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Evolutionary and Ecological Causes and Consequences of Trophic Niche Variation in Ursids

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Evolutionary and ecological causes and consequences of trophic niche variation in ursids

By

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Individual variation and fitness are the cornerstones of evolution by natural selection. The trophic niche represents an important source of phenotypic variation on which natural selection can act. Although individual variation is fundamental to species-level ecological and evolutionary change, individual variation is often ignored in population-level approaches to wildlife ecology, conservation and management. Failing to link individual resource use to fitness or to biological outcomes related to fitness limits us to managing for the average resource needs of a population, which may be insufficient for protecting the diversity of resource use within populations and the underlying eco-evolutionary processes that generate that diversity. My goals were to provide insights into the mechanisms that generate and constrain intrapopulation trophic niche variation, evaluate whether linkages exist between individual biological outcomes and variation in food habits across the range of resources consumed within generalist consumer populations and examine how that variation manifests in population-level responses.

I investigated the causes and physiological consequences of intrapopulation trophic niche variation in two generalist consumers, the American black bear (*Ursus*

americanus) and brown bear (*U. arctos*) across three sites in British Columbia, CAN and at one site in Alaska, USA. My primary tools included stable isotope analysis to estimate diet, enzyme-linked immunoassay of hair to quantify the hormone cortisol for indexing physiological stress, and genetic analyses to identify individuals, species, and sex and to estimate ancestry. I found that individual differences in resource use can result in similar biological outcomes and that similar resource use can result in different biological outcomes. Intra- and interspecific competition, sex-based differences in nutritional and social constraints and annual variation in food availability all influenced trophic niche variation and the resultant biological outcomes. I also found evidence of a link between intrapopulation trophic niche variation and population genetic structure. My results highlight the diverse ecological drivers and diverse consequences of trophic niche variation, which further illuminates why the trophic niche is a nexus for eco-evolutionary dynamics.

DEDICATION

To my family, friends and James Lafferty, I am forever grateful for your patience, love and support.

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CHAPTER I

GENERAL INTRODUCTION

Introduction

The trophic niche is a nexus of eco-evolutionary dynamics because individual variation in trophic niche represents a source of phenotypic variation on which natural selection can act. Indeed, due to the costs and benefits associated with every food type, the survival and reproductive success of all consumers are effected by the food they eat (Estes et al. 2003). Thus, natural selection is thought to favor a diet that maximizes fitness (i.e., optimal diet) and select against all others (Svanbäck and Bolnick 2007). However, myriad studies provide overwhelming evidence of extensive intrapopulation trophic niche variation across diverse taxa (reviewed in Bolnick et al. 2003, Araújo et al. 2011). In fact, one of Darwin's (1859) greatest insights was recognizing that individuals differ in traits such as sex, age, morphology, physiology, behavior and competitive ability, and thus species are not homogeneous units of ecologically equivalent individuals. Therefore, among-individual differences in food resource use may reflect among-individual differences in resource needs as well as the constraints imposed by inter- and intraspecific competition. Despite our fundamental understanding that organisms are unique, intrapopulation variation is often ignored in the population-level approach to wildlife research and management, resulting in failure to consider the role of

individual variation in observed population-level responses on which management decisions are based (Ayers 2014).

Van Valen (1965), however, recognized the eco-evolutionary significance of intrapopulation trophic niche variation in his formulation of the niche variation hypothesis (NVH). Van Valen (1965) posited that (1) among-individual trophic niche variation is adaptive by serving to mitigate intraspecific competition when the constraint of interspecific competition is reduced and (2) that populations occupying wider niches should exhibit greater among-individual phenotypic variation compared to populations occupying narrower niches (Van Valen 1965). While renewed interest in testing the NVH has motivated much research over the past several decades in regards to the role of among-individual variation in population-level niche widths (reviewed in Bolnick et al. 2003, Araújo et al. 2011), there have been few attempts to link individual trophic niche variation to fitness (Belant et al. 2006). Yet individual differences in resource use can result in similar fitness and similar resource use can result in differences in fitness (Ayers 2014). However, fitness often cannot be measured directly, thus linking resource use to individual-based biological outcomes (i.e., metrics related to fitness; Clutton-Brock et al. 1982) is necessary for understanding of the mechanisms that drive and maintain intrapopulation variation in resources use and the consequences of that variation to population-level ecology (Ayers 2014).

My goals were to provide insights into the mechanisms that generate and constrain intrapopulation trophic niche variation within populations characterized as generalist consumers and to evaluate linkages between individual biological outcomes and variation in food habits across the range of resources consumed. To achieve these

goals I used American black bear (*Ursus americanus*) and brown bear (*U. arctos*) as ecological models. The biological outcomes I considered included percentage body fat, which is critical for ursid reproduction and survival during dormancy (Hilderbrand et al. 2000, Belant et al. 2006) and stress hormone levels (i.e., cortisol) that are tied closely to individual performance and fitness (Romero and Wikelski 2001, Sheriff et al. 2009) and are linked to population-level health and dynamics (Boonstra et al. 1998, Kitaysky et al. 2007, Charbonnel et al. 2008, Sheriff et al. 2009). In addition, I explored the role of trophic niche divergence in fostering population genetic structure. My primary tools included stable isotope analysis to estimate diet, enzyme-linked immunoassay of stress hormone levels, genetic analyses to identify individuals, species, and sex and to estimate ancestry as well as isotopic niche modeling. Each of the four proceeding chapters will independently provide greater insight into the mechanisms, including constraints (e.g., intra- and interspecific competition) that influence trophic niche variation in black bear and brown bears as well as the biological consequences of individual trophic niche variation. Together, these chapters will improve our understanding of some of the eco-evolutionary processes generating, maintaining and shaping ursid trophic niches (Figure 1.1). My research objectives were to:

1. Test the niche variation hypothesis with a measure of body condition in black and brown bears;
2. Evaluate the relative influence of individual, environmental and anthropogenic factors on the long-term stress burden experienced by brown bears over multiple years;

3. Investigate the relative influence of diet, sex and the social environment on black bear physiological stress; and
4. Examine the linkage between genetic population structure and trophic niche divergence in a brown bear population across a coastal-interior ecological transition zone.

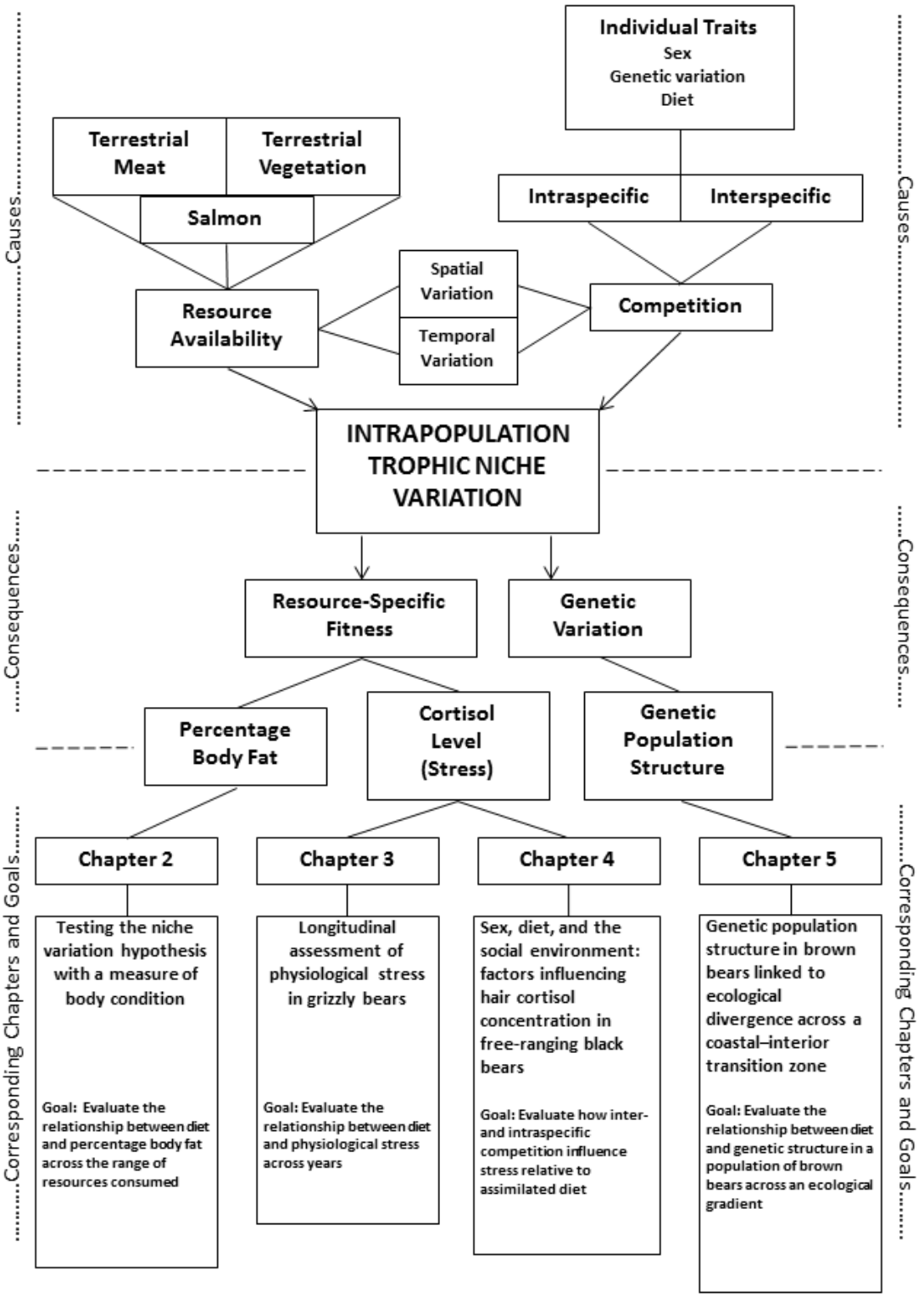

Figure 1.1 Conceptual model of the causes and consequences of intrapopulation trophic niche variation addressed in this dissertation.

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CHAPTER II

TESTING THE NICHE VARIATION HYPOTHESIS WITH A MEASURE OF BODY CONDITION

Previously published in Lafferty, D. J., Belant, J. L., and Phillips, D. L. 2015. Testing the niche variation hypothesis with a measure of body condition. Oikos.

Introduction

One of Darwin's (1859) greatest insights was recognizing that species were not homogeneous units of ecologically equivalent individuals but conspecifics that differ in traits such as sex, age, morphology, physiology and behavior. It is this variation among individuals that is the cornerstone of evolution by means of natural selection (Darwin 1859). Although individual variation can lead to species-level evolutionary and ecological change, observed variation does not ensure the outcome is beneficial. For instance, phenotypic variation among individuals, whether behavioral or morphological, may be selected for if that variation enables individuals to exploit under-used or novel resources, thereby reducing intraspecific competition (Bolnick et al. 2007). However, if exploitation of those resources reduces fitness (e.g., junk-food hypothesis, [Grémillet et al. 2008]), phenotypic traits that promote the use of those resources may be maladaptive and selected against. As such, understanding the relationship between individual variation and fitness is fundamental to evolutionary biology and ecology.

The niche variation hypothesis (NVH) posits that 1) populations occupying wider niches should exhibit greater among-individual variation compared to populations occupying narrower niches and 2) individual variation in dietary niche should confer an adaptive advantage (Van Valen 1965). Theoretically, when the constraint of interspecific competition is relaxed, intraspecific competition should drive niche expansion by selection favoring the use of novel resources. However, niche expansion at the population-level could be achieved by all individuals using the full set of available resources or by each individual using a unique subset of available resources as proposed in the NVH, thereby increasing among-individual variation. Recent studies from across diverse taxa (e.g., three-spine stickleback [*Gasterosteus aculeatus*], whelk [*Nucella* spp.], anolis lizards [*Anolis sagrei*], and wolves [*Canis lupus*]) have found support for the NVH, in that more generalist consumer populations tend to be more ecologically variable (Bolnick et al. 2007, Darimont et al. 2009). Despite overwhelming evidence that individual variation in dietary niche is common (*reviewed in* Bolnick et al. 2003, Araújo et al. 2011), the effects of individual diet variation on biological outcomes such as physiological condition remains largely unexplored (*but see* Both and Visser 2000, Kitaysky et al. 2007). Within a population of generalist consumers, for example, individuals may exist along a dietary gradient ranging from individuals that consume a broad range of food resources to individuals that specialize on subsets of food resources consumed by the population (Bearhop et al. 2004). If the NVH holds, individuals along this dietary gradient would be expected to exhibit similar measures of fitness across a broad range of resources used.

Sympatric American black bears (*Ursus americanus*) and brown bears (*U. arctos*) provide a good model system to test the NVH in relation to individual diet variation and measures of fitness. Both species are generalist omnivores with extensive dietary overlap (Hilderbrand et al. 1996, Mowat and Heard 2006, Zager and Beecham 2006) and their digestive and metabolic efficiencies are similar (Pritchard and Robbins 1990). Evidence from recent studies suggests that a mixed diet of plant and animal matter containing about 15% daily protein is optimal for maximum mass gain in ursids and that insufficient or excess protein may increase the cost of physiological maintenance and reduce mass gain efficiency (Robbins et al. 2007). Black and brown bears also exhibit hyperphagia during the summer and fall, gaining fat by consuming high calorie foods such as Pacific salmon (*Oncorhynchus* spp.), terrestrial meat (e.g., moose [*Alces alces*]), and berries (e.g., blueberries [*Vaccinium* spp.]) before entering a den for the winter (Hilderbrand et al. 2000, Belant et al. 2006). For example, Belant et al. (2006) reported that most lean body mass was accumulated during spring (May–June), whereas 75% of annual mass gains occurred after 1 July, coinciding with the approximate onset of annual salmon runs and berry production in the Denali, Alaska region. Previous studies also have demonstrated a direct relationship between salmon consumption, body condition and reproductive output in both species (Hilderbrand et al. 1999b, Belant et al. 2006). Because fat deposition in ursids is critical for meeting the costs of hibernation and reproduction, percentage body fat can be used to index physiological condition and has been used to infer individual fitness (Hilderbrand et al. 2000, Belant et al. 2006, Ayers et al. 2013).

I tested the NVH by evaluating the relationship between dietary niche and percentage body fat in sympatric female black and brown bears. My objectives were to:

(1) estimate the relative contribution of vegetation, terrestrial meat, and salmon to the diet of black and brown bears using stable isotope ratios derived from claw keratin, (2) assess the extent of intraspecific diet variation in both species, and (3) determine whether percentage body fat was independent of dietary niche, specifically percentage salmon in bear diets. Because brown bears are dominant to black bears and can exclude them from preferred food resources (McLellan 1993, Jacoby et al. 1999, Belant et al. 2006), I expected population-level food resources partitioning between black and brown bears to result in a lower proportional contribution of salmon to the diet of black bears compared to brown bears. I also expected this social dominance relationship to result in less among-individual diet variation within the black bear population due to black bears being constrained to use foods of lower nutritional value. Furthermore, I hypothesized that if the NVH held, percentage body fat would be similar for individuals of the same species across much of the dietary range observed in regards to differences in the proportional contribution of salmon to the diet of individual bears. Alternatively, if percentage body fat was not independent of dietary niche I expected that individuals of either species that consumed relatively more salmon would have greater percentage body fat than individuals consuming a diet comprised of predominantly vegetation.

Methods

Study area

The study area included the southeastern portion of Denali National Park and Preserve and Denali State Park, south-central Alaska (62°15’ to 62°43’N, 149°46’ to 151°26’W). Elevations in this area range from 180 to 1650 m, with an average of 762 mm of rain and 4,572 mm of snow annually (Alaska Department of Natural Resources

2013). At lower elevations white spruce (*Picea glauca*), black spruce (*P. mariana*), white birch (*Betula papyrifera*), and alder (*Alnus* spp.) are common (Pojar et al. 1994), as well as numerous wet meadows containing bear forage species such as sedges (Carex spp.), horsetail (*Equisetum* spp.) and grasses (*Elymus* spp.) (Belant et al. 2006, 2010). Blueberries (*Vaccinium* spp.), an important bear forage item, also occur in low density in association with spruce woodlands (Belant et al. 2006, 2010). Mid-elevations (i.e., 400-800 m) are dominated by shrubs including dwarf birch (*B. glandulosa*) and willow (*Salix* spp.), although blueberry and crowberry (*Empetrum nigrum*) also occur at these elevations (Belant et al. 2006, 2010). Above 800 m, habitat is predominantly tundra including barren rock and glaciers, yet riparian areas may contain shrubs or small trees (Belant et al. 2006, 2010). In addition to plant-based bear foods, five species of Pacific salmon occur within the study area (Denali National Park and Preserve, unpublished data). Spatial and temporal distributions of salmon vary by species, but salmon are available from early July through September (Belant et al. 2006). Moose (*Alces alces*) is the only ungulate in the study area and may serve as food for bears through predation or scavenging, although Arctic ground squirrels (*Spermophilus undulates*) and ants also were present and may have been consumed opportunistically (Jonkel 1984, Mattson 2001).

Animal capture and sample collection/preparation

Adult female black and brown bears were captured from 28–30 June 1999–2000 and again from 20–24 September 1999–2000 (Belant et al. 2006, 2010). Initially, bears were located by spotters in fixed-wing aircraft, and the presence of dependent young was noted when present; adult bears subsequently were anesthetized using immobilizing darts

fired from a helicopter (Belant et al. 2006, 2010). While bears were anesthetized, body temperature, respiration, and heart rate were monitored, bears also were weighed with an electronic scale (± 0.5 kg) and bioelectric impedance analysis (BIA) was used to estimate percentage body fat (Farley and Robbins 1994, Hilderbrand et al. 1998, Belant et al. 2006, 2010). Previous studies have demonstrated that BIA can be an accurate measure of percentage body fat for bears (Farley and Robbins 1994, Hilderbrand et al. 1998, Harlow et al. 2002) and it has been used to estimate body condition (Hilderbrand et al. 1999a, Belant et al. 2006, McLellan 2011,). During initial captures in June, a battery-operated hand-held grinder with a 3 mm diameter cutting bit was used to inscribe a semi-circular arc across the top half of the claw at the hairline on the third digit of the front paws of each individual (Belant et al. 2006). Upon recapture in September, the grinder was used to remove keratin in 3–5 mm increments from the claw on the third digit of the front paw of each individual between the inscribed semi-circular arc and the hairline, thus providing a biological sample representing claw growth between capture events that was used to derive carbon and nitrogen stable isotope ratios to estimate summer bear diet (Belant et al. 2006). Although keratin growth varies seasonally (Belant et al. 2006) and between distinct claw regions (Ethier et al. 2010), it is a metabolically inert tissue similar to hair (Hilderbrand et al. 1996) and can provide a reliable record of assimilated diet over the growth period of the claw when growth pattern is known (Ethier et al. 2010). During both capture events, care was taken to avoid contacting the vein located in the proximal portion of the claw (Belant et al. 2006). The Institutional Animal Care and Use Committee at the University of Alaska, Fairbanks approved all animal capture and handling procedures (Belant et al. 2006, 2010).

Keratin samples were dried at room temperature for 14–30 days, freeze dried, and stored in paper envelopes at room temperature until ground to a fine powder, loaded into standard tin boats containing 0.1−0.4 mg of dried sample and analyzed for stable carbon and nitrogen isotopes at the University of Alaska, Fairbanks using a Finnigan MAT Conflo II interface (Finnigan MAT, Bremen, Germany) with a Finnigan Delta+ mass spectrometer (Belant et al. 2006). During mass spectrometry samples were combusted, resulting in the separation of CO_2 and N_2, which were measured to calculate isotope ratios (Fry 2006). I report isotopic signatures in delta (δ) notation such that $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively. The standards are PeeDee Belemnite limestone for carbon and atmospheric N_2 for nitrogen, and the δ units are parts per thousand or per mil (‰). Although sample sizes were too small to be analyzed in duplicate, between 8 and 25 3–5 mm incremental keratin samples were analyzed per individual and subsequently the isotope values for the increments were averaged for each individual.

Estimating diet

Keratin samples from bear claws were analyzed for stable isotope ratios to index the proportional contribution of three major food categories – salmon, terrestrial meat, and vegetation – to the summer assimilated diet of 23 female black bears and 15 female brown bears from south-central Alaska (Belant et al. 2006, 2010). I estimated the proportional contribution of each food category to the diet of ursids at population and individual-levels by comparing carbon (δ13C) and nitrogen (δ15N) stable isotope values derived from keratin samples with generalized stable isotope values of the three major dietary components derived from the primary literature (Table 2.1). Generalized stable

isotope values of the three major food categories were obtained from previous studies conducted in northern North America (Table 2.1), thus increasing the likelihood that I captured the full range of isotopic variation within each food category in the study area. I used a Bayesian multi-source stable isotope mixing model (Stable Isotope Analysis in R [SIAR]; Parnell et al. 2010) that integrated δ13C and δ15N isotope values for individual bears as well as mean δ13C and δ15N isotope values, standard deviations, and trophic enrichment factors for each food category. Using SIAR, I transformed brown and black bear isotopic values into dietary estimates representing the most likely set of proportions of potential food sources and whole probability distributions for the set of possible food sources consumed by each individual and species (Milakovic and Parker 2011, Phillips 2012).

Evaluating interspecific and intraspecific diet variation

I generated population metrics using a multivariate ellipse-based approach (Stable Isotope Bayesian Ellipse in R [SIBER]; Jackson et al. 2011) to evaluate population-level food resource partitioning between female black and brown bears and to assess the extent of among-individual diet variation within species. I first calculated the standard ellipse area corrected for small sample size (SEA_c) for each species. Each SEA_c contained about 40% of the bivariate isotope data, representing the core dietary niche for each species, which is not sensitive to sample size (Jackson et al. 2011). I then generated 95% credible intervals for each estimated SEA_c to quantify the size of the core dietary niche of each species and determine whether black and brown bear core dietary niches overlapped. To quantify relative differences in the degree of among-individual diet variation between female black and brown bears, I calculated mean nearest neighbor distance (MNND) and

the standard deviation of the nearest neighbor distance (SDNND), which is less influenced by sample size (Layman et al. 2007). The MNND is a measure of Euclidean distance between a bivariate isotopic coordinate ($\delta^{13}C$ and $\delta^{15}N$), which represents an individual's isotopic niche, relative to other individuals within the population (Jackson et al. 2011). As such, MNND provides a relative measure of density and clustering within a population and SDNND provides a measure of evenness of spatial density among individuals in isotopic space (Layman et al. 2007). Smaller values for these population metrics indicate greater redundancy and a more even distribution of dietary niches within a population, thus indicating less intrapopulation dietary niche variation relative to a population with larger MNND and SDNND values (Layman et al. 2007, Jackson et al. 2011). Additionally, I repeated the procedures outlined above to evaluate dietary niche differences between female reproductive classes (i.e., absence or presence of dependent young) within species.

Examining percentage body fat relative to diet

I used linear regression to examine the relationship between multiple factors and percentage body fat in female black and brown bears. Using all subsets, I specified percentage body fat as the response variable and, species, dependent young (absent or present [0, 1]), and percentage salmon in diet as fixed effects. I initially included year (1999, 2000) as a random effect to account for potential variation between years. By fitting both a linear regression model and a linear mixed effects model using restricted maximum likelihood, I were able to apply the likelihood ratio test to determine whether the random intercept was warranted. Inclusion of year did not improve ($P > 0.61$) model fit and subsequently was excluded from additional models. Models were ranked using

Akaike's Information Criterion with a small sample size correction (AIC_c) to compare the weight of evidence for the aforementioned fixed effects on percentage body fat in black and brown bears (Burnham and Anderson 2002). I considered models as competing when ≤ 2 ΔAIC_c from the top model, provided that models within 2 AIC_c units did not include the addition of an uninformative parameter (Burnham and Anderson 2002, Arnold 2010). I used model averaging for competitive models and examined coefficients with 85% confidence intervals for interpretation of covariate effects if intervals excluded zero (Arnold 2010). I used 85% confidence intervals to increase the power of my results, as it would be more detrimental to my study to fail to reject a false null (Type 2 error) than to reduce the risk of committing a Type 1 error by using 95% confidence intervals (Gotelli and Ellison 2004). All statistical analyses were carried in R v. 3.0.1 (R Foundation for Statistical Computing [R Development Core Team 2009]).

Results

Diet estimation

Isotope values ranged from those characteristic of assimilated diets composed predominantly of vegetation to largely salmon (Figure 2.1). At the population-level, black and brown bears diverged in the mean proportional contributions of salmon (26 ± 1.9% SE and 49 ± 4.2% SE, respectively), vegetation (60 ± 1.7% SE and 30 ± 4.5% SE, respectively) and terrestrial meat (14 ± 1.0% SE and 21 ± 0.9% SE, respectively) to the diet (Figure 2.2). For both species, terrestrial meat contributed less to the diet than salmon or vegetation and the contribution of terrestrial meat to individual diets was relatively consistent within species (Figure 2.2).

Inter- and intraspecific dietary variation

Mixing model results revealed considerable variation in the proportional contribution of salmon and vegetation to the diet of female black and brown bears at the population level (Figure 2.2). Species core dietary niches did not overlap, suggesting that black and brown bears partitioned food resources (Figure 2.1), although the size of the SEA_c representing the core dietary niche of black (SEA_c = 3.02 ‰^{2}) and brown (SEA_c = 4.34 ‰^{2}) bears did not differ (P = 0.93). At the population-level, higher MNND and SDNND values exhibited by brown bears (MNND = 0.58, SDNND = 0.51) compared to black bears (MNND = 0.33, SDNND = 0.20) indicated greater intrapopulation dietary niche variation in brown bears. In addition, brown bears exhibited greater among-individual differences in the ranges of estimated proportional contributions of salmon (11–70%) and vegetation (10–70%) compared to black bears (salmon [8–40%] and vegetation [48–70%]).

Within-population niche variation analysis revealed that the sizes of dietary niches of black bears with (SEA_c = 2.48 ‰^{2}, MNND = 0.91, SDNND = 0.42, n = 6) and without (SEA_c = 3.16 ‰^{2}, MNND = 0.41, SDNND = 0.21, n = 17) dependent young did not differ (P = 0.45) nor did the sizes of dietary niches differ (P = 0.40) between brown bears with (SEA_c = 4.34 ‰^{2}, MNND = 1.20, SDNND = 1.38, n = 5) or without (SEA_c = 3.36 ‰^{2}, MNND = 0.99, SDNND = 1.40, n = 10) dependent young. However, overlap in isotopic niche space between females with and without dependent young was less for brown bears 0.22‰^{2} than for black bears 0.44‰^{2}. Brown bears with dependent young consumed 60% (4.5 SE) whereas those without dependent young consumed 44% salmon

(5.1 SE; $P = 0.06$). Conversely, black bears with dependent young consumed less salmon (21 ± 3.7% SE) than those without dependent young (27 ± 2.2% SE; $P = 0.05$).

Percentage body fat relative to diet

Variation in percentage body fat among female bears was best explained by species, presence of dependent young, and percentage salmon in the diet (Table 2.2; Figure 2.3). Salmon had a small but positive effect on percentage body fat; for every percentage increase in salmon in the diet, percentage body fat increased by 0.19 (Table 2.3). However, the interaction between salmon and the presence of dependent offspring had a slightly larger but negative effect of percentage body fat compared to salmon alone (Table 2.3). In addition, female brown bears had an estimated 5.99% lower body fat compared to female black bears, whereas female brown bears with dependent young had 8.52% lower body fat compared to female black bears without dependent young (Table 2.3).

Discussion

In this study, the proportional contribution of salmon to black bear diets ranged from 0–40% and from 11–70% for brown bears, yet within each species individual female bears achieved similar ranges of percentage body fat at various levels of salmon in the diet (Figure 2.3). This result is likely due to a small amount of salmon having a positive effect on percentage body fat but that increased energetic demands of rearing young can reduce this effect. In bears, fat deposition during the late summer and early fall is critical for meeting the costs of hibernation and reproduction (Hilderbrand et al. 2000, Belant et al. 2006) and previous studies have shown a direct relationship between salmon

consumption, body condition and reproductive output in both black and brown bears (Hilderbrand et al. 1999b, Belant et al. 2006). I hypothesized that if the NVH held, percentage body fat would be similar for individuals of the same species across much of the range in variation observed in the proportional contributions of salmon to individual bear diets. Although individual bears in this study only were sampled during a single year (i.e., 1999 or 2000), my results are consistent with recent studies from across diverse taxa showing that populations characterized as generalist consumers, such as black and brown bears, may be comprised of individuals whose dietary niches are small subsets of the total population niche width (Bearhop et al. 2004, Bolnick et al. 2007, Araújo et al. 2011).

I acknowledge my results may appear in contrast with Hilderbrand et al. (1999b), Belant et al. (2006), and others that have demonstrated the importance of salmon to black and brown bear nutritional health and reproductive success. Although the study area was > 200 km from the coast, relatively high salmon content in the diet of female brown bears in this study area is within the range of salmon consumption estimates from studies of coastal brown bear populations in North America that have access to abundant salmon resources (Hilderbrand et al. 1999b, Jacoby et al. 1999, Mowat and Heard 2006, Van Daele et al. 2013). However, my dietary estimates are based on a generalized salmon isotope baseline as well as generalized isotope values for terrestrial meat and vegetation derived from a much wider geographic region than the study area. Although this likely had little effect on mean dietary estimates, this may have led to less certainty in the estimated range in the distribution of the proportional contribution of the three major food categories to the diet of bears in this study, making my estimates conservative. In addition, because the sample sizes for both species were small, it is possible that female

bears on the extreme ends of the dietary gradient were not sampled, and thus any relationship that may exist between percentage body fat and salmon was not evident in either species (Figure 2.3). This is unlikely, however, because the range of proportional contributions of salmon to the diet of individuals of both black bears (8–40%) and brown bears (11–70%) was quite broad, particularly for female brown bears. Alternatively, there may be a non-linear relationship (i.e., threshold effect) between percentage body fat and proportion salmon in the diet, although small sample sizes of bears with and without dependent offspring precluded this analyses. Both species may exhibit a broad optimal dietary range in which small to moderate amounts of animal matter in combination with plant matter high in soluble carbohydrates, such as blueberries (*Vaccinium* spp.), is sufficient for obtaining the necessary calories and energy needed for gaining fat stores. As such, I contend that results from this short-term study conform to NVH because individual female black and brown bears exhibited similar physiological condition across the range of food resources used, which is a central tenant of the NVH. However, I acknowledge that longitudinal data could provide a more robust test of the NVH by providing additional context regarding long-term trends in physiological condition linked to the range of food resources used through time.

Factors including age, sex, morphology, social dominance, reproductive status, and heritable components of food resource preferences can influence among-individual dietary niche variation (Bolnick et al. 2003, Ben-David et al. 2004, Rode et al. 2006). For example, Ben-David et al. (2004) hypothesized that reproductive status was an important factor contributing to intrapopulation diet variation among a high-density brown bears from Chichagof Island, AK, USA and posited that adult female brown bears with

dependent young could reduce the risk of infanticide by avoiding salmon spawning streams where adult male bears are present or by avoiding areas where bear densities are higher due to bears congregating at abundant food sources. Although infanticide may be a risk to offspring at any bear density, brown bear density is considerably lower in the Denali region of Alaska than on Chichagof Island, and I found that on average, brown bears with dependent young consumed more salmon than those without dependent young and percentage body fat was lower for female brown bears with dependent offspring than without. Conversely, black bears without dependent young consumed more salmon than black bears with dependent young but percentage body fat was similar between female black bear reproductive classes.

At the population level, I found strong evidence of interspecific dietary niche partitioning, particularly in regards to use of salmon and vegetation food resources. Greater consumption of salmon by brown bears compared to black bears, however, was not surprising because brown bears, due to their larger size and more aggressive behavior, are competitively dominant to black bears and can exclude black bears from habitats where preferred, high-quality food resources are available (McLellan 1993, Jacoby et al. 1999, Belant et al. 2006, 2010). Jacoby et al. (1999), for example, showed that a black bear population on the Kenai Peninsula, Alaska that was sympatric with brown bears did not consume salmon, but where black bears were allopatric to brown bears, more than 50% of their assimilated diet was attributed to salmon. In my study area, Belant et al. (2010) found evidence of spatial niche partitioning between black and brown bears during summer, and posited that brown bears displaced female black bears from high-quality habitats where spawning salmon were available. My data support this

assertion in that female black bears appeared to be restricted in their use of salmon resources relative to female brown bears. While my study did not include male bears, female black bears in this system exhibited less among-individual diet variation relative to female brown bears, which suggests that the brown bear population is comprised of individuals that are relatively more specialized in their food habits compared to the black bear population (Flaherty and Ben-David 2010).

Niche partitioning between dominant and subordinate species seems to occur when high-quality resources are spatially constrained and alternative resources can be exploited by the subordinate species (Belant et al. 2010). For example, red foxes (*Vulpes vulpes*) can exclude arctic foxes (*Alopes lagopus*) from high-quality denning habitats associated with greater access to preferred prey of both species, reducing reproductive output of arctic fox pairs manifesting population-level effects for both species (Hersteinsson and Macdonald 1992, Tannerfeldt et al. 2002). Bolnick et al. (2010) demonstrated that competition with cut-throat trout (*O. clarki*) reduced the fundamental niche of the three-spine stickleback (*Gasterosteus aculeatus*). Furthermore, when sticklebacks were released from interspecific competition, population-level dietary niche width expanded via among-individual variation (Bolnick et al. 2010), which is consistent with the NVH (Van Valen 1965). My results, along with research by Belant et al. (2006, 2010), suggest that resource partitioning with brown bears may limit the fundamental niche of black bears. However, as indicated by black bears in this study area having achieved percentage body fat levels at least as high as brown bears (Figure 2.3), black bears appear to be able to meet their nutritional needs by consuming greater proportions of food items of lower nutritional value (i.e., predominantly vegetation), at least during

years when adequate forage is available to black bears and abundant salmon resources are accessible to brown bears. For instance, during this study the high proportional contribution of salmon to female brown bear diets is likely a result of abundant salmon availability during 1999 and 2000 when the estimated number of spawning salmon entering streams in the study area was slightly above the 11 year average (1990–2000) for both years (Belant et al. 2006), thus interspecific competition for high-quality vegetation resources, such as blueberries, likely was limited.

Although a rich and diverse literature exists regarding the effects of wildlife nutrition on various measures of fitness (for a recent example see Lane et al. 2014), to my knowledge, no other study has tested the NVH using actual food resource use (i.e., realized dietary niche) relative to a measure of physiological condition (i.e., percentage body fat) directly related to fitness. Most previous attempts to test the NVH have focused on morphological variation as a proxy for resource use to evaluate whether populations with wider niches also exhibited greater among-individual morphological variation compared to populations with narrower niches (reviewed in Bolnick et al. 2007). I agree with Bolnick et al. (2007) and Darimont et al. (2009) that testing the NVH with data on realized dietary niche is more appropriate than the traditional approach of measuring morphological variation among populations relative to niche width. However, I suggest that merely demonstrating increased among-individual diet variation under conditions of greater niche width is insufficient to support the NVH. To offer support for NVH, one must also show that among-individual fitness or some biological outcome related to fitness (e.g., stress hormone levels; Kitaysky et al. 2007) is similar across some range of food resources consumed among individuals within sampled populations. Furthermore, I

believe that linking individual realized dietary niches to measures of physiological condition related to fitness can provide fertile new ground for testing the NVH, which can provide new insights into eco-evolutionary processes linked to variation in food resource use.

Table 2.1 Generalized isotopic values ± SE and trophic discrimination factors ± SD used to estimate female black (*Ursus americanus*) and brown bears (*U. arctos*) diet.

Food category	$\delta^{13}C$ (‰) ± SE	$\Delta\delta^{13}C_{tissue\text{-}diet}$ (‰) ± SD	$\delta^{15}N$ (‰) ± SE	$\Delta\delta^{15}N_{tissue\text{-}diet}$ (‰) ± SD
Salmon	-19.93[a] ± 0.30[a]	1.20[d] ± 1.00[e]	12.82[a] ± 0.34[a]	2.3[d] ± 0.45[e]
Terrestrial meat	-24.30[b] ± 0.60[b]	4.90[d] ± 1.00[e]	1.70[b] ± 0.50[b]	4.0[d] ± 0.45[e]
Terrestrial vegetation	-26.60[c] ± 0.14[c]	3.30[d] ± 1.00[e]	-2.80[c] ± 0.21[c]	2.0[d] ± 0.45[e]

Data collected in southern Denali National Park and Preserve and Denali State Park, Alaska, 1999–2000.

[a] Generalized Pacific salmon isotopic baseline established by averaging published values for Chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), coho (*O. kisutch*), pink (*O. gorbuscha*), and sockeye (*O. nerka*) sampled throughout the Pacific Northwest, USA (n = 237) and estimated standard error (SE) calculated using data from Bilby et al. (1996); Ben-David et al. (1997); Jacoby et al. (1999); Chaloner et al. (2002); Satterfield and Finney (2002); Ben-David et al. (2004)

[b] Generalized herbivore isotopic baseline averaged from moose (*Alces alces*) red blood cell (n = 87) collected in Denali National Park and Preserve, AK, moose hair samples (n = 5) from Kenai, AK, and ground squirrel (*Urocitellus parryii*) hair samples (n = 20) collected from Kluane Lake, Yukon, Canada and estimated standard error calculated using data from Ben-David et al. (1999, 2001); Jacoby et al. (1999); Adams et al. (2010)

[c] Generalized plant isotopic baseline and estimated standard error calculated from isotopic measurements on bear hair from northern North America where bears consume little meat (Mowat and Heard 2006; [n = 200]), tissue-diet discrimination relationships derived from Hilderbrand et al. (1996)

[d] Tissue-diet discrimination values for C and N from Phillips and Koch (2002)

[e] Standard deviations (SD) around discrimination values reflect uncertainty in these data and were derived from Hilderbrand et al. (1999); Ben-David et al. (2004), Mowat and Heard (2006); Merkle et al. (2011)

Table 2.2 Linear models used in modeling percentage body fat as a function of presence of dependent young, percentage salmon in diet, and species for female black (*Ursus americanus*) and brown (*Ursus arctos*) bears.

Model[a]	K[b]	R^2	AIC_c[c]	ΔAIC_c[d]	W[e]	LL[f]
Dep * Sal + Spec	6	0.41	233.20	0	0.61	-109.24
Dep * Spec + Sal	6	0.39	234.12	.92	0.39	-109.70

Data collected in southern Denali National Park and Preserve and Denali State Park, Alaska, 1999–2000.

[a] Models with interaction terms also include main effects. Models shown include all competing models. Terms include dependent young (Dep), percentage salmon in diet (Sal), and species (Spec).

[b] The number of parameters.

[c] Models ranked in ascending order by 2 Akaike's Information Criterion adjusted for small sample sizes.

[d] The difference between the best model and other competing model.

[e] AIC_c weights.

[f] Maximum log-likelihood value for each model.

Table 2.3 Model averaged coefficients ± SE and 85% confidence limits for parameters in competitive models ($\Delta AIC_c \leq 2$) for female black and brown bears.

			85% Confidence Limits	
Parameter	Estimate	SE	Lower	Upper
Intercept	25.99	2.37	22.50	29.49
Dependent[a]	2.35	3.96	-3.44	8.14
Salmon	0.19	0.07	0.08	0.30
Species (grizzly)[b]	-5.99	2.14	-9.15	-2.84
Dependent[a]:Salmon	-0.23	0.09	-0.36	-0.09
Dependent[a]:Species (grizzly)[b]	-8.52	3.74	-14.03	-3.02

Data collected in Denali National Park and southern Denali National Park and Preserve and Denali State Park, Alaska, 1999–2000.

[a] Absence of dependent young is the reference group.

[b] Black bear is the reference group.

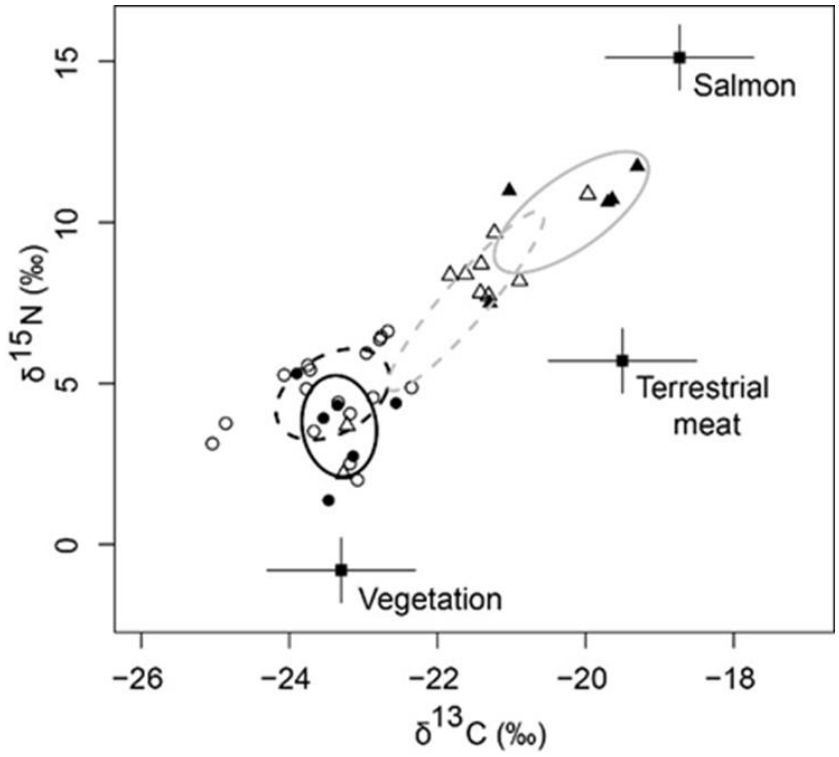

Figure 2.1 $\delta^{13}C$ and $\delta^{15}N$ stable isotope bi-plot for black (*Ursus americanus*) and brown (*U. arctos*) bears, Denali region, USA, 1999−2000.

Trophic enrichment factors were applied to each source and each food sources (mean ± 1 SD deviation) . Standard ellipse areas corrected for small sample size (SEA_c), representing the core (40%) dietary niches of female black bears with (solid black ellipse; $SEA_c = 2.48‰^2$) and without (dashed black ellipse; $SEA_c = 3.16‰^2$) dependent young, and female brown bears with (solid gray ellipse; $SEA_c = 4.34‰^2$) and without (dashed gray ellipse; $SEA_c = 3.36‰^2$) dependent young.

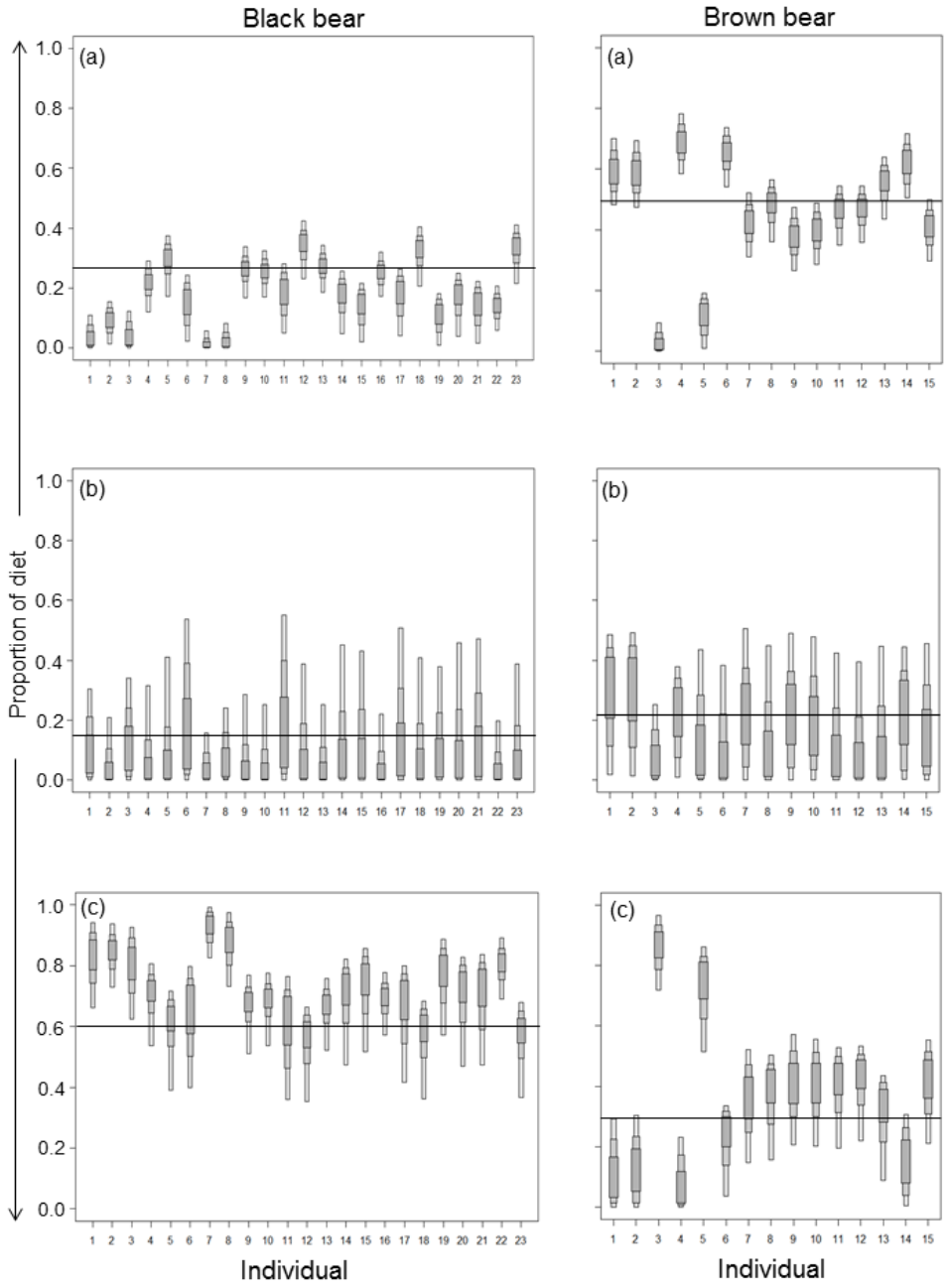

Figure 2.2 Range of estimated proportional contributions of major food categories to female black (*Ursus americanus*) and brown (*U. arctos*) bear diet, Denali region, USA, 1999–2000.

Decreasing bar widths represent 50%, 75%, and 95% Bayesian credible intervals. Solid black lines represent the mean proportional contributions of each food source averaged across individuals for that species. For each species, bears are ordered from least to greatest percentage body fat. Data collected in southern Denali National Park and Preserve and Denali State Park, Alaska, 1999–2000.

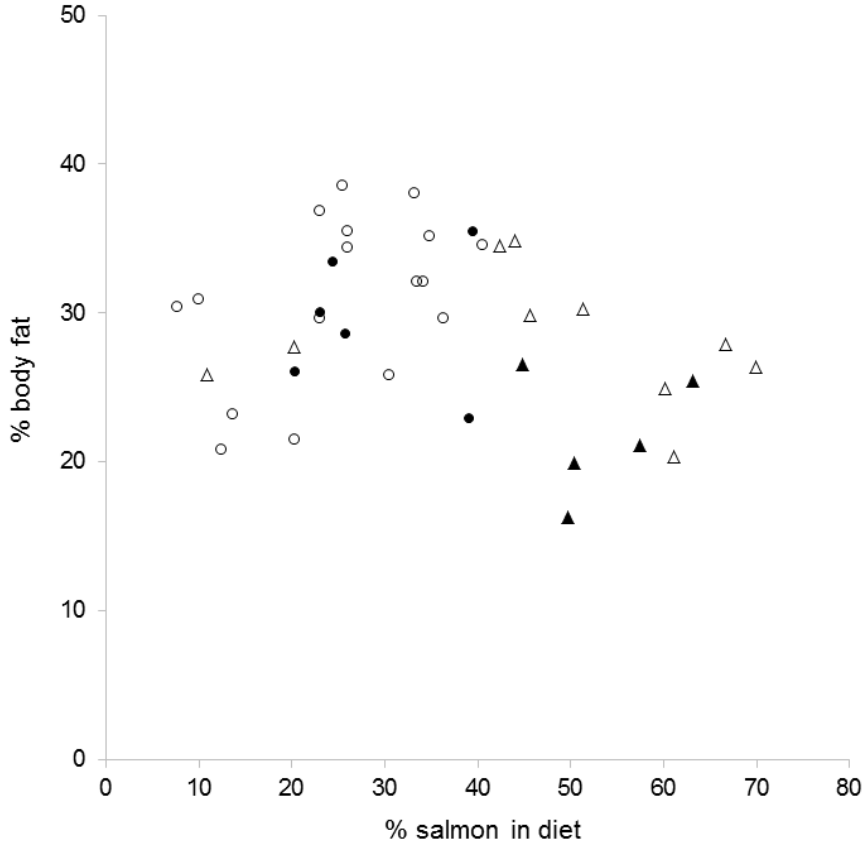

Figure 2.3 Relationship between percentage body fat and proportional contribution of salmon to female black (*Ursus americanus*) and brown (*U. arctos*) bear diet.

Open circles for black bears without dependent young, solid circles for blacks bear with dependent young, open triangles for brown bears without dependent young, solid triangles for brown bears with dependent young. Data collected in southern Denali National Park and Preserve and Denali State Park, Alaska, 1999–2000.

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CHAPTER III

LONGITUDINAL ASSESSMENT OF PHYSIOLOGICAL STRESS IN GRIZZLY BEARS

Introduction

Wildlife receive and process information from their environment in myriad ways. Whether or not predictable, these environmental stimuli can provoke changes in individual behavior, morphology and physiology (Post and Forchhammer 2008, Creel 2013a, Zimova et al. 2014), which may be fundamental to evolutionary and life-history adaptations (Boonstra 2013). As one of the most conserved evolutionary processes in vertebrates (Sheriff et al. 2009, Wingfield 2013), the 'stress response', defined as the neural-endocrine activity that helps restore homeostasis (Sapolsky 1987, Sapolsky 2000, Reeder and Kramer 2005), is perhaps one of the most important responses by wildlife to diverse environmental stimuli. The stress response is initiated through a physiological cascade mediated in part by activation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in the secretion of stress hormones (e.g., glucocorticoids) (Sapolsky 1992, Wingfield and Romero 2001, Sheriff et al. 2009, Creel et al. 2013b, Wingfield 2013). Activation of the HPA axis may be the result of acute stress such as an attack by a predator, chronic stress due to insufficient nutrition, or in anticipation of seasonal changes in environmental conditions or energetic demands (e.g., reproduction, hyperphagia) (*reviewed in* Bray 1985, Dantzer et al. 2014). Because the stress response is

nonspecific, the body reacts similarly to different types of stimuli (e.g., physical, psychological) (Sapolsky 1992, Wingfield 2013). While short-term increases in glucocorticoids may be critical to mounting an immediate survival response such as the fight or flight response, or preparing for future conditions, continuous HPA activation resulting in chronically elevated glucocorticoid levels can negatively affect immune response, muscle maintenance and future reproduction (Boonstra and Singleton 1993, Charbonnel et al. 2008, Sheriff et al. 2009). Thus, by facilitating adaptive behavioral and morphological responses (Sheriff et al. 2009, Creel et al. 2013a) or changes in life-history strategies (Wingfield et al. 1998, Ricklefs and Wikelski 2002, Boonstra 2005), glucocorticoids are fundamental to how wildlife integrate information from their environment (Dantzer et al. 2014). As such, glucocorticoids are linked closely to individual performance and fitness (Romero and Wikelski 2001, Sheriff et al. 2009) and recent studies have demonstrated a link between glucocorticoids and population-level health and dynamics (Boonstra et al. 1998, Kitaysky et al. 2007, Charbonnel et al. 2008, Sheriff et al. 2009).

Measures of glucocorticoid levels, particularly cortisol, increasingly are being assayed from biological matrices including blood, urine, saliva and feces. However, cortisol derived from hair provides an integrative measure of past HPA activity over the growth period of the hair (i.e., weeks to years), thereby indexing the long-term stress burden experienced by the individual (*reviewed in* Sheriff et al. 2011). Thus, hair cortisol concentration can provide meaningful biological information regarding long-term stress in mammals including ungulates (Ashley et al. 2011), non-human primates (Behie et al. 2010, Dettmer et al. 2012), rodents (Dantzer et al 2010, Mastromonaco et al. 2014), hyrax

(Koren et al. 2008) and ursids (Macbeth 2010, Bourbonnais et al. 2013, Malcolm et al. 2013, Bryan et al. 2013, Catett et al. 2014). Hair samples also can be obtained via noninvasive sampling (e.g., ursids: Mowat and Strobeck 2000, felids: Weaver et al. 2005, mustelids: Zielinski et al. 2006, cervids: Belant et al. 2007), thereby eliminating the possible impact of an acute stress response associated with capture and handling that can contaminate or contribute to the long-term stress signal (Cattet et al. 2014). One of the most promising applications for quantification of hair cortisol may be to provide value to large-scale, longitudinal studies that enable concurrent or retrospective assessments of population-level health associated with environmental change (Cattet et al. 2014).

As a long-lived species sensitive to environmental change (Carroll et al. 2004, Mattson and Merrill 2002), the grizzly bear (*Ursus arctos*) may be an excellent ecological model for the application of hair cortisol quantification to index long-term population-level stress. For example, grizzly bears are affected by top-down (i.e., human caused mortality [McLellan 1999, Schwartz et al. 2006]) and bottom-up (i.e., food availability [McLellan 1994, Hilderbrand et al. 1999, Ripple et al. 2014]) factors. Previous studies have shown that where salmon (*Oncorhynchus* spp.) are available, a direct relationship exists between salmon consumption, physiological condition, reproductive output and population density (Stringham 1980, Hilderbrand 1999, Belant et al. 2006). However, key food resource for interior grizzly bears without access to salmon are fruits, particularly huckleberry (*Vaccinium membranaceum*), buffaloberry (*Shepherdia canadensis*), and serviceberry (*Amelanchier alnifolia*) (McLellan and Hovey 1995, Welch 1997, McLellan 2011, McCall et al. 2013). Fruits are important foods during hyperphagia, when grizzly bears consume high calorie foods to gain fat stores necessary for reproduction and

hibernation (Welch 1997, McLellan 2011), although fruit production can vary substantially from year to year (Martin 1983, Krebs et al. 2009, Holden et al. 2012). Moreover, huckleberry and buffaloberry production are highly correlated (McCall et al. 2013) and incidents of grizzly bear-human conflict are inversely correlated with the abundance of natural food sources (Gunther et al. 2004). Grizzly bears also are highly sensitive to anthropogenic disturbances (Carroll et al. 2004, Mattson and Merrill 2002, Bourbonnais et al. 2013) including the direct and indirect effects of harvest (Wieglus and Bunnell 1994, Swenson et al. 1997, but see McLellan 2005). Consequently, complex interactions linked to top-down and bottom-up factors likely contribute to the stress burden experienced by grizzly bears across their range.

In southeastern British Columbia, where grizzly bear hunting is in high demand (Mowat et al. 2013ab), grizzly bear hair samples have been collected and archived annually from 1995–present as part of a long-term population monitoring effort (Mowat et al. 2013a). My goal was to evaluate the long-term stress burden experienced by this grizzly bear population. I used enzyme-linked immunoassay of hair cortisol to quantify the stress responses of free-ranging grizzly bears relative to sex, diet, abiotic environmental conditions associated for forage production and human-induced stress associated grizzly bear harvest. I hypothesized that cortisol levels would (i) be inversely related to the proportional contribution of animal matter in the diet, (ii) decrease with indices associated with increased fruit production, and (iii) increase with reduced hunter effort, which is associated with greater grizzly bear harvest. I further hypothesized that cortisol levels would be higher in males than females due to either the physiological constraints of maintaining a larger body size or as a consequence of greater social stress

associated with competition for access to mates. Understanding the extent to which individual (e.g., sex, diet), environmental and anthropogenic factors influence cortisol in free-ranging wildlife populations is essential for interpreting cortisol as a physiological biomarker for the stress response to enhance long-term population monitoring programs.

Methods

Study area

The study area comprised about 12,000 km^2 in southeastern British Columbia, Canada (49°30'N, 115°4'W; Figure 3.1). Elevation ranges from ~700 m to more than 3,000 m and mean monthly temperature ranges from -7.3°C in January to 16.3°C in July, with an annual average of 702 mm of rain and 3,730 mm of snow (Poole and Stuart-Smith 2002). This area is dominated by western red cedar (*Thuja plicata*) and western hemlock (*Tsuga heterophylla*), but contains additional conifer species (e.g., Douglas fir [*Pseudotsuga menziessii*], Pacific yew [*Taxus brevifolia*]), as well as hardwoods such as American white birch (*Betula papyrifera*) and quaking aspen (*Populus tremuloides*) (Meidinger and Pojar 1991). Skunk cabbage (*Lysichiton americanus*) and devil's club (*Oplopanax horridus*) dominate the understory of forested wetlands, whereas willows (*Salix* spp.), sedges (*Carex* spp.) and horsetail (*Equisetum arvense*) are common in non-forested riparian areas (MoE 1996). In the southern portion of the study area huckleberry are abundant at higher elevations in non-forested areas where wildfires removed the overstory (McLellan and Hovey 2001, Apps et al. 2004), whereas buffaloberry are more common in the northern portion of the study area. Yellow sweetvetch (*Hedysarum sulphurescens*) also occurs sporadically from the floodplain to mountain ridges (McLellan and Hoovey 1995). Potential animal prey includes six species of ungulates,

ground squirrels (*Spermophilus columbianus*), marmot (*Marmota caligata*) and insects (McLellan and Hovey 1995, Hobson et al. 2000).

Sample collection and selection

I used grizzly bear hair samples that were collected annually from 1995 through 2014 using bear hair collection stations spaced systematically using a 16 km^2 grid overlain across the study area (Proctor et al. 2010, Mowat et al. 2013ab). Hair collection stations consisted of a scent lure surrounded by a single strand of barbed-wire and hair collection occured during two 14-day sampling sessions beginning in late June and ending in late July (Mowat et al. 2013ab). Samples were subjected to DNA analysis for species, sex and individual identification (Mowat et al. 2013ab). From this archived collection, I selected the highest quality hair samples available (> 7 guard hairs) representing 7–24 female and male grizzly bears sampled from 2006 to 2012. Because guard hair provides an integrative record of diet and HPA activity over the growth period of the hair, and because I selected only mature guard hairs, I assumed hair samples represented annual assimilated diet and hair cortisol levels during the year before sample collection, thus representing diet and cortisol levels from 2005–2011 (Hilderbrand et al. 1996, Jones et al. 2006, Bryan et al. 2013, Bryan et al. 2014a, Cattet et al. 2014).

Cortisol immunoassay

Whole grizzly bear hair samples were prepared and analyzed for cortisol analysis at the Behavioral Immunology and Endocrinology Laboratory at the University of Colorado Denver Anschutz Medical Campus (Aurora, Colorado, USA). Samples were weighed and placed in pre-weighed 2 ml cryovials (Wheaton, Millville, NJ, USA),

washed three times in 100% isopropanol and dried following methods described by D'Anna-Hernandez et al. (2011). Hair samples then were re-weighed in the cryovials on a high sensitivity electronic balance (Mettler Toledo Model MS105, Switzerland). Next, a single 4.76 mm cleaned stainless steel ball bearing was added to the cryovial to facilitate grinding. Cryovials containing samples were subsequently placed in aluminum cassettes and submerged in liquid nitrogen for three to six minutes to freeze hair samples. Using a ball mill (Retsch, Haan, Germany), frozen samples were ground for four to five minutes and powdered hair was extracted within the cryovial in 0.33-1.0 ml (depending on sample mass) high pressure liquid chromatography (HPLC) grade methanol for 24 hours at room temperature on a side-to-side shaker platform. Cryovials were spun for three minutes in a centrifuge at 1700g to pellet the hair. Next, 133 µl of supernatant from each cryovial was transferred to microcentrifuge tubes and dried using a stream of nitrogen in a drying rack under a fume hood. Based on hair weight, dry extracts were reconstituted with assay diluent. Finally, hair cortisol concentration was quantified using a commercial high sensitivity Enzyme Immunoassay (EIA) kit (Salimetrics LLC, State College, PA, USA) per manufacturer's protocol. Assay cross validation with liquid chromatograph-mass spectrometry methods were reported by Russell et al. (2015). For detailed methods on immunoassay validation for grizzly bears see Appendix A.

Stable isotope analysis

Whole grizzly bear hair samples were prepared and analyzed for stable isotope analysis at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, Ontario, Canada) using standard methods. In short, follicles were removed from guard hair samples and hair was washed in a solution of chloroform:methanol (2:1) via a

sonicator bath at 30 degrees for 20 minutes to remove surface debris and oils. Hair samples subsequently were rinsed twice with distilled water then washed again with distilled water in a sonicator bath for 20 minutes, then oven-dried at 40 degrees Celsius for 24 hours. Whole hair samples were weighed, measured and analyzed for carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios using an Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS).

I report isotopic signatures in delta (δ) notation such that $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively; standards are PeeDee Belemnite limestone for carbon and atmospheric N_2 for nitrogen (Peterson and Fry 1987). Analyses of internal laboratory standards indicate precision of 0.08‰ and 0.17‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively and NIST standards suggested an analytical accuracy of the instruments of 0.06‰ and 0.13‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Following Hopkins et al. (2012), I corrected grizzly bear $\delta^{13}C$ values by –0.022‰ per year for all samples collected before 2012 to account for the dilution of heavy carbon isotopes (^{13}C and ^{14}C) in Earth's atmospheric CO_2 resultant from the last 150 years of burning large amounts of fossil fuel, which is depleted in ^{13}C and contains no ^{14}C (i.e., Seuss effect) (Tans et al. 1979).

Environmental variables

Weather conditions during the growing season and the flowering period have been identified as important factors in the development and subsequent production of forage plants, including fruit-producing shrubs (Krebs et al. 2009, Kelly et al. 2013). I used data from four weather stations (Fernie 1152850; Fording River Cominco 1152899, and Sparwood 1157630 and 1157631; Meteorological Services of Canada, Environment

Canada) to derive metrics associated with forage production. First, I averaged daily temperature readings across weather stations to establish a representative measure of weather conditions across the study region (C. Lamb, University of Alberta, *unpublished data*). From these averaged data, I calculated two metrics identified as effecting fruit production (e.g., huckleberry, buffaloberry, serviceberry) near the study area (Kasworm et al. 2008, Holden et al. 2012, McCall et al. 2013). First, I calculated annual growing-degree day units (hereafter, GDD) as $T_{min} + T_{max}/2 - 5°C$ for April–June (Holden et al. 2012, Reeves et al. 2013). During this study, GDD ranged between 71.63 and 285.71. Next, because July is the flowing period for fruiting shrubs in this area and when weather influences subsequent fruit production (Yudina and Maksimova 2005, Krebs et al. 2009), I calculated July temperature range as the difference between T_{min} and T_{max} (Holden et al. 2012), which ranged between 23.35 and 33.43. Together, GDD and July temperature range account for 70% of the inter-annual variability in huckleberry productivity near the study area (Holden et al. 2012). In addition, huckleberry production is highly correlated with buffaloberry production ($r = 0.70$; data from Kasworm 2008).

Fruit production index

An index of annual huckleberry production was established by surveying major huckleberry shrub-fields (i.e., ~ 5–12 km^2) annually in the southern portion of the study area every year from 1978 to 2014 with the exception of 1995, and all surveys were conducted by the same observer (B. McLellan, Ministry of Forest, Lands and Natural Resource Operations (MFLNRO), British Columbia, *unpublished data*). A 5-point ordinal scale was used to classify huckleberry production each year subjectively; 1 = very low (little to no berry production [i.e., crop failure]), 2 = low, 3 = moderate, 4 = high, and

5 = very high [B. McLellan, *unpublished data*]). During this study, huckleberry production ranged from very low to moderate. Although this index was established for assessing annual changes in huckleberry production, which is the most common fruit producing shrub in the southern portion of the study area, I acknowledge that buffaloberry is more common in the northern portion of the study area. However, because of the high correlation between huckleberry and buffaloberry production, I use this index as measure of fruit production for the whole study area.

Hunter effort

To index the potential impact of anthropogenic effects associated with grizzly bear harvest activities on population-level stress responses, I used hunter activity and grizzly bear mortality data from the MFLNRO, British Columbia. I calculated hunter effort by summing the total number of days hunted by each individual or hunting party (hunting party = 1 hunter) for both resident and non-resident hunts, as reported by hunters to MFLNRO, divided by the total number of animals harvested. Annual hunter effort ranged from 38.5 to 75.1 days across the study period. Because hunter effort and total grizzly bear mortality were highly negatively correlated ($r = 0.90$, $p < 0.001$), I assumed that any direct or indirect effects of anthropogenic hunting activities on population-level stress would be indexed by hunter effort. However, my estimate of hunter effort may be conservative because all unsuccessful hunts may not have been reported to MFLNRO.

Diet estimation

I simultaneously evaluated how isotopic variation was structured throughout the population and estimated diet using the Bayesian stable isotope mixing model MixSIAR

(Stock and Semmens 2013). Within the MixSIAR framework, I included $\delta^{13}C$ and $\delta^{15}N$ values from analysis of grizzly bear guard hairs and mean $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for major (i.e., plant matter, animal matter) dietary components reported in the primary literature from locations as close as possible to the study area (Appendix A). Trophic discrimination factors for generalized plant and animal matter were calculated using equations described by Felicetti et al. (2003). Additionally, I created a candidate set of four models to test support for including effects of year and sex for modeling isotopic variation. I also included a random effect for individual in all models and model error structure followed Moore and Semmens (2008). I specified conventional uninformative priors for model parameters and for random effect standard deviation (Stock and Semmens 2013). I sampled models from three parallel Markov chains (length = 300,000) with JAGS (Plummer 2013), burning the first 200,000 iterations and thinning by 100. I assessed convergence by visually inspecting trace plots and with the Gelman-Rubin diagnostic ($\hat{R} < 1.01$ indicating convergence; Gelman and Rubin 1992). I used deviance information criterion (DIC; Speigelhalter et al. 2002) to evaluate which model in the candidate set was most supported; the most parsimonious model having the least DIC value (Hadfield 2010). Accordingly, I estimated proportional contributions of plant and animal matter to the diet of grizzly bears using the most parsimonious model. Analyses were carried out in R statistical software (R Development Core Team, 2011) and all data included in the candidate model set are presented in Appendix A.

Cortisol analyses

I fit Bayesian general linear models to examine the influences of individual, environmental and anthropogenic factors on grizzly bear cortisol levels on the natural log

scale. I developed a candidate set of 11 models that included combinations of fixed (i.e., diet [$\delta^{15}N$ to index consumer trophic position associated with consumption of animal matter (Layman et al. 2007)], berry index [factor variable with three levels], GDD + July temperature range, hunter effort) and random effects (i.e., individual, year). All continuous variables were standardized to a mean of zero and standard deviation of one before analysis (Zuur et al. 2009). I examined Cook's distance (Cook 1977) as an indicator of influential observations and subsequently removed three samples from further analysis (see Bryan et al. 2013). I assessed variables for collinearity using variance inflation factors (VIF), with collinearity considered ≥ 3 (Zuur et al. 2010). Diet and sex were highly correlated; I subsequently retained diet as a covariate in my models. No other explanatory variables were correlated (VIF = 1.03–2.85). I tested for differences in mean cortisol levels between females and males using an independent sample *t*-test.

I estimated model parameters from the joint posterior distribution of each model by sampling from three Markov chains for 60,000 iterations after burning the first 2,000 using JAGS version 3.3.0 (Plummer 2013) in the package *R2jags* (Su and Yajima 2013). Visual inspection of trace plots indicated that thinning was unnecessary. I visually examined chains for convergence and with the Gelman-Rubin diagnostic ($\hat{R} < 1.1$ indicating convergence; Gelman and Rubin 1992), which compares within- and between-chain variation (Gelman et al. 2014). I specified conventional uninformative priors for model parameters, including $\beta \sim N(0,1000)$ for fixed effects and $\sigma \sim Unif(0,20)$ for random effects standard deviations. I assessed model goodness-of-fit using Bayesian *p*-values, which compare fit statistics for the observed data and the predicted values ($p > 0.05$ and < 0.95 indicating adequate fit; Gelman and Meng 2004). I ranked models using

DIC and considered models competing if ≤ 5 DIC from the top model (Speigelhalter et al. 2002). I examined coefficients with 95% credible intervals for interpretation of important covariate effects if intervals excluded zero. Also, I assessed the amount of variance explained by model random effects following Ntzoufras (2009). All statistical analyses were carried out in R statistical software (R Development Core Team, 2011).

Results

I obtained stable isotope and cortisol values from 227 grizzly bear hair samples, representing 177 individuals (female = 95, males = 82); 36 individuals were represented in multiple years (between 2–4 years).

Isotopic variation and diet estimation

Grizzly bear $\delta^{13}C$ and $\delta^{15}N$ values ranged from –24.67 to –22.36 (2.31‰) and 0.66 to 7.91 (7.25‰), respectively (Figure 3.1a). All models in the candidate set converged to the posterior distribution. The most parsimonious model (DIC = 1410.82) included sex as the only fixed effect (Table 3.1), which suggests that the low isotopic variation observed was driven by differences in food assimilated between the sexes. Mean posterior diet estimates indicated that plant matter accounted for 72% [67–77 CI] of the annual diet of female grizzly bears, whereas animal matter contributed about 28% [23–33 CI]. Plant matter (67% [62–72 CI]) contributed a greater proportion to the annual diet of male grizzly bears than animal matter (33% [28–38 CI]).

Cortisol variation

Grizzly bear hair cortisol values ranged from 1.61 to 18.67 pg/mg (median = 6.19 ± 1.12 median absolute deviation [MAD]; Figure 3.1b) and did not differ between sexes

(p = 0.28, t = 1.11, df = 225). All models adequately fit the data (Bayesian p = 0.48−0.49). Two covariate models were better supported than the null model (Table 3.2). The top-ranked model included the effect of fruit production with moderate fruit production associated with a 1.65 pg/mg (24%) decrease in cortisol (β = 1.64, 95% CI = 1.46−1.83) compared to low (β = 1.92, 95% CI = 1.74−2.10) and 1.48 pg/mg (22%) decrease in cortisol compared to very low (β = 1.89, 95% CI = 0.74−2.04) years of fruit production (Table 3.3). However, among-individual variation accounted for a substantial proportion of the observed variance (σ = 0.89). The second ranked model (ΔDIC = 5.0) included GDD (β = 0.02, 95% CI = -0.20−0.24) and July temperature range (β = 0.03, 95% CI = -0.22−0.27), although credible intervals broadly overlapped zero. Among-individual variation again accounted for most of the observed variance (σ = 0.71). The null model was less supported with ΔDIC = 7.6; all other models ranked below the null.

Discussion

My hypothesis that hair cortisol levels would be inversely related to the proportional contribution of animal matter to the diet was not supported, although previous studies have shown a direct relationship between cortisol and diet in diverse taxa (e.g., seabirds: Kitaysky et al. 2007, non-human primates: Behie et al. 2010) including grizzly bears (von der Ohe et al. 2004, Wasser et al. 2004, Bryan et al. 2013ab). For example, Bryan et al. (2013, 2014a) found a weak but significant relationship between hair cortisol and the proportional contribution of salmon to the diet of grizzly bears from coastal British Columbia. However, the extent of intrapopulation diet variation required to detect a link between cortisol and diet is unknown and my analysis revealed little diet variation within sexes and only slight difference between sexes.

Because male grizzly bears are larger than females, slightly greater contributions of animal matter to the diet of male bears in this study may have been substantial enough to compensate for the physiological costs of maintaining larger body sizes (Welch et al. 1997, Rode et al. 2001).

Although I did not find a direct link between hair cortisol levels and diet, my results indicated that grizzly bears may exhibit a physiological stress response to changes in food availability across the landscape. My top model, for instance, suggested that population-level stress was lower during years of moderate fruit production compared to years of low and very low (i.e., crop failure) fruit production. As weather indices are a less direct measure of resource availability (Ayers et al. 2013), it was not surprising that the second ranked model included GDD and July temperature range, which account for a large proportion of inter-annual variation in fruit production near the study area (Kasworm et al. 2008, Holden et al. 2012, McCall et al. 2013). However, parameter estimates for GDD and July temperature range overlapped zero, suggesting no significant effects of these two weather metrics on cortisol levels. Bryan et al. (2013, 2014a) found that cortisol was higher in a coastal grizzly bear population in years after reduced salmon availability compared to years following greater salmon availability. In addition, Bourbonnais et al. (2013b) observed a distinct spatial pattern in grizzly bear hair cortisol levels that was linked to the abundance and spatial distribution of energy-rich, anthropogenic food. Moreover, earlier studies have shown that elevated cortisol levels can be an adaptive response to changes in food availability. For example, slight increases in cortisol can influence the amount and types of foods consumed (Epel et al. 2001). Moderately elevated cortisol levels can promote innovative foraging behaviors (Reader

2003), increase exploratory behavior (Reneerkens et al. 2002) and increase foraging efficiency by enhancing cognitive spatial memory (Pravosudov 2003). Thus, the slightly elevated cortisol levels I observed during years of very low and low fruit production may have played a role in minimizing nutritional stress.

Although human caused mortalities represent acute stress events and not prolonged exposure to stressors (Bourbonnais et al. 2013), activities associated with harvest efforts could result in small-scale perturbations over the hunting period (e.g., several days) regardless of whether a hunt is successful (Vaisfeld and Pazhetnov 1992, Kilgo et al. 1998, Swenson 1999). However, I found no evidence that hunter activities influenced cortisol levels. My results, in conjunction with McLellan (2005, 2011), suggest that grizzly bear harvest over the seven year study period did not result in increased population-level stress. I acknowledge that my index of hunter effort was likely conservative because all unsuccessful hunts might not have been reported and a hunting party was equal to a single individual. Furthermore, stress responses resultant from demographic disruption associated with harvest, particularly male-biased harvest (Wieglus and Brunnell 1994, Swenson 2003), may be more localized than what can be detected at a population-level.

Lack of differences in hair cortisol levels between female and male grizzly bears may be emerging as a pattern. For example, no differences in hair cortisol levels were detected between females and males in the aforementioned coastal non-invasively sampled population (Bryan et al. 2014a), in my study population, or in a live-captured population from Alberta (Macbeth et al. 2010). In a non-invasively sampled population in Alberta, Bourbonnais et al. (2013ab) also found no sex-based differences in hair cortisol

at the population-level. However, Bourbonnais et al. (2013ab) did find difference in the spatial distribution of cortisol levels for males and females, which suggests that females and males have different stressors (Bourbonnais et al. 2013b). If spatial differences are inherent in the long-term stress burdens experienced by females and males, understanding how these differences are linked to natural environmental processes and anthropogenic disturbances represents potential opportunities to enhance grizzly bear conservation and management and warrants further exploration.

Although physiological biomarkers such as cortisol derived from hair are increasingly used to index the long-term stress burden experienced by free-ranging wildlife to enhance population monitoring programs, lack of knowledge regarding the many factors that influence HPA activity limits our ability to interpret population-level patterns. Interestingly, hair cortisol values in my study (median = 6.2 pg/mg, range 1.6 to 18.7) were slightly lower than those reported in a non-invasively sampled coastal grizzly bear population with access to spawning salmon (median = 8.1 pg/mg, range 5.3 to 26.1; Bryan et al. 2014a) and higher than values reported in a live-captured population from Alberta (2.8 pg/mg, range 0.6 to 43.3, Macbeth et al. 2010). While this apparent decreasing trend in cortisol levels from west to east could be methodological, it is possible that among-population differences might relate to among-individual differences in resource use, resource availability, differences in social dynamics associated with population densities, genetics or differences in the ecosystem dynamics and human-mediated stressors (Bryan et al. 2014a). Higher cortisol levels in the coastal population relative to both interior populations is likely due to the social environment influencing the stress burden experienced by coastal grizzly bears. For example, coastal population

densities can exceed interior population densities by an order of magnitude (Miller et al. 1997, Mowat et al. 2006, Mowat et al. 2013) and because salmon are a spatially constrained resource, foraging on salmon may facilitate frequent social interactions (Egbert et al. 1976, Rode et al. 2006). Alternatively, interior grizzly bears forage on dispersed vegetative food sources that may limit social interactions, resulting in less social stress than coastal populations.

Despite substantial changes in annual weather as well as inter-annual variability in fruit production and anthropogenic activities across the seven year study, population-level stress was relatively consistent with the exception of decreased cortisol during years of moderate fruit production. Grizzly bears appear able to tolerate a broad range of environmental change without expressing a significant stress response at the population-level, at least within the range of environmental change during my study. However, extremes in the range of environmental conditions may manifest a more pronounced population-level stress response. Further analysis of hair samples across a longer time period that incorporates all levels of fruit production (i.e., huckleberry index 1−5) could provide greater insight into the possible effects of annual fluctuations in fruit production on grizzly bear stress burdens and possibly capture environmental conditions beyond what was observed during my study. An important note is that extensive among-individual variation in cortisol levels appeared to mask my ability to detect a population-level response to environmental change. I believe our understanding of stress physiology in grizzly bears would benefit from longitudinal studies that would enable the evaluation of within-individual variation in cortisol across years relative to environmental change. Similarly, evaluating the cortisol levels of grizzly bears of known age and breeding

condition would be advantageous as this may provide greater context for understanding among-individual variation in stress responses (Cattet et al. 2014). I also recommend future studies consider the spatial distribution of cortisol responses among individuals across a landscape, which have been shown to differ by sex, following the framework provided by Bourbonnais et al. (2013ab). Knowledge about the spatial distribution of differences in cortisol responses throughout a population may inform our understanding of stress across a heterogeneous landscape. Understanding the extent to which individual variation in cortisol levels contribute to measures of stress at population-levels is critical for applying and interpreting cortisol as a physiological biomarker to enhance long-term population monitoring programs for grizzly bears and other species of conservation concern.

Table 3.1 Stable isotope mixing models explaining grizzly bear (*Ursus arctos*) isotopic, southeastern British Columbia, Canada, 2005–2011.

Model[a]	*K*[b]	pD[c]	DIC[d]	ΔDIC[e]
Sex	4	125.78	1410.82	0
Null	2	129.44	1415.35	4.53
Sex + Year	6	147.88	1420.47	9.65
Year	4	137.87	1425.53	14.71

[a] Individual and process error were included as random effects in all models.
[b] Number of fixed effects.
[c] Effective number of parameters.
[d] Models ranked by Deviance Information Criterion.
[e] Distance from the top model.

Table 3.2 Candidate mixed models used to assess grizzly bear (*Ursus arctos*) cortisol levels, southeastern British Columbia, Canada, 2005−2011.

Model[a]	K[b]	pD[c]	DIC[d]	Δ DIC[e]	R^2
Berry Index	3	72.2	287.4	0	12
Growing degree day units + July temperature range	3	78.4	292.4	5.0	11
Null	1	81.2	295.0	7.6	11
Berry Index + Hunter	4	85.6	297.6	10.2	11
Growing degree day units + July temperature range + Hunter	4	84.3	297.9	10.5	10
Berry Index + $\delta^{15}N$	4	86.7	299.3	11.9	11
Growing degree day units + July temperature range + Hunter + $\delta^{15}N$	5	86.9	300.1	12.7	11
Hunter + $\delta^{15}N$	3	88.1	302.4	15.0	11
Meat	2	89.5	303.0	15.6	11
Growing degree day units + July temperature range + $\delta^{15}N$	4	91.6	304.7	17.3	11
Hunter	2	94.8	307.4	20.0	11

[a] Individual and year were included as random effects in all models.
[b] Number of fixed effects.
[c] Effective number of parameters.
[d] Models ranked by Deviance Information Criterion.
[e] Distance from the top model.

Table 3.3 Parameter estimates for models explaining cortisol levels in grizzly bears (*Ursus arctos*), southeastern British Columbia, Canada, 2005−2011.

Model 1	Mean	SD	95% CI
Berry index (very low)	1.89	0.08	1.74−2.04
Berry index (low)	1.92	0.09	1.74−2.10
Berry index (moderate)	1.64	0.10	1.46−1.83
Model 2 (ΔDIC = 5.0)			
Growing degree day units	0.02	0.11	-0.20−0.24
July temperature range	0.03	0.13	-0.22−0.27

Note: mean (natural log-transformed data), SD and 95% credible intervals for parameter estimates also shown.

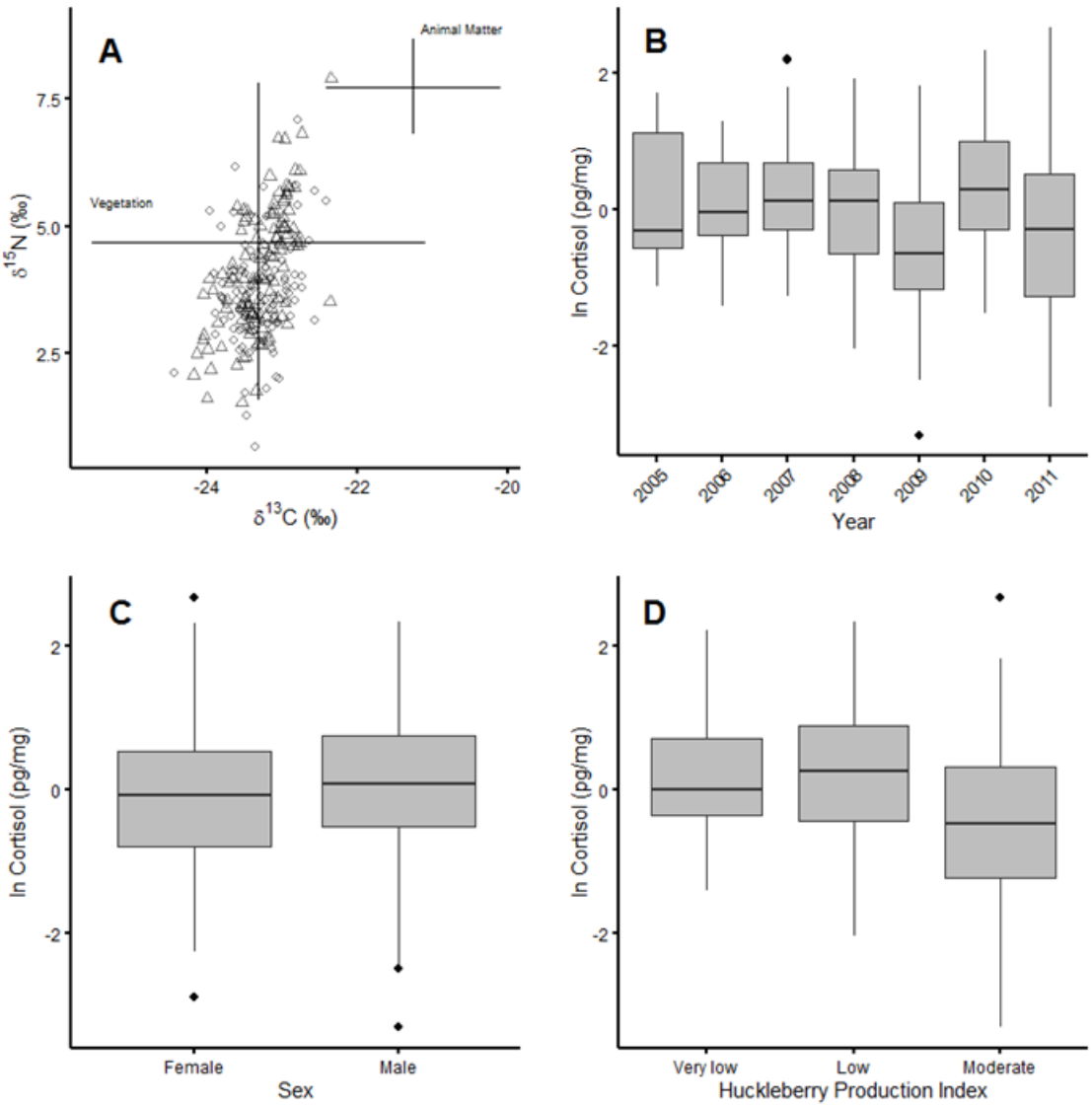

Figure 3.1 Grizzly bear (*Ursus arctos*) stable isotope bi-plot and cortisol plots by sex, year, and huckleberry production index, southeastern British Columbia, Canada, 2005−2011.

(A) Stable isotope bi-plot showing the distribution of grizzly bears in isotopic space relative to food resources; female (open circles) and male (open triangles); trophic discrimination factors were applied to food sources.; (B) median cortisol levels across years; (C) median cortisol by sex; (D) median cortisol relative to the fruit production index.

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CHAPTER IV

SEX, DIET, AND THE SOCIAL ENVIRONMENT: FACTORS INFLUENCING HAIR CORTISOL CONCENTRATION IN FREE-RANGING BLACK BEARS

Introduction

Understanding the physiological response of wildlife to their environment is fundamental to evolutionary biology, ecology and conservation. Arguably, one of the most important physiological responses by wildlife to environmental stimuli is activation of the hypothalamic-pituitary-adrenal (HPA) axis, which results in the release of stress hormones such as glucocorticoids (e.g., cortisol) (Sheriff et al. 2011). Activation of the HPA axis may occur in response to environmental challenges such as resource competition or in anticipation of seasonal environmental changes resulting in increased energetic demands (e.g., hyperphagia, reproduction, migration) (Dantzer et al. 2014). Moreover, short-term elevated cortisol levels can facilitate adaptive behavioral responses (e.g., fight or flight) (Wingfield et al. 1998, Creel et al. 2013) or shifts in life-history strategies (Wingfield et al. 1998, Boonstra et al. 2005), whereas chronic activation of the HPA axis can have deleterious effects including suppression of immune function, muscle wasting, weight loss, and the reduction or absence of reproduction (Boonstra and Singleton 1993, Moberg 1999, Creel et al. 2002, Charbonnel et al. 2008).

Increasingly, measures of cortisol are used to quantify past HPA activity to index psychological and physiological stress experienced by wildlife (Sheriff et al. 2011, Meyer

and Novak 2012, Dantzer et al. 2014). Although cortisol can be assayed from biological matrices including blood, urine and feces (Sheriff et al. 2011, Meyers and Novak 2012, Creel et al. 2013a), hair cortisol concentration (HCC) provides an integrative measure of past HPA activity over the growth period of the hair (e.g., weeks to months) (Sheriff et al. 2011, Russell et al. 2012). As such, hair provides a matrix in which to measure chronic stress rather than acute hormonal fluctuations, which are influenced by, for example, circadian rhythms or foraging activities (Bechshøft et al. 2012).

Myriad intrinsic and extrinsic factors as well as predictable and unpredictable environmental changes can influence stress responses in wildlife. For example, age, sex, social status and past experience can influence how animals respond to environmental challenges (Dantzer et al. 2014, Boonstra 2013, Creel et al. 2013a). In addition, the abundance and quality of food (Boonstra and Singleton 1993, Bryan et al. 2014), the social competitive environment within and between species (Bryan et al. 2014), extreme weather events (Romero and Wikelski 2010) and anthropogenic disturbances (Creel et al. 2013b) can influence HPA activity. These factors also may interact, confounding our ability to interpret HCC as a proxy to stress in wildlife (Dantzer et al. 2014). Understanding how different factors influence HCC is critical for interpreting observed HCC patterns in free-ranging wildlife populations and for advancing our knowledge about the ecological and evolutionary importance of intrapopulation variation in HCC throughout wildlife populations.

Using American black bears (*Ursus americanus*) as model species, I tested multiple hypotheses regarding the influence of diet, sex and the social environment on HCC in a free-ranging population that spanned adjoining ecoregions with differing

absolute and relative black bear and grizzly bear (*U. arctos*) densities. First I hypothesized that if a link exists between diet and HPA activity, then differences in HCC would be associated with dietary niche differences. For example, individuals or population segments (e.g., sex-class) with greater $\delta^{15}N$ values, which reflects foraging at a higher trophic level (e.g., eating animal matter), should have lower HCC because consumption of high-quality resources should confer a nutritional benefit resulting in lower nutritional stress (Bryan et al. 2013). Alternatively, if females and males have similar dietary niches, then males may exhibit higher overall HCC due to greater nutritional stress associated with the physiological constraints of maintaining a larger body size. However, if nutritional requirements are met across the range of dietary niches observed, then I would expect no differences in HCC associated with dietary niche differences.

Second, I hypothesized that female black bears, which are competitively subordinate to males, would exhibit greater among-individual variation in HCC due also to differences in reproductive states (i.e., with or without dependent young), which can influence female foraging behavior and social interactions (Ben-David et al. 2004) as well as physiological condition (Hilderbrand et al. 1999). Alternatively, my third hypothesis was that if the social competitive environment was an important driver of HCC in black bears, then differences in HCC would be associated with differences in black bear and grizzly bear densities across the study area, which would be most pronounced in male black bears. Specifically, male dominance hierarchies associated with breeding as well as potential variability in interactions with grizzly bears could result in higher and more variable HCC among males. For example, if interspecific

interactions with grizzly bears influences HCC among male black bears, I would expect male black bears in an ecoregion with higher grizzly bear density to have higher and more variable HCC. However, if intraspecific dominance hierarchies have a greater influences on black bear HCC, I would expect higher and more variable HCC in an ecoregion with higher black bear densities.

Materials and methods

I used an archived collection of black bear hair samples collected non-invasively during 30 May–2 August 2000 that were previously subjected to DNA analysis for species, sex and individual identification (Mowat et al. 2005). Hair samples represented a black bear population in central-eastern British Columbia, Canada (54°39'N, 122°36'W) that spanned two adjoining ecoregions: Parsnip Plateau (hereafter, plateau: 3,016 km^2) and Hart Ranges of the Rocky Mountains (hereafter, mountains: 6,436 km^2). Ecoregions varied in black bear and grizzly bear densities (mountains: 100 black/49 grizzly/1000 km^2; plateau: 257 black/17 grizzly/1000 km^2) and in the extent of anthropogenic disturbances (Mowat et al. 2005). For example, the plateau was subjected to industrial development, extensive transportation infrastructure including a major highway, human settlements, and widespread logging over the previous several decades. Anthropogenic disturbance in the mountain ecoregion was less pervasive because logging operations had been restricted to lower elevations and there were no permanent human settlements (Ciarnello 2006). In addition, both ecoregions had similar relative abundances of terrestrial prey (D. Heard, Ministry of the Environment, BC, pers. comm.), whereas Chinook salmon (*Oncorhynchus tshawytscha*) are only available in a small portion of the mountain ecoregion. From this archived black bear hair collection, I selected samples

representing 32 female and 32 male bears from each ecoregion (n = 128). Samples subsequently were subjected to stable carbon and nitrogen isotope analysis to estimate diet and for HCC analysis to assay past HPA activity. Because hair provides an integrative record of diet and HPA activity over the growth period of the hair, and because I selected mature guard hairs for analysis, I assumed hair samples represented diet and hormones assimilated in the previous year (i.e., 1999) during the hair growth period (Hilderbrand et al. 1996, Jones et al. 2006, Bryan et al. 2013, 2014). Standard laboratory procedures were employed for stable isotope analysis and for black bear hair cortisol extraction and immunoassay validation procedures (Appendix B).

Assessing intraspecific dietary niche variation

To assess intraspecific dietary niche variation between sexes and ecoregions, I used a multivariate Bayesian ellipse technique (Stable Isotope Bayesian Ellipses in R [SIBER]; Jackson et al. 2011). Standard ellipse areas (SEA) and small sample size corrected ellipses (SEAc) were estimated using approximately 40% of the bivariate isotope data that best explained covariance, and by an error term associated with each ellipse that was generated by resampling the bivariate data 10^6 times (Jackson et al. 2011). From the proportional outcome of repeated sampling, I generated 95% Bayesian credible intervals (CI) for each SEA_C, enabling me to compare SEAc sizes between sexes within and between ecoregions (Jackson et al. 2011).

I used generalized linear models to evaluate effects of sex, diet ($\delta^{13}C$ and $\delta^{15}N$) and ecoregion on black bear HCC. Ecoregion and sex were assigned as factor variables, $\delta^{13}C$ and $\delta^{15}N$ were included as continuous covariates and were not correlated (r = 0.03), and hair mass (mg) was included as a continuous covariate to account for potential

variation in HCC when extracting cortisol from hair of variable weight (D'Anna-Hernandez et al. 2011). I scaled $\delta^{13}C$, $\delta^{15}N$, and hair mass and regression assumptions were met by natural-log (ln) transforming the response variable, HCC. I ranked models using Akaike's Information Criterion with small sample correction (AICc) and considered models competing if ≤ 2 AICc from the top model (Burnham and Anderson 2002).

Results

I obtained stable isotope values and HCC from 116 individuals (29 female and 28 males from the mountain ecoregion, and 29 females and 30 males from the plateau ecoregion). Stable δ13C and δ15N values ranged from –25.6 to –22.8 (2.8‰) and 2.0 to 6.2 (4.2‰), respectively (Figure 4.1). The narrow range of black bear stable isotope values relative to generalized stable isotope values representing three potential major dietary components (Table B.1) reflected a predominately herbivorous diet (Figure 4.1).

Core dietary niches overlapped between sexes within and between ecoregions, although I found intrapopulation dietary niche differences (Figure 4.2a). Specifically, the size of the dietary niche of females from the mountain ecoregion (1.73‰^{2}) was greater than the size of the dietary niche of females (0.68‰^{2}; p = 0.008) and males (0.76‰^{2}; p = 0.003) from the plateau ecoregion (Figure 4.2b). Similarly, the size of the dietary niche of males from the mountain ecoregion (1.42‰^{2}) was greater than the size of the dietary niche of females (p = 0.020) and males (p = 0.016) from the plateau (Figure 4.2b) ecoregion. However, sizes of dietary niches did not differ between sexes within the mountain (p = 0.709) or plateau (p = 0.630) ecoregions (Figure 4.2b).

I had 16 competing models explaining observed HCC variation (Table 4.1) and I used model averaging to examine coefficients using 95% confidence limits (Table 4.2). Based on analysis of ln-transformed data, HCC varied by sex (Figure 4.3; untransformed data); males had 1.40 pg/mg (back-transformed) greater HCC levels than females, although sex had low explanatory power (adjusted $R^2 = 0.06$). Among females, HCC ranged from 0.6 to 10.7 pg/mg (untransformed median = 4.5 ± 1.2 mean absolute deviation [MAD]), whereas variation was greater among males (untransformed range = 0.5–35.1 pg/mg; median = 6.2 ± 2.6 MAD). However, among-individual HCC variation in females did not differ between the mountain and plateau ecoregions, nor did among-individual HCC variation in males differ between ecoregions. I also found a three-way interaction among sex, δ13C and ecoregion that had low explanatory power (adjusted $R^2 = 0.10$; Table 4.1, Figure B.3). For example, black bears inhabiting the plateau ecoregion had slightly enriched carbon values relative to black bears from the mountain ecoregion, whereas the diet of black bears in the mountains spanned a wider range f carbon values and male black bears, overall had higher and more variable HCC values than females.

Discussion

Throughout the population, male black bears generally exhibited higher HCC than females, a pattern also documented in a black bear population in coastal British Columbia, Canada, that co-occurs with grizzly bears (Bryan et al. 2014) as well as in humans (Feller et al. 2014). Recent studies that measured fecal cortisol levels in mammalian taxa, including red squirrel (*Tamiasciurus hudsonicus*) and African lions (*Panthera leo*), also have found that males typically have higher cortisol levels than females, and have suggested that multiple factors may contribute to observed sex-based

difference in stress hormone levels (Dantzer et al. 2010, 2014, Creel et al. 2013b). In my study, the composition and size of the dietary niches of black bears differed between ecoregions, likely a consequence of a greater range in plant δ13C values along elevation gradients in the mountain ecoregion compared to the plateau (Van de Water et al. 2002). However, diet and the sizes of dietary niches of females and males within ecoregions did not differ, although males exhibited higher HCC than females, possibly due to nutritional stress associated with the physiological constraints of maintaining a larger body size than females on a predominantly plant-based diet. As such, I was unable to reject my hypothesis that diet was influencing black bear HCC.

My second hypothesis, which stated that females would exhibit greater among-individual variability in HCC due to differences in reproductive states was not supported. Male bears not only had higher mean HCC but also greater among-individual HCC variation. In addition, my third hypothesis, which posited that among-individual HCC variation in black bears, particularly males, may be driven by the social competitive environment associated with differences in black bear and grizzly bear densities across the study area also was not supported. For example, despite marked differences in black bear and grizzly bear densities between ecoregions, I found no differences in the mean or variance of HCC between males (or between females) from the two ecoregions. If interspecific social interactions with brown bears was an important factor in black bear HCC (Bryan et al. 2014), I expected to find higher HCC and greater among-individual HCC variation, particularly among males, in the mountain ecoregion where grizzly bear density was much higher and there were fewer trees to provide escape cover for black bears. Similarly, if intraspecific dominance hierarchies were influencing HCC and

driving greater among-individual HCC variation among black bears due to agonistic behaviour between males associated with breeding, then I expected to find higher and more variable HCC among black bears on the plateau where black bear density was quite high (Mowat et al. 2005).

A limitation of my study was the absence of behavioral observations regarding social dominance hierarchies within the population that are likely driven by differences in sex-age class and body size (Chi 1999). Recent studies have demonstrated a link between social rank and stress hormone levels with basal cortisol levels in social mammals including non-human primates, carnivores, and ungulates often highest in dominant individuals (Creel et al. 1996, 1997, Barrett et al. 2002, Mooring et al. 2006, Koren et al. 2008, but see Sapolsky 1993). As black bears exhibit social dominance hierarchies and adult males are the dominant social class within a black bear population (Chi 1999), male black bears may have had higher and more variable HCC due to social dominance relationships that could not be assessed with my data.

Another factor that might contribute to higher and more variable HCC among male black bears is that they may be more sensitive to human disturbances than females. From a meta-analysis comprising four vertebrate classes (i.e., Amphibia, Aves, Mammalia, Reptilia), Dantzer et al. (2014) suggested that males of a species may be more sensitive overall to human activities than females, regardless of disturbance type. As both mountain and plateau ecoregions were subject to human disturbances during the sampling period, males may have been disproportionally affected, and thus mounted a stronger stress response than females resulting in higher and possibly more variable HCC.

My results, particularly the finding of an interaction effect among sex, δ13C and ecoregion, support previous assertions that observed differences between sexes in HCC are likely a consequence of multiple interacting factors. For example, Bryan et al. (2014) found no evidence that grizzly bear density influenced black bear HCC directly, although HCC in black bears was affected by salmon availability, which was thought to mediate food resource competition between black bears and grizzly bears. Belant et al. (2006) found that grizzly bears displaced female black bears from high-quality habitats containing salmon spawning streams and suggested that where black bears are sympatric with grizzly bears, black bears can reduce interspecific competition and meet their nutritional requirements by consuming a diet dominated by vegetation. Lafferty et al. (2015) subsequently found that black bears in a population inhabiting the Denali region of Alaska achieved similar percentage body fat, which is perhaps a higher-order index of fitness than HCC (Macbeth et al. 2012, Ayers et al. 2013), across the range of food resources consumed, indicating that black bears that co-occur with grizzly bears can achieve their nutritional needs on a predominantly herbivorous diet. As such, higher and more variable HCC in male black bears is likely a result of variation in the nutritional needs of individuals as well as the social environment.

The study of HCC for applications in wildlife health and conservation physiology is in its infancy with existing methods requiring additional testing (see Sheriff et al. 2011, Meyer and Novak 2012, Cattet et al. 2014, Dantzer et al. 2014). For instance, I excluded 12 samples from statistical analyses due to HCC detection issues arising from low mass hair samples. However, mounting evidence suggests that as a retrospective biomarker of endocrine activity in wildlife, including four species of bears (Bechshøft et al. 2012;

Macbeth et al. 2010, 2012, Bryan et al. 2013, 2014, Malcolm et al. 2013, Cattet et al. 2014) measures of HCC can provide meaningful insight into the long-term physiological responses of individuals to their environment. Greater understanding of observed HCC patterns in free-ranging wildlife can help build our understanding of the eco-evolutionary significance of intrapopulation differences in HCC, which also can inform conservation and management planning. However, for HCC to be a useful tool, factors that contribute to intrapopulation variability in HCC must be identified. As such, in addition to measures of diet, sex and densities of conspecific and heterospecific competitors, future studies would benefit from incorporating measures of reproductive condition (e.g., testosterone, estradiol), identifying presence of young and age data. Moreover, studies that evaluate linkages between HCC and measures of fitness (e.g., survival, reproduction) in wildlife would enhance the utility of HCC as a conservation tool. Macbeth et al. (2012) found preliminary evidence that HCC in polar bears (U. maritimus) was inversely related to measures of growth (i.e., length, mass, body condition index [BCI; Cattet et al. 2002]) and previous research provides evidence of a direct relationship between growth and fitness in polar bears (Atkinson and Ramsey 1995, Derocher and Stirling 1996, Derocher 2010). However, few studies have linked HCC in mammals to measures of fitness. Thus, I contend that once the drivers of intrapopulation differences in HCC and how HCC is related to measures of fitness is understood, HCC will have tremendous potential to inform our understanding of the physiological stress burden experienced by wildlife due to diverse environmental challenges and the eco-evolutionary consequences of that stress burden to individual and population-level well-being.

Table 4.1 Linear models for explaining black bear (*Ursus americanus*) hair cortisol concentration, Parsnip Plateau and Hart Ranges of the Canadian Rocky Mountains, British Columbia, Canada, 1999.

Model[a]	k [b]	AICc [c]	ΔAIC	LL [d]	Wt [e]	Adj. R^2 [f]
sex	3	235.30	0.00	-114.54	0.11	0.06
sex + $\delta^{13}C$	4	235.33	0.03	-113.48	0.10	0.07
sex + $\delta^{13}C$ * ecoregion	6	235.60	0.31	-111.42	0.09	0.08
sex + $\delta^{13}C$ + $\delta^{15}N$	5	236.17	0.87	-112.81	0.07	0.07
sex + $\delta^{15}N$	4	236.19	0.90	-113.92	0.07	0.06
sex + ecoregion	4	236.28	0.98	-113.96	0.07	0.06
sex * $\delta^{13}C$	5	236.38	1.09	-112.92	0.06	0.07
sex + $\delta^{13}C$ + hair mass	5	236.46	1.16	-112.96	0.06	0.07
sex + hair mass	4	236.65	1.35	-114.14	0.05	0.05
sex + $\delta^{13}C$ * ecoregion + $\delta^{15}N$	7	236.70	1.41	-110.83	0.05	0.08
sex * $\delta^{13}C$ + $\delta^{13}C$ * ecoregion	7	236.90	1.60	-110.93	0.05	0.08
sex + $\delta^{13}C$ * ecoregion + hair mass	7	236.97	1.67	-110.97	0.05	0.08
sex + ecoregion + $\delta^{15}N$	5	236.98	1.68	-113.22	0.05	0.06
sex * $\delta^{13}C$ + hair mass	6	237.01	1.72	-112.12	0.05	0.07
sex * $\delta^{13}C$ * ecoregion	9	237.17	1.87	-108.73	0.04	0.10
sex + $\delta^{13}C$ + ecoregion	5	237.25	1.95	-113.35	0.04	0.06
null	1	240.92	5.62	-118.40	0.00	0.00

[a] Models with interaction terms also include main effects.
[b] The number of parameters.
[c] All competing models are shown and are ranked in ascending order by Akaike's information criterion (AIC) adjusted for small sample size.
[d] maximum log likelihood.
[e] Model weight.
[f] Measure of model fit for each model.

Table 4.2 Model averaged coefficients for parameters in competitive models explaining cortisol levels in black bears (*Ursus americanus*), Parsnip Plateau and Hart Ranges of the Canadian Rocky Mountains, British Columbia, Canada, 1999.

			95% confidence limits	
Parameter	Estimate	SE	Lower	Upper
Intercept	1.44	0.10	1.23	1.64
$\delta^{13}C$	-0.13	0.09	-0.30	0.04
sex (male)[a]	0.34	0.13	0.08	0.61
ecoregion (plateau)[b]	-0.10	0.15	-0.40	0.19
$\delta^{13}C$ * ecoregion (plateau)[b]	0.23	0.19	-0.14	0.61
$\delta^{15}N$	0.07	0.06	-0.05	0.19
sex (male)[a] * $\delta^{13}C$	0.09	0.16	-0.22	0.41
hair mass	-0.06	0.07	-0.20	0.06
sex (male)[a] * ecoregion (plateau)[b]	-0.12	0.28	-0.67	0.43
sex * $\delta^{13}C$ * ecoregion (plateau)[b]	0.60	0.30	0.02	1.19

Note: also shown are parameter estimate SE and 95% confidence limits for competitive models (ΔAICc < 2) explaining cortisol levels.

[a] Female is the reference group.

[b] Mountain ecoregion is the reference group.

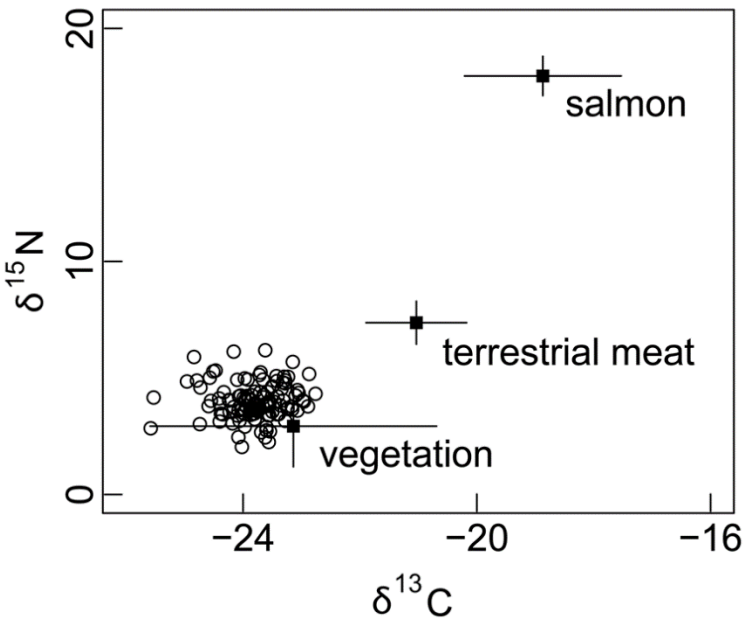

Figure 4.1 Black bear (*Ursus americanus*) $\delta^{13}C$ and $\delta^{15}N$ values, Parsnip Plateau and Hart Ranges of the Rocky Mountains, British Columbia, Canada, 1999.

Black bear (n = 116); also shown are mean $\delta^{13}C$ and $\delta^{15}N$ (± SD) for three primary food categories available in the study area. Trophic discrimination factors were applied to each food category.

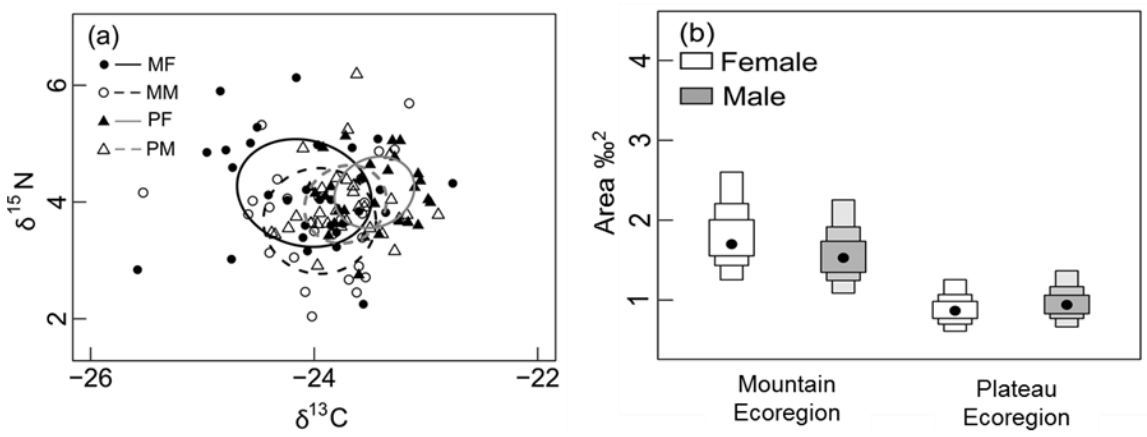

Figure 4.2 Black bear (*Ursus americanus*) isotopic niches and corresponding density plots, Parsnip Plateau and Hart Ranges of the Rocky Mountains, British Columbia, Canada, 1999.

(a) Standard ellipse areas corrected for small sample size (SEAc), representing the black bear core (40%) dietary niches. Dietary niches of females (MF) and males (MM) from the mountain ecoregion and females (PF) and males (PM) from the plateau ecoregion. (b) Density plot representing the posterior probability distribution of SEAc sizes Black dots correspond to means and decreasing bar widths represent 50%, 75% and 95% Bayesian credible intervals.

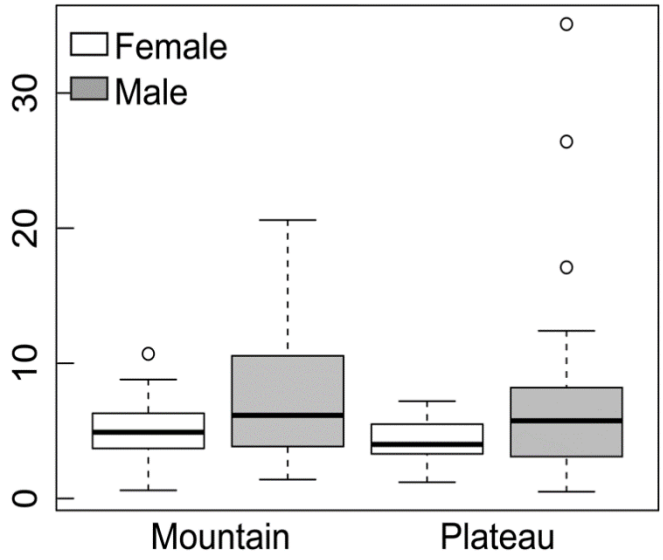

Figure 4.3 Median cortisol concentration from black bear (*Ursus americanus*) guard hair samples, Parsnip Plateau and Hart Ranges of the Rocky Mountains, British Columbia, Canada, 1999.

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CHAPTER V

GENETIC POPULATION STRUCTURE IN BROWN BEARS LINKED TO ECOLOGICAL DIVERGENCE ACROSS A COASTAL–INTERIOR TRANSITION ZONE

Introduction

The hypothesis that ecological niche variation can foster genetic differentiation without the need for physical separation or the cessation of interbreeding relates to a fundamental debate on the mechanisms that create biological diversity. There is mounting evidence that ecological divergence can lead to population genetic structure in regions where environmental changes are gradual and gene flow is uninhibited by geographical barriers (Doebeli and Dieckmann 2003, Pilot et al. 2006). As such, populations that span ecological transition zones are candidates for studying the role of ecological niche variation in generating and maintaining genetic population structure.

Genetic population structure linked to ecological niche differences has been observed in in diverse taxa, including large, wide-ranging mammals (Shafer and Wolf 2013). For example, ecological factors (e.g., hunting behavior, diet, habitat use) can influence gene flow and genetic population structure in wolves (*Canis lupus*) (Carmichael et al. 2001, 2007, Pilot et al. 2006, Musiani et al. 2007,), killer whales (*Orcinus orca*) (Hoelzel et al. 1998), mule deer (*Odocoileus hemionus*) (Pease et al. 2009), and North American lynx (*Lynx Canadensis*) (Rueness et al. 2003).

Along portions of the Pacific coast of North America, the range of brown bears (*Ursus arctos*) spans a coastal–interior ecological transition zone, separating the 'brown bears' of the coast from interior 'grizzly bears'. While traditionally recognized as subspecies (*U. a. middendorffi* on the coast, *U. a. horribilis* in the interior) (Kurtaen 1973), this classification is not supported by population genetic data (Paetkau et al. 1998). Gene flow among isolated pockets of brown bears along coastal Alaska appears to pass through the interior grizzly bear populations of British Columbia and Yukon, where geographic barriers to movement are less severe (Paetkau et al 1998). The morphological similarities among coastal populations are presumed to reflect convergence driven by common ecological forces, rather than the existence of a cohesive evolutionary group suitable for taxonomic recognition (Paetkau et al. 1998).

Morphological and ecological differences across this coastal–interior ecological transition zone can be dramatic. Mean adult body mass in coastal populations can easily double those of nearby interior populations (Hilderbrand et al. 1999a), while population densities in more productive coastal ecosystems can exceed interior population densities by an order of magnitude (Miller et al. 1997, Mowat et al. 2005, Mowat et al. 2013b). A plausible driver of observed ecological differences between coastal and interior brown bear populations is the availability of spawning salmon (*Oncorhynchus* spp.), an important food source in coastal regions (Hilderbrand et al. 1999ab). By contrast, the diet of interior grizzly bears is generally dominated by plant matter (Miller et al. 1997, Mowat and Heard 2006, Mowat et al. 2013).

I sampled brown bears across a coastal–interior ecological transition zone, and used genetic analysis, stable isotope analysis and isotopic niche modeling to investigate

the occurrence of, and association between, genetic and niche divergence. As anadromous salmon enter fresh water river systems, salmon availability to bears occurs along a coastal–inland gradient, but also across finer-scale gradients in elevation, creating a matrix of strata with and without access to spawning salmon. Because topographical features across this coastal−interior transition zone are not expected to inhibit brown bear movement, I hypothesized that any marked population genetic structure would be linked to ecological differentiation between salmon-eating 'brown bears' and non-salmon-eating, predominantly plant-supported 'grizzly bears'.

Field and laboratory methods

Study area

My study area (Figure 5.1), the upper Stikine watershed (57°07'N, 131°27'W), covered about 10,119 km^2 in northwestern British Columbia, a transition zone between coastal and interior climate regimes. This region is characterized by rugged topography including glaciers with elevations ranging from 30 to 1828 m, multiple watersheds, and two ecologically relevant strata: marine and terrestrial. The marine strata encompasses streams that contain marine-derived resources including five species of Pacific salmon that spawn in streams from early summer through late fall, as well as harbor seals (*Phoca vitulina*), whereas the terrestrial strata lacks salmon spawning streams but supports abundant huckleberry (*Vaccinium membranaceum*). Both strata contain abundant plant-based foods including grasses (*Elymus* spp.), sedges (*Carex* spp.) and horsetails (*Equisetum* spp.), and animal-based foods such as moose (*Alces alces*), mountain goats (*Oreamnos americanus*) and marmots (*Marmota caligata*).

Sample collection

Hair traps consisting of a single strand of barbed-wire surrounding a scent lure were distributed systematically throughout the marine and terrestrial strata to collect hair samples from bears. In 2004, samples were collected from late-July and early-September and I used only these samples for isotope analysis. During 2005, samples were collected from late May to early October and these samples were used to examine movements of individual bears in this study (G. Mowat, *unpublished data*). Although brown bears begin molting in May and new hair may begin growing soon thereafter (Felicetti et al. 2003), mature guard hairs may be present throughout the year (B. McLellan, *pers. comm.*). To reduce potential bias that might be introduced by using immature guard hairs that represent a relatively short time frame, the longest (> 10 cm) guard hairs were selected for estimating annual assimilated diet whenever possible; I assumed these samples represented bear diet over the entire previous year's growing season. Evidence to support this assumption was demonstrated by an analysis of individual diet relative to hair length in brown bears from my study system (G. Mowat, *unpublished data*).

Genetic and stable isotope analysis

From brown bear hair follicles, DNA was extracted using DNeasy Blood and Tissue Kits (QIAGEN Inc., Mississauga, Ontario, Canada). Using the quality assurance protocols of Paetkau (2003) and Kendall et al. (2009), samples were analyzed at 6 microsatellite markers to establish individual identity in the context of an abundance estimation project (Mowat et al. 2013). One representative sample from each individual was selected to extend the genotype to a set of 15 microsatellite markers for population genetic analyses. These 15 markers, and the methods used to analyze them, were used

previously to assess population structure across British Columbia (Proctor et al. 2012). Additionally, an amelogenin marker was used for sex determination (Ennis and Gallagher 1994).

For stable isotope analysis, hair follicles were removed from whole guard hairs and hairs subsequently were washed for two hours in a 2:1 chloroform and methanol solution at room temperature to remove surface debris, rinsed four times with ultrapure water, and air dried at room temperature for at least 72 hours. Hair samples were analyzed for carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios at the University of British Columbia-Okanagan, Kelowna, Canada and for sulfur ($^{34}S/^{33}S$) stable isotope ratios at the United States Geological Survey Laboratory in Boulder, Colorado. I report isotopic signatures in delta (δ) notation such that $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$ ratios of the sample and standard, respectively. The standards are PeeDee Belemnite limestone for carbon, atmospheric N_2 for nitrogen, and Vienna Canõn Diablo meteorite troilite for sulfur.

Analytical techniques

Population structure analyses

To assess population genetic structure among the sampled individuals (n = 117), I used the Bayesian model-based clustering algorithm in program Structure v. 2.3.3 (Pritchard et al. 2000), which uses multilocus genotype data to estimate the proportion of each individual's ancestry that derives from each of K populations, without reference to geographic origin. To ensure that both larger-bodied, salmon-eating bears and smaller-bodied, non-salmon-eating bears were represented with adequate sample sizes in the

analysis, training data from adjacent areas where larger-bodied coastal brown bears and smaller-bodied interior grizzly bears had been genotyped previously were included in the analysis. Training data consisted of 14 genotypes from the lower Stikine watershed in Alaska, USA (R. Flynn, Alaska Department of Fish and Game, *unpublished data*) and 29 interior genotypes from the Spatsizi Plateau, British Columbia, Canada (Proctor et al. 2012). Fifteen genotypes from Admiralty Island, Alaska, USA also were included as an outgroup (Paetkau et al. 1998). Inference was based on 10^6 Markov chain Monte Carlo (MCMC) iterations, after discarding the initial 50,000 iterations as burn-in. *K* was allowed to vary from 1 to 4, and a total of 10 independent simulations were run for each *K*. Without using population information, I used an admixture model with correlated allele frequencies (Falush et al. 2003) and I assessed *K* based on the greatest change in log-likelihood [L(*K*)] value (Evanno et al. 2005), and also by relevance to my study questions.

Having grouped individuals into the Structure-defined populations to which the largest portion of their ancestry was assigned, I used program GENEPOP (v. 3.4; Raymond and Rousset 1995) to test for non-random associations of alleles within (Hardy-Weinberg Equilibrium [HWE]) and between ('linkage disequilibrium') loci, which is expected in the presence of population structure. I also tested the significance and magnitude (F_{ST}) of allele frequency differences between the *K* populations defined using Structure (Pritchard et al. 2000).

Estimating diet and assessing isotopic structure throughout the population

Using brown bears sampled during 2004 (n = 89), I modeled isotopic variation throughout the population to estimate diet via the Bayesian stable isotope mixing model

MixSIAR (Stock and Semmens 2013). I included $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope values for individual brown bears as well as mean $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ stable isotope values, standard deviations, and trophic discrimination factors for each food category (i.e., plant matter, terrestrial meat, salmon) (Table C.1–C.3). See Appendix C for detailed data used for diet estimation.

I created a candidate set of 10 models to examine how isotopic variation was structured throughout the population. I modeled strata and sex as fixed effects and explored the integration of ancestry as both a continuous covariate (Francis et al. 2011) and as a categorical fixed effect. Strata was putatively assigned as 'marine' or 'terrestrial' based on hair capture location, proximity to salmon streams during spawning season, and when available, movements of individuals based on detection data from both 2004 and 2005. Thus, strata indexed whether an individual had access to salmon, which was independent of diet analysis. Ancestry was included as a continuous covariate based on the proportion of ancestry of each individual ascribed to one of the genetic clusters identified using program Structure (i.e., proportion interior ancestry ranging from 0 to 1.0), or as a categorical fixed effect (e.g., > 50% interior ancestry = interior). The purpose of evaluating ancestry as a continuous covariate and categorical fixed effect was to examine whether ancestry measured at a fine (i.e., continuous) or coarse (i.e., categorical) resolution better informed our understanding of how isotopic variation was structured throughout the population. I also included a random effect for individual in all models as well as a term for process error (see Moore and Semmens 2008). To avoid model overparameterizing, I ran models with each fixed and continuous effect individually and

all two-way combinations of fixed and continuous effects, resulting in 10 candidate models (Table 5.1).

I used deviance information criterion (DIC) to evaluate which models were most supported with the most parsimonious model having the lowest DIC value (Speigelhalter et al. 2002). I specified conventional uninformative priors for all model parameters (Stock and Semmens 2013). I ran models on three parallel MCMC chains with JAGS (Plummer 2013) for 160,000 iterations, burning the first 10,000 iterations and thinning by 10. I assessed convergence by visually inspecting trace plots and with the Gelman-Rubin diagnostic ($\hat{R} < 1.05$ indicating convergence; Gelman and Rubin 1992).

Quantifying ecological divergence between genetic clusters

I estimated niche widths of identified genetic clusters using a multivariate Bayesian ellipse-based technique (Stable Isotope Bayesian Ellipses in R [SIBER]; Jackson et al. 2011). For each genetic group I estimated the standard ellipse area (SEA) and a corrected measure for small sample size (SEA_C) associated with each bivariate isotopic pair ($\delta^{13}C$:$\delta^{15}N$, $\delta^{13}C$:$\delta^{34}S$, $\delta^{15}N$:$\delta^{34}S$). To represent the core isotopic niche of each genetic cluster, which is robust to differences in sample sizes and potential outliers, I defined SEA_C using approximately 40% of the bivariate isotope data that best explained covariance, and by an error term associated with each SEA_C that was generated by resampling the bivariate data 10^6 times. From the proportional outcome of repeated sampling, I generated 95% Bayesian credible intervals (CI) for each SEA_C for each isotopic pair within each genetic group, enabling us to compare the sizes of each SEA_C and assess isotopic divergence between genetic clusters.

Results

Population structure

Cluster analysis using Structure revealed improved model fit at $K = 2$ (Δ L[K] = 245), with brown bears from Admiralty Island assigned a mean of 96% ancestry in one cluster, while mainland brown bears were assigned a mean of 58% ancestry in the second cluster. At $K = 3$ (Δ L[K] = 169), Admiralty Island brown bears had 93% (98% excluding 1 apparent hybrid) of their ancestry assigned to one 'population', to which just 3% of the ancestry of mainland bears was assigned (with 2 apparent hybrids contributing disproportionately to that mean). The remaining 97% of the ancestry of mainland bears was bimodally apportioned between the other 2 populations (Table 5.1). At $K \geq 4$ (Δ L[K] = 21), the ancestry of mainland bears was apportioned such that all individuals were effectively hybrids, indicating that $K < 4$ in my study system.

Because my interest was in whether there was evidence of population genetic differentiation between 'brown bears' and 'grizzly bears' in the upper Stikine watershed, I used the best (i.e., greatest likelihood) of 10 runs at $K = 3$ (i.e., Admiralty brown bears, mainland brown bears and grizzly bears) for my estimates of the ancestry of the 160 mainland bears, excluding the 15 Admiralty Island bears from subsequent analyses. This sample included 117 brown bears from my study area (i.e., upper Stikine watershed, BC, Canada) and the training data from the lower Stikine watershed in Alaska, USA (R. Flynn, *unpublished data*; $n = 14$) and from the Spatsizi Plateau Wilderness Provincial Park, BC, Canada (Proctor et al. 2012, $n = 29$). The 29 grizzly bears from the Spatsizi Plateau were assigned a mean of 86% ancestry in one population, which I defined as 'interior ancestry' (Table 5.1). Fifty brown bears from my study area were assigned >

50% interior ancestry. The ancestry of the14 lower Stikine watershed brown bears in the training set was apportioned 2% to Admiralty ancestry, 12% interior ancestry, and 86% ancestry in the third population, which I defined as 'coastal ancestry'. Sixty-seven brown bears from my study population were assigned > 50% coastal ancestry, whereas 10% of the ancestry of the Spatsizi Plateau bears was assigned to the coastal cluster. The test for population differentiation between individuals from my study area assigned > 50% interior ancestry and those assigned > 50% coastal ancestry differed for 10 of 15 markers ($p < 0.001$) (Figure 5.2), consistent with the strongly bimodal clustering of individuals by estimated ancestry. Estimated F_{ST} between these groups was 0.046.

Isotopic structure and diet estimation

Brown bears showed considerable isotopic variation (Figure 5.3). All models converged to the posterior distribution and three models were ranked above the null model (Table 5.2). The top model, in which strata was the only fixed effect, suggested that most isotopic variation was driven by dietary differences between bears inhabiting different strata. Mean posterior dietary estimates for brown bears inhabiting the marine strata were 63% vegetation (CI = 44–91%), 19% salmon (CI = 9–31%) and 18% terrestrial meat (CI = 5–34%). For brown bears inhabiting the terrestrial strata, vegetation (94% [CI = 91–99%]) also dominated the diet but terrestrial meat (5% [CI = 0–18%]) and salmon (1% [CI = 1–2%]) contributed little (Table 5.3). Although there is less certainty in lower-ranked models, by accounting for strata and sex (2nd ranked model), males in the marine strata consumed 1.7 times more salmon than females, whereas, by accounting by strata and ancestry (3rd ranked model), the estimated proportion of salmon to the diet of

brown bears of coastal ancestry occupying marine strata was 2.5 times greater than for brown bears of interior ancestry occupying marine strata.

Ecological divergence between genetic clusters

The SEA_C representing the core bivariate isotopic niches of each genetic cluster indicated that the sizes of the isotopic niches of brown bears from the coastal and interior genetic clusters did not differ ($\delta^{13}C$:$\delta^{15}N$ [$p = 0.28$], $\delta^{13}C$:$\delta^{34}S$ [$p = 0.76$], $\delta^{15}N$:$\delta^{34}S$ [$p = 0.32$]; Figure 5.4). I also found evidence of extensive isotopic niche overlap between coastal and interior genetic clusters for all three bivariate isotope pairs (Figure 5.4). However, by accounting for strata, evidence of a link between ancestry and isotopic niche emerged despite some overlap between brown bears of different ancestry occupying the same strata type. Specifically, the size of the SEA_C representing the core $\delta^{13}C$:$\delta^{15}N$ isotopic niche of brown bears occupying marine strata with coastal ancestry (SEA_C = 10.06‰^{2}) was larger ($p = 0.01$; 2.4 times larger) compared to brown bears occupying marine strata with interior ancestry (SEA_C = 4.17‰^{2}) (Figure 5.4). Similarly, for brown bears occupying terrestrial strata the size of the SEA_C representing the core $\delta^{13}C$:$\delta^{15}N$ isotopic niche of brown bears with interior ancestry (SEA_C = 3.73‰^{2}) was larger ($p = 0.05$; 1.8 times larger) compared to brown bears with coastal ancestry (SEA_C = 2.06‰^{2}) (Figure 5.4). In contrast, the sizes of SEA_C representing the core $\delta^{13}C$:$\delta^{34}S$ isotopic niche for brown bears of coastal (37.71‰^{2}) and interior (37.20‰^{2}) ancestry occupying marine strata did not differ ($p = 0.54$) nor did the sizes of SEA_C representing the core $\delta^{13}C$:$\delta^{34}S$ isotopic niches for brown bears of coastal (7.44‰^{2}) or interior (11.83‰^{2}) ancestry occupying interior strata ($p = 0.10$). Similarly, the sizes of SEA_C representing the core $\delta^{15}N$:$\delta^{34}S$ isotopic niche for brown bears of coastal (59.25‰^{2}) and interior (71.43‰^{2})

ancestry occupying marine strata did not differ ($p = 0.28$) nor did the sizes of SEA_C representing the core $\delta^{13}C$:$\delta^{34}S$ isotopic niche for brown bears of coastal (5.61‰^{2}) or interior (5.74‰^{2}) ancestry occupying interior strata ($p = 0.49$).

Discussion

The population of brown bears inhabiting the coastal–interior ecological transition zone consisted of two well defined genetic groups, one associated with coastal brown bears (i.e., lower Stikine watershed, AK) and the other with interior grizzly bears (Spatsizi Plateau, British Columbia). Genetic differentiation between these groups is remarkable, in terms of the bimodality of individual ancestry estimates and in population-level variance in allele frequencies ($F_{ST} = 0.05$). Proctor et al. (2012) observed $F_{ST} \approx 0.02$ between the Spatzisi reference group and study areas centered 300 to 400 km north (Nahanni) and south (Skeena North), respectively, whereas my study area was less than 100 km wide. Although $F_{ST} > 0.10$ has been observed between neighboring populations separated by anthropogenic disturbance zones (Proctor et al. 2012) or breadths of ocean several km wide (Paetkau et al. 1989), there are no natural or anthropogenic barriers to explain the substantial genetic structure observed within my study area.

As expected, I observed extensive variation in isotopic values, and thus in the estimated proportional contributions of major food categories to diet. That strata as the best predictor of salmon in the diet was unsurprising given that strata classification was based on proximity to salmon spawning streams. Brown bears sampled in marine strata had greater among-individual isotopic variation than those in terrestrial strata, possibly due to a greater combination of potential foods associated with the availability of salmon and subsequent sex-based dietary differences. On average, females consumed less salmon

than males, which may be evidence of a tradeoff between access to high-quality food and avoidance of infanticidal males (e.g., Ben-David et al. 2004). Annual measures of diet may also underestimate some food categories based on the timing of hair growth and sample collection. For instance, because brown bears begin molting in May it is possible that some hair samples represented the current year's hair growth and diet (May−collection date) rather than the entire previous year's diet; although this likely had little effect on my results because I selected guard hairs > 10 cm in length whenever possible to ensure capturing the previous year's diet (G. Mowat, *unpublished data*). However, because both marine and terrestrial strata were sampled simultaneously, any shortcomings in diet estimation methodology were consistent throughout the population and would account for the observed isotopic differences or estimated dietary differences between strata.

Brown bears whose ancestry corresponded with their strata (e.g., marine strata and coastal ancestry) accessed a wider range of foods than those whose ancestry and strata were mismatched (e.g., a bear of interior ancestry in marine strata). For instance, whether inhabiting marine or terrestrial strata, segments of the population whose ancestry and strata are mismatched exhibited smaller niches, indicating a more constrained diet than brown bears whose ancestry and strata are matched. However, the mechanisms and implications of the documented genetic subdivision, and the relationship between genetic subdivision and niche width, are unclear. A simple explanation might be that body size reduces the capacity of brown bears to make a living in mismatched strata (Welch et al. 1997, Rode et al. 2001). For example, small grizzly bears of interior ancestry might be unable to compete for salmon due to the physical threat posed by larger-bodied coastal

bears (Egbert et al. 1976, Gende et al. 2004). At the same time, the energetic cost of wide-ranging foraging, which is needed to fully exploit dispersed interior food resources, may confer a competitive advantage to smaller individuals, or render some resources energetically unprofitable for larger individuals (Welch et al. 1997, Rode et al. 2001).

The observed bimodal distribution of ancestries is at odds with the integrated distribution of strata across the study area, indicating that mating is limited between ecotypes. Population genetic theory suggests that even a few matings between ecotypes per generation would reduce F_{ST} below 0.05 (Wright 1931), and those matings could occur anywhere in the regional interface between ecosystems, not just in the region I sampled. The implication is that the overwhelming majority of matings are within-ecotype. The simplest explanation for this mating bias would be that body size encourages brown bears to occupy the strata to which their body size is best suited for exploiting food resources within the constraints of intraspecific competition (Welch et al. 1997, Rode et al. 2001), thereby reducing encounter rates, and thus mating opportunities between ecotypes (Willis et al. 2011). In addition, considerations of physical security or mate choice might encourage size-assortative mating (Baldauf et al. 2009), reinforcing the experiential and heritable components of niche adaptation. Irrespective, my data suggest that two ecotypes of brown bears occur in this coastal-interior transition zone, resulting in larger-bodied salmon-eating bears co-occurring with smaller-bodied bears that predominantly plant resources, and that these two ecotypes rarely interbreed.

If the mechanisms driving the observed isotopic and genetic divergences are unclear, the implications are even less obvious. Perhaps the topographic severity of the southeast Alaskan coast, where large, heavily glaciated mountain ranges generally

separate coastal and interior populations, have reduced gene flow such that the mechanisms postulated here can act to generate ecologically divergent populations. For instance, along south coastal British Columbia, where migrating salmon are accessed by brown bears and topography is less severe, morphological divergence among coastal brown bears and interior grizzly bears can be less pronounced. Thus, if the existence of a distinct coastal brown bear population depends on a balance between gene flow and selection, I speculate that interior grizzly bears would numerically dominate the coastal brown bear population in the event of increased connectivity in response to rapid deglaciation, as seen in and near Glacier Bay National Park and Preserve, Alaska (Lewis 2012). This would be similar to, though less dramatic than, the apparent recapture of a southeast Alaskan polar bear population by brown bears since the Pleistocene (Miller et al. 2012). Perhaps more importantly in the face of climate change, my results provide evidence that populations that occur across ecological transition zones may have an important role in generating and maintaining ecological and genetic diversity in populations with limited barriers to gene flow.

Table 5.1 Estimated membership coefficients to three genetic clusters for brown bears (*Ursus arctos*) sampled across a coastal–inland ecological zone, Pacific Northwest, North America.

		Average membership coefficient to clusters ($K = 3$)		
Sampling location	n	1	2	3
Admiralty Island, AK	15	0.055	0.934	0.010
Lower Stikine Watershed, AK	14	0.123	0.017	0.860
Upper Stikine Watershed, BC	117	0.413	0.025	0.562
Spatsizi Plateau Wilderness Provincial Park, BC	29	0.864	0.035	0.101

Table 5.2 Candidate stable isotope mixing models used to explain annual isotopic variation among brown bears (*Ursus arctos*), upper Stikine watershed, British Columbia, Canada, 2005.

Model[a]	K[b]	pD[c]	DIC[d]	ΔDIC
strata	6	140.7	1150.0	0.0
strata + sex	12	147.8	1158.0	8.0
strata + ancestry (categorical)	18	149.9	1161.1	11.1
null	3	162.8	1176.2	26.2
strata + ancestry (continuous)	278	163.7	1177.8	27.8
sex	6	167.0	1180.3	30.3
ancestry (categorical)	12	166.6	1181.8	31.8
sex + ancestry (continuous)	278	163.3	1183.2	33.2
sex + ancestry (categorical)	18	168.2	1183.7	33.7
ancestry (continuous)	272	183.7	1204.7	54.7

[a] Individual ($n = 89$) and process error were included as random effects in all models.
[b] Number of fixed effects.
[c] Effective number of parameters.
[d] Models ranked by Deviance Information Criterion.

Table 5.3 Mean isotopic values ± SD and estimated brown bear (*Ursus arctos*) diet (95% Bayesian credible interval), upper Stikine watershed, British Columbia, Canada, 2005.

Model 1: Strata		$\bar{x}$ isotopic value (‰)			$\bar{x}$ dietary proportions (95% CI)		
Strata	*n*	$\delta^{13}C \pm sd$	$\delta^{15}N \pm sd$	$\delta^{34}S \pm sd$	terrestrial meat	salmon	vegetation
Marine	50	-21.04 ± 2.30	7.20 ± 4.29	-4.60 ± 7.91	18 (5–34)	18 (9–31)	63 (44–81)
Terrestrial	39	-22.86 ± 1.31	3.56 ± 1.18	-1.22 ± 2.92	5 (0–18)	1 (0–2)	94 (81–99)

Model 2: Strata + Sex			$\bar{x}$ isotopic value (‰)			$\bar{x}$ dietary proportions (95% CI)		
Strata	Sex	*n*	$\delta^{13}C \pm sd$	$\delta^{15}N \pm sd$	$\delta^{34}S \pm sd$	terrestrial meat	salmon	vegetation
Marine	Female	25	-21.20 ± 2.10	6.81 ± 3.98	3.06 ± 7.83	14 (3–32)	14 (5–27)	72 (50–89)
Marine	Male	26	-20.85 ± 2.46	7.44 ± 4.56	6.23 ± 7.68	19 (3–46)	24 (10–43)	56 (31–80)
Terrestrial	Female	29	-23.35 ± 0.34	3.34 ± 0.75	1.68 ± 2.59	7 (0–23)	1 (0–2)	92 (75–99)
Terrestrial	Male	9	-21.56 ± 2.03	3.64 ± 0.56	0.81 ± 2.05	6 (0–24)	4 (0–15)	90 (68–99)

Model 3: Strata + Ancestry			$\bar{x}$ isotopic value (‰)			$\bar{x}$ dietary proportions (95% CI)		
Strata	Ancestry	*n*	$\delta^{13}C \pm sd$	$\delta^{15}N \pm sd$	$\delta^{34}S \pm sd$	terrestrial meat	salmon	vegetation
Marine	Coastal	28	-20.29 ± 2.38	8.31 ± 4.41	7.09 ± 6.81	15 (4–32)	25 (12–32)	61 (41–79)
Marine	Interior	23	-21.92 ± 1.81	5.70 ± 3.76	1.75 ± 6.81	20 (3–47)	10 (3–20)	70 (43–90)
Terrestrial	Coastal	20	-23.24 ± 0.91	3.50 ± 0.69	-2.10 ± 2.48	1 (0–23)	1 (0–4)	92 (76–99)
Terrestrial	Interior	18	-22.58 ± 1.51	3.31 ± 0.75	-0.79 ± 2.35	2 (0–20)	2 (0–7)	92 (77–99)

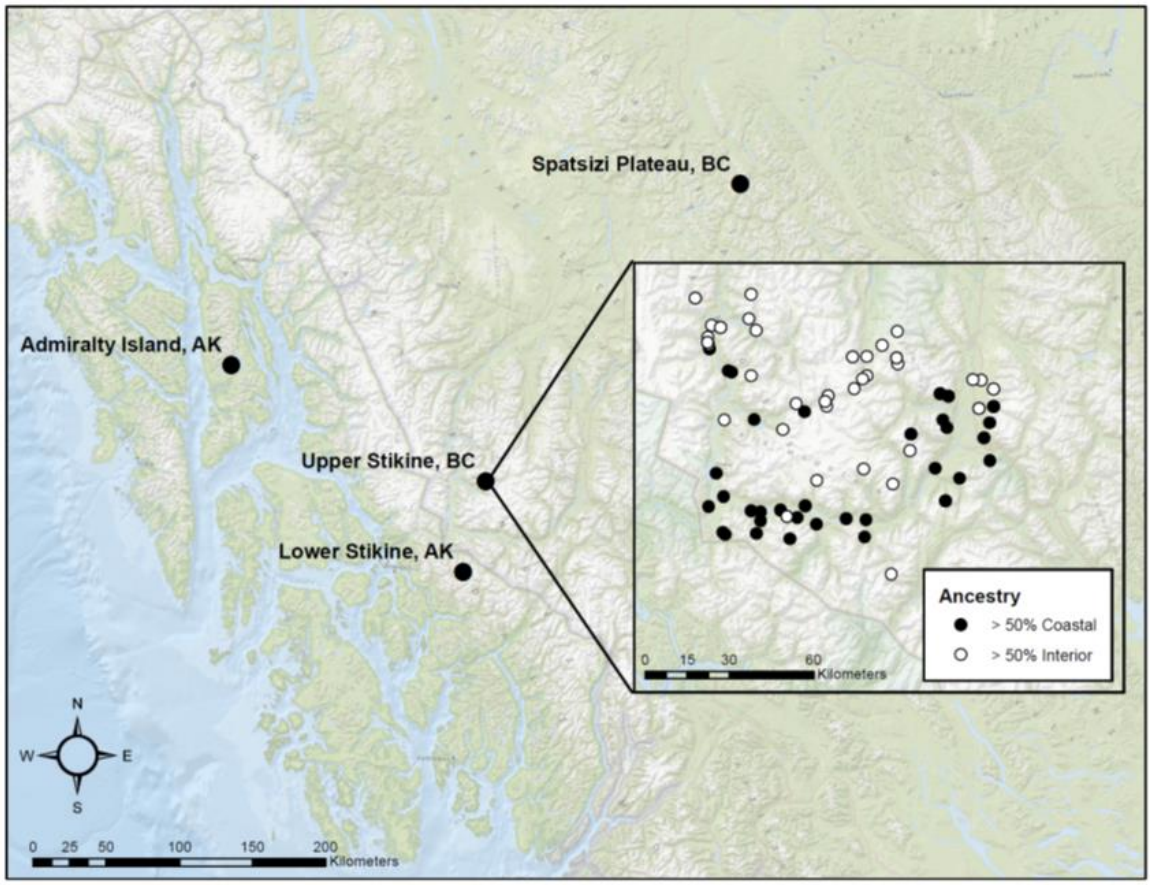

Figure 5.1 Upper Stikine watershed, British Columbia, Canada.

Upper Stikine shown relative to locations from where training data were acquired to assess brown bear ancestry. Inset map showing the distribution of brown bears (*Ursus arctos*) with > 50% coastal and > 50% interior ancestry sampled across the study area (*n* = 117).

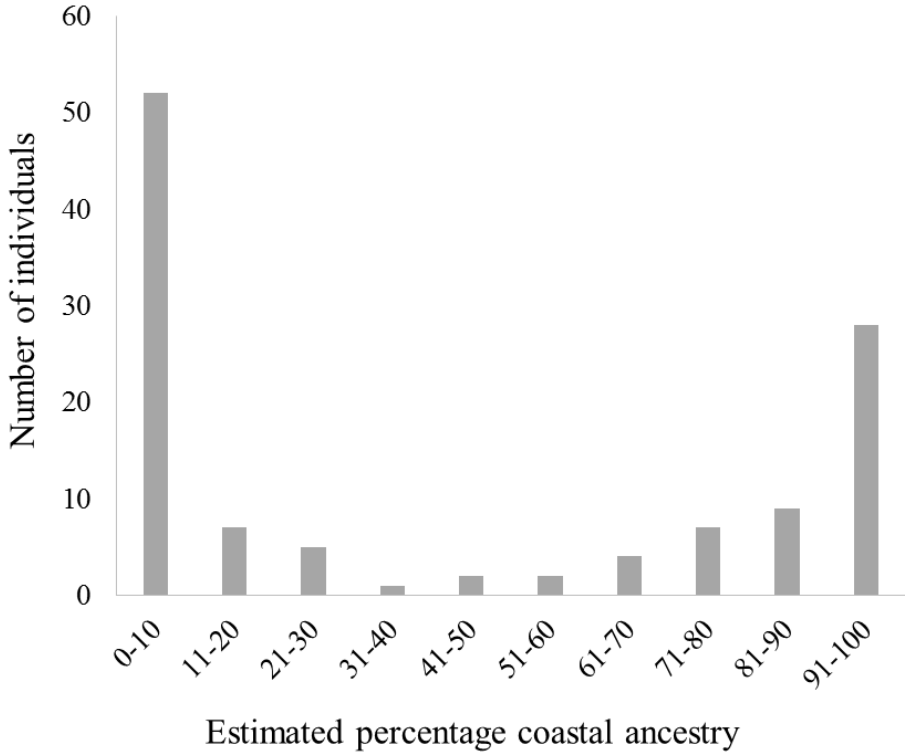

Figure 5.2 Structure results showing amount of ancestry ascribed to 1 of 2 mainland brown bear (*Ursus arctos*) genetic clusters at $K = 3$ ($n = 117$), upper Stikine watershed, British Columbia, Canada..

Training data from Admiralty Island, AK ($n = 15$), lower Stikine watershed ($n = 14$) Alaska, and Spatsizi Plateau, British Columbia ($n = 29$) are not shown.

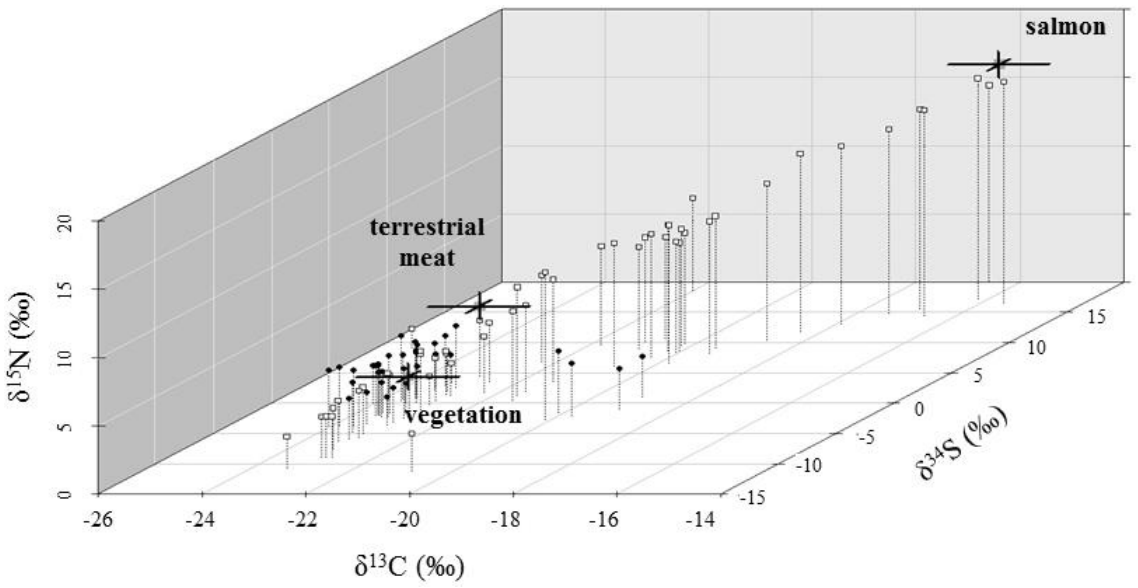

Figure 5.3 $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotopic values for brown bears (*Ursus arctos*) sampled in the upper Stikine watershed, British Columbia, Canada, 2005.

White boxes represent brown bears sampled from the marine strata (salmon present), black dots represent brown bears sampled from the terrestrial strata (salmon absent). Brown bear (n = 89) guard hair isotope values are presented relative to published mean isotope values (± 1 s.d.) derived from tissues of salmon, terrestrial meat, and terrestrial vegetation from known local prey items, represented by gray boxes (mean) and cross-bars (± 1 s.d.) and labeled accordingly. Trophic discrimination factors were applied to each food source.

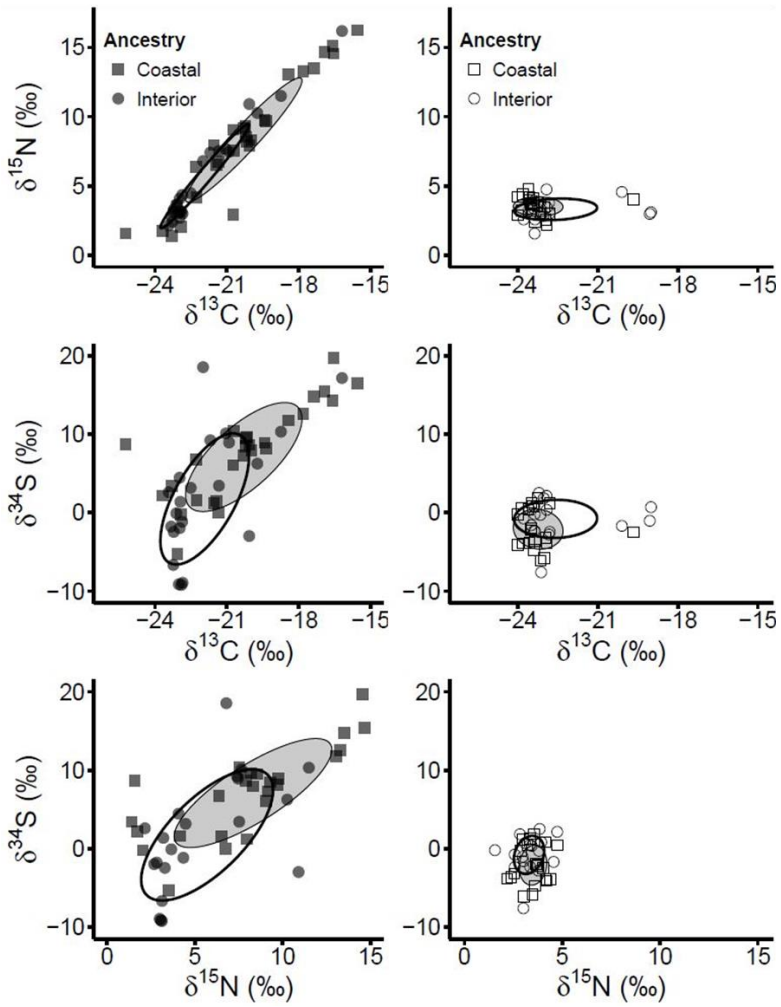

Figure 5.4 Standard ellipse areas representing core dietary niches of brown bears (*Ursus arctos*), upper Stikine watershed, British Columbia, Canada, 2005.

Standard ellipse areas corrected for small sample size (SEAc) representing the core (40%) dietary niche of brown bears. Bears assigned to the coastal cluster (> 50% coastal ancestry, $n = 50$) are represented by gray-shaded ellipses and brown bears assigned to the interior cluster (> 50% interior ancestry, $n = 39$) are represented by black-outlined ellipses. Left column represents brown bears sampled in marine strata (salmon present; larger isotopic niches), right column represents brown bears sampled in terrestrial strata (salmon absent; smaller isotopic niches). The core $\delta^{13}C$:$\delta^{15}N$ niche of brown bears occupying marine strata with coastal ancestry ($SEA_C = 10.06‰^2$) is larger ($p = 0.014$; 2.4 times larger) compared to brown bears occupying marine strata with interior ancestry ($SEA_C = 4.17‰^2$). The core $\delta^{13}C$:$\delta^{15}N$ niche of brown bears occupying terrestrial strata with interior ancestry ($SEA_C = 3.73‰^2$) was larger ($p = 0.049$; 1.8 times larger) than brown bears with coastal ancestry occupying terrestrial strata ($SEA_C = 2.06‰^2$). No differences were detected in the sizes core $\delta^{13}C$:$\delta^{34}S$ or $\delta^{15}N$:$\delta^{34}S$ niches between brown bears of different ancestry occupying the same strata.

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CHAPTER VI

GENERAL CONCLUSIONS

Understanding the causes and consequences of intrapopulation trophic niche variation is necessary for advancing our ecological and evolutionary understanding of species' resource use. But, perhaps of more immediate importance is the need to link individual resource use to fitness or to measures of fitness (i.e., biological outcomes; Clutton-Brock et al. 1982) to develop a more mechanistic understanding of the diverse resource needs of a population, which is the biological level at which management decisions typically are made. Specifically, understanding the mechanistic relationships between individual-based resource use and biological outcomes will inform our understanding how and why populations change and enhance our ability to forecast population dynamics. After all, it is the collective fitness of individuals that determines long-term population persistence (Homyack 2010).

Generalist consumer populations often consist of individuals that exist along a dietary gradient ranging from individuals that consume a broad range of food resources to individuals that focus on subsets of the resources consumed by the population (e.g., Bearhop et al. 2004, Darimont et al. 2009). Whether these differences result in similar biological outcomes is rarely considered. However, the results of my study demonstrate that individual differences in resource use can result in similar biological outcomes and that similar resource use can result in different biological outcomes. In Chapter 2, my test

of the niche variation hypothesis (Van Valen 1965) using a measure of body condition related directly to fitness showed that percentage body fat was similar for individuals of the same species (i.e., American black bear [*Ursus americanus*], brown bear [*U. arctos*]) across a broad observed dietary range in the Denali region of Alaska, USA. Brown bears also exhibited a wider population-level dietary niche and greater among-individual trophic niche variation compared to black bears. My results along with other recent studies of diverse taxa demonstrate the importance of understanding the link between individual diet variation and biological outcomes that manifest in population-level effects because this knowledge improve conservation and management (Both and Visser 2000, Kitaysky et al. 2007, McLoughlin et al. 2007).

In Chapters 3 and 4, I demonstrated contrasting patterns of biological outcomes relative to resource availability and use. For instance, in Chapter 3, my longitudinal assessment of brown bear cortisol levels an indirect measure of fitness (i.e., physiological stress) showed a weak negative relationship between cortisol and fruit production, with cortisol levels decreasing during years of increased fruit production in southeastern British Columbia, Canada. Moreover, I found extensive intrapopulation variation in stress levels but no discernable differences in diet among females or among males; although males consumed slightly more animal matter than females. However, I found no evidence that stress levels differed between sexes. These data provided an example of similar resource use among individuals with highly variable biological outcomes, which may indicate that resource use and availability are less important than other factors (e.g., reproductive state) in mediating the stress response of brown bears in this population. However, within a black bears population spanning adjoining ecoregions in central-

eastern British Columbia, Canada, I found that stress levels were on average about 2.0 pg/mg higher among males than females (Chapter 4), yet I found no discernable differences in diet between sexes. As such, larger-bodied male bears may experience nutritional stress when consuming the same diet as smaller-bodied female bears or possibly be subject to greater social stress associated with resource acquisition (e.g., mates, food). The link between resource availability and social stress has been demonstrated in other ursid populations and may have an important role in the long-term stress burden experience by individuals in some brown bear and black bear populations (Bryan et al. 2014).

In Chapter 5, I highlighted the central role of trophic niche variation in the genetic structuring of a brown bear populations across a coastal-interior ecological transition zone in northwestern British Columbia, Canada. Specifically, I found evidence of two partially distinct genetic groups inhabiting a coastal-interior ecological transition zone about 80 km wide and evidence linking these genetic groups to food resource use mediated by the availability of salmon (*Oncorhynchus* spp.). These results provides strong support for the central role of trophic niche variation as a driver of eco-evolutionary processes and highlights the importance of further studies aimed at understanding the causal mechanisms that link individual ecology to population ecology to conservation genetics (see Huber et al. 2007).

Given the extent of trophic niche variability among individuals throughout my study populations and the differences in observed biological outcomes, my work illustrates the diversity of ecologies within population and highlights the need for additional studies linking resource use to measures of fitness. For example, although

long-term stress may be influenced numerous factors (e.g., food availability, sex, anthropogenic disturbance), at the population level, physiological stress may be the primary mechanism linking wildlife health to landscape change (Macbeth et al. 2010, 2011). In addition, management strategies directed at safeguarding the most common food resources used by a population may not adequately protect the diversity of foods used by diverse individuals within that population or the underlying ecological processes that maintain dietary variation among individuals (Darimont et al. 2009). Consequently, only when I understand the factors that influence trophic niche variation and the subsequent consequences of that variation to fitness or measures of fitness, can I establish more efficient and effective wildlife conservation and management programs that protect the diverse resource needs of diverse populations.

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APPENDIX A

SUPPLEMENTARY MATERIAL

Immunoassay Validation Procedures and Results

Hair samples from three grizzly bears were used for assay validation with a commercially available high sensitivity Enzyme Immunoassay (EIA) kit (Salimetrics LLC, State College, PA, USA) according to previous protocols (Hoffman et al. 2013, Fairbanks et al. 2011, Russell et al. 2012). Cortisol extraction efficiency was assessed based on five determinations of recovery for each grizzly bear following a 0.938 microgram/dl spike with subsequent serially dilutions. Samples from each grizzly bear also were individually spiked with cortisol concentrations of 0.11, 0.33, 1.0, and 3.0 microgram/dl and mean spiking recovery for each individual was 107.35% ± 0.09 SD, 94.31% ± 0.08 SD, and 91.75% ± 0.4 SD (Figure A.1). High and low quality controls provided with each kit ran within expected ranges. To evaluate variability among hair samples due to assay procedures (e.g., weighing, extraction, assay), an internal laboratory control sample that consisted of a pooled hair sample that was ground, mixed and extracted as previously described was run to quantify intra-assay (1.9%) and inter-assay (11.6%) coefficients of variation. Parallelism was tested by spiking extracted grizzly bear hair samples with 0.938 microgram/dl and serially diluting this sample for comparison with assay standards provided in the kit (Figure A.2). Simple linear regression was used to assess parallelism between serially diluted hair extracts and cortisol standards in the same assay. Visual inspection and regression results suggested high parallelism for diluted extracts of grizzly bear hair and assay standards (Lee et al. 2006; Figure A.2).

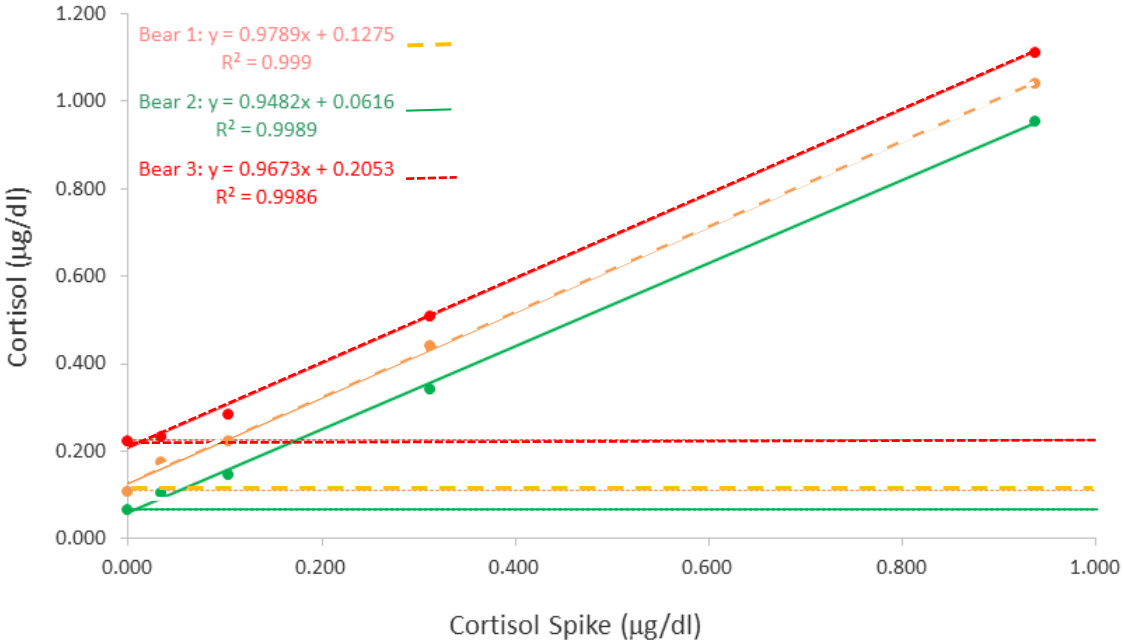

Figure A.1 Cortisol extraction efficiency for grizzly bear (*Ursus arctos*) hair.

Cortisol extraction efficiency based on five determinations of recovery from a 0.938 microgram/dl spike of serially diluted extracts of three different grizzly bear hair samples. Extraction efficiency for bear 1 =107.35% ± 0.09 SD, bear 2 = 94.31% ± 0.08 SD, bear 3 = 91.75% ± 0.4 SD). Horizontal lines represents the grizzly bear hair sample without the spike.

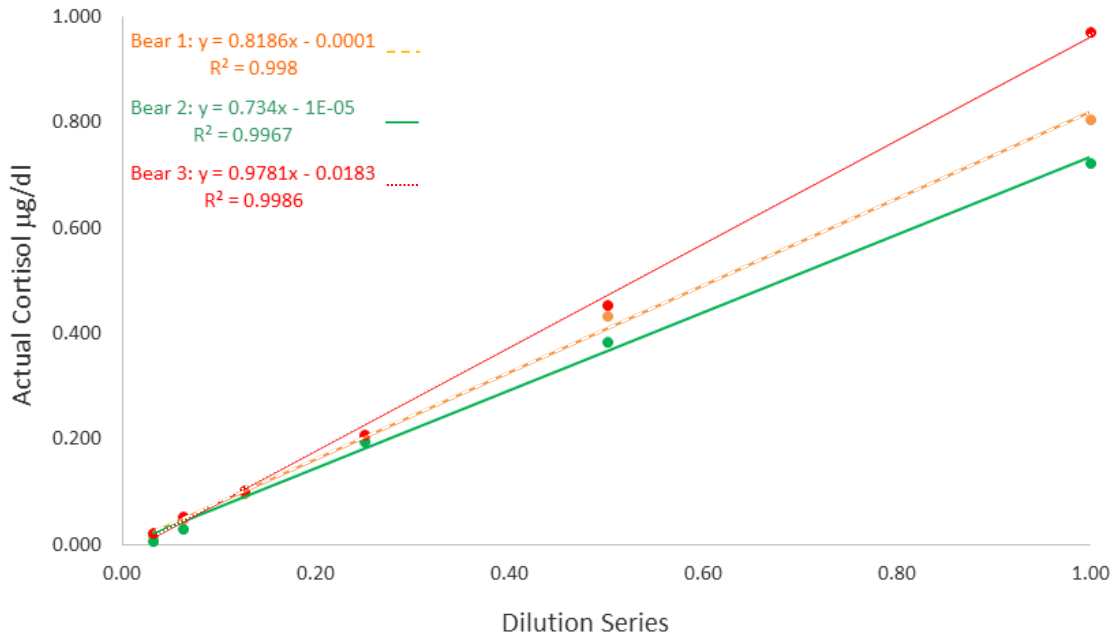

Figure A.2 Relationships between serially diluted extracted grizzly bear (*Ursus arctos*) hair samples.

Serially diluted (1:1, 1:2, 1:4, 1:8, 1:16, 1:32) extracted grizzly bear hair was spiked with 0.938 microgram/dl. Assay standards provided in the commercial high sensitivity Enzyme Immunoassay kit (Salimetrics LLC, State College, PA, USA).

Table A.1 Cross-reactivity for antibodies used in grizzly bear (*Ursus arctos*) cortisol assay.

Antibody Specificity		
Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Cortisol EIA
Prednisolone	100	0.568
Prednisone	1000	ND
Cortisone	1000	0.13
11-Deoxycortisol	500	0.156
21-Deoxycortisol	1000	0.041
17α-Hydroxyprogesterone	1000	ND
Dexamethasone	1000	19.2
Triamcinolone	1000	0.086
Corticosterone	10,000	0.214
Progesterone	1000	0.015
17β-Estradiol	10	ND
DHEA	10,000	ND
Testosterone	10,000	0.006
Transferrin[a]	66,000	ND
Aldosterone[a]	10,000	ND

[a] ND is reported for compounds where cross-reactivity was not detected (< 0.0004).

Table A.2 Mean $\delta^{13}C$ and $\delta^{15}N$ isotopic values ± SD and tissue-diet discrimination values ($\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ ± SD) used to estimate grizzly bears (*Ursus arctos*) diet.

Food category	$\delta^{13}C$ (‰) ± SD	$\Delta\delta^{13}C_{tissue\text{-}diet}$ (‰) ± SD	$\delta^{15}N$ (‰) ± SD	$\Delta\delta^{15}N_{tissue\text{-}diet}$ (‰) ± SD
Plant matter[a]	-29.01 ± 1.88[a]	5.97 ± 1.09[c]	-1.58 ± 2.30[a]	5.47 ± 0.28[c]
Animal matter[b]	-24.92 ± 0.94[b]	3.59 ± 0.54[c]	2.80 ± 1.27[b]	4.94 ± 0.15[c]

Grizzly bears sampled from southeastern British Columbia, Canada, 2005–2011.

[a] Generalized plant matter isotopic baseline was derived from the primary literature and based on previously identified plant forage species consumed by grizzly bears in the study area. Plant species used to estimate plant matter isotopic baseline include spring beauty (*Claytonia lanceolata*, *n* = 7), fireweed (*Epilobium angustifolium*, *n* = 5), soapberry (*Shepherdia Canadensis*, *n* = 7), and glacier lily (*Erythronium montanum*, n = 7) sampled from the Columbia River basin, British Columbia (Hobson et al. 2000); skunk cabbage (*Lysichiton americanus*, *n* = 8) and thinleaf huckleberry (*Vaccinium membranaceum*, *n* = 4) sampled from the Stikine River watershed, British Columbia (G. Mowat, Ministry of the Environment, British Columbia, *unpublished data*); devil's club (*Oplopanax horridus*, sample size not reported) from Chichagof Island, Alaska (Ben-David et al. 1998); averaged isotope value from above-ground foliage from *Festuca* spp., *Carex* spp., *Elymus* spp., *Equisetum* spp., *Epilobium a*ngustifolium, *Heracleum maximum* and roots and bulbs of *Hedysarum* spp., *Astragalus* spp., and *Oxytropis* spp. samples (*n* = 91) from the Besa-Prophet region, British Columbia (Milakovic and Parker 2012); and dandelion (*Taraxacum* spp., *n* = 1), blueberry (*Vaccinium* spp., *n* = 4) sampled from Gustavus, Alaska (K. White, Alaska Department of Fish and Game, *unpublished data*).

[b] Generalized animal matter isotopic baseline was derived from the primary literature and based on previously identified prey species consumed by grizzly bears in the study area. Isotopic values of animal biological samples (i.e., hair, muscle, and red blood cells) used to estimate animal matter isotope baseline include moose (*Alces alces*, *n* = 2), elk (*Cervus elaphus*, *n* = 1), mountain goat (*Oreamnos americanus*, *n* = 2), white-tailed deer (*Odocoileum virginianus*, *n* = 1), mule deer (*O. hemionus*, *n* = 1) and ants (Formicidae bulk samples representing multiple individuals, *n* = 4) sampled from the Columbia River basin (Hobson et al. 2000); moose (*n* = 21), elk (*n* = 15) and caribou (*Rangifer tarandus*, *n* = 36) sampled from the Besa-Prophet region, British Columbia (Milakovic and Parker 2012); moose (*n* = 30), elk (*n* = 26), white-tailed deer (*n* = 31), and mule deer (*n* = 30) sampled from northwestern Montana (Derbridge 2010); Columbian ground squirrel (*Spermophilus columbianus*, *n* = 16) sampled from northern North America (Roth et al. 2007); and marmot (*Marmota calgata*, *n* = 16) sampled from the Stikine River watershed, British Columbia (G. Mowat, Ministry of the Environment, British Columbia, *unpublished data*).

[c] Carbon and nitrogen discrimination factors for plant and animal matter were calculated using equations described by Felicetti et al. (2003).

Table A.3 Data used to evaluate the influence of individual, environmental and anthropogenic factors on grizzly bear (*Ursus arctos*) cortisol concentration, southeastern British Columbia, Canada, 2006−2011.

Individual	Sex	Year	$\delta^{13}C$	$\delta^{15}N$	Cort (pg/mg)	Berry Index	July Range	GDD	Hunter Effort	Meat Proportion
225	F	2005	-23.40	3.82	3.99	1	23.35	151.10	38.54	0.28
138	F	2005	-23.02	3.58	5.36	1	23.35	151.10	38.54	0.28
127	F	2005	-23.18	3.09	5.40	1	23.35	151.10	38.54	0.28
634	F	2005	-23.57	3.87	5.54	1	23.35	151.10	38.54	0.28
2958	F	2005	-23.22	4.62	5.89	1	23.35	151.10	38.54	0.28
2958	F	2005	-23.59	3.71	6.45	1	23.35	151.10	38.54	0.28
105	F	2005	-22.94	3.73	6.71	1	23.35	151.10	38.54	0.28
641	F	2005	-23.90	4.06	9.77	1	23.35	151.10	38.54	0.28
2940	F	2005	-23.87	3.28	10.20	1	23.35	151.10	38.54	0.28
275	F	2005	-23.49	1.73	10.96	1	23.35	151.10	38.54	0.28
654	F	2005	-23.64	4.14	11.84	1	23.35	151.10	38.54	0.28
Mammy	F	2006	-23.63	2.98	3.51	1	25.29	285.71	75.14	0.28
3561	F	2006	-23.47	3.65	3.82	1	25.29	285.71	75.14	0.28
15147-13b	F	2006	-23.36	2.61	4.22	1	25.29	285.71	75.14	0.28
15039-1v	F	2006	-22.87	4.86	4.48	1	25.29	285.71	75.14	0.28
Pam	F	2006	-22.95	3.92	5.52	1	25.29	285.71	75.14	0.28
225	F	2006	-23.53	2.62	5.68	1	25.29	285.71	75.14	0.28
10940	F	2006	-23.28	3.47	5.75	1	25.29	285.71	75.14	0.28
4489	F	2006	-23.52	3.73	5.79	1	25.29	285.71	75.14	0.28
3666	F	2006	-23.76	3.16	5.84	1	25.29	285.71	75.14	0.28
3777	F	2006	-23.73	3.46	6.09	1	25.29	285.71	75.14	0.28
3825	F	2006	-22.82	3.54	6.24	1	25.29	285.71	75.14	0.28
79349	F	2006	-23.78	3.57	6.58	1	25.29	285.71	75.14	0.28
15202-7s	F	2006	-23.11	2.50	7.45	1	25.29	285.71	75.14	0.28
4152	F	2006	-23.22	4.34	8.36	1	25.29	285.71	75.14	0.28
Trix	F	2006	-23.06	3.12	8.56	1	25.29	285.71	75.14	0.28
15181-8x	F	2006	-23.43	3.17	9.22	1	25.29	285.71	75.14	0.28
3461	F	2006	-23.09	4.14	9.31	1	25.29	285.71	75.14	0.28
3900	F	2007	-23.32	3.94	4.59	1	33.43	178.16	45.65	0.28
5EVSA-F15	F	2007	-23.21	1.81	5.21	1	33.43	178.16	45.65	0.28
5488	F	2007	-22.92	3.52	5.44	1	33.43	178.16	45.65	0.28
3537	F	2007	-23.41	5.21	5.44	1	33.43	178.16	45.65	0.28
5060	F	2007	-23.12	3.00	6.08	1	33.43	178.16	45.65	0.28
4152	F	2007	-23.27	3.61	6.16	1	33.43	178.16	45.65	0.28
4570	F	2007	-23.36	2.95	6.35	1	33.43	178.16	45.65	0.28

Table A.3 continued

3024	F	2007	-22.81	5.82	6.50	1	33.43	178.16	45.65	0.28
5128	F	2007	-23.22	3.53	6.54	1	33.43	178.16	45.65	0.28
5117	F	2007	-23.87	2.88	6.65	1	33.43	178.16	45.65	0.28
5181	F	2007	-23.12	2.58	6.89	1	33.43	178.16	45.65	0.28
5545	F	2007	-23.24	2.72	7.18	1	33.43	178.16	45.65	0.28
4605	F	2007	-22.88	3.24	8.08	1	33.43	178.16	45.65	0.28
3123	F	2007	-23.20	5.18	8.24	1	33.43	178.16	45.65	0.28
5560	F	2007	-23.04	2.01	9.07	1	33.43	178.16	45.65	0.28
4500	F	2007	-23.38	4.37	9.38	1	33.43	178.16	45.65	0.28
4449	F	2007	-23.16	3.38	15.41	1	33.43	178.16	45.65	0.28
3177	F	2007	-23.56	2.57	15.44	1	33.43	178.16	45.65	0.28
7143	F	2008	-22.83	3.81	3.28	2	28.73	71.63	53.72	0.28
8104	F	2008	-22.78	7.10	3.56	2	28.73	71.63	53.72	0.28
174387-a	F	2008	-22.64	4.72	3.60	2	28.73	71.63	53.72	0.28
125	F	2008	-22.74	3.78	3.67	2	28.73	71.63	53.72	0.28
7035	F	2008	-23.35	0.66	4.08	2	28.73	71.63	53.72	0.28
3373	F	2008	-23.50	4.20	4.22	2	28.73	71.63	53.72	0.28
7575	F	2008	-23.53	2.99	4.66	2	28.73	71.63	53.72	0.28
7709	F	2008	-23.27	3.30	4.91	2	28.73	71.63	53.72	0.28
5470	F	2008	-23.08	3.22	5.20	2	28.73	71.63	53.72	0.28
275	F	2008	-23.05	3.08	5.86	2	28.73	71.63	53.72	0.28
5560	F	2008	-23.81	3.61	5.97	2	28.73	71.63	53.72	0.28
343	F	2008	-23.55	3.38	6.77	2	28.73	71.63	53.72	0.28
5893	F	2008	-23.62	6.17	6.96	2	28.73	71.63	53.72	0.28
5EVSA-F15	F	2008	-23.07	2.04	6.96	2	28.73	71.63	53.72	0.28
5010	F	2008	-23.16	4.47	7.36	2	28.73	71.63	53.72	0.28
7593	F	2008	-23.33	3.09	7.68	2	28.73	71.63	53.72	0.28
3825	F	2008	-22.94	3.83	8.08	2	28.73	71.63	53.72	0.28
4097	F	2008	-23.81	5.00	13.68	2	28.73	71.63	53.72	0.28
11410	F	2009	-22.42	5.49	2.50	3	29.47	150.55	61.81	0.28
114557	F	2009	-23.55	3.35	3.12	3	29.47	150.55	61.81	0.28
5280	F	2009	-22.82	4.05	3.45	3	29.47	150.55	61.81	0.28
525	F	2009	-23.42	2.50	3.78	3	29.47	150.55	61.81	0.28
987	F	2009	-22.74	4.01	3.88	3	29.47	150.55	61.81	0.28
8120	F	2009	-23.79	3.59	4.44	3	29.47	150.55	61.81	0.28
3177	F	2009	-23.39	2.92	4.49	3	29.47	150.55	61.81	0.28
4449	F	2009	-23.25	3.12	5.22	3	29.47	150.55	61.81	0.28
8109	F	2009	-23.14	3.96	5.56	3	29.47	150.55	61.81	0.28
15174-11x	F	2009	-23.60	3.54	5.67	3	29.47	150.55	61.81	0.28
6909	F	2009	-23.11	3.63	5.96	3	29.47	150.55	61.81	0.28

Table A.3 continued

520	F	2009	-23.56	2.94	6.56	3	29.47	150.55	61.81	0.28
3900	F	2009	-23.00	4.33	10.32	3	29.47	150.55	61.81	0.28
5545	F	2009	-23.19	2.84	11.20	3	29.47	150.55	61.81	0.28
6066	F	2010	-23.45	3.46	4.22	2	27.68	162.25	48.59	0.28
11423	F	2010	-23.43	2.97	4.35	2	27.68	162.25	48.59	0.28
MOE4313	F	2010	-23.64	5.27	4.78	2	27.68	162.25	48.59	0.28
14837-21f	F	2010	-22.96	3.99	5.60	2	27.68	162.25	48.59	0.28
10165	F	2010	-23.14	3.98	6.19	2	27.68	162.25	48.59	0.28
7035	F	2010	-23.47	1.27	6.93	2	27.68	162.25	48.59	0.28
14837	F	2010	-22.88	4.54	7.46	2	27.68	162.25	48.59	0.28
6221	F	2010	-23.49	3.24	7.48	2	27.68	162.25	48.59	0.28
10765	F	2010	-23.30	2.76	7.74	2	27.68	162.25	48.59	0.28
4152	F	2010	-23.35	3.80	8.29	2	27.68	162.25	48.59	0.28
4449	F	2010	-23.44	3.46	9.82	2	27.68	162.25	48.59	0.28
8804-k	F	2010	-23.48	3.20	9.85	2	27.68	162.25	48.59	0.28
4929	F	2010	-23.22	4.19	10.88	2	27.68	162.25	48.59	0.28
3820	F	2010	-23.21	4.18	12.93	2	27.68	162.25	48.59	0.28
845	F	2010	-23.42	3.67	16.19	2	27.68	162.25	48.59	0.28
CI79546	F	2011	-23.48	4.64	1.92	3	26.35	123.43	72.43	0.28
CI106029	F	2011	-22.78	5.42	2.85	3	26.35	123.43	72.43	0.28
10743-I	F	2011	-24.42	2.10	3.20	3	26.35	123.43	72.43	0.28
CI78793	F	2011	-23.64	3.98	3.37	3	26.35	123.43	72.43	0.28
CI105891	F	2011	-23.24	5.77	3.54	3	26.35	123.43	72.43	0.28
4292	F	2011	-23.48	3.29	3.76	3	26.35	123.43	72.43	0.28
CI105817	F	2011	-22.91	3.68	3.85	3	26.35	123.43	72.43	0.28
CI107996	F	2011	-23.40	4.53	3.87	3	26.35	123.43	72.43	0.28
7624	F	2011	-23.96	5.30	4.72	3	26.35	123.43	72.43	0.28
9583	F	2011	-23.49	4.06	5.07	3	26.35	123.43	72.43	0.28
CI79310	F	2011	-23.78	3.88	5.57	3	26.35	123.43	72.43	0.28
CI104866	F	2011	-22.56	3.15	6.06	3	26.35	123.43	72.43	0.28
CI79312	F	2011	-23.10	3.46	7.12	3	26.35	123.43	72.43	0.28
5470	F	2011	-23.52	4.12	7.12	3	26.35	123.43	72.43	0.28
12929	F	2011	-23.46	3.01	7.76	3	26.35	123.43	72.43	0.28
CI119862	F	2011	-22.56	5.69	7.78	3	26.35	123.43	72.43	0.28
8196	F	2011	-23.63	2.75	7.96	3	26.35	123.43	72.43	0.28
CI85225	F	2011	-23.68	3.22	18.67	3	26.35	123.43	72.43	0.28
154	M	2005	-23.80	2.62	4.27	1	23.35	151.10	38.54	0.33
374	M	2005	-23.71	3.89	4.56	1	23.35	151.10	38.54	0.33
530	M	2005	-23.54	4.92	4.56	1	23.35	151.10	38.54	0.33
3141	M	2005	-23.17	2.80	4.96	1	23.35	151.10	38.54	0.33

Table A.3 continued

384	M	2005	-23.73	3.55	5.04	1	23.35	151.10	38.54	0.33
2913	M	2005	-24.17	2.08	5.43	1	23.35	151.10	38.54	0.33
365	M	2005	-24.04	3.66	11.28	1	23.35	151.10	38.54	0.33
3144	M	2005	-23.94	2.19	12.72	1	23.35	151.10	38.54	0.33
999	M	2006	-23.10	4.95	4.08	1	25.29	285.71	75.14	0.33
3333	M	2006	-23.99	1.62	4.27	1	25.29	285.71	75.14	0.33
332	M	2006	-23.44	3.42	4.66	1	25.29	285.71	75.14	0.33
3819	M	2006	-23.65	4.26	5.04	1	25.29	285.71	75.14	0.33
154	M	2006	-24.03	2.86	5.20	1	25.29	285.71	75.14	0.33
374	M	2006	-24.05	2.77	5.44	1	25.29	285.71	75.14	0.33
3578	M	2006	-23.29	4.02	5.52	1	25.29	285.71	75.14	0.33
15038-3a	M	2006	-23.02	4.65	5.52	1	25.29	285.71	75.14	0.33
Harry	M	2006	-22.96	3.49	6.24	1	25.29	285.71	75.14	0.33
2913	M	2006	-23.66	4.14	6.28	1	25.29	285.71	75.14	0.33
14808-6a	M	2006	-22.76	6.10	6.34	1	25.29	285.71	75.14	0.34
Derrick	M	2006	-22.93	4.83	6.48	1	25.29	285.71	75.14	0.33
15205-4x	M	2006	-23.53	1.54	6.93	1	25.29	285.71	75.14	0.33
3224	M	2006	-23.12	4.57	8.46	1	25.29	285.71	75.14	0.33
4875	M	2006	-23.39	3.24	8.52	1	25.29	285.71	75.14	0.33
3040	M	2006	-23.07	4.79	9.16	1	25.29	285.71	75.14	0.33
15050-30r	M	2006	-22.93	3.07	9.92	1	25.29	285.71	75.14	0.33
3890	M	2006	-22.85	4.61	10.06	1	25.29	285.71	75.14	0.33
2925	M	2006	-22.79	4.71	10.75	1	25.29	285.71	75.14	0.33
3040	M	2007	-23.04	5.67	3.76	1	33.43	178.16	45.65	0.33
4951	M	2007	-23.22	3.94	4.08	1	33.43	178.16	45.65	0.33
4666	M	2007	-22.85	4.96	4.19	1	33.43	178.16	45.65	0.33
530	M	2007	-22.93	5.80	5.15	1	33.43	178.16	45.65	0.34
5486	M	2007	-23.74	3.38	5.56	1	33.43	178.16	45.65	0.33
154	M	2007	-23.00	3.23	5.56	1	33.43	178.16	45.65	0.33
Harry	M	2007	-23.40	2.97	5.68	1	33.43	178.16	45.65	0.33
644	M	2007	-23.16	5.99	5.84	1	33.43	178.16	45.65	0.33
4456	M	2007	-23.48	4.43	6.76	1	33.43	178.16	45.65	0.33
5441	M	2007	-23.36	3.20	6.77	1	33.43	178.16	45.65	0.33
3333	M	2007	-23.98	2.58	7.60	1	33.43	178.16	45.65	0.33
5434	M	2007	-23.27	2.67	7.90	1	33.43	178.16	45.65	0.33
5210	M	2007	-22.36	3.52	8.43	1	33.43	178.16	45.65	0.33
667	M	2007	-22.73	6.84	10.49	1	33.43	178.16	45.65	0.34
8801-u-5423	M	2007	-23.25	3.96	11.95	1	33.43	178.16	45.65	0.33
5118	M	2007	-23.26	2.99	12.92	1	33.43	178.16	45.65	0.33
5433	M	2007	-23.34	1.77	13.10	1	33.43	178.16	45.65	0.33

Table A.3 continued

6372	M	2008	-23.52	3.40	2.72	2	28.73	71.63	53.72	0.33
8028	M	2008	-23.12	4.38	4.54	2	28.73	71.63	53.72	0.33
6276	M	2008	-23.96	3.97	4.56	2	28.73	71.63	53.72	0.33
5486	M	2008	-23.18	2.73	4.80	2	28.73	71.63	53.72	0.33
5522-4835	M	2008	-23.43	2.89	5.06	2	28.73	71.63	53.72	0.33
5449	M	2008	-23.02	4.95	5.87	2	28.73	71.63	53.72	0.33
2913	M	2008	-23.41	3.29	5.89	2	28.73	71.63	53.72	0.33
3021	M	2008	-23.05	6.74	6.13	2	28.73	71.63	53.72	0.34
332	M	2008	-23.31	4.44	6.16	2	28.73	71.63	53.72	0.33
5601	M	2008	-23.51	2.41	6.56	2	28.73	71.63	53.72	0.33
7952	M	2008	-23.30	3.54	6.65	2	28.73	71.63	53.72	0.33
5854	M	2008	-23.31	2.70	6.72	2	28.73	71.63	53.72	0.33
7078	M	2008	-23.79	4.06	7.00	2	28.73	71.63	53.72	0.33
7654	M	2008	-23.50	3.98	7.05	2	28.73	71.63	53.72	0.33
728	M	2008	-23.00	5.07	7.31	2	28.73	71.63	53.72	0.33
5057-5201	M	2008	-24.12	2.50	8.45	2	28.73	71.63	53.72	0.33
7641	M	2008	-23.49	3.46	8.97	2	28.73	71.63	53.72	0.33
6357	M	2008	-23.60	2.27	9.04	2	28.73	71.63	53.72	0.33
10784-e	M	2008	-23.36	5.13	9.36	2	28.73	71.63	53.72	0.33
3879	M	2008	-23.92	3.74	9.76	2	28.73	71.63	53.72	0.33
154	M	2008	-23.11	3.19	10.53	2	28.73	71.63	53.72	0.33
5712	M	2008	-22.82	6.11	10.78	2	28.73	71.63	53.72	0.34
3805	M	2008	-23.02	4.54	11.60	2	28.73	71.63	53.72	0.33
4875	M	2008	-23.21	3.22	11.98	2	28.73	71.63	53.72	0.33
11448	M	2009	-22.76	4.66	1.61	3	29.47	150.55	61.81	0.33
2989	M	2009	-23.60	5.40	2.24	3	29.47	150.55	61.81	0.33
7654	M	2009	-23.85	3.10	2.32	3	29.47	150.55	61.81	0.33
6207	M	2009	-23.32	3.28	2.57	3	29.47	150.55	61.81	0.33
3805	M	2009	-22.97	5.55	4.00	3	29.47	150.55	61.81	0.33
148	M	2009	-23.11	3.91	4.53	3	29.47	150.55	61.81	0.33
667	M	2009	-22.84	5.76	4.54	3	29.47	150.55	61.81	0.33
339	M	2009	-23.11	5.32	4.77	3	29.47	150.55	61.81	0.33
4492	M	2009	-22.97	4.99	4.80	3	29.47	150.55	61.81	0.33
7319	M	2009	-22.94	5.62	4.80	3	29.47	150.55	61.81	0.33
5814	M	2009	-22.35	7.91	5.29	3	29.47	150.55	61.81	0.34
644	M	2009	-22.91	5.77	5.37	3	29.47	150.55	61.81	0.34
3224	M	2009	-22.98	5.19	6.44	3	29.47	150.55	61.81	0.33
15038-3a	M	2009	-22.79	4.61	7.12	3	29.47	150.55	61.81	0.33
8062	M	2009	-23.47	2.43	7.13	3	29.47	150.55	61.81	0.33
4431	M	2009	-22.91	5.33	7.51	3	29.47	150.55	61.81	0.33

Table A.3 continued

180	M	2009	-23.25	4.64	8.44	3	29.47	150.55	61.81	0.33
4666	M	2009	-22.95	4.99	11.76	3	29.47	150.55	61.81	0.33
3578	M	2009	-23.29	4.36	13.20	3	29.47	150.55	61.81	0.33
MOE4	M	2010	-23.51	5.33	3.36	2	27.68	162.25	48.59	0.33
728	M	2010	-22.98	5.48	4.56	2	27.68	162.25	48.59	0.33
10703-a	M	2010	-23.37	3.87	4.90	2	27.68	162.25	48.59	0.33
10629	M	2010	-23.34	3.99	5.31	2	27.68	162.25	48.59	0.33
3890	M	2010	-23.29	5.02	5.50	2	27.68	162.25	48.59	0.33
3040	M	2010	-22.94	5.67	5.60	2	27.68	162.25	48.59	0.34
10209	M	2010	-23.39	3.28	6.06	2	27.68	162.25	48.59	0.33
6276	M	2010	-23.47	5.31	6.52	2	27.68	162.25	48.59	0.33
5963	M	2010	-23.50	5.10	6.80	2	27.68	162.25	48.59	0.33
5983	M	2010	-23.65	3.98	6.85	2	27.68	162.25	48.59	0.33
9698	M	2010	-23.48	3.61	6.94	2	27.68	162.25	48.59	0.33
11448	M	2010	-22.76	4.77	7.11	2	27.68	162.25	48.59	0.33
10739-c	M	2010	-23.02	4.69	7.12	2	27.68	162.25	48.59	0.33
6037	M	2010	-23.39	3.97	7.20	2	27.68	162.25	48.59	0.33
4875	M	2010	-23.53	3.16	7.85	2	27.68	162.25	48.59	0.33
644	M	2010	-23.15	5.26	9.03	2	27.68	162.25	48.59	0.33
15248-3x	M	2010	-22.96	6.72	9.48	2	27.68	162.25	48.59	0.33
6151	M	2010	-23.16	4.41	9.52	2	27.68	162.25	48.59	0.33
332	M	2010	-23.44	3.46	9.77	2	27.68	162.25	48.59	0.33
10169	M	2010	-23.07	4.77	10.01	2	27.68	162.25	48.59	0.33
5338	M	2010	-23.47	5.18	11.47	2	27.68	162.25	48.59	0.33
10309	M	2010	-23.16	3.71	16.28	2	27.68	162.25	48.59	0.33
148	M	2011	-23.33	4.77	3.36	3	26.35	123.43	72.43	0.33
644	M	2011	-23.12	5.24	5.11	3	26.35	123.43	72.43	0.33
10629	M	2011	-23.05	3.59	5.69	3	26.35	123.43	72.43	0.33
260	M	2011	-23.53	3.46	6.17	3	26.35	123.43	72.43	0.33
11677	M	2011	-22.98	4.19	7.75	3	26.35	123.43	72.43	0.33
10739-c	M	2011	-23.13	3.39	8.48	3	26.35	123.43	72.43	0.33
3890	M	2011	-23.10	5.43	11.36	3	26.35	123.43	72.43	0.33

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APPENDIX B

SUPPLEMENTARY MATERIAL

Stable isotope analysis

One sample from each genetically identified individual was selected. Follicles were removed from whole guard hairs and the hair washed in a chloroform:methanol (2:1) solution using a sonicator bath at 30 degrees for 20 minutes, rinsed twice with distilled water, washed again with distilled water in a sonicator bath for 20 minutes and dried in an oven at 40 degrees Celsius for 24 hours. Whole hair samples were weighed, measured and analyzed for carbon (13C/12C) and nitrogen (15N/14N) stable isotope ratios at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, Ontario, Canada) using an Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS). I report isotopic signatures in delta (δ) notation such that δ13C or δ15N = [(Rsample/Rstandard) – 1] x 1000, where Rsample and Rstandard are the 13C/12C or 15N/14N ratios of the sample and standard, respectively. The standards are PeeDee Belemnite limestone for carbon and atmospheric N2 for nitrogen (Peterson and Fry 1987). Analysis of internal laboratory standards suggested precision of 0.08‰ and 0.17‰ for δ13C and δ15N, respectively and NIST standards suggested an analytical accuracy of the instruments of 0.06‰ and 0.13‰ for δ13C and δ15N, respectively.

Table B.1 Generalized $\delta^{13}C$ and $\delta^{15}N$ isotopic values ± SD and $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ discrimination factors ± SD used to examine the black bear (*Ursus americanus*) isotopic values, Parsnip Plateau and Hart Ranges of Rocky Mountains, British Columbia, Canada, 1999.

Food category	$\delta^{13}C$ (‰) ± SD	$\Delta\delta^{13}C_{tissue\text{-}diet}$ (‰) ± SD	$\delta^{15}N$ (‰) ± SD	$\Delta\delta^{15}N_{tissue\text{-}diet}$ (‰) ± SD
Plant	-29.23 ± 2.44[a]	6.09 ± 1.42[d]	-2.67 ± 1.74	5.60 ± 0.21[d]
Terrestrial meat	-24.12 ± 0.87[b]	3.26 ± 0.51[d]	2.21 ± 0.99	4.85 ± 0.12[d]
Salmon	-19.06 ± 1.07[c]	0.19 ± 0.62[d]	14.19 ± 0.76	3.56 ± 0.09[d]

[a] Vegetation isotopic baseline from common bear forage plants (n = 91) sampled in the Besa–Prophet region, BC (Milakovic and Parker 2013) and from the upper Columbia River basin (n = 26), BC (Hobson et al. 2000), and from *Vaccinium* spp (n = 7) collected from Gustavus, AK (K. White *unpublished data*).
[b] Generalized terrestrial meat isotopic baseline averaged from caribou (*Rangifer tarandus*, n = 34) and moose (*Alces alces*, n = 36) whole hair samples from the Greater Caribou Recovery Area, British Columbia (Steenweg 2011), caribou whole hair samples (n = 24), caribou red blood cell samples (n = 12), moose whole hair samples (n = 10), moose hair tip samples (n = 6) and moose meat (n = 11) from the Besa–Prophet region, BC (Milakovic and Parker 2013), and from ant (Formicidae) bulk samples (n = 4) representing several individuals from the Upper Columbia River Basin, BC (Hobson et al. 2000).
[c] Generalized chinook (*Oncorhynchus tshawytscha*) isotopic baseline from throughout the Pacific Northwest from Johnson and Schindler (2009, n = 51). *Note*: chinook salmon are only available to black bears in the mountain ecoregion.
[d] Carbon and nitrogen discrimination factors for all food categories were calculated using equations described by Felicetti et al. (2003).

Extraction of cortisol from hair

Cortisol concentration analysis was conducted at University of Colorado Denver Anschutz Medical Campus (Aurora, Colorado, USA) as previously described (D'Anna-Hernandez et al. 2011). Each sample was placed in a pre-weighed 2 ml cryovial (Wheaton, Millville, NJ, USA), washed three times in 100% isopropanol and dried. After washing, drying and re-weighing samples on a high sensitivity electronic balance (Mettler Toledo Model MS105, Greifense, Switzerland), hair was ground in the same cryovial using a ball mill (Retsch, Haan, Germany) after adding a 4.76 mm carefully cleaned stainless steel ball bearing. Specially milled aluminum cassettes were designed to

hold three cryovials. The cassettes, containing the cryovials, were submerged in liquid nitrogen for 3 to 6 minutes to freeze hair samples to facilitate grinding. Samples subsequently were ground for 4 to 5 minutes. Powdered hair was extracted in the same cryovial in 0.33-1.0 ml (depending on sample mass) high pressure liquid chromatography (HPLC) grade methanol for 24 hours at room temperature on a side-to-side shaker platform. Confining hair to the same cryovial during these initial steps allowed for working with smaller samples (e.g., lower weights) as there was no loss of hair during aforementioned weighing steps. Following methanol extraction, cryovials were spun for three minutes in a centrifuge at 1700g to pellet the hair and 133 µl of the extraction supernatant was removed, placed into a microcentrifuge tube and dried under a stream of nitrogen in a drying rack in a fume hood. The dried extracts were then reconstituted with assay diluent based on hair weight; cortisol levels were determined using a commercial high sensitivity Enzyme Immunoassay (EIA) kit (Salimetrics LLC, State College, PA, USA) per manufacturer's protocol (D'Anna-Hernandez et al. 2011). Assay cross validation with liquid chromatograph-mass spectrometry (LC-MS/MS) methods and cross reactivity were described by Russell et al. (2015).

Immunoassay Validation Procedures and Results

A commercially available EIA kit (Salimetrics LLC, State College, PA, USA) was used for hair cortisol assay validation according to protocols previously reported (D'Anna-Hernandez et al. 2011, Fairbanks et al. 2011, Russell et al, 2012). Briefly, cortisol assay parallelism was evaluated by spiking an extracted black bear hair sample with 0.938 microgram/dl and serially diluting this sample for comparison with assay standards provided in the kit. Simple linear regression was used to assess parallelism

between serially diluted hair extracts and cortisol standards in the same assay. Visual inspection and regression results suggested high parallelism for diluted extracts of black bear hair and assay standards (Lee et al. 2006; Figure B.1). Cortisol extraction efficiency was 95.01% ± 0.06 SD based on five determinations of recovery from a 0.938 microgram/dl spike of serially diluted extracts of black bear hair (Figure B.2). High and low quality controls provided with each kit ran within expected ranges. As an additional control to assess variability among hair samples due to immunoassay procedures, a pooled hair sample (i.e., internal laboratory control sample) was run in duplicate for each assay after being ground, mixed and extracted as described above to quantify intra-assay (1.9%) and inter-assay (11.6) coefficients of variation. Assay cross-reactivity to multiple non-target compounds was provided with the immunoassay kits (Salimetrics LLC, State College, PA, USA) (Table B.2). Cross-reactivity for cortisol was provided by the manufacturer (Table B.2).

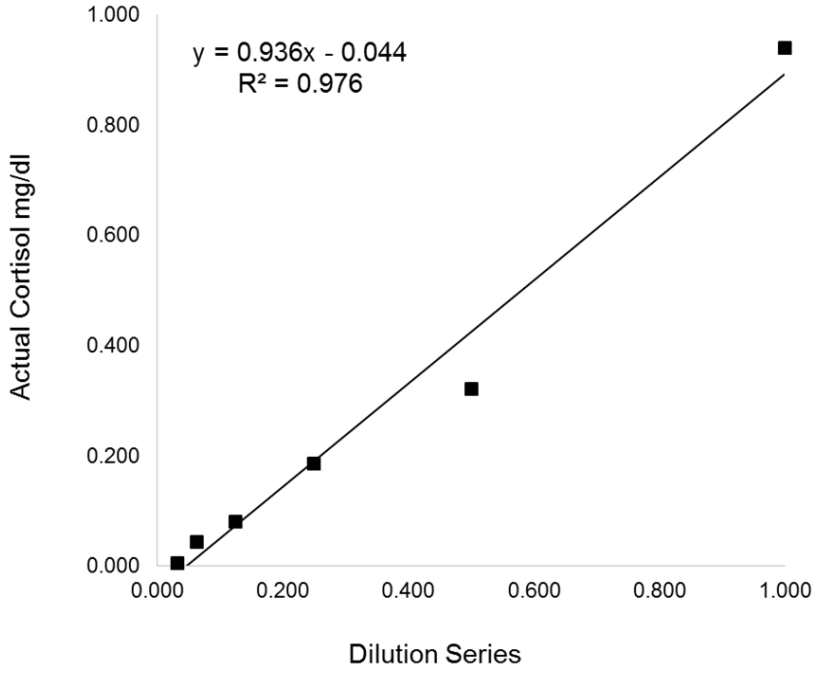

Figure B.1 Relationship between serially diluted extracted black bear (*Ursus americanus*) hair and cortisol spike.

Serially diluted (1:1, 1:2, 1:4, 1:8, 1:16, 1:32) extracted black bear hair was spiked with 0.938 microgram/dl. Assay standards provided in the commercial high sensitivity Enzyme Immunoassay kit (Salimetrics LLC, State College, PA, USA).

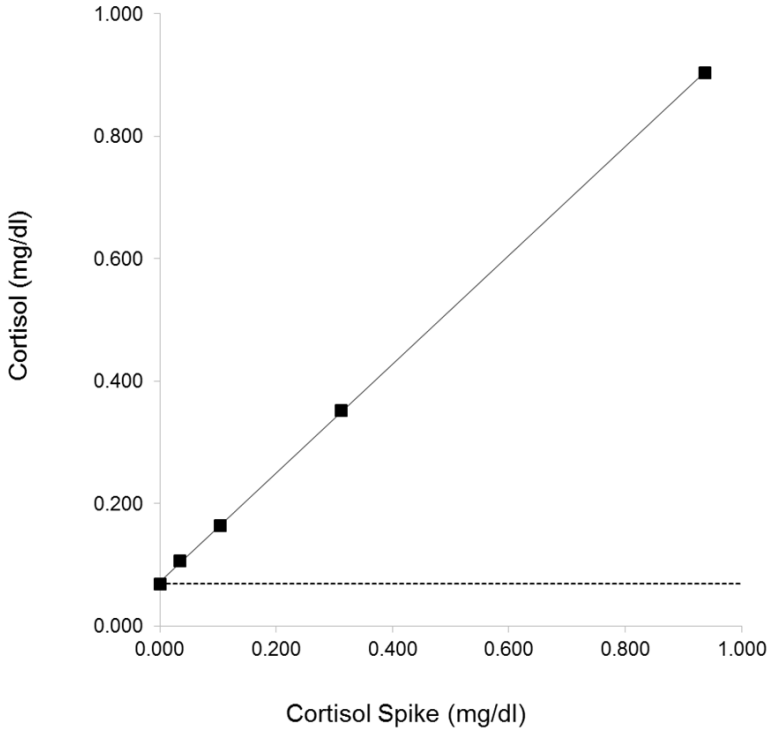

Figure B.2 Black bear (*Ursus americanus*) cortisol extraction efficiency based on five determinations of recovery.

Extraction efficiency (95.01% ± 0.06 SD) based on five determinations of recovery from a 0.938 microgram/dl spike of serially diluted extracts of black bear hair. Dashed line represents black bear hair sample without the spike.

Table B.2 Cross-reactivity for antibodies used in black bear (*Ursus americanus*) hair cortisol assay.

Antibody Specificity		
Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Cortisol EIA
Prednisolone	100	0.568
Prednisone	1000	ND
Cortisone	1000	0.13
11-Deoxycortisol	500	0.156
21-Deoxycortisol	1000	0.041
17α-Hydroxyprogesterone	1000	ND
Dexamethasone	1000	19.2
Triamcinolone	1000	0.086
Corticosterone	10,000	0.214
Progesterone	1000	0.015
17β-Estradiol	10	ND
DHEA	10,000	ND
Testosterone	10,000	0.006
Transferrin[a]	66,000	ND
Aldosterone[a]	10,000	ND

[a] ND is reported for compounds where cross-reactivity was not detected (< 0.0004).

Table B.3 Data used to evaluate the influence of diet, sex, and social environment (represented by ecoregion) on black bear (*Ursus americanus*) hair cortisol concentration, Parsnip Plateau and Hart Ranges of the Rocky Mountains, Canada, 1999.

Ecoregion	Sex	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Hair Weight (mg)	Cortisol Concentration (pg/mg)
Mountains	F	-23.60	3.84	3.46	0.60
Mountains	F	-23.97	4.98	10.22	1.50
Mountains	F	-23.36	3.82	28.00	2.10
Mountains	F	-23.95	4.04	12.35	2.30
Mountains	F	-24.06	3.16	12.63	3.20
Mountains	F	-23.80	3.23	16.77	3.50
Mountains	F	-23.85	4.04	8.73	3.60
Mountains	F	-24.96	4.85	7.80	3.70
Mountains	F	-22.76	4.32	28.23	3.70
Mountains	F	-24.10	3.39	17.91	3.70
Mountains	F	-24.07	4.21	19.71	4.00
Mountains	F	-24.41	4.12	23.30	4.40
Mountains	F	-24.74	3.02	10.24	4.60
Mountains	F	-23.80	3.48	19.28	4.80
Mountains	F	-25.58	2.84	34.66	4.90
Mountains	F	-24.51	5.28	27.94	5.30
Mountains	F	-24.84	5.90	20.79	5.50
Mountains	F	-24.24	4.03	16.82	5.60
Mountains	F	-23.56	2.25	21.91	5.60
Mountains	F	-23.41	4.21	10.26	5.60
Mountains	F	-23.74	3.63	32.45	6.00
Mountains	F	-24.16	6.13	17.75	6.30
Mountains	F	-23.85	3.60	37.93	7.10
Mountains	F	-23.43	5.08	27.63	7.70
Mountains	F	-24.57	5.01	13.30	7.90
Mountains	F	-24.08	3.60	14.83	7.90
Mountains	F	-23.66	4.93	8.14	8.50
Mountains	F	-24.73	4.59	22.26	8.80
Mountains	F	-24.79	4.89	43.59	10.70
Mountains	M	-23.60	2.90	9.59	1.40
Mountains	M	-24.08	2.46	12.05	1.90
Mountains	M	-23.28	4.90	27.62	2.50
Mountains	M	-23.57	3.39	10.91	3.10
Mountains	M	-24.47	5.32	7.48	3.30

Table B.3 continued

Mountains	M	-23.76	3.70	20.80	3.60
Mountains	M	-23.96	4.08	10.17	3.80
Mountains	M	-23.58	4.40	21.39	3.90
Mountains	M	-24.59	3.79	12.89	4.00
Mountains	M	-23.54	2.71	7.22	4.60
Mountains	M	-23.57	3.80	18.50	4.70
Mountains	M	-23.55	3.99	13.40	5.80
Mountains	M	-24.02	3.65	12.76	6.00
Mountains	M	-24.02	3.65	12.76	6.00
Mountains	M	-23.83	3.45	17.79	6.30
Mountains	M	-23.62	2.45	13.55	7.10
Mountains	M	-23.62	2.45	13.55	7.10
Mountains	M	-23.69	2.67	7.58	7.50
Mountains	M	-24.33	4.39	2.95	7.90
Mountains	M	-24.02	2.04	12.29	8.00
Mountains	M	-25.53	4.16	17.46	8.50
Mountains	M	-24.00	3.50	5.09	10.20
Mountains	M	-23.42	4.87	7.77	10.90
Mountains	M	-24.40	3.91	10.07	11.10
Mountains	M	-24.40	3.13	4.49	11.10
Mountains	M	-23.15	5.69	21.23	12.50
Mountains	M	-24.24	4.06	1.85	16.20
Mountains	M	-24.18	3.05	14.19	18.90
Mountains	M	-24.55	4.02	1.62	20.60
Plateau	F	-22.96	3.99	16.56	1.20
Plateau	F	-23.46	3.97	17.79	1.30
Plateau	F	-23.16	3.65	19.40	1.50
Plateau	F	-23.30	5.05	10.82	2.10
Plateau	F	-23.78	3.86	24.97	2.40
Plateau	F	-23.42	3.44	26.03	2.90
Plateau	F	-23.23	5.05	15.92	3.10
Plateau	F	-23.87	3.42	20.82	3.30
Plateau	F	-23.85	4.26	3.96	3.40
Plateau	F	-23.73	3.85	15.55	3.40
Plateau	F	-22.87	5.17	14.47	3.50
Plateau	F	-23.72	5.13	21.35	3.60
Plateau	F	-23.92	4.93	7.94	3.60
Plateau	F	-23.50	4.64	19.83	4.00
Plateau	F	-22.98	4.04	7.72	4.00
Plateau	F	-23.07	4.48	13.31	4.20

Table B.3 continued

Plateau	F	-23.07	3.60	9.38	4.30
Plateau	F	-23.24	3.67	19.56	4.70
Plateau	F	-23.90	4.10	23.13	4.80
Plateau	F	-23.55	3.40	27.38	5.10
Plateau	F	-23.28	4.77	18.18	5.30
Plateau	F	-23.99	4.15	14.61	5.50
Plateau	F	-24.04	4.25	6.55	5.70
Plateau	F	-23.10	4.25	4.88	5.90
Plateau	F	-23.05	4.36	13.37	6.30
Plateau	F	-23.58	4.40	19.72	6.50
Plateau	F	-23.60	2.75	10.52	7.00
Plateau	F	-23.82	3.63	17.51	7.10
Plateau	F	-23.34	4.54	7.73	7.20
Plateau	M	-23.88	3.63	6.99	0.50
Plateau	M	-23.80	4.41	16.99	2.10
Plateau	M	-24.16	3.75	9.64	2.30
Plateau	M	-24.23	3.55	9.80	2.50
Plateau	M	-23.97	2.91	18.72	2.90
Plateau	M	-23.39	3.45	19.58	3.00
Plateau	M	-23.71	4.38	23.24	3.10
Plateau	M	-23.71	3.67	23.65	3.10
Plateau	M	-23.93	4.23	6.70	3.50
Plateau	M	-24.10	4.92	21.44	3.70
Plateau	M	-23.24	3.71	14.40	3.70
Plateau	M	-23.76	3.58	16.06	4.70
Plateau	M	-23.50	3.55	2.34	4.70
Plateau	M	-24.38	3.47	18.87	4.80
Plateau	M	-24.35	3.44	11.39	5.30
Plateau	M	-23.70	5.24	15.15	6.20
Plateau	M	-22.89	3.78	14.31	6.30
Plateau	M	-24.03	3.63	10.11	6.60
Plateau	M	-23.32	4.81	4.51	6.90
Plateau	M	-23.62	6.19	15.01	7.60
Plateau	M	-23.17	3.78	21.69	8.00
Plateau	M	-23.55	3.91	6.57	8.10
Plateau	M	-23.95	3.81	12.24	8.20
Plateau	M	-23.80	3.85	5.38	9.90
Plateau	M	-23.97	3.62	5.26	10.60
Plateau	M	-23.65	4.17	4.09	11.80
Plateau	M	-23.28	3.16	16.83	12.40

Table B.3 continued

Plateau	M	-23.65	4.27	3.37	17.10
Plateau	M	-23.31	4.04	1.20	26.40
Plateau	M	-23.60	3.91	1.66	35.10

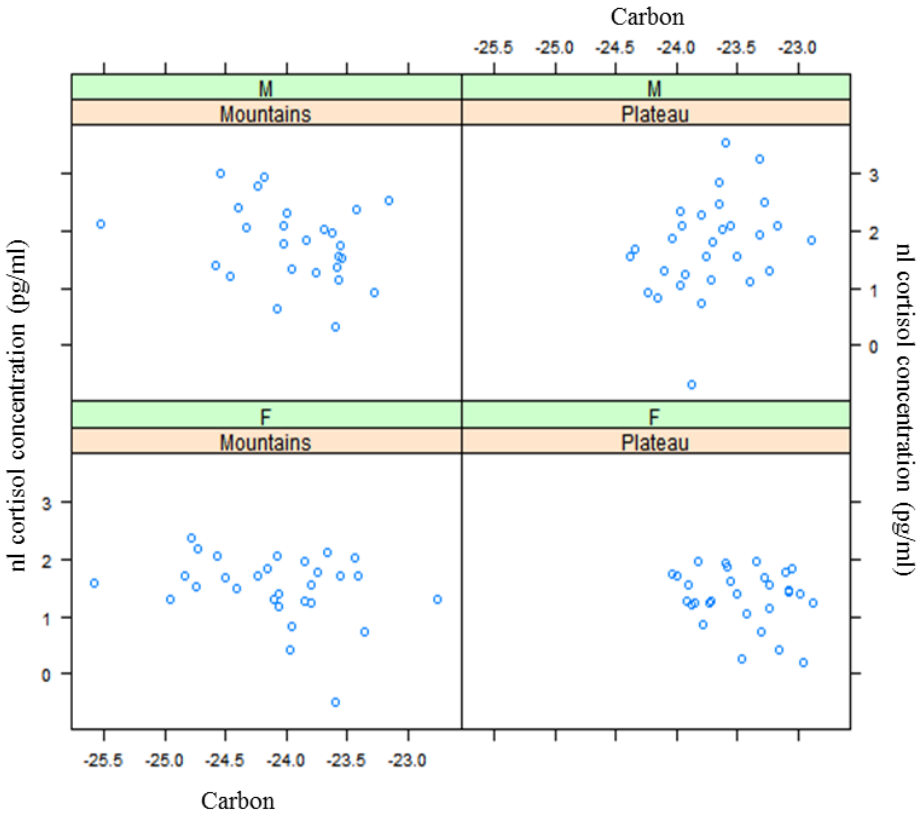

Figure B.3 Three-way interaction among carbon, sex, and ecoregion associated with hair cortisol concentration in black bears (*Ursus americanus*), Parsnip Plateau and Hart Ranges of the Rocky Mountains, Canada, 1999.

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APPENDIX C

SUPPLEMENTARY MATERIAL

Empirical data

Table C.1 Brown bear (*Ursus arctos*) data used to estimate diet and structure of isotopic variation, upper Stikine watershed, British Columbia, Canada, 2004.

$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	Strata	Sex	Proportion Interior Ancestry
-25.222	1.572	8.693	Coastal	F	0.006
-21.343	6.732	-0.001	Coastal	F	0.007
-20.224	9.292	8.462	Coastal	M	0.007
-23.820	4.375	-3.908	Interior	F	0.010
-18.413	13.066	11.750	Coastal	M	0.011
-20.173	8.205	9.639	Coastal	F	0.012
-23.676	1.740	2.182	Interior	F	0.013
-23.562	4.153	-3.881	Interior	M	0.013
-20.711	7.533	10.425	Coastal	F	0.014
-23.492	3.652	-1.976	Interior	F	0.015
-23.490	3.756	-2.036	Interior	F	0.015
-22.945	2.544	-3.173	Interior	F	0.015
-23.391	3.618	-4.713	Interior	F	0.015
-16.535	14.545	19.703	Coastal	M	0.015
-22.261	4.156	1.620	Coastal	M	0.016
-21.438	6.502	1.537	Coastal	F	0.017
-15.535	16.249	16.450	Coastal	M	0.017
-20.726	2.919	-11.558	Coastal	M	0.021
-21.531	7.951	1.225	Coastal	M	0.021
-23.144	3.045	-6.107	Interior	F	0.022
-19.417	9.756	8.903	Coastal	F	0.024
-19.963	8.308	7.935	Coastal	F	0.025
-20.058	7.900	8.647	Coastal	F	0.025
-22.920	2.180	-3.811	Interior	F	0.028
-23.990	4.217	-4.068	Interior	F	0.032
-16.918	14.681	15.432	Coastal	F	0.036
-16.585	15.098	14.306	Coastal	F	0.043
-19.364	9.691	8.181	Coastal	M	0.047
-22.908	2.046	-0.188	Coastal	F	0.054
-23.351	2.400	-3.660	Interior	F	0.064
-17.374	13.493	14.779	Coastal	M	0.065
-19.662	4.031	-2.487	Interior	M	0.070
-23.297	1.404	3.439	Coastal	M	0.071

Table C.1 continued

-23.417	4.145	0.840	Interior	F	0.080
-20.208	8.548	9.495	Coastal	M	0.085
-23.498	3.731	-2.237	Interior	F	0.096
-20.732	9.045	6.050	Coastal	F	0.114
-22.286	6.379	6.735	Coastal	M	0.127
-23.486	3.337	1.273	Interior	M	0.136
-17.806	13.264	12.571	Coastal	M	0.158
-23.059	3.518	-5.290	Coastal	M	0.170
-23.850	3.219	0.551	Interior	F	0.187
-23.994	2.885	-0.252	Interior	F	0.198
-20.297	9.138	7.304	Coastal	F	0.250
-23.262	3.554	1.857	Interior	F	0.252
-23.601	4.752	0.445	Interior	F	0.260
-23.019	3.483	-5.805	Coastal	F	0.263
-21.989	6.793	18.531	Coastal	M	0.493
-22.805	3.001	1.192	Interior	F	0.497
-23.324	2.828	-1.768	Coastal	M	0.584
-19.006	3.098	0.707	Interior	M	0.603
-23.109	3.661	-0.083	Coastal	M	0.641
-20.903	7.460	8.937	Coastal	M	0.650
-21.329	7.525	3.433	Coastal	M	0.711
-22.504	4.474	3.165	Coastal	M	0.726
-21.686	7.406	9.213	Coastal	M	0.747
-18.736	11.492	10.314	Coastal	F	0.767
-23.568	3.339	0.452	Interior	F	0.770
-23.778	2.573	-0.746	Interior	F	0.775
-19.726	10.262	6.248	Coastal	F	0.795
-23.204	3.848	2.513	Interior	M	0.808
-23.026	2.842	1.842	Coastal	F	0.836
-16.187	16.178	17.149	Coastal	M	0.842
-23.354	2.573	-2.346	Interior	F	0.853
-22.912	3.445	0.360	Interior	F	0.854
-23.272	2.410	-10.940	Coastal	F	0.919
-22.939	3.205	1.379	Coastal	M	0.924
-21.034	7.657	10.094	Coastal	F	0.928
-23.122	3.016	-7.591	Coastal	F	0.929
-22.970	4.073	4.452	Coastal	M	0.934
-22.802	3.028	-2.446	Interior	M	0.939
-23.150	3.812	-0.279	Interior	M	0.941
-23.494	3.024	-1.592	Interior	F	0.943

Table C.1 continued

-23.234	3.132	-6.657	Coastal	F	0.946
-22.846	3.000	-8.932	Coastal	F	0.948
-23.394	3.360	-2.314	Interior	F	0.950
-22.897	3.119	-9.204	Coastal	F	0.951
-23.418	2.156	2.602	Coastal	M	0.955
-23.367	1.561	-0.191	Interior	F	0.961
-22.968	2.683	-1.970	Coastal	F	0.962
-22.921	4.745	2.145	Interior	F	0.965
-22.827	3.781	-2.793	Interior	F	0.967
-20.063	10.907	-2.974	Coastal	F	0.969
-23.381	3.930	0.824	Interior	F	0.972
-23.002	3.064	-9.128	Coastal	F	0.972
-20.098	4.561	-1.682	Interior	M	0.973
-19.053	2.979	-1.039	Interior	M	0.975
-23.216	3.290	-2.445	Coastal	M	0.979
-22.862	4.344	-1.156	Coastal	F	0.986

Table C.2 Food source data used to estimate brown bear (*Ursus arctos*) diets and structure of isotopic variation, upper Stikine watershed, British Columbia, Canada, 2004.

	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$	Mean $\delta^{34}S$	SD $\delta^{34}S$	n
Salmon	-19.90	1.00	12.50	1.00	19.1	0.50	21
Meat	-25.80	0.74	1.70	1.33	0.60	4.81	31
Vegetation	-26.60	2.00	-2.80	3.00	-2.00	4.20	44

(G. Mowat, *unpublished data*)

Table C.3 Isotopic fractionation data used to estimate brown bear (*Ursus arctos*) diets and structure of isotopic variation, upper Stikine watershed, British Columbia, Canada, 2004.

	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$	Mean $\delta^{34}S$	SD $\delta^{34}S$
Salmon	3.70	0.20	3.78	0.60	0	0
Meat	3.70	0.20	5.07	0.50	0	0
Vegetation	3.70	0.20	5.62	0.50	0	0

(G. Mowat, *unpublished data*)