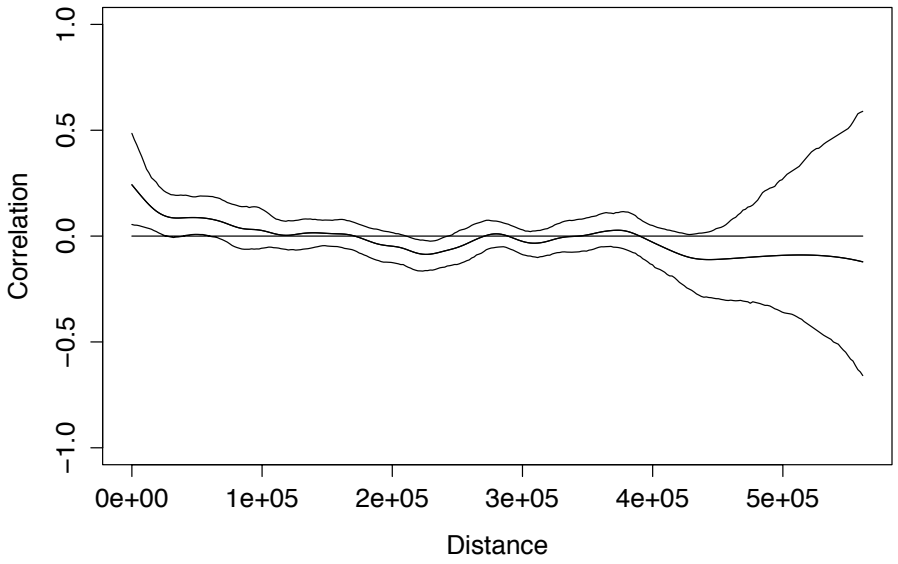

A
B
Animal matter
Human-derived food
Native vegetation
Nuisance removal
Vehicle collisions
Hunter-harvested
Density
Proportion
δ15N
δ13C

Table A1. Stable isotope signatures of potential diet sources collected throughout Colorado in 2012, grouped by region and broad diet category. Different superscripts denote significance of K nearest-neighbor randomization at $P < 0.05$.

		δ^{13}C (‰)		δ^{15}N (‰)	
Diet Groups	*n*	Mean	SD	Mean	SD
Northeast	*139*				
Berries	40	-27.60	1.63	0.64	2.48
Herbaceous	36	-28.84	1.18	-0.08	2.96
Insects	13	-23.32	0.94	3.46	2.69
Ungulates	39	-24.10	0.68	4.35	1.45
Rabbits	11	-25.26	1.30	2.15	2.57
Northwest	*75*				
Berries	28	-27.18	1.40	2.12	1.98
Hard Mast	1	-26.70		3.95	
Herbaceous	37	-27.46	1.33	0.91	2.54
Insects	7	-23.27	0.60	4.35	1.89
Ungulates	2	-24.66	1.36	4.13	0.01
Southeast	*60*				
Berries	11	-26.37	0.86	2.29	2.54
Herbaceous	38	-27.82	1.58	1.61	1.92
Insects	9	-22.56	1.43	5.37	1.36
Rabbits	2	-23.02	0.82	6.42	1.45
Southwest	*129*				
Berries	27	-26.45	1.34	1.98	2.05
Hard Mast	12	-26.83	1.35	2.75	2.87
Herbaceous	57	-26.93	1.43	0.29	1.49
Insects	14	-22.95	1.48	5.54	1.38
Ungulates	15	-24.03	0.50	5.01	1.13
Rabbits	4	-24.40	0.96	3.18	1.53

		δ^{13}C (‰)		δ^{15}N(‰)	
Diet Groups	Region	Mean	SD	Mean	SD
Animal matter	NE[a]	-24.17	1.03	3.71	2.05
(insects, ungulates,	NW[ab]	-23.58	0.94	4.30	1.64
rabbits)	SE[b]	-22.64	1.32	5.56	1.37
	SW[ab]	-23.62	1.20	5.01	1.45
Native vegetation	NE[a]	-28.19	1.56	0.30	2.72
(berries, hard mast,	NW[ab]	-27.33	1.35	1.47	2.38
herbaceous plants)	SE[ab]	-27.49	1.57	1.76	2.07
	SW[b]	-26.78	1.40	1.07	2.09

Figure A1. Spline correlogram run with 1,000 permutations on $\delta^{13}C$ global model residuals, testing for spatial autocorrelation (bear harvest locations), shown with 95% confidence intervals. Though some correlation was detected at the smaller distances, it was not sufficient to warrant additional analyses (Bjørnstad and Falck, 2001).



Environmental, not individual, factors drive biological aging in black bears

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Abstract

Aging negatively affects individual survival and reproduction; consequently, characterizing the factors behind aging can enhance our understanding of fitness in wild populations. The drivers of biological age are diverse, but often related to factors like chronological age or sex of the individual. Recently, however, environmental factors have been shown to strongly influence biological age. To explore the relative importance of these influences on biological aging in a free-ranging and long-lived vertebrate, we quantified the length of telomeres—highly conserved DNA sequences that cap the ends of eukaryotic chromosomes and a useful molecular marker of biological age—for black bears sampled throughout Colorado, and measured a variety of environmental variables (habitat productivity, human development, latitude, elevation) and individual characteristics (age, sex, body size, genetic relatedness). Our extensive sampling of bears (n = 245) revealed no relationships between telomere length and any individual characteristics. Instead, we found a broad-scale latitudinal pattern in telomere length, with bears in northern Colorado possessing shorter telomeres. Our results suggest that environmental characteristics overwhelm individual ones in determining biological aging for this large carnivore.

Keywords: Biological aging, landscape variation, stress, telomere, *Ursus americanus*

Introduction

Age-related differences in fitness can influence the dynamics of populations. Older individuals tend to experience reduced physical stamina, cognitive function, and immunocompetence (Cichoń et al. 2003; Punzo and Chavez 2003). Such aspects of senescence are exhibited by a diversity of wild animals (Nussey et al. 2013), ranging from fruit flies (Mackenzie et al. 2011) to elephants (Robinson et al. 2012). Consequently, older animals typically exhibit decreased reproductive (Broussard et al. 2003) and survival rates (Bryant and Reznick 2004). Thus, understanding such age-related differences in individual condition can be important for developing conservation and management strategies (Tarlow and Blumstein 2007). Tools to estimate individual condition and predict survival in wild populations are diverse, including field techniques using body condition indices (Stevenson and Woods 2006), physiological measures of stress hormones (Bonier et al. 2009), and genetic markers like MHC (e.g. Bonneaud et al. 2004). Such approaches are limited though, because they either capture relatively brief periods of an individual's life, or a very limited aspect of condition. Telomeres, however, have emerged as a molecular marker to quantify biological age (Aydos et al. 2005; Houben et al. 2008; Monaghan 2010a; Pauliny et al. 2006; Young et al. 2015), and consequently capture accumulated life stress (Finkel and Holbrook 2000), which can provide a broader insight into individual condition and fitness.

Telomeres are repetitive and highly conserved DNA sequences $(T_2AG_3)_n$ (Monaghan and Haussmann 2006; Meyne et al. 1989) that cap the ends of eukaryotic chromosomes, providing chromosomal stability and an elegant solution to the "end replication problem" (Watson 1972). Telomeric repeats are lost during cellular replication, and attrition increases due to DNA damage, particularly oxidative damage (Epel et al. 2004; Kotrschal et al. 2007; von Zglinicki

2002). Telomerase, a reverse transcriptase, counteracts this degradation in the germline, but is far less active in somatic cells, likely evolved to be a barrier against developing cancer-causing "immortal cells" (Gomes et al. 2011). Consequently, telomeres tend to shorten with cellular replication and organismal age (Haussmann et al. 2003; Pauli et al. 2011).

However, in most species telomere length is still highly variable within age groups (Monaghan and Haussmann 2006). Besides chronological age, individual characteristics can drive telomere dynamics (Benetos et al. 2011). For example, the sex of an individual often explains some of this variation due to differing life histories; among mammals, females tend to have longer telomeres, potentially due to ameliorating effects of estrogen on telomere attrition (Barrett and Richardson 2011; Olsson et al. 2011). Telomere length is also partially heritable, though the strength of its heritability varies across species (Horn et al. 2011). Variation in telomere length can sometimes be attributed to body size (Ringsby et al. 2015; Scott et al. 2006)—larger animals tend to have shorter telomere lengths, which has been attributed to lower telomerase activity (Seluanov et al. 2007). In addition to the effects of such individual characteristics, chronic life stressors can lead to increased oxidative stress (Patel et al. 2002), resulting in shorter telomeres (Angelier et al. 2013; Cassidy et al. 2010; Shi et al. 2007) and amplified cellular aging (Buffenstein et al. 2008). Much of our research and understanding of telomere dynamics have been focused on these characteristics inherent to an individual, regardless of its environment.

Increasingly, though, research is identifying environmental factors as relevant in driving telomere dynamics; factors such as habitat and forage quality (Angelier et al. 2013; Mizutani et al. 2013; Young et al. 2013, 2015), as well as behavioral correlates like hibernation (Turbill et al. 2012, 2013) and social status (Lewin et al. 2015). Habitat quality and associated behaviors can

modify individual stress, resulting in changes in telomeres and individual condition or fitness (e.g. Angelier et al. 2013; Young et al. 2015). Research that concurrently examines both individual and environmental drivers of biological aging is currently uncommon, but can provide insight into the relative importance of each to fitness and aging.

To better understand how individual and environmental characteristics influence chronic stress and biological aging in a wild and long-lived vertebrate, we quantified relative telomere length (RTL) in American black bear (*Ursus americanus*) endothelial tissues sampled throughout Colorado. We examined telomere length in relation to chronological age, and other individual characteristics such as sex and body size. We further examined environmental characteristics of each sample location to ascertain relative influences on telomere length. Bears are large-bodied hibernators that have evolved to survive with seasonal resource extremes, and are sufficiently long-lived to show evidence of reproductive senescence (Schwartz et al. 2003). Increased time spent in torpor has recently been shown to slow biological aging (as measured by telomeres) in rodents using daily torpor or seasonal hibernation (Turbill et al. 2012, 2013). However, it is unknown whether large hibernators will respond similarly. Like small hibernators, black bears also demonstrate increased oxidative stress as part of metabolic depression (Chauhan et al. 2002), but suppress their metabolic rate independent of body temperature (Tøien et al. 2011). Additionally, bears show strong individual differences in daily activity and heart rate, indicating idiosyncratic behavioral and physiological strategies to hibernation (Laske et al. 2011). Bears also exhibit strong demographic differences with females providing all parental care. As opportunistic omnivores, black bears have plastic foraging strategies (Jacoby et al. 1999; Robbins et al. 2004), and food availability is the primary predictive factor for their behavior, particularly denning chronology and reproduction (Baldwin and Bender 2010; Costello et al.

2003; Hilderbrand et al. 1999; Noyce and Garshelis 1994; Rogers 1987). Across Colorado, black bears experience varying conditions of habitat quality, and previous work found bear diet correlated with aspects of human development (Kirby et al. 2016).

We hypothesized that telomere length should reflect not only characteristics unique to individual bears, but also be influenced by environmental conditions. We predicted that age and sex would strongly influence telomere lengths, like most mammals, with older and male bears having shorter telomere lengths. Further, we predicted that environmental characteristics, particularly those related to habitat and hibernation would also influence biological age. Specifically, bears with access to better habitat and food should be under less stress, and thus have relatively longer telomeres. Additionally, if the consequences of hibernation in bears are similar to those of small hibernators (Turbill et al. 2013), bears with longer and deeper hibernation bouts (presumably at higher elevations and latitudes) should experience attenuated telomere attrition. Because multiple factors may affect biological aging in black bears, we further evaluated the relative influences of each of these individual and environmental characteristics.

Materials and methods

Sample preparation

We opportunistically sampled guard hairs with intact follicles from hunter-harvested black bears (n = 245) throughout the state of Colorado during fall hunting season in 2011. Given that humans are the single most important cause of adult mortality in bears (from harvest, nuisance removal, and vehicle collisions, Baldwin and Bender 2009; Hebblewhite et al. 2003) our cross-sectional analysis of telomere length should not overrepresent age-specific telomere lengths and provides the strength of a broad-scale spatial analysis to capture potentially relevant environmental variables. Collected samples were stored at -20°C until we extracted DNA with standard procedures (QIAGEN DNeasy Blood and Tissue Extraction Kit; QIAGEN Inc., Valencia, CA). DNA concentration was determined with Qubit 2.0 Fluorometer (Life Technologies) and DNA quality assessed using gel-electrophoresis.

Quantitative PCR assay

Primer optimization

We quantified relative length of telomeres using real-time quantitative polymerase chain reaction (qPCR) (Cawthon 2002). This approach has been found to be highly accurate, in particular for within species comparison (Cawthon 2002; Nakagawa et al. 2004). Although relative telomere length estimates from qPCR quantify both terminal and interstitial telomere repeats, other studies have shown them to be robust and highly correlated with mean telomere length as estimated using terminal restriction fragment analysis (Bize et al. 2009). The method determines relative telomere length by comparing the ratio of telomere repeat copy number (T) to single copy gene number (S) in a particular DNA sample. Relative differences in telomere length between

individuals then, is exhibited by contrasting the T/S ratio of one individual to that of another (RTL). Any reliably amplified single copy (or non-variable copy) gene sequence can be employed for standardization (Olsen et al. 2012). We performed conventional PCRs on each primer set to assess amplification via gel electrophoresis, and then performed a series of qPCR reactions to test primer concentrations, annealing temperatures and template DNA concentrations. We tested three single copy gene primer sets previously applied in multiple taxa: 36B4 (Callicott and Womack 2006), albumin (Cawthon 2009), beta-globin (Cawthon 2009), and three primer sets specifically used in black bears: GADPH (Gilbert et al. 2007), IRBP (Yu et al. 2004), and HNRPF (Fedorov et al. 2009). We also tested both sets of telomere primers developed by Cawthon (2002, 2009). To select the best single copy gene for this study, we assessed melting curves and correlations between each primer pair, as suggested by Smith et al. (2011). Although albumin and HNRPF were correlated and both exhibited appropriate single-peak melting curves, the most consistently and reliably amplified single copy primer pairs were for HNRPF: HNRPF-f (CAAAGCCACAGAGAACGACA) and HNRPF-r (ACCCGTCACTCTTCCATCAG). The telomere primers developed by Cawthon (2009), telg (ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT) and telc (TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA), generate a short, fixed length product, and also showed reduced variability within sample replicates. These primer sets were used for all analyses, and are hereafter referred to as “telomere” and “single copy.”

qPCR reaction conditions

Telomere and single-copy gene PCR were conducted on separate 96-well plates, with identical preparation except for primers. Immediately prior to reaction setup, samples were diluted to 3

ng/µl. Each reaction then contained 8 µl sample DNA, 10 µl SYBR Select Master Mix (Life Technologies - Applied Biosystems), telomere primers (250 nM each final concentration) or single copy gene primers (500 nM each final concentration), and distilled water to total 20 µl reaction volume. Samples were analyzed in triplicate within a plate and the average used in subsequent statistical analyses (each set of telomere and single copy plates here is referred to as a "batch"). Real-time PCR was conducted with an Eppendorf Mastercycler ep realplex, with the following thermocycling conditions: 50°C for 2 min, 95°C for 5 min, followed by 2 cycles of 94°C for 15 sec and 49°C for 15 sec, and then 35 cycles of 95°C 15 sec, 62°C 10 sec, 74°C 15 sec (telomere) or 95°C 15 sec, 62°C 15 sec, 72°C 45 sec (single copy); both protocols ended with a melting curve from 60°C to 95°C with a resolution of 0.5°C.

Quantitative methods

We initially examined amplification curves visually in the Eppendorf Mastercycler ep realplex software, and then performed baseline correction on raw fluorescence data in the program LinRegPCR (Ruijter et al. 2009) using its automatic strict baseline correction. After baseline correction, we quantified telomere and single copy genes using three methods from Pfaffl (2001), Ruijter et al. (2009), and comparative Cq (Olsen et al. 2012). We ran 76 samples in triplicate within plate and across 2-3 separate batches (coefficient of variations for T: within-plate = 13%, between-plate = 19%; S: within-plate = 11%, between-plate = 9%). We found mean RTL (T/S) was highly correlated regardless of method (>0.8), but the lowest coefficient of variation for RTL sample estimates was from comparative Cq (13%, as opposed to 20%), and we therefore proceeded using comparative Cq with all subsequent analyses. Samples within a batch were excluded or rerun if their efficiency fell 2.5% outside the mean. All batch mean efficiencies

for telomere as well as single copy gene reactions ranged from 1.79-1.81 (as calculated within LinRegPCR), similar to Olsen et al. (2012); batches that exhibited means outside this range were rerun. Mean RTL for each sample were used in subsequent analyses.

Predictors of telomere length

We examined how potential variables could influence relative telomere length in black bears. Specifically, we considered two groups of variables: individual and environmental. Individual variables measured included age, sex, body size, and heritability. Teeth (first premolar) from each carcass were used to determine age by counting the cementum annuli (Matson's Lab, Milltown, MT), and sex and body size (approximated by zygomatic width) were determined at time of sampling. Hunters provided GPS locations used to extract environmental characteristics (Fig. 1).

Although a single panmictic population was determined using 8 hyper-variable microsatellites during a previous project conducted in Colorado (Alldredge et al. 2008), we also explored genetic structure within our sampled individuals. To that end, we genotyped a subset of bear samples, stratified by latitude (n = 100), at 4 previously described bear-specific microsatellite loci (G1A, G1D, G10C, G10L; Paetkau and Strobeck 1994). Unlabeled reverse primers and fluorescent-labeled forward primers were obtained from Integrated DNA Technologies or Life Technologies- Applied Biosystems. All reactions were carried out in singleplex according to protocols in Brown et al. (2009) before combined into panels and submitted for fragment analysis at UW Biotechnology using a 3730xl DNA Analyzer (Applied Biosystems). Alleles were scored using GeneMapper v.4.1, and PCR was repeated for any sample or marker that produced an ambiguous genotype. We used program STRUCTURE v.2.3

to estimate whether there were genetically distinct populations in the samples across Colorado (Pritchard et al. 2000). We ran STRUCTURE for K = 1-10 populations and evaluated each value of K using the log-likelihood of the data given K[lnPr(X|K)]. We used 10,000 "burn-in" iterations followed by 50,000 iterations for analyses.

We characterized environmental characteristics of bear locations via measures of both vegetative productivity and human development. Though bear home ranges can vary widely, Colorado bears typically range less than 50 km^2 (Baldwin 2008; Baruch-Mordo et al. 2014). Thus, we buffered each bear harvest location by approximately 50 km^2 (a radius of 4 km) to analyze environmental variables and elevation, which we calculated in ArcGIS (ESRI, v.10). We considered mean growing season Normalized Difference Vegetation Index (NDVI) (Wiegand et al. 2008) in 2011, with higher NDVI values representing greater primary productivity. We took monthly composites (0.1 degrees) collected by Terra/MODIS (NASA Earth Observations) and averaged them across the growing season (April – October) in ArcGIS (ESRI, v.10). We extracted mean growing season NDVI of each buffered bear location using Geospatial Modelling Environment (GME, v. 0.7.2.1). Within each buffer, we considered human development indexed by housing density from 2010 Block Level Housing Density (Radeloff et al. 2010). We also considered latitude and longitude in UTMs of each bear location, and calculated elevation from the National Elevation Dataset (USGS 2009).

Analyses were conducted in R package v 3.1.1. We first explored effects of continuous variables on telomere length with simple linear regression analyses. We tested for differences in telomere length between age and sex classes using Welch's 2-sample t-test, and explored relationships among covariates with Pearson correlations. Housing density was log-transformed to meet assumptions of normality. We excluded highly correlated variables (≥ 0.6), or those that

increased a variance inflation factor > 2, from further analyses. We then considered a suite of linear models with RTL as the response variable, and compared all possible combinations of individual and environmental covariates. Because latitude and elevation were slightly correlated with vegetative productivity and human development (< 0.6, but significant at $P < 0.01$), they were examined in separate models. We considered only individuals for which we had complete data on all variables (n =195) and used Akaike's Information Criterion to select the best models.

Results

Colorado black bears exhibited wide variation in relative telomere lengths (RTL); hunter-harvested bears aged 1 – 21 (152 males and 93 females) averaged 3.43 (range: 1.28 – 6.99). Of the individual variables investigated, we did not detect any relationship with telomere length, including age ($F_{1,217} = 0.39$, $P = 0.54$), age class ($t_{219} = 0.57$, $P = 0.57$), zygomatic width ($F_{1,223} = 0.59$, $P = 0.44$), or sex ($t_{186} = 0.49$, $P = 0.63$; Fig. 2a). Heritability was also unrelated to telomere length—genetic structure analysis for the subset of sampled bears indicates that the most likely number of genetic populations was $K = 1$, confirming little or no genetic structure for black bears throughout the state (see Online Appendix Table 1A for microsatellite descriptive statistics).

In contrast, telomere length of black bears exhibited patterns with several environmental variables. First, telomere lengths declined with increasing latitudes ($F_{1,193} = 21.87$, $P < 0.0001$); bears harvested in northern Colorado tended to have shorter telomere lengths (Fig. 2b). Second, telomere length declined in bears harvested in areas with higher vegetative productivity ($F_{1,193} = 9.45$, $P = 0.002$). Telomere length did not, however, exhibit a relationship with elevation ($F_{1,193} = 0.24$, $P = 0.62$) or housing density ($F_{1,193} = 0.31$, $P = 0.58$) (Fig. 2b).

Model selection comparing individual and environmental influences on telomere length revealed that environmental variables had the strongest relationship with telomere length. The top models all included latitude as an influential covariate (Table 1). Bears harvested in northern Colorado had shorter relative telomere lengths than those harvested in southern Colorado regardless of individual characteristics.

Discussion

Our results suggest that individual factors do not strongly influence biological aging in Colorado black bears. Instead, the emergent patterns we detected were latitudinal: latitude of bear harvest was negatively correlated with telomere length. We suggest that this pattern reflects differences in important environmental conditions that are overwhelming potential relationships of individual variables to biological aging.

Though initial research into telomeres suggested they shorten with cellular replication (and chronological age), an increasing number of studies have illustrated that telomere length is not always an effective marker of chronological age (Dunshea et al. 2011; Horn et al. 2010; Monaghan 2010b; Ujvari and Madsen 2009). In black bears, we found a slight negative, but nonsignificant, decline in telomere length with age. Furthermore, we found that size, as measured by zygomatic width, was also unrelated to telomere length. As zygomatic width was correlated with chronological age, an alternative size measure such as body mass might have yielded more information. Levels of telomerase activity tend to scale with body mass in rodents (Seluanov et al. 2007), but whether this pattern holds in bear somatic cells is unknown. Further, as black bears fluctuate in mass, gaining and losing over 20% of their weight seasonally as part of their hibernation phenology (McLellan 2011), biological age would more likely reflect lifetime changes in body condition, rather than a single timepoint measure. Finally, we found no sex-specific differences in telomere length in our population. In most mammals, females have higher telomerase activity thought to be due to estrogen (Leri et al. 2000), as well as lower adult mortality (Liker and Szekely 2005). Though sex can influence telomere dynamics, no single theory yet explains the complex relationship among telomere length, sex, and survival (Barrett

and Richardson 2011). In black bears then, sex does not appear to be influential on biological aging.

Instead, we found that telomere lengths were influenced by broad-scale environmental variables. First, vegetative productivity, as predicted by NDVI, was negatively correlated with telomere length, suggesting that bears in areas of greater natural food abundance had shorter telomere lengths and greater chronic stress. At first glance, this pattern seems counterintuitive. However, NDVI may not adequately capture natural food availability, as it could miss important bear foods such as berries (Wiegand et al. 2008), or alternatively over-represent conifers. Further, natural food may not be the only important measure of habitat quality, but rather food availability in general. We recently showed that diet of Colorado black bears varies directly with human influence (Kirby et al. 2016); bears consume more human-derived foods in areas of higher housing density, as well as forage at a higher trophic level. Though we found no relationship between telomeres and human development in this study, the relationship between food availability and telomere length is likely more complex than measuring only vegetative productivity.

The strongest correlation we found was that bears at higher latitudes have shorter telomere lengths, suggesting they are biologically older, or under more chronic stress than bears living in southern Colorado. Though telomeres are heritable, we can discount this as simply a consequence of heritability because bears exhibited no genetic structure throughout Colorado. Northern Colorado is also associated with higher NDVI, as well as cooler temperatures and higher precipitation, hence both of these geographical and environmental variables measured may be capturing similar patterns. At this scale, location explains the most variation in telomere length.

If telomere length accurately reflects underlying stress, bears experiencing less stress throughout their lives should have longer telomeres. As food availability drives much of bear body condition and reproduction, as well as hibernation length, we suspect that these latitudinal patterns are linked to the complex influences of habitat quality. In particular, hibernation has been linked to increased annual survival and longevity across a diverse assemblage of species (Lyman et al. 1981; Melvin and Andrews 2009; Turbill et al. 2011; Wilkinson and South 2002), and has recently been shown to slow telomere attrition in rodents (Turbill et al. 2012, 2013). If large-bodied hibernators respond similarly to rodents, hibernation length should decelerate bear telomere attrition. Unfortunately, this study does not have a direct measure of hibernation length. However, as habitat quality and food availability determine denning chronology (Johnson and Pelton 1980), bears with better access to food tend to hibernate for shorter periods (Baldwin and Bender 2010; Bridges et al. 2004) and, consequently they might exhibit amplified cellular aging, despite possible trade-offs with enhanced body condition and fecundity.

Specifically, bears residing at higher latitudes in Colorado, and higher NDVI, likely have access to more food, and thus may hibernate for shorter periods of time, reflected in shorter telomeres and accelerated biological aging. A further complication to this story, however, is human activity—black bears that overwinter near urban areas can also exhibit shorter denning periods, presumably due to supplemental human food (Baldwin and Bender 2009; Beckmann and Berger 2003). As we did not find a significant relationship between human development and telomere length in this study, the relative contribution of overall food availability to bear stress and hibernation length remains unknown.

Our pattern-based cross-sectional analysis suggests that emergent environmental properties are driving telomere length in black bears. Though determining the mechanism behind

biological aging from this data set is not possible, this latitudinal pattern is strongly suggestive that extrinsic environmental conditions, rather than simply individual characteristics, drive biological aging in black bears. We attribute these results to bear habitat parameters, likely food availability and hibernation. Further work should incorporate survival and telomere dynamics of individuals at a fine scale to investigate the particular influences of habitat conditions.

Acknowledgements

Funding for this project was provided by Colorado Parks and Wildlife and an American Society of Mammalogists Grant-in-Aid. We thank all of the Colorado wildlife managers and bear hunters for generously collecting and providing samples. We are also grateful to Cristina Vaughan, Garrett Johnson, and Samantha Paddock for laboratory help.

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Table 1. Top models to predict relative telomere length (RTL). Covariates included individual variables (age, sex) and environmental variables (latitude, elevation, growing season NDVI, log-transformed housing density), comparing highly correlated variables separately. Elevation, NDVI, and housing density were estimated for a 50 km^2 buffer around each bear harvest location. Models were ranked using AIC (only < 2 ΔAIC are shown).

	AIC	Δ AIC	weight	Adj. R^2
$-2.97*10^{-6}$(Latitude)[a] + 16.29	12.09	0.00	0.32	0.10
-0.02(Age) + $-3.04*10^{-6}$(Latitude)[a] + 16.73	12.48	0.38	0.27	0.10
0.07(Male) + $-3.00*10^{-6}$(Latitude)[a] + 16.37	13.91	1.82	0.13	0.09
$-3.00*10^{-6}$(Latitude)[a] + $-5.2*10^{-5}$(Elevation) + 16.38	14.02	1.92	0.12	0.09

[a]significant at $P < 0.001$

Figure Legends

Figure 1. Sample locations of black bears (*n* = 195) harvested in 2011 throughout Colorado (GPS coordinates provided by hunters). Shown with elevation (lighter = higher elevation).

Figure 2. Relationship of relative telomere length (RTL) with potential influences on biological aging in Colorado black bears: **(a)** intrinsic characteristics **(b)** environmental characteristics. Regressions shown for significant relationships.

Figure 1.

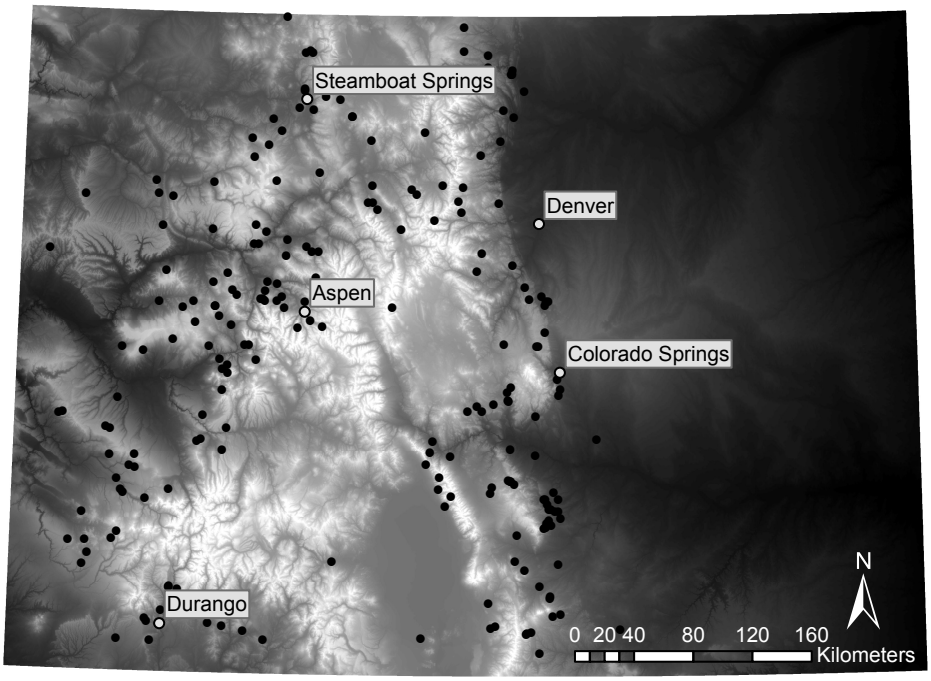
Steamboat Springs
Denver
Aspen
Colorado Springs
Durango
N
0 20 40 80 120 160
Kilometers

Figure 2.

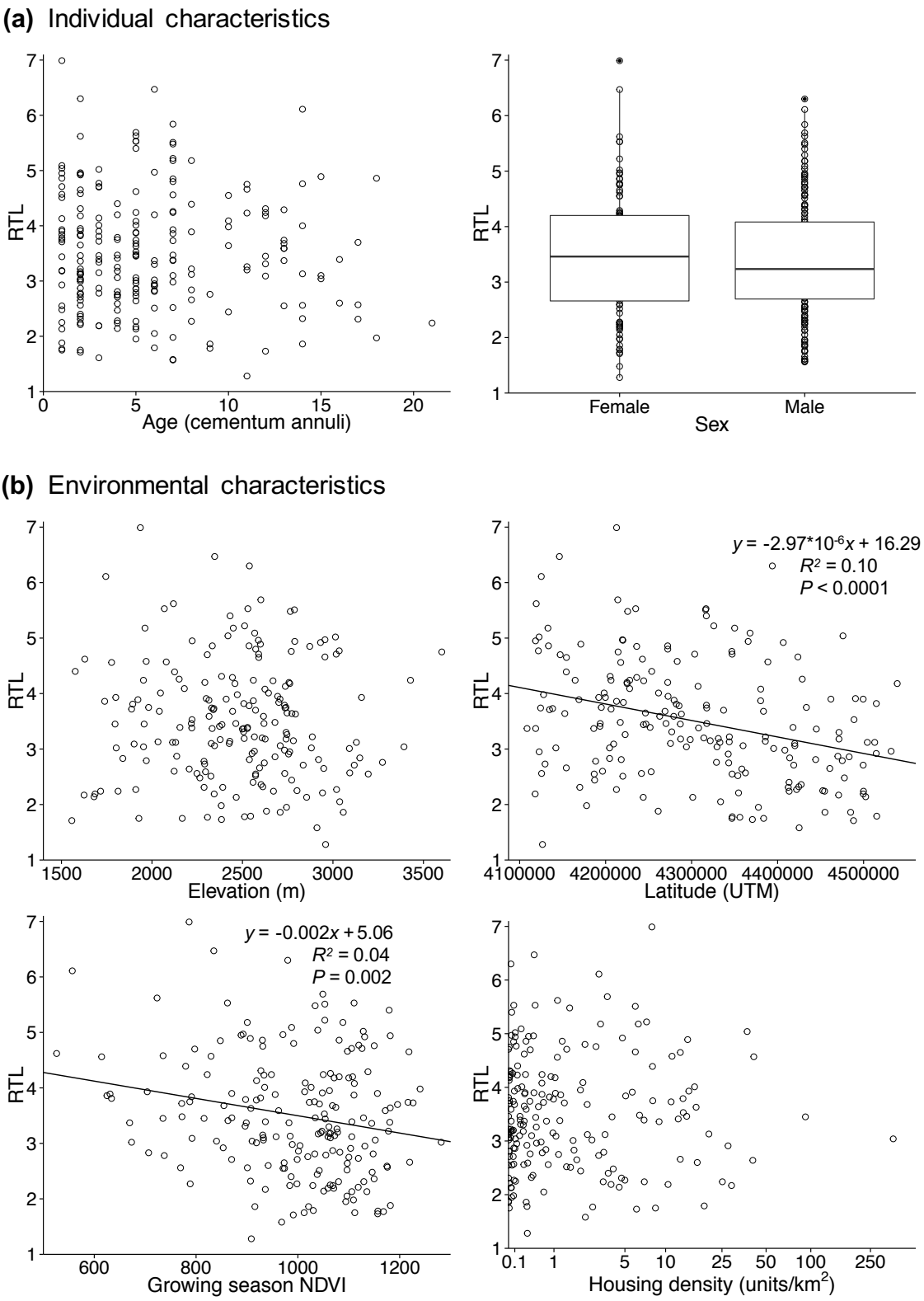
(a) Individual characteristics
RTL
Age (cementum annuli)
Female
Male
Sex
(b) Environmental characteristics
Elevation (m)
y = -2.97*10-6x + 16.29
R2 = 0.10
P < 0.0001
Latitude (UTM)
y = -0.002x + 5.06
R2 = 0.04
P = 0.002
Growing season NDVI
Housing density (units/km2)

Table A1. Descriptive statistics (allelic richness, expected and observed heterozygosity) for microsatellites by loci and population of black bears sampled latitudinally throughout Colorado bear range (n = 100). Loci described in Paetkau and Strobeck 1994.

	Alleles	H_e	H_o
G1A	7	0.53	0.55
G1D	6	0.76	0.72
G10C	3	0.31	0.33
G10L	11	0.81	0.76
Population mean	6.75	0.60	0.59

Unintended effects of intentional food subsidies for a behaviorally plastic carnivore

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Summary

1. Animal consumption of human foods has become commonplace, and these subsidies can dramatically alter a species' behavior, population growth, interspecific interactions, and even enhance conflicts with humans. Intentional food subsidies, including the feeding or baiting of wildlife for viewing or hunting, can represent a large caloric resource, yet are rarely studied.

2. We explored consumption of intentionally deployed bait for an American black bear (*Ursus americanus*) population in northern Wisconsin. Given the state's permissive baiting policy, we hypothesized that bear baits would be highly available, and both harvested and unharvested bears would become habituated to such baits. We documented the abundance of bear bait on forestlands, and quantified the assimilated diet of black bears using stable isotopes and Bayesian mixing models to determine the relative contribution of human foods to individual and population diet.

3. Baits occurred at $\geq 0.25/km^2$ on public lands, and bears ($n = 180$) were heavily subsidized by these baits, with human foods contributing to >40% of their diet. Reliance on subsidies was found not only in the year of harvest, but also in preceding years. Patterns of bait consumption were primarily influenced by age-sex class – adult males were the most reliant on human foods followed by adult females.

4. *Synthesis and applications.* We found a remarkably high level of food subsidization in this black bear population. We posit that the regionally high density of bears in northern Wisconsin may be due in part to this reliance on subsidies. Our results reveal how intentionally deployed baits used for hunting can unintentionally become one of the most important dietary resources for free-ranging wildlife, and highlight the importance of considering unexpected consequences when supplemental feeding is used for management.

Keywords: bait, black bear, diet composition, foraging, management policy, stable isotopes, supplemental feeding, *Ursus americanus*

Introduction

Food subsidies from human sources are a near-global phenomenon (Oro *et al.* 2013). The high availability and predictability of calorically-rich subsidies can affect almost every aspect of an animal's biology: altering behavior (Yirga *et al.* 2012; Newsome *et al.* 2015), shifting space use (Newsome *et al.* 2013), enhancing survival (Oro *et al.* 2008), increasing population densities (Fedriani, Fuller & Sauvajot 2001), changing interspecific interactions (Rodewald, Kearns & Shustack 2011), and even structuring communities (Newsome *et al.* 2014b). Human foods are predominantly available to wildlife unintentionally, in the form of garbage and agriculture, but the effects of deliberately deployed food subsidies have been less examined.

Intentional subsidies have been used to support declining or endangered populations (supplemental feeding, Ewen et al. 2014), reduce conflict with humans (diversionary feeding, Kubasiewicz et al. 2016), or to enhance hunting or viewing of wildlife (Maljković & Côté 2011). In relatively intact landscapes, intentional subsidies principally take the form of baiting, a common method to increase success and selectivity of hunting game species (Putnam & Staines 2004). In the U.S., an estimated 2.8×10^{12} tonnes of bait is provided each year for such hunting (Oro *et al.* 2013). Balancing hunter success and satisfaction with management goals is a challenge faced by natural resource agencies (Milner *et al.* 2014). As baiting in excess can enhance reproduction (Ballari *et al.* 2015), increase human-wildlife conflicts (Steyaert *et al.* 2014), and spillover to non-target species (Bowman *et al.* 2015), understanding how a population responds to bait can be crucial to conservation and management.

Bears (*Ursus spp.*) are opportunistic omnivores that use human foods when available (Merkle *et al.* 2013; Kirby, Alldredge & Pauli 2016). Reliance on subsidies, predominantly from garbage and crops, tends to increase in years of low natural food production (Baruch-Mordo *et*

al. 2014; Johnson *et al.* 2015), which can buffer populations by enhancing fecundity, but can also lead to increased conflict with humans and elevated adult mortality (Beckmann & Lackey 2008). American black bears (*Ursus americanus*) are hunted in 30 U.S. states, 12 of which allow baiting as the primary hunting method (see Table S1 in Supporting Information). Typically, baits are high-caloric foods, which may include meat or fish, but often high-sugar foods, such as cookies, donuts, and candies. Bear baits are deployed on both private and public lands on average 23 days prior to the hunting season, and in seven states hunters are limited in the number of baits they can deploy and maintain. Wisconsin is unique, however, in its extended 145 days of baiting prior to the 35-day hunting season, from mid-April through early October, which spans the entire active period for bears. Also, the state restricts bait to non-animal products. The Wisconsin Department of Natural Resources (WDNR) estimates >1 million gallons of bait deployed throughout the state annually (Rees, Dhuey & MacFarland 2014). In addition to bait piles intended for bears, shelled corn for deer baiting (Bowman et al. 2015) is also accessible to bears in the fall, but how much bears rely on either form of bait is unknown. It has been suggested that the high availability of subsidies could be affecting Wisconsin's bear population (Johnson 2007). Indeed, in northern Wisconsin, the bear population is large ($\widehat{N}$ = 20,400); it has grown on average 3.4% annually since 1988, leveling off in the last few years, and currently features bear densities twice that compared to northeastern Minnesota or the Upper Peninsula of Michigan (Fig. 1), both of which have more restrictive baiting laws.

Given the duration and scale of baiting in Wisconsin, we hypothesized that bears rely largely on bait, and that bait is consumed by both harvested and un-harvested bears, ultimately supporting the high population density. To quantify the importance of bait, we estimated the density of baiting on public lands and used stable isotope analysis of tissues from black bears in

northern Wisconsin collected during the 2011-2013 hunting seasons to determine the extent to which the bear population relies on bait, as well as temporal variations in bear diet. Our results reveal the importance of bait to individual bear diet and some of the unintended effects of provisioning a highly flexible omnivore with intentionally deployed human food resources.

Materials and methods

Food availability

We restricted our sampling to management units in northern Wisconsin that are primarily forested with minimal crop cover (<5% crop cover from National Landcover Database, NLCD 2006) and principally national forest lands (Fig. 1B). Within public lands, we opportunistically sampled native bear foods monthly from May-September 2012 for isotopic analyses. Native forage in northern Wisconsin included known bear foods (Noyce, Kannowski & Riggs 1997; Payne *et al.* 1998) grouped broadly into vegetation (herbaceous plants, grasses/sedges, berries, acorns, $n = 122$) and animal matter (ants, deer, $n = 34$). We also sampled bear bait throughout the forest ($n = 27$). We estimated available bait in late August 2012, one week prior to the start of the bear hunting season, by searching fifteen 5 km forest road transects for bait stations. Transects were designated by overlaying a 2.5×2.5 km grid onto forest lands, randomly selecting 15 grids, and a point within each grid as the starting locations for the transects. We drove and walked each transect searching for well-worn foot trails. Although we designed our study area to minimize crop cover, we also accounted for the amount of agricultural corn that bears consumed by examining 2012 USDA Census of Agriculture field crop data for the 6 counties surrounding the core sampling area (Ashland, Bayfield, Iron, Price, Rusk, Sawyer), combined with corn damage reports from the Wisconsin Department of Natural Resources. Because deer bait is not deployed until mid-September, it was not included in our sampling.

Black bear diet reconstruction

We opportunistically sampled hair, blood, and bone from hunter-harvested black bears at hunting registration stations in northern Wisconsin during the fall hunting seasons of 2011 ($n = 11$), 2012

(*n* = 129), and 2013 (*n* = 40). We recorded sex of the individual at the time of sampling, and determined age from counts of cementum annuli (Matson's Lab, Milltown, MT). Because corn and cane-sugar dominated foods (i.e. human foods) are enriched in ^{13}C relative to C_3 native plant base (Jahren *et al.* 2006; Jahren & Kraft 2008), stable isotope analysis has been previously used to reconstruct human food contributions to bear diets (Hopkins *et al.* 2014; Kirby, Alldredge & Pauli 2016). Hair growth in black bears occurs from the spring into fall, and its isotopic signature is representative of assimilated diet (Hilderbrand *et al.* 1996; Jacoby *et al.* 1999). Whole blood represents recent bear diet, 1-2 months, whereas bone assimilates diet throughout the lifetime of the bear (Hilderbrand *et al.* 1996). Though there is some overlap in the time periods captured by each tissue, we refer to spring/summer, fall, and lifetime for hair (*n* = 159), blood (*n* = 158), and bone (*n* = 43), respectively.

Bear hair and animal prey samples were rinsed three times with 2:1 chloroform:methanol solution to remove surface oils, homogenized with surgical scissors, and dried for 72 hours at 56°C (Pauli *et al.* 2009). Whole bear blood samples, vegetation and bear bait were dried at 56°C for a minimum of 72 hours and homogenized with a spatula or in a ball mill (Mixer Mill MM200, Restch Inc. Newton, PA, USA). We extracted collagen from rib bone by decalcifying samples with HCl and extracting lipids with 2:1 chloroform:methanol (Ambrose 1990). We weighed all samples (>50% in duplicate) into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis at the University of Wyoming's Stable Isotope Facility using a Costech 4010 and Carlo Erba 1110 Elemental Analyzer (Costech, Valencia, CA) attached to a Thermo Finnigan Delta Plus XP Continuous Flow Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA); bone collagen was analyzed at University of New Mexico's Center for Stable Isotopes.

Results are provided as per mil (‰) ratios relative to the international standards of Vienna Peedee Belemnite for C and atmospheric N_2 for N, with calibrated internal laboratory standards.

We tested the isotopic difference for biologically distinct diet groups of 1) native vegetation; 2) animal matter; 3) non-corn bear bait; and 4) corn (Fig. 2A and Table S2, corn signatures from Ditmer et al. 2016) with a K nearest-neighbor randomization test (KNN; Rosing et al. 1998). We estimated proportional importance of each forage group with Bayesian mixing models in Stable Isotope Analysis in R package (SIAR; Parnell et al. 2008). Because samples did not differ isotopically between years (MANOVA, Wilk's $\lambda = 0.99$, $P = 0.41$), we pooled them together. To test age and sex based differences in bait use in bears, we compared three groups: adult male, adult female, and subadults (1-2 year olds). We ran two sets of mixing models to estimate bear diet for each tissue type. In the first set, we used an informative prior probability for animal matter estimated from previous studies in Minnesota and Wisconsin (Payne *et al.* 1998; Ditmer *et al.* 2016). Black bears in the upper Midwest consume minimal animal matter, so we used a conservatively high prior estimate of 10% (with a standard deviation of 0.05), and kept the remaining priors flat (uniformly distributed across the three groups). In the second model set, we used uninformative priors (uniform across all groups) to explore the potential influence of our prior probability on dietary estimates. All models included trophic discrimination and concentration dependence (mean digestible elemental concentrations for each diet group; Hopkins and Ferguson 2012, Table S3). We applied hair and blood discrimination factors developed for omnivorous mammals (Kurle *et al.* 2014). As discrimination of bone collagen is similar to hair (Hilderbrand *et al.* 1996), bone samples were corrected to hair by adding 0.7 $\delta^{13}C$ and 0.3 $\delta^{15}N$ (Hopkins et al. 2014), and analyzed within the hair mixing space. Data are expressed as medians of the probability density functions with 95% credible intervals, which

represent each forage group's likely contribution to bear diet (Parnell *et al.* 2010). We present all three tissue types, but we focus on spring/summer diet because hair is the most commonly examined tissue in isotopic bear studies and the largest sample size in this study.

Results

Subsidy availability

We encountered active bear bait stations during the first week of July, 2012 on forest service lands, though we found remnant stations as early as May. Northern Wisconsin public lands have a high road density of 0.98 km/km^2. During the peak of baiting just prior to the hunting season, we encountered 42 bait stations along transects, 19 of which were filled at the time. This translates to 0.25 active (0.75 total) bait stations per km^2 (or linear km of forest road). In Wisconsin, the average female bear home range is 17 km^2, and the average male home range is 85 km^2 (Johnson 2007; Sadeghpour & Ginnett 2011). Within public lands, then, the average female bear theoretically encountered 4-13 baits and the average male would have access to 21-63 baits.

Our study area was selected to avoid confounding results with agriculturally-derived foods. Agricultural corn in 2012 constituted only 0.67% of the landcover within the 6 counties of north-central Wisconsin surrounding our study area. Bear damage reports confirmed that only 0.7% of that crop (or 0.85 km^2) was damaged by bears in 2012, suggesting minimal agricultural corn consumption in our study area. Consequently, we assume that all corn in the diet was likely bait placed for bear, or possibly deer, and refer to it as “corn bait”.

Black bear diet

Potential forage groups for bears were isotopically distinct, and bear signatures varied throughout the mixing space (Fig. 2A). Spring/summer diet of black bears as a population consisted of just over half native foods (44% vegetation, 13% animal matter) and somewhat less of human foods (29% non-corn bear bait, 13% corn bait) (Table 1). Hair samples from adult

males were significantly enriched in ^{13}C and ^{15}N compared to adult females or subadults (MANOVA, Wilk's $\lambda = 0.78$ $P < 0.001$, Table S4), which translated to this group consuming the most human subsidies. Although consumption of non-corn bear bait by adult males was similar (31% of diet) compared to adult females and subadults, adult males consumed more corn bait (23% of diet) (Fig. 2B). Adult females also consumed more corn bait than subadults. Adult males consumed slightly more animal matter than other age-sex classes, and substantially less native vegetation during the spring/summer (Fig. 2B and Table S5). Both model sets yielded similar median estimates for population diet, with the informed model primarily reducing variation around the estimates (95% CI), particularly for non-corn bear bait and animal matter. When comparing age-sex classes, the informative model median estimate for adult males did have slightly higher estimates of non-corn bear bait and lower estimates of animal matter than the uninformed model; however, the credible intervals substantially overlapped (Table S5).

Tissue samples were more similar within individuals than across tissue types (RM-MANOVA, Pillai's trace, $F_{4,168} = 0.88$, $P < 0.001$), indicating any changes in diet were consistent across individuals, and that an individual enriched in ^{13}C and ^{15}N in current seasons was more likely to be enriched throughout its lifetime. Indeed, diet changed little across time periods analyzed, with consistent high consumption of human foods regardless of time period (Table 1), suggesting that bears consume subsidies in years prior to their harvest. The forage group with the greatest seasonal variation was animal matter, which showed increased consumption in the fall (for all age-sex classes), though there was substantial overlap with other seasons.

Discussion

We found black bears in northern Wisconsin are heavily subsidized by intentionally deployed human foods. Over 40% of bear diet consisted of subsidies in the form of bait – both non-corn bear bait, but also corn bait likely intended for bear or deer. Bait is highly available on the landscape throughout active bear season; we found minimally one bait site every four square kilometers. In other populations, human foods including trash, bird feed, fruit trees, and agricultural crops (Merkle *et al.* 2013) contributed to bear diet. However, our study area is primarily composed of public forestlands with minimal agricultural corn, so such unintentional subsidies should be negligible. As the purpose of baiting is to habituate bears for harvest (Bischof *et al.* 2008), it is not surprising that harvested bears consumed bait. However, the substantial use of subsidies by this population is notable, and even greater than the historic Yosemite population widely regarded as one of the most highly food-conditioned black bear populations in North America (35% of bear diet was from human foods, Hopkins et al. 2014). Our findings also reveal that these food subsidies are being consumed throughout the lifetime of a bear, not just immediately prior to being harvested.

Surprisingly, we did not detect strong inter-annual or inter-seasonal diet differences within this bear population. Previous work in Wisconsin similarly found that bears did not alter their bait visitations with changes in natural food phenology (Johnson 2007). This finding was unexpected as bears inhabiting developed landscapes tend to increase use of developed areas according to physiological demands for food (e.g. hyperphagia, natural food shortage years, Baruch-Mordo et al. 2014; Johnson et al. 2015), and we expected to find a similar increased consumption of bait in the fall. It seems likely that the lack of seasonal difference could have been reinforced by our isotopic method of diet reconstruction. We parameterized each seasonal

model with the same diet sources (animal matter, native vegetation, non-corn bear bait, and corn bait). Bears, however, tend to have more access to animal matter (e.g. ants, deer fawns) in the spring and early summer and some studies exclude animal matter as a potential source from fall models (Ditmer *et al.* 2016). A fall model excluding animal matter would yield a higher estimate of non-corn bear bait consumption (>80%). However, although we used tissue-specific discrimination factors, there is temporal overlap in foraging periods in hair and blood samples. Recent work in polar and brown bears found that whole blood integrates over a longer period of time (>6 months) than was previously demonstrated in black bears (Rode *et al.* 2016). Thus, due to the overlap between tissue types, our conservative approach maintained the potential diet sources in all seasonal models. Future work could incorporate segmenting hair samples to provide a finer timescale analysis of diet changes within the same tissue type.

Nevertheless, we found less variability within each individual than among tissue types, suggesting that some bears specialize more on food subsidies than others. As seen in other populations (Merkle *et al.* 2013; Ditmer *et al.* 2016), age and sex of the animals primarily drive these differences with adults and males tending to consume the most subsidies. Adult males typically access the best food sources (Beckmann & Berger 2003a), and are also more likely to try unfamiliar diet items. Interestingly, after exposure to novel foods, females will also preferentially seek out such food sources (Ditmer, Burk & Garshelis 2015). Most of the demographic differences in diet contributions appear to be derived from corn bait, rather than non-corn bear bait. These adult males may be using deer bait sites of shelled corn, or this corn bait signature may also come from additional bear bait sites not represented in the sampled isotopic signature. As deer baiting is not permitted until mid-September, already ten days into the bear hunting season and our sampling period, bear consumption of deer bait should be minimal

prior to sample collection, however, could be important in lifetime consumption. Thus, we suspect our sampling of bear baits did not fully capture some of the bait items that are more highly derived from corn (such as candies), and would be more similar to our corn bait signature (Jahren *et al.* 2006).

Bears are using human foods extensively in northern Wisconsin, and this use may be contributing to Wisconsin's high density of bears compared to neighboring states. As female consumption of high caloric food subsidies can augment population size (Graber 1982; Beckmann & Berger 2003b; Beckmann & Lackey 2008) and bias cub foraging towards subsidies (Mazur & Seher 2008), regular long-term supplementation can increase a population above its natural carrying capacity (Gray, Vaughan & Mcmullin 2004; Kavčič *et al.* 2015) and create a population reliant on food subsidies (Gunther *et al.* 2004; Robbins, Schwartz & Felicetti 2004). In northern Wisconsin, humans are influencing the ecosystem not only through top-down forces via hunting (Dorresteijn *et al.* 2015) but also through bottom-up forces by subsidizing the food base (Newsome *et al.* 2014a). Baiting certainly increases hunter success – Wisconsin has about a 50% harvest success rate (Rolley *et al.* 2015). However, the regular subsidization of the un-harvested animals may be artificially bolstering the bear population above its natural carrying capacity. Our findings add to the growing recognition of the substantive role that food subsidies can play in structuring communities (Oro *et al.* 2013). Consequently, this work emphasizes the need to identify potential unintended, and sometimes contradictory, effects on a population from conservation and management strategies that feature human subsidies.

Acknowledgements

Funding was provided by Wisconsin Department of Natural Resources, the American Society of Mammalogists, and the Department of Forest and Wildlife Ecology at the University of Wisconsin-Madison. We thank Sonia Petty, Samantha Paddock, Christina Vaughan, Garrett Johnson, Jenna Carlson, and Phil Manlick for laboratory and field assistance, and Seth Newsome for helpful discussions. We especially thank the many student volunteers and bear hunters for collecting and providing samples.

Supporting Information

Table S1. Bear baiting regulations.

Table S2. Mixing model parameters.

Table S3. Estimating digestible concentrations of bear bait.

Table S4. Mean isotopic signatures of age-sex classes.

Table S5. Assimilated dietary estimates for age-sex classes of bears across seasons.

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Table 1. Assimilated dietary estimates for black bears across spring/summer, fall, and lifetimes, estimated from isotopic signatures of hair, blood, and bone, respectively, using Bayesian mixing models. Informed model is parameterized with informative animal matter prior of 0.1 (± 0.05) estimated from previous bear diet studies. Uninformed model is parameterized with uniform priors.

Diet group	Population Median (95% CI)	
Time period	Uninformed model	Informed model
Native vegetation		
Spring/summer	0.44 (0.28-0.58)	0.44 (0.37-0.50)
Fall	0.30 (0.21-0.38)	0.29 (0.24-0.34)
Lifetime	0.43 (0.29-0.57)	0.42 (0.34-0.49)
Animal matter		
Spring/summer	0.13 (0.03-0.22)	0.13 (0.09-0.18)
Fall	0.31 (0.15-0.45)	0.26 (0.18-0.34)
Lifetime	0.10 (0.01-0.18)	0.09 (0.04-0.14)
Bear bait (non-corn)		
Spring/summer	0.30 (0.00-0.63)	0.29 (0.16-0.44)
Fall	0.24 (0.00-0.52)	0.30 (0.16-0.44)
Lifetime	0.28 (0.00-0.54)	0.30 (0.17-0.44)
Corn bait		
Spring/summer	0.13 (0.04-0.20)	0.13 (0.09-0.17)
Fall	0.15 (0.08-0.22)	0.14 (0.10-0.18)
Lifetime	0.20 (0.12-0.27)	0.19 (0.15-0.24)

Figure Legends

Figure 1. A) Regional bear density estimates for core black bear range in northeastern Minnesota, the Upper Peninsula of Michigan, and northern Wisconsin. B) Study area with public lands: national forests in dark grey, state/county/other public lands in light grey. Primary sampling area (deer management units) is outlined in black and the maximum sampling area (county limits) is outlined in grey. C) Northern Wisconsin black bear population size estimates and harvest numbers from 1988-2014 (Rolley *et al.* 2015).

Figure 2. A) Isotopic signatures of potential forage groups and black bear hair samples. Diet groups include native vegetation, animal matter, bear bait (non-corn), and corn bait. B) Assimilated dietary estimates shown as proportional density distributions for summer/fall bear diet, and grouped by age-sex class (informed model).

Figure 1.

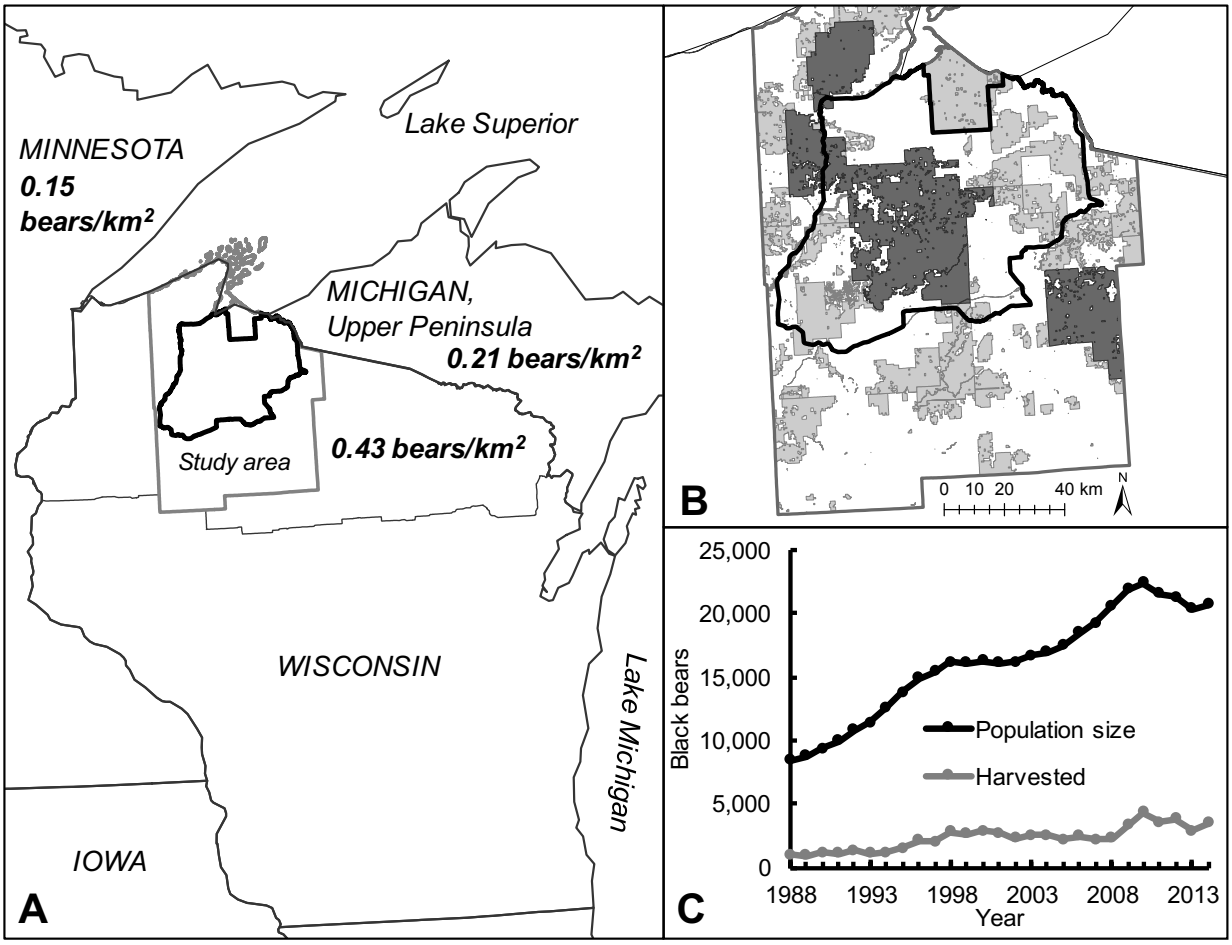
MINNESOTA
0.15
bears/km²
Lake Superior
MICHIGAN,
Upper Peninsula
0.21 bears/km²
Study area
0.43 bears/km²
WISCONSIN
Lake Michigan
IOWA
A
B
0 10 20 40 km
N
C
Black bears
25,000
20,000
15,000
10,000
5,000
0
Population size
Harvested
1988
1993
1998
2003
2008
2013
Year

Figure 2.

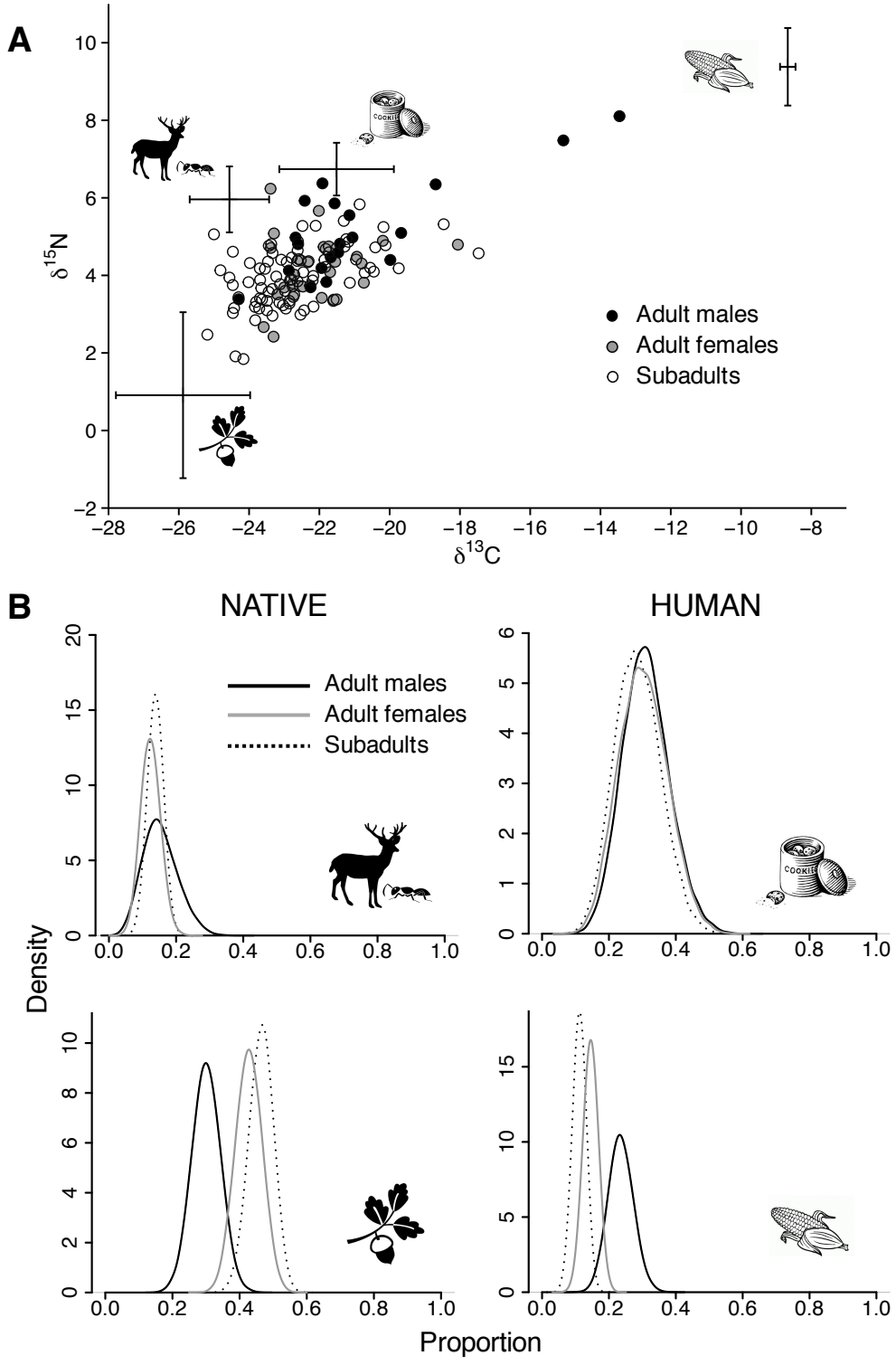
A
δ15N
δ13C
Adult males
Adult females
Subadults
B
NATIVE
HUMAN
Density
Proportion
Adult males
Adult females
Subadults

Table S1. Baiting regulations for black bear hunting in the United States. Data summarized from compilation by Wisconsin Department of Natural Resources.

State	Days prior to season	Quantity per hunter	Volume restrictions	Content restrictions	Registration required	Spring Season	Fall Season
Alaska	0	2	None	Biodegradable products only	Yes	Yes	Yes
Arkansas	30	Unlimited	None	None	No	No	Yes
Idaho	7	3	None	No parts of game animals or fish	Yes	Yes	Yes
Maine	30	Unlimited	None	Natural or agricultural products	Yes	No	Yes
Michigan	31	3	Unrestricted on meat, fish, confectionary items; up to 2 gallons of grains and 2 gallons of all other materials	Advised to avoid chocolate	No	No	Yes
Minnesota	17	3	None	Readily biodegradable; only less than 25% intact mammal carcass; no swine except cured pork	Yes	No	Yes
New Hampshire	0	2	None	Chocolate/cocoa not permitted	Yes	No	Yes
New Jersey	0	Unlimited	None	None	No	No	Yes
North Carolina	0	Unlimited	None	Processed foods prohibited	No	No	Yes
Utah	14	2	None	No game fish/protected wildlife species	Yes	Yes	Yes
Wisconsin	145	Unlimited	10 gallons	No animal parts or by-products; advise against chocolate	No	No	Yes
Wyoming	7	2	8 ft^3; 55 gallon drum	Processed food restrictions in certain areas	Yes	Yes	Yes

State	Regulation Link
Alaska	http://www.adfg.alaska.gov/index.cfm?adfg=bearbaiting.requirements https://www.adfg.alaska.gov/static/regulations/wildliferegulations/pdfs/bear.pdf
Arkansas	http://www.agfc.com/resources/GuidebookDocs/hunt_p34-50.pdf
Idaho	https://fishandgame.idaho.gov/public/docs/rules/bgBear.pdf
Maine	http://www.eregulations.com/maine/hunting/bear-hunting/
Michigan	http://www.michigan.gov/documents/dnr/Bear_Hunting_Digest_454168_7.pdf
Minnesota	http://files.dnr.state.mn.us/recreation/hunting/bear/bear_regs.pdf
New Hampshire	http://www.eregulations.com/newhampshire/hunting/general-hunting-regulations/ http://www.eregulations.com/newhampshire/hunting/bear-hunting/
New Jersey	http://www.eregulations.com/newjersey/hunting/bear-hunting/ ; http://njfishandwildlife.com/pdf/2015/dighnt15.pdf
North Carolina	http://www.ncwildlife.org/Portals/0/Regs/Documents/Hunting-Regulations.pdf
Utah	http://wildlife.utah.gov/guidebooks/2015_pdfs/2015_bear.pdf
Wisconsin	http://dnr.wi.gov/files/pdf/pubs/wm/wm0197.pdf
Wyoming	https://wgfd.wyo.gov/Regulations/Regulation-PDFs/REGULATIONS_CH3_BROCHURE

Table S2. Mixing model parameters for diet groups: mean isotopic value, discrimination factors, and digestible concentrations (standard deviations).

Diet group	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Discrimination factors (hair, bone) Δ C, Δ N	Discrimination factors (blood) Δ C, Δ N	Digestible concentrations C, N
Native vegetation	*122*	-29.28 (1.90)	-1.49 (0.13)	3.4 (0.2), 2.4 (0.2)	1.3 (0.1), 1.9 (0.3)	0.47 (0.03), 0.04 (0.03)
Animal matter	*34*	-26.66 (1.13)	2.06 (0.80)	2.1 (0.1), 3.9 (0.3)	0.6 (0.1), 3.0 (0.3)	0.52 (0.01), 0.12 (0.02)
Bear bait (non-corn)	*27*	-25.61 (1.60)	3.94 (0.65)	4.1 (0.3), 2.8 (0.2)	2.5 (0.3), 2.4 (0.2)	0.51 (0.05), 0.01 (0.01)
Corn bait	*24*	-12.07 (0.08)	6.98 (0.98)	3.4 (0.2), 2.4 (0.2)	1.3 (0.1), 1.9 (0.3)	0.46 (0.00), 0.02 (0.00)

Table S3. Digestible concentrations of N and C for inclusions in mixing models calculated from most frequently encountered dietary items in bear bait. Data from USDA National Nutritional Database (NDB).

Bait item	NDB #	Energy kcal	Water	Prot.	Lip.	Carb.	Ash	Prot.	Lip.	Carb.	Ash	Prot. N	Prot. C	Lip. C	Carb. C	Dig. Prot. C	Digest [C]	Digest [N]
			gm / 100 g wet weight					decimal % dry weight								gm / 100 gm dry weight		
Corn flakes	08020	357	3.76	7.50	0.40	84.10	4.00	0.08	0.00	0.88	0.04	0.01	0.04	0.00	0.39	0.04	0.43	0.01
Chex mix	19033	424	2.64	8.83	10.00	74.77	3.75	0.09	0.10	0.77	0.04	0.01	0.05	0.08	0.35	0.04	0.47	0.01
Granola	08218	412	3.39	9.66	10.38	74.67	1.90	0.10	0.11	0.77	0.02	0.02	0.05	0.08	0.35	0.05	0.48	0.01
Honey roasted nuts	12206	594	1.70	18.17	49.90	27.90	2.33	0.18	0.51	0.28	0.02	0.03	0.10	0.38	0.13	0.09	0.59	0.03
Jelly donuts	18256	340	35.60	5.90	18.70	39.00	0.80	0.09	0.29	0.61	0.01	0.01	0.05	0.22	0.27	0.04	0.53	0.01
Cake donuts	18250	426	19.60	5.20	22.90	50.80	1.50	0.06	0.28	0.63	0.02	0.01	0.03	0.21	0.28	0.03	0.53	0.01
Waffle cones	28156	424	2.00	6.50	5.30	87.70	0.00	0.07	0.05	0.88	0.00	0.01	0.03	0.04	0.40	0.03	0.47	0.01
Chocolate chip cookies	28058	517	2.80	5.50	28.30	62.70	0.00	0.06	0.29	0.65	0.00	0.01	0.03	0.22	0.29	0.03	0.54	0.01
Sugar cookies	18205	436	14.08	4.00	19.48	61.22	1.20	0.05	0.23	0.71	0.01	0.01	0.02	0.17	0.32	0.02	0.51	0.01
Chocolate candy	19081	507	0.50	3.90	34.20	60.40	1.00	0.04	0.34	0.61	0.01	0.01	0.02	0.26	0.27	0.02	0.55	0.01
																Mean	**0.51**	**0.01**
																SD	**0.05**	**0.01**

Equations used from Hopkins and Ferguson 2012

Macronutrient dry weight (Robbins 1993):
Protein N = Protein (% dry weight) x 0.16
Protein C = Protein (% dry weight) x 0.52
Lipid C = Lipid (% dry weight) x 0.75
Carbohydrate C = Carbohydrate (% dry weight) x 0.45

Assume 100% digestibility for all sources for Lipid C and Carbohydrate C
Assume 90% for plant foods (Koch and Phillips 2002)
Digest Protein C (nuts, cereals, pastries, candies) = Protein C x 0.9
Digest [C] = Digest Protein C + Lipid C + Carb C
Digest [N] (nuts, cereals, pastries, candies) = Protein N x 0.9

Table S4. Mean isotopic signatures of black bear tissues, separated by age-sex class.

Tissue type			$\delta^{13}C$ (‰)		$\delta^{15}N$(‰)	
	Age-sex class	*n*	Mean	SD	Mean	SD
Hair						
	Adult male	*20*	-20.90	2.59	5.15	1.23
	Adult female	*30*	-22.08	1.16	4.14	0.81
	Subadult male	*51*	-22.50	1.49	4.01	0.88
	Subadult female	*31*	-22.95	1.33	3.93	0.57
Blood						
	Adult male	*20*	-23.21	1.73	5.17	0.94
	Adult female	*30*	-23.66	1.35	4.40	0.88
	Subadult male	*51*	-23.80	1.67	4.37	0.80
	Subadult female	*31*	-24.39	1.04	4.00	0.59
Bone						
	Adult male	*5*	-19.75	1.22	5.59	1.42
	Adult female	*15*	-20.65	1.39	4.35	0.89
	Subadult male	*14*	-20.31	1.97	4.33	0.99
	Subadult female	*8*	-21.63	1.43	4.08	0.83

Adult includes individuals 3+ years of age
Subadult includes individuals 1-2 years

Table S5. Assimilated dietary estimates for black bear age-sex classes across spring/summer, fall, and lifetime, estimated from isotopic signatures of hair, blood, and bone, respectively, using Bayesian mixing models. Uninformed model is parameterized with uniform priors, informed model parameterized with informative animal matter prior of 0.1 (± 0.05) estimated from previous bear diet studies.

Diet group	Adult males		Adult females		Subadults	
Time period	Uninformed model	Informed model	Uninformed model	Informed model	Uninformed model	Informed model
Native vegetation						
Spring/summer	0.30 (0.17-0.32)	0.30 (0.22-0.38)	0.42 (0.28-0.58)	0.43 (0.35-0.50)	0.42 (0.28-0.60)	0.47 (0.39-0.53)
Fall	0.09 (0.00-0.22)	0.23 (0.14-0.32)	0.30 (0.19-0.41)	0.31 (0.24-0.38)	0.33 (0.23-0.43)	0.33 (0.28-0.38)
Lifetime	0.26 (0.04-0.44)	0.31 (0.20-0.42)	0.43 (0.28-0.58)	0.41 (0.33-0.50)	0.44 (0.30-0.59)	0.43 (0.35-0.51)
Animal matter						
Spring/summer	0.25 (0.07-0.42)	0.15 (0.06-0.26)	0.13 (0.03-0.23)	0.12 (0.07-0.18)	0.12 (0.04-0.21)	0.14 (0.09-0.18)
Fall	0.41 (0.15-0.70)	0.24 (0.12-0.36)	0.30 (0.12-0.46)	0.22 (0.13-0.32)	0.26 (0.12-0.40)	0.24 (0.17-0.31)
Lifetime	0.25 (0.02-0.46)	0.12 (0.03-0.24)	0.10 (0.01-0.20)	0.09 (0.03-0.15)	0.09 (0.01-0.18)	0.08 (0.03-0.14)
Bear bait (non-corn)						
Spring/summer	0.23 (0.00-0.47)	0.31 (0.17-0.45)	0.31 (0.00-0.60)	0.30 (0.16-0.44)	0.37 (0.04-0.66)	0.28 (0.16-0.42)
Fall	0.33 (0.00-0.65)	0.32 (0.19-0.48)	0.24 (0.00-0.54)	0.30 (0.17-0.45)	0.27 (0.00-0.57)	0.30 (0.16-0.43)
Lifetime	0.25 (0.00-0.49)	0.30 (0.17-0.44)	0.28 (0.00-0.54)	0.30 (0.17-0.44)	0.28 (0.00-0.28)	0.30 (0.16-0.44)
Corn bait						
Spring/summer	0.22 (0.12-0.32)	0.23 (0.16-0.31)	0.13 (0.05-0.22)	0.15 (0.10-0.19)	0.08 (0.01-0.17)	0.11 (0.07-0.15)
Fall	0.15 (0.05-0.25)	0.20 (0.14-0.27)	0.16 (0.07-0.23)	0.16 (0.11-0.20)	0.13 (0.05-0.20)	0.13 (0.09-0.17)
Lifetime	0.25 (0.13-0.35)	0.25 (0.18-0.34)	0.19 (0.10-0.27)	0.19 (0.14-0.25)	0.18 (0.08-0.27)	0.19 (0.13-0.24)

The tension between foraging and hibernation shapes biological aging in bears

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Summary

1. Senescence, or biological aging, results from physiological declines associated with aging, but also from accumulated life stress that is mediated by individual and environmental conditions. Certain activities, such as hibernation, appear to counteract the aging process. Hibernation characteristics, however, are driven by local foraging and individual physiological condition.

2. We investigated how foraging and hibernation interact to affect biological aging and stress in a large-bodied hibernator, black bears (*Ursus americanus*). We quantified relative telomere length, a molecular marker for biological age, and hypothesized that longer hibernation would decelerate telomere attrition and decrease stress, but hibernation length would shorten with increased food availability.

3. We compared 30 adult female bears longitudinally sampled 2-5 times during summer or winter. For each sample, we examined oxidative stress, diet, and hibernation length with simple and linear mixed effects models. We found that oxidative stress was not reflected in telomere length, but instead was mediated predominantly by season and breeding status – bears were under increased oxidative stress during hibernation and decreased stress when they had cubs.

4. Telomere length change, an index of biological aging, was primarily related to age class and hibernation length, with older bears and longer hibernators on average showing decelerated biological aging. In addition, bears that foraged more on human food subsidies hibernated for shorter periods of time the following winter. Together, these results suggest that hibernation ameliorates biological aging even among large-bodied hibernators, but that foraging on human food subsidies counteract this by shortening hibernation length. Our findings highlight that in

addition to expected direct effects on behavior and survival, there can be cryptic consequences of global changes at the molecular level, including accelerated biological aging.

Keywords: food subsidies, oxidative stress, senescence, telomeres, *Ursus americanus*

Introduction

Senescence, the reduction in survival or reproduction with age from physiological declines, is found in a range of wild animals across taxa (Nussey *et al.* 2013). These age-related changes can emerge in a variety of traits and species: diminished foraging performance in honey bees (Dukas 2008), reduced immunocompetence in swallows (Palacios *et al.* 2011), litter failures in common lizards (Massot *et al.* 2011), muscle loss in seals (Hindle *et al.* 2009), and decreased predatory success in wolves (MacNulty *et al.* 2009). Physiologists have studied senescence to understand the mechanisms underpinning aging and mortality; ecologists have become increasingly interested in how altered individual fitness can affect population- and community-level dynamics. Recent work suggest that senescence and biological aging can be profoundly influenced by environmental conditions, such as habitat quality (Angelier *et al.* 2013; Mizutani *et al.* 2013), and associated behavior, such as foraging (Young *et al.* 2015), social status (Lewin *et al.* 2015), and even overall individual quality (Le Vaillant, Viblanc & Saraux 2015). Global changes – principally from altered climate and landcover – are creating novel environments, altering abiotic conditions, resource availabilities, and interspecies interactions (Thomas *et al.* 2004). These new and unique conditions have known consequences on animal behavior, including range shifts, altered phenology, decreased activity levels, and increased stress (Ditchkoff, Saalfeld & Gibson 2006; Shochat *et al.* 2006). While these changes can overtly affect survival, they may also have subtle long-term fitness consequences by changing central aspects of biological aging, although these changes are rarely quantified.

Certain life history strategies are associated with increased longevity and reduced biological aging (Promislow & Harvey 1990). One of the more dramatic is hibernation, which has evolved as an adaptive response in birds and mammals that cannot migrate to escape harsh

seasonal environmental conditions (Ruf & Geiser 2015). By lowering body temperatures and extremely reducing metabolic rates, animals incur significant energetic savings from relaxing homeothermy, which has direct implications for longevity and survival (Lyman *et al.* 1982). Recently, increased time spent in daily and seasonal torpor was linked to decelerated biological aging in rodents (Turbill *et al.* 2012, 2013). Hibernation, then, not only conserves energy expenditure, but may also be adaptive in slowing biological aging (Lyman *et al.* 1981). Although the exact mechanism for this is unknown, it appears to be associated with a reduction in cell turnover rates (Koizumi *et al.* 1992). Because the process of metabolic depression itself increases oxidative stress, repeated cycles of metabolic depression coupled with bouts of arousal will induce maladaptive oxidative stress in small mammalian hibernators (Buzadžić *et al.* 1990; Carey, Andrews & Martin 2003). This suggests, then, that hibernating would only be effective in retarding aging if torpor and arousal cycles are minimized within a hibernation season (Turbill *et al.* 2012). Hibernators that either remain dormant for shorter time periods, or arouse more frequently, may counteract the otherwise long-term biological aging benefits of hibernation. As hibernation is modulated primarily by local environmental conditions and behavior (Melvin & Andrews 2009; Baldwin & Bender 2010; Ruf & Geiser 2015), such conditions, especially food availability, could indirectly govern senescence.

To date, the vast majority of our knowledge on hibernation is derived from small-bodied mammals. Whether hibernation similarly affects biological aging in large hibernators, however, is unknown. As large-bodied hibernators, bears are long-lived enough to exhibit senescence (Schwartz *et al.* 2003; Turbill & Ruf 2010), but unlike small hibernators remain near-euthermic during hibernation in spite of their reduced metabolic rate (Tøien *et al.* 2011). Bears generally hibernate for 4-6 months, and denning chronology is generally driven by forage availability –

populations with access to more food tend to enter hibernation later and emerge earlier (Johnson & Pelton 1980; Baldwin & Bender 2010). Furthermore, human-derived food subsidies are now highly available and predictable resources that bears often use to supplement their diet, especially in years of natural food shortages (Johnson *et al.* 2015; Kirby, Alldredge & Pauli 2016), and consequently this availability can also influence denning timing (Beckmann & Berger 2003). In addition to the role of resource availability in structuring hibernation characteristics, bears show individual variation in activity levels, particularly those that experience disturbances and females with cubs (Laske, Garshelis & Iaizzo 2011). Preliminary research suggests that biological aging in black bears may be driven principally by environmental conditions found at different latitudes, such as vegetative productivity, rather than individual characteristics such as age or sex (Kirby et al. in review). We aimed to investigate how these conditions interact with hibernation, to influence biological aging in this long-lived, large-bodied hibernator.

To determine the influences of hibernation and foraging on biological aging, we examined telomeres, repetitive DNA sequences on the ends of eukaryotic chromosomes (Meyne, Ratiliff & Moyzis 1989; Monaghan & Haussmann 2006), that have become a biomarker for biological age (Finkel & Holbrook 2000; Bize *et al.* 2009). Telomeres are lost during cellular replication, but also erode due to oxidative damage (von Zglinicki 2002), so individual and environmental conditions influence telomere dynamics (Houben *et al.* 2008). We quantified relative telomere lengths, rates of telomeric change, and oxidative stress in an American black bear population sampled over several seasons. Black bears show increased activity in exurban areas (Goad *et al.* 2014), and those with access to human supplemental food tend to grow faster and mature earlier (McLean & Pelton 1989). Bears in areas of higher human use then, may have better body condition, but may also hibernate for shorter periods (Beckmann & Berger 2003),

confounding the relative importance on biological aging. Further, as bears experience some form of increased oxidative stress as part of metabolic suppression (Chauhan *et al.* 2002), those that either respond with increased antioxidant capacity or have overall lower lifetime stress would likely exhibit reduced cellular aging. Thus, we predicted that biological aging in black bears is driven by the interaction between food availability and hibernation lengths, and this may be mediated by oxidative stress.

Materials and methods

Sample collection

Black bears were captured near Durango, Colorado from summer 2011 through winter 2015 as part of a larger research project led by Colorado Parks and Wildlife. Adult females were fitted with GPS collars, which were used to relocate each bear in its winter den. Thirty bears were included in this study that were sampled a minimum of twice throughout the study period, twenty-six were sampled ≥ 3 times. Sampling primarily took place first during a summer capture and then was repeated during winter den visits in subsequent years, but only 18 of the bears had samples taken in both the summer and the following winter within the same year.

During capture, bears were immobilized with a combination of butorphanol, azaperone, and medetomidine in the summer (Wolfe, Goshorn & Baruch-Mordo 2008) and Telazol in the winter. Guard hair and blood samples were collected from anesthetized bears for molecular analyses. At first captures, a premolar was removed to determine chronological age by counting cementum annuli (Matson's Lab, Milltown, MT) (Willey 1974) and body condition was scored using the bone prominence method, with a higher score indicating increased body fat and overall condition (Noyce, Coy & Garshelis 2002). Breeding status was also identified by the presence/absence of cubs (or lactation during summer captures when cubs were not visible) or yearlings, and subsequently categorized as "with yearlings," "with cubs," or "without young."

We used the collar acceleration sensor data (Vectronics Globalstar collars) to determine den entry and exit dates for each bear on an annual basis. Acceleration data were summed over the orthogonal axes and averaged over a 60-minute time frame to create hourly activity averages; bears were considered "active" when their activity levels exceeded 22.9 and "inactive" below that threshold (Gervasi, Brunberg & Swenson 2006). We considered the start of the denning

period when a bear was active ≤ 3 hours/day for at least 14 days (Laske *et al.* 2011). Conversely, we considered the end of the denning period to be when a bear was active ≥ 3 hours/day that was sustained for ≥ 14 days. In 11 observations, activity data were not available to estimate denning dates. In those cases, we used hourly GPS locations to define den entry as the first day of a 6-day period when a bear was exclusively located within 135 m of her den, and den emergence as the first day of a 6-day period when a bear remained 135 m away from her den (Waller *et al.* 2012). Hibernation length (in days) was considered to be the period between den entrance and emergence.

Laboratory analyses

Blood samples for DNA extraction were stored in EDTA tubes; those for oxidative stress analyses were kept in serum-separating tubes. All samples were stored at -20°C until analyses. We extracted DNA with standard procedures (QIAGEN DNeasy Blood and Tissue Extraction Kit; QIAGEN Inc., Valencia, CA). DNA concentration was determined with Qubit 2.0 Fluorometer (Life Technologies) and DNA quality assessed using gel-electrophoresis.

We quantified relative telomere lengths using real-time quantitative polymerase chain reaction (qPCR) (Cawthon 2002). This method determines relative telomere lengths by comparing the ratios of telomere repeat copy number (T) to a single copy/non-variable copy gene (S) in a DNA sample. Contrasting the T/S ratio allows the comparison of the relative differences in telomere length between individuals (relative telomere length, RTL). Though any reliably amplified non-variable copy gene can be employed for standardization (Olsen *et al.* 2012), we previously optimized this method using HNRPF gene (Fedorov *et al.* 2009) and telomere primers telg and telc (Cawthon 2009, Kirby et al. in review). Telomere and single-copy gene PCR were

conducted on separate 96-well plates, with identical preparation except for primers (see Kirby et al. in review for details). Each sample was analyzed in triplicate within a plate and the average used in subsequent analyses (coefficient of variations for T: within-plate = 14%, between-plate = 17%; S: within-plate = 8%, between-plate = 9%). Standard curves were generated from a mixture of 6 randomly chosen bear samples run in triplicate on each plate and diluted to 0.5, 1, 2.5, 6, and 10 ng/µl. Real-time PCR was conducted using an Eppendorf Mastercycler ep realplex, followed by baseline correction in LinRegPCR, and sample quantification using the standard curve method (Ruijter *et al.* 2009). We examined relative telomere lengths at first sampling for each bear and calculated the rate of telomere length change (per month) within each individual between sampling periods and throughout the entire study (n = 30).

We measured oxidative stress in bear serum samples, using the d-ROM and the oxy-adsorbent tests (Diacron International, Italy). Although developed for humans, these tests have been validated in multiple wildlife species (Beaulieu *et al.* 2011; Stier *et al.* 2012). The d-ROM test measures oxidative damage via the concentration of hydroperoxide, a reactive oxygen metabolite (ROM) that results from an attack of reactive oxygen species on organic substrates (e.g. nucleotides, proteins). Following the manufacturer's protocol, 1.5 µl of bear serum was mixed with 300 µl of an acidic buffer solution, 3 µl of a chromogenic mixture, and incubated for 90 minutes at 37 °C. In these acidic conditions, iron is released from proteins catalyzing hydroperoxide to generate alkoxyl and peroxyl radicals, which react with the chromogenic mixture to produce a color intensity that is proportional to its concentration and read at 505 nm with a spectrophotometer. The concentration of hydroperoxide (expressed as mg H_2O_2 dl^{-1}) was calculated by comparison with a calibrator solution with an oxidative activity of 0.08 mg dl^{-1} (equivalent to that of H_2O_2). The oxy-adsorbent test measures the total antioxidant capacity of

the sample by measuring the ability of the serum to oppose the massive oxidative action of a hypochlorous acid (HClO) solution. Briefly, serum was first diluted 1:100 with distilled water, 2 µl of the diluted sample was mixed with 200 µl of the oxidant (HClO-based) solution, and incubated at 37 °C for 10 minutes. After incubation, 2 µl of the chromogenic solution was added and the resulting color read with a microplate spectrophotometer at 505 nm, with the color intensity inversely related to the antioxidant capacity, expressed as µmol HClO ml^{-1} neutralized. For each assay, all samples were analyzed in triplicate and the averages were compared to standard solutions. The inter-assay coefficients of variation were 0.10 and 0.06 for d-ROM and oxy-adsorbent tests, respectively.

Hair samples for stable isotope analyses were rinsed three times with 2:1 chloroform:methanol solution, homogenized with surgical scissors, and dried to 72 hours at 56°C (Pauli *et al.* 2009). Samples were then weighed into tin capsules and analyzed at University of New Mexico's Center for Stable isotopes using a Costech 4010 and Carlo Erba 1110 Elemental Analyzer (Costech, Valencia, CA) attached to a Thermo Finnigan Delta Plus XP Continuous Flow Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). Results are provided as per mil (‰) ratios relative to the international standard of Vienna Peedee Belemnite, with calibrated internal laboratory standards. Individual foraging was represented by $\delta^{13}C$ of hair samples; specifically enrichment in $\delta^{13}C$ signifies increased human food in bear diet (Hopkins *et al.* 2014; Kirby *et al.* 2016), and hair samples represent assimilated diet during hair growth from spring through fall (or up until the time of sampling) (Hilderbrand *et al.* 1996).

Data analyses

We compared longitudinally sampled individual adult female bears to explore how hibernation length and foraging on human foods influenced both telomere length change and oxidative stress. We first examined the relationship between foraging and hibernation by restricting our examination to bears sampled in the summer and then the immediately following winter ($n = 15$) using linear regression with hibernation length as the response variable and foraging on human foods as the explanatory variable.

We then considered the relationship between hibernation length and biological aging. We first examined the relationship between either RTL or monthly rate of telomere change and the chronological age or age class (binned as 2-4, 5-9, and ≥ 10 years old) of each individual with linear regression and ANOVA. We tested the hypothesis that hibernation slows telomeric attrition by regressing each bear's overall rate of telomere change on their average hibernation length ($n = 30$).

Finally, we examined how oxidative stress reflects age, season, and breeding status using linear mixed models with individual bear as a random effect. We considered oxidative damage and antioxidant capacity separately, and included only samples for which we had complete data (groups = 28, observations = 84). We compared each suite of additive models using Akaike's Information Criteria (AIC). Furthermore, including only the most recent winter samples ($n = 17$), we compared oxidative stress to the rate of change in telomere length with linear regression.

Results

Black bears ranged from 2 through 24 years old at first sampling. We found that bears that were enriched in $\delta^{13}C$ in the summer were likely to hibernate for a shorter period the following winter (Figure 1a, $F_{1,13} = 11.08$, $P = 0.005$). Bear relative telomere lengths averaged 0.93 (range: 0.57 to 2.36), and we did not detect a relationship between RTL at first sampling and age ($F_{1,28} = 2.31$, $P = 0.14$). Telomere lengths on average decreased at a rate of 0.001/month throughout the study period, but inconsistently, as almost half the bears increased telomere lengths during the study. Rate of telomere length change (per month) throughout the study was associated with the individual's starting telomere length, with longer telomeres shortening more quickly ($F_{1,28} = 16.13$, $P < 0.001$). Though the rate of telomere length change was not significantly related to individual age ($F_{1,28} = 3.19$, $P = 0.08$), it was related to age classes – bears that were 10 years and older on average exhibited telomeres that shortened at a slower rate or, in some cases, lengthened, compared to those less than 5 years old, (Figure 1b, $F_{2,27} = 3.66$, $P = 0.039$, Tukey's HSD $P = 0.033$). Finally, the rate of telomere change was related to mean hibernation lengths. Bears that hibernated longer on average experienced a slower rate of telomere attrition or even experienced telomere lengthening throughout the study (Figure 1c, $F_{1,28} = 4.86$, $P = 0.036$).

The greatest support was for the global model of oxidative damage being driven by age, season, and breeding status, although age was not a significant effect (Table 1). Bears exhibited increased oxidative damage during hibernation compared to the summer, and bears that had cubs and were currently lactating exhibited reduced oxidative damage (Figure 2). Although the top model for antioxidant capacity was also the global model, none of the effects were significant (Table 1). The additional examination of the winter season samples showed that oxidative stress was unrelated to the rate of telomeric change (all $P > 0.25$).

Discussion

We found that for this large-bodied mammal, biological aging is influenced by two opposing yet interacting forces. First, hibernation slows biological aging in black bears, but foraging on human food subsidies is associated with shorter hibernation. Consequently, bears with access to greater food subsidies may hibernate for shorter periods of time, counteracting some of the benefits of hibernation.

Denning chronology is generally based on the energy balance of each individual bear (Schooley *et al.* 1994), which is strongly linked to food availability (Baldwin & Bender 2010), and bears with access to more foods typically den later. Our finding that bears enriched in $\delta^{13}C$ exhibited shortened hibernation the following winter supports the concept that bears near human development den for shorter periods of time primarily as a result of their access to food subsidies (Beckmann & Berger 2003; Baldwin & Bender 2010). Altered behavior from bear reliance on food subsidies, then, is not only negative because it increases their risk of conflict with humans (Kirby *et al.* 2016), but also because it alters their seasonal activity.

Bears display a remarkable suite of adaptations allowing them to remain chiefly immobile during hibernation, yet avoid negative side effects such as bone loss (Fedorov *et al.* 2012) and muscle atrophy (Harlow *et al.* 2001). An additional advantage of hibernation appears to be delayed biological aging – we found longer mean hibernation lengths are associated with reduced rates of telomere shortening, and in some cases, telomere lengthening. This finding corroborates recent work in small hibernators that effectively demonstrated that longer and deeper bouts of torpor slowed biological aging (Turbill *et al.* 2012, 2013). However, the mechanism linking hibernation length to telomeric shortening remains speculative. Certainly the reduction in metabolic rate and cellular turnover may plausibly have a role, although metabolic

rate and telomere attrition do not exhibit a straightforward linear relationship (Turbill *et al.* 2012).

Telomere dynamics were also related to age classes, supporting previous findings that telomeres shorten at a greater rate in younger animals, likely as a cost to growth (Heidinger *et al.* 2012; Turbill *et al.* 2012). Telomeric shortening can be counteracted by high activity levels of the enzyme telomerase, and some animals maintain unusually high levels (Munshi-South & Wilkinson 2010), though it does tend to be less active in somatic cells. Hibernation, though, may be an enhanced form of somatic maintenance (Iaizzo *et al.* 2012; Turbill *et al.* 2013), with additional adaptations that counter aging. Indeed, one bat species exhibits increased telomerase activity during hibernation (Wang, McAllan & He 2011). Future work could compare telomerase activity in black bears between the active and hibernation seasons.

Oxidative damage, however, is a known accelerant of telomere attrition in other species (von Zglinicki 2002; Monaghan, Metcalfe & Torres 2009; Hammers *et al.* 2015). Because metabolic suppression and arousal involves increased oxidative stress, disrupted hibernation could result in increased oxidative stress and accelerated aging (Turbill *et al.* 2013). However, we did not find a relationship between telomere dynamics and biomarkers of oxidative stress. In fact, bears exhibited increased oxidative damage during hibernation compared to the active season (Chauhan *et al.* 2002), but the amount did not vary with hibernation length or telomere change. Some species avoid telomere loss with enhanced antioxidant capacity (Beaulieu *et al.* 2011). However, we did not detect a concurrent increased antioxidant capacity in winter-sampled bears either. According to these stress measures, it appears that hibernation ameliorates biological aging in spite of increased oxidative damage, perhaps due to reduced metabolic rate or enhanced somatic maintenance. However, the variation in sampling day within each season

among individuals may have contributed to the lack of significant relationships. Future work can more directly examine these relationships by comparing stress and telomeres sampled immediately pre- and post-hibernation, ideally combined with heart rate and temperature monitors to ascertain individual differences in hibernation activity levels, rather than simply length (Ditmer *et al.* 2015; Støen *et al.* 2015). Nevertheless, our findings suggest that altered oxidative damage is not the primary mechanism by which hibernation slows biological aging.

In addition to seasonal differences, oxidative damage differed among breeding status – females with cubs showed less damage, which was unexpected. Reproduction, and lactation in particular, is energetically expensive (LeBlanc *et al.* 2001), and resulting oxidative stress is typically regarded as a cost of reproduction (Reichert *et al.* 2014). However, data to support that stress is a tradeoff with reproduction has been equivocal across species, particularly when examined without experimental manipulation of reproductive effort (Metcalfe & Monaghan 2013; Xu *et al.* 2014; Blount *et al.* 2016). Still, we did not find an association between current breeding status and telomere change, suggesting that a single reproductive event does not drive telomeric attrition. However, is it still possible that lifetime reproductive success may be ultimately related to biological aging (Hammers *et al.* 2015).

Our findings demonstrate that hibernation slows biological aging in large-bodied mammals, corroborating the aging benefits of hibernation found in small-bodied species (Turbill *et al.* 2013). However, reliance on human food subsidies reduces hibernation duration, and counteracts the aging advantages associated with hibernating. Furthermore, increasing and more variable winter temperatures also decrease hibernation and survival (Turbill & Prior 2016). Thus, the consequences of shortened winters and altered resource availability associated with human

development not only have important implications for animal behavior and survival, but also cryptic consequences including accelerated biological aging.

Acknowledgements

This work was funded by Colorado Parks and Wildlife, University of Wisconsin-Madison, and an American Society of Mammologists Grant-in-Aid. We thank numerous field technicians involved with the Durango bear project, as well as student lab technicians, especially Sonia Petty and Samantha Paddock.

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Table 1. Fixed-effect coefficients from the top models for oxidative damage and antioxidant capacity in black bear serum.

	Oxidative damage			Antioxidant capacity		
Variable	*β*	SE	*P*	*β*	SE	*P*
Intercept	9.63	1.43	<0.0001	488.93	43.46	<0.0001
Age	0.03	0.11	0.7467	0.78	3.47	0.8241
Season (winter)						
Summer	-4.96	1.29	0.0003	-5.60	29.30	0.8492
Breeding status (with cubs)						
No young	3.65	1.31	0.0074	27.00	29.88	0.3705
With yearlings	5.01	1.60	0.0028	7.50	35.73	0.8347

Figure Legends

Figure 1. a) Hibernation length regressed on the $\delta^{13}C$ signature of bear hair sampled in the preceding summer (n = 15), showing a negative relationship between increased enrichment in $\delta^{13}C$ and shorter hibernation lengths. **b)** Telomere length rate of change (per month) compared between age classes of bears (2-4 year olds, 5-9 year olds, and 10-24 year olds), indicating that younger bears tend to exhibit a decrease in telomere lengths, whereas older bears are more likely to shorten at a slower rate, or show an increase. **c)** Telomere length rate of change (per month) regressed on mean hibernation lengths (days) for each bear (n = 30), exhibiting a positive relationship between hibernation length and slower rate of telomere shortening, as well as telomere lengthening.

Figure 2. Oxidative damage and antioxidant capacity of bear serum samples, compared between active (summer) and hibernating (winter) seasons, and among reproductive status (at summer sampling, yearlings had already dispersed). Bears show increased oxidative damage in the winter, and decreased damage with cubs. Antioxidant capacity did not differ among categories.

Figure 1.

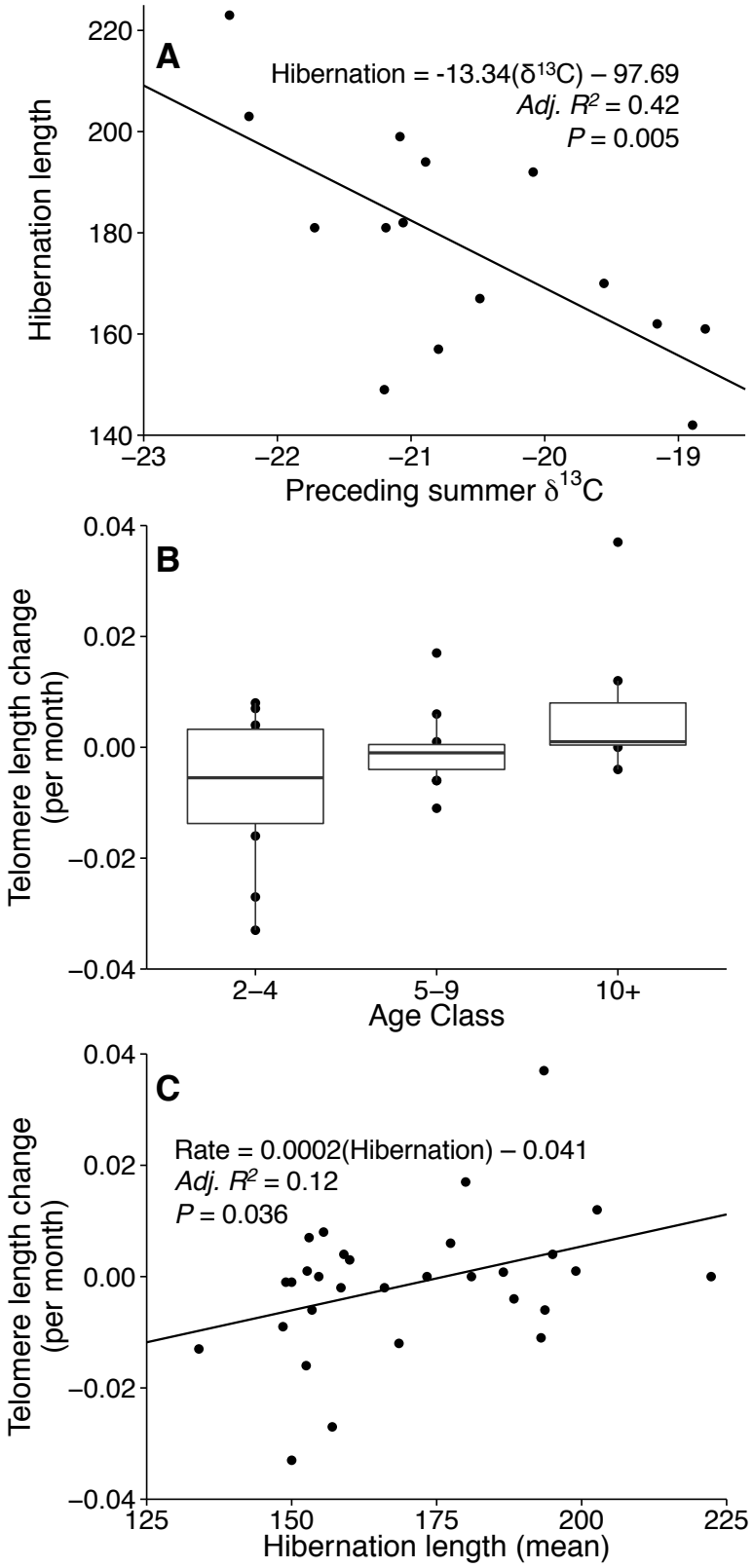
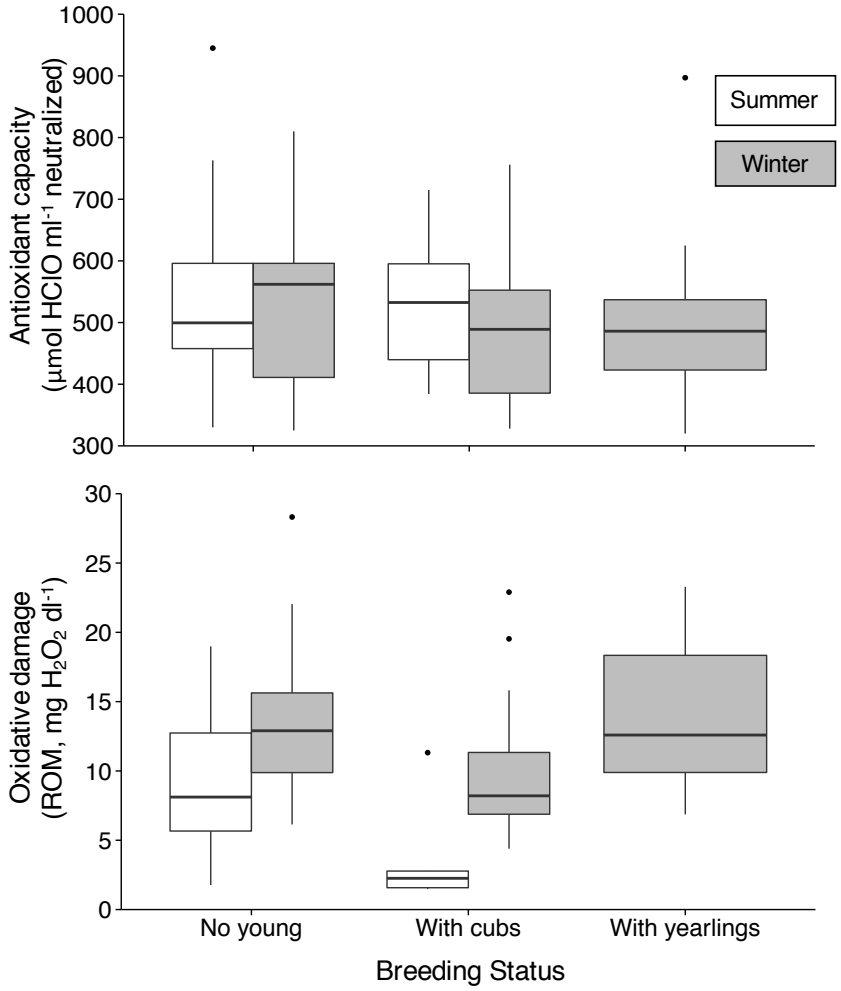

A
Hibernation = -13.34(δ13C) – 97.69
Adj. R2 = 0.42
P = 0.005
Hibernation length
Preceding summer δ13C
B
Telomere length change (per month)
2–4
5–9
10+
Age Class
C
Rate = 0.0002(Hibernation) – 0.041
Adj. R2 = 0.12
P = 0.036
Telomere length change (per month)
Hibernation length (mean)

Figure 2.


Antioxidant capacity
(µmol HClO ml-1 neutralized)
1000
900
800
700
600
500
400
300
Summer
Winter
Oxidative damage
(ROM, mg H2O2 dl-1)
30
25
20
15
10
5
0
No young
With cubs
With yearlings
Breeding Status