TRACING CARBON SOURCES OF SOUTHERN BEAUFORT SEA AND CHUKCHI SEA POLAR BEARS USING STABLE ISOTOPE ANALYSES

By

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Abstract

The Southern Beaufort Sea (SBS) polar bear (*Ursus maritimus*) subpopulation has declined in response to sea ice loss, while the Chukchi Sea (CS) subpopulation appears stable. The substantial population decline in the SBS subpopulation in recent years is concurrent with increases in the proportion of polar bears coming on shore, and the duration they spend there. Both of these changes have been associated with the loss of access to their primary sea ice habitat, which is mainly used as a platform to hunt seals. The first objective of this study was to determine if the SBS and CS polar bear subpopulations could be distinguished based on stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$ values) of bone collagen. The second objective was to examine patterns in SBS polar bear trophic level and terrestrial carbon sources over a 65-year time period, as polar bears have increasingly used coastal environments. We analyzed 112 SBS and CS polar bear bones (predominantly mandibles) from 1954–2019 that had been archived at the University of Alaska Museum of the North, as well as bones from subsistence-harvested polar bears. In addition to $\delta^{13}C$ and $\delta^{15}N$, samples from the SBS bears were analyzed for compound-specific stable carbon isotopes of amino acids ($\delta^{13}C_{AA}$ values). Another 50 bone collagen samples from terrestrial mammals and pinnipeds from northern Alaska were analyzed for $\delta^{13}C_{AA}$ values to provide a regional comparative dataset. Our study showed a significant difference in bulk $\delta^{13}C$ ($p<0.001$) values, but not $\delta^{15}N$ ($p=0.654$) values between the CS (-13.0‰±0.3‰ and 22.0‰±0.9‰, respectively) and the SBS bears (-14.7‰±1.3‰ and 22.2‰±1.0‰, respectively). We performed a logistic regression analysis (LR) using bulk $\delta^{13}C$ and $\delta^{15}N$ values of the polar bears to predict their placement into these two subpopulations. Using Icy Cape, AK as the geographical boundary, LR correctly placed polar bears in their respective subpopulations 82% of the time. Overall accuracy of placement changed to 84% when using the current geographical boundary at Utqiaġvik, AK. Bone collagen has a slow turnover rate, providing long-term, potentially life-long stable isotope signatures. Our findings could be used to determine the association of harvested polar bears to Alaska subpopulations, thus aiding in harvest quota management. The LR predicted samples collected from the Wainwright, AK region to be 58% CS and 42% SBS polar bears. This indicates that the area between Wainwright and Icy Cape is a polar bear mixing zone that includes bears from both subpopulations. Over the 65-year study period, two distinct groups of SBS polar bears were identified based on their $\delta^{13}C$ values of the amino acid proline: a high $\delta^{13}C_{Pro}$ group (1.8‰±2.3‰, n=45) and a low $\delta^{13}C_{Pro}$ group (-15.7‰±1.9‰, n=26). The high proline polar bear group had $\delta^{13}C_{Pro}$ values similar to those of Arctic brown bears (*Ursus arctos*; 0.4‰±1.6‰), while the low group had $\delta^{13}C_{Pro}$ values similar to ice seals (-15.3‰±1.2‰). Among the available samples, there were more high proline/pelagic bears (n=17) after the 2007 sea ice minimum than high proline/coastal bears (n=12), which is opposite of what we expected. This study provides evidence that two distinct ecotypes in Southern Beaufort Sea polar bears, pelagic and coastal, have existed since at least the 1950's. Overall, our results represent a detailed isotopic view of the Alaskan polar bear subpopulations, demonstrating the possibility of distinguishing and categorizing individuals as either SBS or CS, while also highlighting the existence of two ecotypes in the SBS subpopulation.

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Chapter 1: General Introduction

Arctic sea ice has declined substantially since the beginning of satellite-based observations in 1979, causing repercussions for the wildlife inhabiting and depending on it. There have been several recent major sea ice minima in the Arctic, e.g., 2007, 2012, and 2019 (Fetterer et al. 2017), and the current seasonal sea ice in the Bering Sea is at a minimum compared with anything shown for at least the last ~5000 years (Jones et al. 2020). The Chukchi and Beaufort seas, specifically, are experiencing some of the highest rates of sea ice loss in the Arctic (Onarheim et al. 2018). Sea ice is critical habitat for a variety Arctic animals that depend on sea ice for feeding, resting, and breeding (Martin and Jonkel 1983; Freitas et al. 2008; Laidre et al. 2015). As this habitat continues to decline, these animals will have to adapt and adjust their life histories accordingly (Kovacs et al. 2011; Laidre et al. 2015).

Declining sea ice is problematic for polar bears (*Ursus maritimus*), as it is their main habitat and provides access to their preferred ice seal prey. Reduced availability of sea ice has forced polar bears in many parts of their range to spend more time on land (Stirling et al. 1999; Atwood et al. 2016). With less seasonal sea-ice habitat available as a hunting platform, it is more difficult for polar bears to capture prey, and, as a result, several studies attest to prey shifts (Mckinney et al. 2009, 2013, 2017; Stirling and Derocher 2012) and changes in body condition (Stirling et al. 1999; Rode et al. 2010, 2012; Obbard et al. 2016). Projections of polar bear abundance indicate anticipated summer habitat loss of almost 70% by the end of the 21st century (Durner et al. 2009; Hunter et al. 2010; Regehr et al. 2010). In response to changing habitat and concerns of limited adaptive response, polar bears in Alaska were listed as threatened under the U.S. Endangered Species Act in 2008 (U.S. Fish and Wildlife Service 2008).

Polar bears consume a high fat diet, mainly consisting of the blubber of ice seals (Stirling et al. 1977). This lipid-rich diet is calorically dense (Stirling and McEwan 1975), which helps to maintain their high metabolic rate (Pagano et al. 2018a) and supports the high total energy costs associated with their large body size (Rode et al. 2015). A high fat diet also provides the energy needed to support high-energetic costs of locomotion (Pagano et al. 2018b). However, with declining summer sea ice extent, polar bears have been spending increasing durations on land in many parts of their range (Stirling et al. 1999; Rode et al. 2015; Atwood et al. 2016).

Polar bears are separated into 19 subpopulations shared among Canada, Russia, United States, Greenland/Denmark, and Norway (IUCN/SSC Polar Bear Specialist Group 2019). The most recent population assessment categorizes five of these subpopulations as stable, two as increasing, eight as data deficient, and four as declining along with sea ice extent (IUCN/SSC Polar Bear Specialist Group 2019). The estimated population size for all polar bears is 22,000–31,000 worldwide (Regehr et al. 2016).

Polar bear subpopulations have been designated using a variety of life history information, genetics, and habitat use. For example, the border separating the Chukchi Sea (CS) and Southern Beaufort Sea (SBS) subpopulations has been determined using both radio-collared animals, as well as mark-recapture data (Amstrup et al. 1986; Amstrup and DeMaster 1988; Derocher and Stirling 1990, 1995; Lunn et al. 1997; Stirling et al. 1977, 1988; Taylor and Lee 1995). However, female polar bears are known to migrate between these two subpopulations, creating overlap (Scharf et al. 2018). Further, only females are tracked with radio collars, because males have necks that are wider than their heads, causing the radio collars to slip off (Amstrup et al. 2001; Wilson et al. 2014). There is some evidence that males and females exhibit different movement patterns (Laidre et al. 2013). Thus, distribution patterns based solely on movement patterns of adult females may not be representative of distribution patterns of all sex and age classes. Finally, a lack of genetic differentiation between CS and SBS polar bears limits the utility of genetics for distinguishing the two Alaska subpopulations (Paetkau et al. 1999).

The observed overlap in female polar bear movement patterns can make it difficult to define the SBS/CS geographical lines. The eastern boundary of the CS subpopulation, defined under the U.S.-Russia Bilateral Agreement (Obbard et al. 2010, United States T. Doc. 107–10), which assigns harvest levels across Alaska communities, is currently under review due to uncertainty in bear movement patterns between the current boundary at Utqiaġvik, AK (formerly Barrow) and Icy Cape, AK, which is the proposed new boundary (Amirkhanov et al. 2018; IUCN/SSC Polar Bear Specialist Group 2019). Further, Icy Cape was proposed by the Alaska Nunnut Co-Management Council to be reinstated as the western biological boundary for the SBS (Amirkhanov et al. 2018). Subpopulation boundaries to date were based in part on Amstrup et al. (2001), which documented that polar bears captured near Wainwright, AK had an 80-90% chance of being a CS bear, and those captured closer to Utqiaġvik had an increasingly higher probability of being SBS bears.

The SBS polar bear range covers the northern portion of Alaska into Canada. There has been a 40% decrease in population size for the SBS polar bears since 2001 (Bromaghin et al. 2015). However, recent demographic analysis suggests that the SBS subpopulation has stabilized (Atwood et al. 2020). The SBS subpopulation has a quota (number of harvested bears permitted), of 70 polar bears, which is shared evenly with Canada under the Inuvialuit-Inupiat Polar Bear Management Agreement (Inuvialuit-Inupiat Polar Bear Commission; Amirkhanov et al. 2018). The harvest quota set by this Native to Native agreement is voluntary. Prey for SBS polar bears are mainly ice seals, though they do have access to flensed bowhead whale (*Balaena mysticetes*) carcasses from Native subsistence hunts.

In recent summers, sea ice has retreated further away from the coast, causing an increasing number of polar bears in the Southern Beaufort Sea to spend the summer on land, including up to 25-30% of collared adult females (Atwood et al. 2016; Pongracz and Derocher 2017). Conversely, some polar bears will retreat with the sea ice habitat in the summer (Pongracz and Derocher 2017). Several studies have indicated that bears in the SBS subpopulation exhibit two general distribution patterns with one group selecting more pelagic habitats over the deep waters of the Arctic basin and a coastal group with a more nearshore range (Rogers et al. 2015; Boucher et al. 2019). Many of the coastal bears also utilize bowhead whale carcasses originating from Alaska Native subsistence harvests along the Alaska coastline (Schliebe et al. 2008; Herreman and Peacock 2013; Rogers et al. 2015).

The CS polar bear subpopulation ranges between the western coast of Alaska and eastern Russia and is currently considered to be a stable population of ~3,000 bears (Regehr et al. 2018). CS bears are in better body condition, are of larger body size, and exhibit higher reproductive success in comparison to SBS bears (Rode et al. 2014), despite 40% of adult females summering on shore in the Chukchi Sea (Rode et al. 2015). CS polar bears also have greater access to gray whales (*Eschrichtius robustus*) from beach cast carcasses along the Chukotkan coast and Wrangel Island (Laidre et al. 2018) as well walruses (*Odobenus rosmarus divergens*; Fischbach et al. 2009; Jay et al. 2012; Monson et al. 2013). The CS quota is shared with Russia as specified under the Alaska-Chukotka Bilateral Agreement (IUCN/SSC Polar Bear Specialist Group).

The environments and ecosystems in which the CS and SBS polar bear subpopulations reside have several differences. The high productivity of the Chukchi Sea allows for large phytoplankton blooms (Arrigo et al. 2012, 2014) and high benthic productivity, which supports bottom-feeding marine mammals, such as walruses and bearded seals (*Erignathus barbatus*). CS polar bears have further increased access to walruses (Fischbach et al. 2009; Jay et al. 2012; Monson et al. 2013) due to common haul-outs in the Point Lay, AK area, and on the Chukotka Peninsula (CS subpopulation territory; U.S. Fish and Wildlife Service 2019). There is also a higher abundance of ice seals, namely ringed (*Pusa hispida*) and bearded seals in the Chukchi Sea than in the Beaufort Sea (Stirling and McEwan 1975; Boveng et al. 2017). These ecological and environmental differences between the areas occupied by SBS and CS polar bears suggest that differences in stable isotopic concentrations in tissues may be a useful method for distinguishing bears from these two subpopulations.

Stable isotope analysis of carbon and nitrogen is a well-established tool in animal ecology (West et al. 2006). Trophic position of organisms can be studied using stable nitrogen isotopes ($\delta^{15}N$ values; Fry 2006), and stable carbon isotopes ($\delta^{13}C$ values) are useful for comparing carbon sources, such as terrestrial or aquatic, benthic or pelagic, and nearshore or offshore (DeNiro and Epstein 1978; Newsome et al. 2010). Stomach content analysis can be biased and only identifies the most recently consumed prey (Bowen and Iverson 2013). Alternatively, stable isotopes turnover at different rates in different tissues, ranging from days (e.g., blood) to years or even an animal's lifetime (e.g., bone collagen), and thereby can provide temporal windows into diets over longer periods (Tieszen et al. 1983; Hare et al. 1991). Two common approaches for applying stable isotope analyses include examining isotope values of 'bulk' tissues or in specific compounds within those tissues (e.g., fatty acids and amino acids). Bulk stable isotope analysis provides the total isotope values of the tissue sample analyzed (Philp 2007). However, this picture can then be refined by compound-specific stable isotope analysis (CSIA) of carbon and nitrogen of individual amino acids (AA). AAs are excellent indicators of the regional isotopic baseline from primary producers of the originating ecosystem (Whiteman et al. 2019). Thus, using CSIA-AA can more readily distinguish between food sources, e.g., terrestrial or marine (Bowes and Thorp 2015) than bulk stable isotope analysis alone.

The overall goals of this thesis are to determine if stable isotopes can distinguish between the SBS and CS polar bear subpopulations, and if there is evidence of an increased reliance on terrestrial carbon sources for the SBS subpopulation over time. In Chapter 2, we investigate if SBS and CS polar bear subpopulations can be differentiated using bulk δ^{15}N and δ^{13}C values from analyses of bone collagen, as it has a lifetime integrated isotopic signature. Based on previous work on stable isotopes in ice seals, bowhead whales, and zooplankton in this region (Saupe et al. 1989; Schell et al. 1989, 1998; Dehn et al. 2007), we hypothesized that the two polar bear subpopulations can be distinguished using δ^{15}N and δ^{13}C values. Adding a stable isotope approach to the management toolkit for polar bear biological boundary analysis will be helpful in resolving Alaskan polar bear subpopulations boundary and thus quota issues.

Chapter 3 focuses on polar bear bone collagen extracted from samples collected within the boundaries of the SBS subpopulation to examine long-term patterns in bulk and compound-specific stable isotopes over a 65-year period from 1954–2019. Specifically, we sought to determine, if there were changes in dietary trophic level and in the degree of incorporation of terrestrially derived carbon after an Arctic sea ice minimum in September 2007. We used bulk δ^{15}N and δ^{13}C values to identify changes in dietary trophic level and δ^{13}C values in AA to identify marine versus terrestrially derived carbon by comparing polar bear collagen values with those of a variety of marine and terrestrial mammal species. Given the lifetime isotopic signature from bone collagen, the use of polar bear bone archives allows the study of past and present in this subpopulation. There have been several major sea ice minimum years (Fetterer et al. 2017) in the Arctic, which are likely to have potentially lasting effects on polar bear ecology. Looking into several years of changing habitat in the Southern Beaufort Sea, and how dietary carbon and nitrogen sources may have changed, allows for a better understanding of their resiliency to continued sea ice loss.

1.1 References

Amirkhanov A, Kavry S, Siekaniec G, Brower C (2018) Report of the proceedings of the 10th meeting of the Russian-American Commission on polar bears. Egvekinot, Russian Federation.

Amstrup S, Stirling I, Lentfer J (1986) Past and present status of polar bears in Alaska. Wildl Soc Bull 14: 241–254.

Amstrup S, DeMaster D (1988) Polar bear–*Ursus maritimus*. In: Lentfer J (eds) Selected marine mammals of Alaska: Species accounts with research and management recommendations. Marine Mammal Commission, Washington, DC, USA, pp 39–56.

Amstrup S, Durner G, Mulcahy D, Garner G (2001) Comparing movement patterns of satellite-tagged male and female polar bears. Can J Zool 79: 2147–2158. https://doi.org/10.1139/z01-174

Arrigo K, Perovich D, Pickart R, Brown Z, van Dijken G, Lowry K, Mills M, Palmer M, Balch W, Bahr F, Bates N, Benitez-Nelson C, Bowler B, Brownlee E, Ehn J, Frey K, Garley R, Laney S, Lubelczyk L, Mathis J, Matsuoka A, Mitchell B, Moore G, Ortega-Retuerta E, Pal S, Polashenski C, Reynolds R, Schieber B, Sosik H, Stephens M, Swift J (2012) Massive phytoplankton blooms under Arctic sea ice. Science 336: 1408. https://doi.org/10.1126/science.1215065

Arrigo K, Perovich D, Pickart R, Brown Z, van Dijken G, Lowry K, Mills M, Palmer M, Balch W, Bates N, Benitez-Nelson C, Brownlee E, Frey K, Laney S, Mathis J, Matsuoka A, Mitchell B, Moore G, Reynolds R, Sosik H, Swift J (2014) Phytoplankton blooms beneath the sea ice in the Chukchi Sea. Deep Res Part II Top Stud Oceanogr 105: 1–16. https://doi.org/10.1016/j.dsr2.2014.03.018

Atwood T, Peacock E, McKinney M, Lillie K, Wilson R, Douglas D, Miller S, Terletzky P (2016) Rapid environmental change drives increased land use by an Arctic marine predator. PLoS One 11: 1–18. https://doi.org/10.1371/journal.pone.0155932

Atwood TC, Bromaghin JF, Patil VP, Durner GM, Douglas DC, and Simac KS (2020) Analyses on subpopulation abundance and annual number of maternal dens for the U.S. Fish and Wildlife Service on polar bears (*Ursus maritimus*) in the southern Beaufort Sea, Alaska: U.S. Geological Survey Open-File Report 2020-1087, pp 16. https://doi.org/10.3133/ofr20201087

Boucher N, Derocher A, Richardson E (2019) Space use patterns affect stable isotopes of polar bears (*Ursus maritimus*) in the Beaufort Sea. Polar Biol 42: 1581–1593. https://doi.org/10.1007/s00300-019-02546-9

Boveng P, Cameron M, Conn P, Moreland E (2017) Abundance estimates of ice-associated seals: Bering Sea populations that inhabit the Chukchi Sea during the open-water period. Final Report. BOEM Report 2016-077. Bureau of Ocean Energy Management, Alaska Outer Continental Shelf Region, Anchorage, Alaska, USA.

Bowen W, Iverson S (2013) Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Mar Mammal Sci 29: 719–754. https://doi.org/10.1111/j.1748-7692.2012.00604.x

Bowes R, Thorp J (2015) Consequences of employing amino acid vs. bulk-tissue, stable isotope analysis: A laboratory trophic position experiment. Ecosphere 6: 1–12. https://doi.org/10.1890/ES14-00423.1

Bromaghin J, Mcdonald T, Stirling I, Derocher A, Richardson E, Regehr E, Douglas D, Durner G, Atwood T, Amstrup S (2015) Polar bear population dynamics in the Southern Beaufort Sea during a period of sea ice decline. Ecol Appl 25: 634–651. https://doi.org/10.1890/14-1129.1

Dehn L, Sheffield G, Follmann E, Duffy L, Thomas D, O'Hara T (2007) Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biol 30: 167–181. https://doi.org/10.1007/s00300-006-0171-0

DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42: 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

Derocher A, Stirling I (1990) Distribution of polar bears (*Ursus maritimus*) during the ice-free period in western Hudson Bay. Can J Zool 68:1395–1403. https://doi-org/10.1139/z90-208

Derocher A, Stirling I (1995) Estimation of polar bear population size and survival in western Hudson Bay. J Wildl Manage 59: 215–221. http://doi.org/10.2307/3808933

Durner G, Douglas D, Nielson R, Amstrup S, McDonald T, Stirling I, Mauritzen M, Born E, Wiig Ø, DeWeaver E, Serreze M, Belikov S, Holland M, Maslanik J, Aars J, Bailey D, Derocher A (2009) Predicting 21st-century polar bear habitat distribution from global climate models. Ecol Monogr 79: 25–58. https://doi.org/10.1890/07-2089.1

Fischbach A, Monson D, Jay C (2009) Enumeration of Pacific walrus carcasses on beaches of the Chukchi Sea in Alaska following a mortality event, September 2009: U.S. Geological Survey Open-File Report 2009- 1291.

Freitas C, Kovacs K, Ims R, Fedak M, Lydersen C (2008) Ringed seal post-moulting movement tactics and habitat selection. Oecologia 155: 193–204. https://doi.org/10.1007/s00442-007-0894-9

Fry B (2006) Stable isotope ecology. Springer Science+Business Media,LLC, New York, NY. https://doi.org/10.1007-0-387-33745-8

Hare E, Fogel M, Stafford Jr T, Mitchell A, Hoering T (1991) The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18: 277–292. https://doi.org/10.1016/0305-4403(91)90066-X

Herreman J, Peacock E (2013) Polar bear use of a persistent food subsidy: Insights from non-invasive genetic sampling in Alaska. Ursus 24: 148–163. https://doi.org/10.2192/URSUS-D-12-00030.1

Hunter C, Caswell H, Runge M, Regehr E, Amstrup S, Stirling I (2010) Climate change threatens polar bear populations: A stochastic demographic analysis. Ecology 91: 2883–2897. https://doi.org/10.1890/09-1641

IUCN/SSC Polar Bear Specialist Group (2019) Status report on the world's polar bear subpopulations. 2019 Status Report, pp 1–31. http://pbsg.npolar.no/export/sites/pbsg/en/docs/2019-StatusReport.pdf

Jay C, Fischbach A, Kochnev A (2012) Walrus areas of use in the Chukchi Sea during sparse sea ice cover. Mar Ecol Prog Ser 468: 1–13. https://doi.org/10.3354/meps10057

Jones MC, Berkelhammer M, Keller KJ, Yoshimura K, Wooller MJ (2020) High sensitivity of Bering Sea winter sea ice to winter insolation and carbon dioxide over the last 5500 years. Sci Adv 6: 1–10. http://doi.org/10.1126/sciadv.aaz9588

Kovacs KM, Lydersen C, Overland JE, Moore SE (2011) Impacts of changing sea-ice conditions on Arctic marine mammals. Mar Biodiv 41:181–194. http://doi.org/10.1007/s12526-010-0061-0

Laidre KL, Born EW, Gurarie E, Wiig Ø, Dietz R, Stern H (2013) Females roam while males patrol: divergence in breeding season movements of pack-ice polar bears (*Ursus maritimus*). Proc R Soc B 280: 20122371. http://doi.org/10.1098/rspb.2012.2371

Laidre KL, Born EW, Heagerty P, Wiig Ø, Stern H, Dietz R, Aars J, Anderson M (2015) Shifts in female polar bear (*Ursus maritimus*) habitat use in East Greenland. Polar Biol 38:879–893. http://doi.org/ 10.1007/s00300-015-1648-5

Lunn N, Andriashek D, Kolenosky G (1997) Re-estimating the size of the polar bear population in western Hudson Bay. Arctic 50: 234–240. https://doi.org/10.14430/arctic1105

Martin S, Jonkel C (1983) Use of sea ice habitat by polar bears. Int Conf Bear Res and Manage 5: 255–259. http://doi.org/10.2307/3872545

McKinney M, Peacock E, Letcher R (2009) Sea ice-associated diet change increases the levels of chlorinated and brominated contaminants in polar bears. Environ Sci Technol 43: 4334–4339. https://doi.org/10.1021/es900471g

McKinney M, Iverson S, Fisk A, Sonne C (2013) Global change effects on the long-term feeding ecology and contaminant exposures of East Greenland polar bears. Glob Chang Biol 19: 2360–2372. https://doi.org/10.1111/gcb.12241

McKinney M, Atwood T, Iverson S, Peacock E (2017) Temporal complexity of southern Beaufort Sea polar bear diets during a period of increasing land use. Ecosphere 8: 1–14. https://doi.org/10.1002/ecs2.1633

Monson D, Udevitz M, Jay C (2013) Estimating age ratios and size of Pacific walrus herds on coastal haulouts using video imaging. PLoS One 8: 1–12. https://doi.org/10.1371/journal.pone.0069806

Newsome S, Clementz M, Koch P (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mammal Sci 26: 509–572. https://doi.org/10.1111/j.1748-7692.2009.00354.x

Obbard ME, Thiemann GW, Peacock E, Debruyn TD (2010) Proceedings of the 15th working meeting of the IUCN/SSC Polar Bear Specialist Group, 29 June-3 July 2009, Copenhagen, Denmark. Gland, Switzerland and Cambridge, UK: IUCN.

Obbard M, Cattet M, Howe E, Middel K, Newton E, Kolenosky G, Abraham K, Greenwood C (2016) Trends in body condition in polar bears (*Ursus maritimus*) from the Southern Hudson Bay subpopulation in relation to changes in sea ice. Arct Sci 2: 15–32. https://doi-org/10.1139/as-2015-0027

Onarheim I, Eldevik T, Smedsrud L, Stroeve J (2018) Seasonal and regional manifestation of Arctic sea ice loss. J Clim 31: 4917–4932. https://doi.org/10.1175/JCLI-D-17-0427.1

Paetkau D, Amstrup C, Born E, Calvert W, Derocher A, Garner G, Messier F, Stirling I, Taylor M, Wiig Ø, Strobeck C (1999) Genetic structure of the world’s polar bear populations. Mol Ecol 8: 1571–1584. https://doi.org/10.1046/j.1365-294x.1999.00733.x

Pagano AM, Durner GM, Rode KD, Atwood TC, Atkinson SN, Peacock E, Costa DP, Owen MA, Williams TM (2018a) High-energy, high-fat lifestyle challenges an Arctic apex predator, the polar bear. Science 359:568-572. http://doi.org/10.1126/science.aan8677

Pagano AM, Carnahan AM, Robbins CT, Owen MA, Batson T, Wagner N, Cutting A, Nicassio-Hiskey N, Hash A, Williams TM (2018b) Energetic costs of locomotion in bears: Is plantigrade locomotion energetically economical? J Exp Biol 221:1-9. http://doi.org/ 10.1242/jeb.175372

Philp RP (2007) The emergence of stable isotopes in environmental and forensic geochemistry studies: A review. Environ Chem Lett 5:57–66. https://doi.org/10.1007/s10311-006-0081-y

Pongracz J, Derocher A (2017) Summer refugia of polar bears (*Ursus maritimus*) in the southern Beaufort Sea. Polar Biol 40: 753–763. https://doi.org/10.1007/s00300-016-1997-8

Regehr E, Hunter C, Caswell H, Amstrup S, Stirling I (2010) Survival and breeding of polar bears in the Southern Beaufort Sea in relation to sea ice. J Anim Ecol 79: 117–127. https://doi.org/10.1111/j.1365-2656.2009.01603.x

Regehr EV, Laidre KL, Akcakaya HR, Amstrup SC, Atwood TC, Lunn NJ, Obbard M, Stern H, Thiemann GW, Wiig Ø (2016) Conservation status of polar bears (*Ursus maritimus*) in relation to projected sea-ice declines. Biol Lett 12:20160556. https://doi.org/10.1098/rsbl.2016.0556

Regehr E, Hostetter N, Wilson R, Rode K, St. Martin M, Converse S (2018) Integrated population modeling provides the first empirical estimates of vital rates and abundance for polar bears in the Chukchi Sea. Sci Rep 8: 1–12. https://doi.org/10.1038/s41598-018-34824-7

Rode K, Amstrup S, Regehr E (2010) Reduced body size and cub recruitment in polar bears associated with sea ice decline. Ecol Appl 20: 768–782. https://doi.org/10.1890/08-1036.1

Rode KD, Peacock E, Taylor M, Stirling I, Born EW, Laidre KL, Wiig Ø (2012) A tale of two polar bear populations: ice habitat, harvest and body condition. Popul Ecol 54:3-18. http://doi.org/ 10.1007/s10144-011-0299-9

Rode K, Regehr E, Douglas D, Durner G, Derocher A, Thiemann G, Budge S (2014) Variation in the response of an Arctic top predator experiencing habitat loss: feeding and reproductive ecology of two polar bear populations. Glob Chang Biol 20: 76–88. https://doi.org/10.1111/gcb.12339

Rode K, Robbins C, Nelson L, Amstrup S (2015) Can polar bears use terrestrial foods to offset lost ice-based hunting opportunities? Front Ecol Environ 13: 138–145. https://doi.org/10.1890/140202

Rogers M, Peacock E, Simac K, O'Dell M, Welker J (2015) Diet of female polar bears in the southern Beaufort Sea of Alaska: Evidence for an emerging alternative foraging strategy in response to environmental change. Polar Biol 38: 1035–1047. https://doi.org/10.1007/s00300-015-1665-4

Saupe S, Schell D, Griffiths W (1989) Carbon-isotope ratio gradients in western arctic zooplankton. Mar Biol 103: 427–432. https://doi.org/10.1038/164914a0

Scharf HR, Hooten MB, Wilson RR, Durner GM, Atwood TC (2018) Accounting for phenology in the analysis of animal movement. arXiv Prepr. arXiv1806.09473. https://doi.org/10.1111/biom.13052

Schell DM, Saupe SM, Haubenstock N (1989) Natural isotope abundances in bowhead whale (*Balaena mysticetus*) baleen: Markers of aging and habitat usage. In: Rundel PW, Ehleringer JR, Nagy KA (eds), Stable Isotopes in Ecological Research. Ecological Studies (Analysis and Synthesis). Springer, New York, NY, pp 260–269. https://doi.org/10.1007/978-1-4612-3498-2_15

Schell D, Barnett B, Vinette K (1998) Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi and Beaufort seas. Mar Ecol Prog Ser 162: 11–23. https://doi.org/10.3354/meps162011

Schliebe S, Rode K, Gleason J, Wilder J, Proffitt K, Evans T, Miller S (2008) Effects of sea ice extent and food availability on spatial and temporal distribution of polar bears during the fall open-water period in the Southern Beaufort Sea. Polar Biol 31: 999–1010. https://doi.org/10.1007/s00300-008-0439-7

Stirling I, Archibald R, DeMaster D (1977) The distribution and abundance of seals in the eastern Beaufort Sea. J Fish Res Board Can 34: 976–988. http://doi.org/10.1139/f77-150

Stirling I, Jonkel C, Smith P, Robertson R, Cross D (1977) The ecology of the polar bear (*Ursus maritimus*) along the western coast of Hudson Bay. Can Wildl Serv 33: 1–64.

Stirling I, Andriashek D, Spencer C, Derocher A (1988) Assessment of the polar bear population in the eastern Beaufort Sea. Final report to the Northern Oil and Gas Assessment Program. Edmonton, Alberta, Canada.

Stirling I, Lunn N, Iacozza J (1999) Long-term trends in the population ecology of polar bears in Western Hudson Bay in relation to climatic change. Arctic 52: 294–306. http://doi.org/10.14430/arctic935

Stirling I, Derocher A (2012) Effects of climate warming on polar bears: a review of the evidence. Glob Chang Biol 18: 2694–2706. https://doi.org/10.1111/j.1365-2486.2012.02753.x

Stirling I, McEwan E (1975) Caloric value of whole ringed seals (*Phoca hispida*) in relation to polar bear (*Ursus maritimus*) ecology and hunting behavior. Can J Zool 53:1021–1027. https://doi.org/10.1139/z75-117

Taylor M, Lee J (1995) Distribution and abundance of Canadian polar bear populations: A management perspective. Arctic 48: 147–154. https://doi.org/10.14430/arctic1236

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57: 32–37. https://doi.org/10.1007/BF00379558

U.S. Fish and Wildlife Service (2008) Endangered and threatened wildlife and plants: Determination of threatened status for the polar bear (*Ursus maritimus*) throughout its range. Fed Regist 73: 28211-28303.

U.S. Fish and Wildlife Service (2019) Pacific walrus: Use of coastal haulouts along the Chukchi Sea coast.

West JB, Bowen GJ, Cerling TE, Ehleringer JR (2006) Stable isotopes as one of nature's ecological recorders. Trends Ecol Evol 21: 408–414. https://doi.org/10.1016/j.tree.2006.04.002

Whiteman J, Smith E, Besser A, Newsome S (2019) A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. Diversity 11: 1–18. https://doi.org/10.3390/d11010008

Wilson R, Horne J, Rode K, Regehr E, Durner G (2014) Identifying polar bear resource selection patterns to inform offshore development in a dynamic and changing Arctic. Ecosphere 5: 1–24. https://doi.org/10.1890/ES14-00193.1

Chapter 2: Stable carbon and nitrogen isotope differences of polar bears in the Southern Beaufort Sea and Chukchi Sea[1]

2.1 Abstract

This study analyzed bulk $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen of polar bears (*Ursus maritimus*) in two subpopulations in Alaska. The Southern Beaufort Sea (SBS) polar bear subpopulation has declined in response to sea ice loss, while the Chukchi Sea (CS) subpopulation appears stable. We analyzed 112 polar bear bones (predominantly mandibles) from 1954–2019 that have been archived in the University of Alaska Museum of the North, as well as modern bone samples from polar bears harvested for subsistence purposes by coastal Alaskan Natives. The purpose of this study was to determine if the SBS and CS subpopulations could be distinguished based on the stable isotope signatures of bone collagen. Our study showed a significant difference in $\delta^{13}C$ values (P<0.001), but not $\delta^{15}N$ values (P=0.654) between the CS (-13.0‰±0.3‰ and 22.0‰±0.9‰, respectively) and the SBS bears (-14.7‰±1.3‰ and 22.2‰±1.0‰, respectively). Our findings indicate that the two subpopulations are consuming similar high trophic level prey, while feeding in ecosystems with different $\delta^{13}C$ baselines. We performed a logistic regression analysis (LR) using $\delta^{13}C$ and $\delta^{15}N$ values of the polar bears to predict their placement into these two subpopulations. Using Icy Cape, AK as the geographical boundary, the LR was able to correctly place the polar bears in their respective subpopulations 82% of the time. Overall accuracy of placement changed to 84% when using the current geographical boundary at Utqiaġvik, AK. Samples collected from the Wainwright, AK region were predicted to be 58% CS and 42% SBS polar bears. This indicates that the area between

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Wainwright and Icy Cape is a polar bear mixing zone that includes bears from both subpopulations. Bone collagen has a long-term, potentially life-long stable isotope turnover rate, and our findings could be used to determine the association of harvested polar bears to Alaska subpopulations, thus aiding in harvest quota management.

2.2 Introduction

Worldwide, 19 polar bear (*Ursus maritimus*) subpopulations are recognized from Canada, Russia, United States, Greenland/Denmark, and Norway (IUCN/SSC Polar Bear Specialist Group 2019). Of these 19 subpopulations, five are stable, two are increasing, eight are data deficient, and four are declining along with sea ice extent (IUCN/SSC Polar Bear Specialist Group 2019). Projections for polar bear abundance indicate anticipated summer habitat loss of almost 70% by the end of the 21st century (Durner et al. 2009; Hunter et al. 2010; Regehr et al. 2010). The Chukchi and Beaufort seas are experiencing some of the highest rates of sea ice loss in the Arctic (Onarheim et al. 2018). The SBS subpopulation shows somewhat differing population trends with the CS subpopulation. The CS subpopulation is currently estimated at 3000 bears and stable (Regehr et al. 2018). Recent demographic analysis suggests that the SBS subpopulation, which declined from 2001–2010 (Bromaghin et al. 2015), has stabilized (Atwood et al. 2020).

The CS and SBS polar bear subpopulations occupy ecosystems that differ environmentally and ecologically. The CS is generally more productive than the SBS, as it occurs over a large, shallow continental shelf (Grebmeier et al. 2006), allowing for large phytoplankton blooms (Arrigo et al. 2012, 2014) and high benthic productivity, which supports bottom-feeding marine mammals, such as Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*). Overall abundance of ice seals, namely ringed (*Pusa hispida*) and bearded seals, which are the preferred prey of polar bears, is greater in the CS than in the SBS (Stirling et al. 1977a; Boveng et al. 2017). Furthermore, walrus haul-outs in the Point Lay, AK area, and on the Chukotka peninsula (CS subpopulation territory; U.S. Fish and Wildlife Service 2019), provide feeding opportunities for CS polar bears (Fischbach et al. 2009; Jay et al. 2012;

Monson et al. 2013). CS polar bears also have a greater access to gray whales (*Eschrichtius robustus*) from beach cast carcasses along the Chukotkan coast and Wrangel Island (Laidre et al. 2018). Conversely, polar bears in the SBS that come on shore during the summer have access to bowhead whale (*Balaena mysticetes*) carcasses from Native subsistence hunts (Miller et al. 2006; Bentzen et al. 2007; Rogers et al. 2015). Thus, differences in prey composition could result in different isotopic concentrations in tissues of polar bears in these two regions.

Environmentally, baseline stable isotope concentrations differ between the SBS and CS polar bear ranges. Zooplankton taxa from the SBS exhibit lower $\delta^{13}C$ values compared to those from the Chukchi Sea due to differences in ocean nutrient circulation patterns and the influence of freshwater from the Mackenzie River in the eastern Beaufort Sea (Saupe et al. 1989; Schell et al, 1998; Lee et al. 2005; Divine et al. 2017). Following this pattern, ringed seals have lower $\delta^{13}C$ values in the Beaufort Sea compared to those in the Chukchi Sea (Dehn et al. 2007). Similarly, bowhead whales that migrate between the Chukchi and Beaufort seas exhibit $\delta^{13}C$ values of their baleen plates that change according to which of the two ecosystems they are primarily feeding in, with the Beaufort Sea having lower $\delta^{13}C$ values (Schell et al. 1989; Lee et al. 2005). Using baseline isotopic concentrations with polar bear tissues could provide insight into any potential differences between the SBS and CS subpopulations. However, the distinction of the polar bear subpopulations themselves are determined by many factors.

Geographic boundary designation for the two subpopulations is based on a variety of life history information, genetics, and habitat use. In Alaska, the SBS subpopulation has been distinguished from the CS subpopulation (shared between the U.S. and Russia) in the west and the Canadian Northern Beaufort Sea (NBS) subpopulation in the east using both radio-collared animals as well as mark-recapture data (Amstrup et al. 1986; Amstrup and DeMaster 1988;

Derocher and Stirling 1995, 1990; Lunn et al. 1997; Stirling et al. 1988, 1977b; Taylor and Lee 1995). Genetics, although a useful approach for delineating stocks in general, has proven less effective for these two subpopulations, as the CS subpopulation only shows a small genetic difference from SBS bears, and there is little to no structure within these two subpopulations based on 16 $(CA)_n$ microsatellite markers (Paetkau et al. 1999).

Migration of polar bear subpopulations across geographical boundaries has been reported between neighboring subpopulations and between those that are several countries apart. It is not uncommon for the radio-collar tracking to show overlap between two neighboring subpopulations, as seen with the SBS and CS (Scharf et al. 2018). It is rare, for polar bears to travel distances that span multiple countries, but it has been reported for an SBS bear recaptured in Greenland (Durner and Amstrup 1995). However, only female polar bears are tracked with radio collars, because males have necks that are larger than their heads, causing the radio collars to slip off (Amstrup et al. 2001; Wilson et al. 2014). This causes subpopulation boundaries to be more biased toward female-based movement patterns (Amstrup et al. 2001). While some bears disperse long distances, geographical boundaries are based on the majority of bears exhibiting discrete movement patterns and home ranges (IUCN/SSC Polar Bear Specialist Group 2019). However, in some areas, movement patterns are less discrete making it difficult to define subpopulation boundaries, for example the SBS/Northern Beaufort Sea (NBS) and SBS/CS geographical lines. Both of these subpopulations are currently facing potential boundary changes (Amirkhanov et al. 2018; IUCN/SSC Polar Bear Specialist Group 2019).

An important potential outcome from a geographical border change of the SBS and CS polar bear subpopulations is the effect on the Native subsistence harvest quotas for both areas. The current annual harvest quotas for the SBS and CS polar bear subpopulations are 70 and 85, respectively (Inuvialuit-Inupiat Polar bear Commission; Amirkhanov et al. 2018). The quota for the SBS polar bears is shared evenly with Canada under the Inuvialuit-Inupiat Polar Bear Management Agreement, while the CS quota is shared with Russia under the Alaska-Chukotka Bilateral Agreement (IUCN/SSC Polar Bear Specialist Group). Polar bears are important cultural, nutritional, and spiritual resources for Inuit communities (Voorhees et al. 2014; Braund et al. 2018).

The geographical boundary separating the two polar bear subpopulations that inhabit Alaska, SBS and CS, is defined under the U.S.-Russia Bilateral Agreement (Obbard et al. 2010, United States T. Doc. 107–10). This agreement places the boundary between the CS and SBS subpopulations at Utqiaġvik, AK (formerly Barrow) based on radio-collar tracking data. Icy Cape, Alaska, the former western boundary, is still recognized as the SBS subpopulation boundary by the IUCN/SSC Polar Bear Specialist Group. The Alaska Nunnut Co-Management Council has proposed to reinstate Icy Cape as the western biological boundary for the SBS. Updated biological boundary analysis for the subpopulations using satellite telemetry suggests that the eastern biological boundary of the CS is located between Icy Cape and Smith Bay (Amirkhanov et al. 2018). Harvest of the subpopulations is managed for the SBS by a Native to Native agreement, the Inuvialuit-Inupiat Polar bear commission, and for the CS the harvest quota is set under the U.S.-Russia Polar Bear Agreement. The CS quota is legally binding, while the SBS harvest quota is voluntary. The geographical boundary is proposed to revert back to Icy Cape, AK at the next U.S.-Russia Commission on Polar Bears meeting (Amstrup et al. 2005; Amirkhanov et al. 2018).

Defining the SBS/CS geographical boundary has been the subject of several studies. Amstrup et al. (2001) provided support for a geographical boundary at Utqiaġvik, finding that bears tagged from the Wainwright, AK area had an 80-90% chance of being a CS bear, and those captured closer to Utqiaġvik had an increasingly higher probability of being SBS bears. Additionally, Scharf et al. (2018) used telemetry data to show that it is possible to separate the subpopulations, though these data supported a hypothetical border between Utqiaġvik and Icy Cape. Both genetics and telemetry studies have only been partly successful distinguishing between the SBS and CS subpopulations.

Stable isotope analysis of carbon and nitrogen is a well-established tool in animal ecology (West et al. 2006). The method is based on the premise "you are what you eat", meaning the stable isotopes of animal tissues are derived from its food source, with a predictable offset (Hobson et al. 1994). Stable nitrogen isotopes ($\delta^{15}N$ values) are commonly used as an indicator of trophic position (Fry 2006), while stable carbon isotopes ($\delta^{13}C$ values) can trace carbon sources, such as terrestrial or aquatic, benthic or pelagic, nearshore or offshore (DeNiro and Epstein 1978; Newsome et al. 2010). Bulk stable isotope analysis is however complicated by differing and poorly understood tissue turnover times ranging from days (e.g., blood) to years or even an animal's lifetime (e.g., bone collagen) (Tieszen et al. 1983; Hare et al. 1991).

We investigated if SBS and CS polar bear subpopulations can be differentiated using $\delta^{15}N$ and $\delta^{13}C$ values from analyses of bone collagen, as it has a lifetime integrated isotopic signature. Our access to numerous archived polar bear bone samples and those provided by Native subsistence hunters allowed for a substantial sample size from both subpopulations. Based on previous work on stable isotopes in ice seals, bowhead whales, and zooplankton in this region (Saupe et al. 1989; Schell et al. 1989, 1998; Dehn et al. 2007), we hypothesized that the two polar bear subpopulations can be distinguished using $\delta^{15}N$ and $\delta^{13}C$ values. Adding a stable isotope approach to the management toolkit for polar bear biological boundary analysis will be helpful in resolving Alaskan polar bear subpopulations boundary and thus quota issues.

2.3 Materials and Methods

2.3.1 Sample Collection

For this study, we analyzed 112 polar bear bone samples, including 41 from the CS and 71 from the SBS subpopulation (subpopulations assigned based on Amstrup et al. 2005), spanning the past 65 years from 1954–2019 (Tables 2.1 and 2.2, Figure 2.1). We used bone collagen for several reasons. First, bone collagen is not degraded or broken down by external factors due to the protection from the bone itself, meaning the isotopic signatures are not influenced or contaminated by storage and handling (Collins et al. 2002). Second, use of hard structures, such as bone, opens up vast archives of polar bear collections spanning decades, centuries, and even millennia. This provided the opportunity to use archived bones from museum collections to assess changes in isotope signatures from the past and present. All samples were obtained from the archives of the Mammalogy Department of the University of Alaska Museum of the North and from polar bears harvested for subsistence purposes by Native polar bear hunters from the North Slope, Alaska (Table 2.2). Bone samples from harvested polar bears were collected during routine post-mortem examination in Utqiaġvik, AK (formerly Barrow) as part of the North Slope Borough Department of Wildlife Management (NSB-DWM) polar bear health assessment program (Table 2.1). Polar bear samples were collected and analyzed under authority of permit number MA80164B-0 issued to the NSB-DWM (Appendix B).

2.3.2 Collagen Extraction

We removed approximately 5 g of cortical bone from each individual polar bear (mainly lower mandibles and bacula). Bone was polished with a Dremel® sand drum attachment to eliminate outside contamination. Using a diamond blade attachment, a piece of cortical bone was extracted. There is no appreciable variation in stable isotope signatures among different skeletal elements except distal limb bones (Clark et al., 2017; Bas et al. 2020). All samples were prepared following bone collagen extraction procedures described by Clark et al. (2019a). Briefly, compact cortical bone was cleaned in an Elmasonic P 300 H sonicator from Elma Schmidbauer GmbH. Lipids were removed using chloroform/methanol (Folch et al. 1957), and bones were demineralized with 6 N hydrochloric acid (HCl) and rinsed to neutral, gelatinized by agitating the sample at 65°C, filtered through a 0.45 um filter, and freeze dried for 48 hours.

The quality of collagen was assessed based on the atomic carbon to nitrogen (C:N) ratios as well as the percentage of collagen extracted (Table 2.2). The atomic C:N ratio was calculated using the formula $\left(\frac{14}{12} \times \frac{\%C}{\%N}\right)$, which takes the atomic mass of each element into consideration. An atomic C:N ration range of 2.9–3.6 is expected for bone collagen with no organic carbon contamination (DeNiro 1985). Only one sample fell outside of this range and did not influence the results whether included or not (Table 2.2).

2.3.3 Bulk Stable Carbon and Nitrogen Isotope Analysis

All polar bear bone collagen samples were submitted for bulk stable isotope analysis at the Alaska Stable Isotope Facility. Samples were analyzed using a Costech ECS 4010 elemental analyzer coupled to a Finnegan DeltaPlus XP. A peptone standard (No. P-7750 bovine-based protein, Sigma Chemical Company, lot #76f-0300 ($\delta^{13}C$, -15.8‰; $\delta^{15}N$, 7.0‰) was analyzed after every 10 bulk samples. Quality-control analysis from all peptone standard runs (n=26) provided an instrument error of ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. Following analysis, bulk $\delta^{13}C$ values were Suess corrected following Misarti et al. (2009) to account for anthropogenic CO_2 in the atmosphere since the Industrial Revolution.

2.3.4 Statistical Analysis

We performed a binary logistic regression (LR) to test which samples were more associated with the SBS or CS polar bear subpopulation. The LR was used with the packages *stats* (R Core Team 2020), *car* (Fox and Weisberg 2019), and *dplyr* (Wickham et al. 2020) in R version 4.0.1 (R Core Team 2020). The assumptions of an LR include a binary dependent variable (SBS or CS), independent observations, little or no multicollinearity, and a large sample size (Starkweather and Moske 2011). This test was chosen because our data were not normally distributed and the LR does not assume normality or homoscedasticity (Starkweather and Moske 2011). The dataset was created assuming a geographical border separating CS and SBS polar bear subpopulations at Icy Cape, AK (Amstrup et al. 2005). The purpose of LR is to find the probability that a polar bear sample is either CS or SBS, independent from the other samples being tested. We defined polar bear samples collected from east of Icy Cape, AK to the eastern border (Canada) as belonging to the SBS subpopulation, and samples collected from the west of Icy Cape, AK to the western border (Russia) as belonging to the CS subpopulation based on the new proposed border to be set at Icy Cape (Amstrup et al. 2005; IUCN/SSC Polar Bear Specialist Group 2019). We then performed an additional LR defining a subpopulation to each sample using a border at Utqiaġvik (Amirkhanov et al. 2018) to test if the predictive power of assigning polar bears to their respective subpopulations was higher or lower depending on the chosen border. We also assessed how the samples from the Wainwright, AK area were placed into each subpopulation to determine if this region could be a potential mixing area between the two subpopulations.

We then tested how, if at all, our data changed over time using a Kruskal-Wallis ranked sum test in R version 4.0.1 using the package *stats* (R Core Team 2020). This same statistical analysis was used to compare the bulk values between the two subpopulations and sex differences within subpopulations (alpha=0.05). All data are reported as mean ± 1 standard deviation (SD) unless otherwise noted. We also performed a linear regression on the $\delta^{13}C$ and $\delta^{15}N$ values of SBS polar bears over time to test for any temporal changes due to a wide range in bulk values for this subpopulation.

2.4 Results

The bulk $\delta^{13}C$ and $\delta^{15}N$ values for CS (-13.0‰±0.3‰ and 22.0‰±0.9‰, respectively) and SBS polar bears (-14.7‰±1.3‰ and 22.2‰±1.0‰, respectively; Figure 2.2) were tested for significant differences (alpha=0.05). The Kruskal-Wallis rank sum test showed a significant difference in $\delta^{13}C$ value of bone collagen (chi-squared=54.093, df=1, $P<0.001$), but not $\delta^{15}N$ values (chi-squared=0.201, df=1, $P=0.654$) between the two subpopulations. The $\delta^{13}C$ values of the SBS subpopulation were more negative than the CS subpopulation, with the largest difference being 5.4‰ (Table 2.2, Figure 2.2). The $\delta^{13}C$ and $\delta^{15}N$ values for the SBS subpopulation declined by about 2‰ and 1‰ from 1954–2019, respectively (Figure 2.3). The mean (±1SD) for the atomic C:N ratios was 3.3±0.1 for the SBS polar bears and 3.2±0.1 for the CS polar bears, with a total mean of 3.3±0.1 (Table 2.2).

Sex and temporal differences were tested within polar bear subpopulations to test if they were influencing the bulk stable isotope data. Within the SBS subpopulation, there were significant sex differences in $\delta^{13}C$ values (chi-squared=7.21, df=2, $P=0.027$) and $\delta^{15}N$ values (chi-squared= 22.14, df=2, $P<0.001$). Within the CS subpopulation, there were no significant sex differences for δ^{13}C values (chi-squared= 3.409, df = 2, $P=0.182$) and δ^{15}N values (chi-squared=0.993, df=2, $P=0.609$). Over time/year of collection, there were significant differences in both δ^{13}C values (chi-squared=56.603, df=28, $P=0.001$) and δ^{15}N (chi-squared=48.197, df=28, $P=0.010$) for the SBS subpopulation, but not for either δ^{13}C values (chi-squared=12.393, df=16, $P=0.717$) or δ^{15}N values (chi-squared=11.741, df=16, $P=0.762$) in CS polar bears. Bulk δ^{15}N and δ^{13}C in SBS polar bear bone collagen declined on average by 1‰ and 2‰, respectively, from 1954–2019 with statistically significant regression coefficients of $R^2=0.26$ ($P<0.001$) and $R^2=0.38$ ($P<0.001$), respectively (Figure 2.3). There were minimal changes in bulk δ^{13}C values and δ^{15}N values for the CS subpopulation from 1955–1983, with non-significant regression correlations of $R^2=0.02$ ($P=0.368$) and $R^2=0.00$ ($P=0.677$), respectively (Figure 2.3).

The LR used bulk δ^{13}C and δ^{15}N values to correctly place 81% of the SBS polar bears and 83% of the CS bears in their corresponding subpopulations based on a geographical border at Icy Cape, AK, with an overall accuracy of 82%. When placing the geographical border at Utqiaġvik and including the Wainwright polar bears as part of the CS subpopulation, the prediction was 90% correct for SBS polar bears and 77% correct for CS polar bears, with an overall accuracy of 84%. The LR placed 58% of the Wainwright individuals in the CS subpopulation and 42% in the SBS subpopulation.

An outlier CS sample with a δ^{13}C value of -19.7‰ and δ^{15}N value of 3.9‰ was removed from all statistical analyses due to the extreme difference in δ^{15}N compared to all other samples (Table 2.2).

2.5 Discussion

This study shows a distinct difference in $\delta^{13}C$ values of bone collagen between the SBS and CS polar bear subpopulations. The separation in $\delta^{13}C$ values of SBS and CS polar bears is likely due to differences in the $\delta^{13}C$ values at lower trophic levels within the food webs of the Beaufort and Chukchi seas. When compared to the variety of pelagic and benthic consumers from the Chukchi Sea Shelf, taxa tested in the Beaufort Sea had lower $\delta^{13}C$ values (Dunton et al. 2006). A similar isotopic difference (up to 6‰ in $\delta^{13}C$ values) between the Chukchi and Beaufort seas is then propagated to bowhead whales and ice seals (Lee et al. 2005; Schell et al. 1998, 1989; Dehn et al. 2007). This is consistent with our data, as the SBS subpopulation had lower $\delta^{13}C$ values than the CS subpopulation (Table 2.2, Figure 2.2)

There are also ecosystem differences between the Chukchi and Beaufort seas that could be associated with isotopic differences. The Chukchi Sea has a higher primary productivity than the Beaufort Sea (Grebmeier et al. 2006), which is reflected in the food web as an increase in available preferred prey, specifically for CS polar bears (Stirling et al. 1977a; Fischbach et al. 2009; Jay et al. 2012; Monson et al. 2013; Boveng et al. 2017). This is further supported by the better body condition and larger body size of the CS polar bears compared to the SBS subpopulation (Rode et al. 2014). Further investigation is needed to determine if differences in productivity affect isotopic concentrations within the food web.

CS polar bear samples were not available after the mid-1980's. However, potential changes in CS polar bear isotopic concentrations between the 1980s–2000s are unlikely to reduce the differences we observed between the SBS and CS subpopulations. Differences in $\delta^{13}C$ values within organisms of these two ecosystems have been known to further differentiate from each other with decreasing longitude (Dehn et al. 2007). A study by Clark et al. (2019a) also compared the $\delta^{13}C$ values of bone collagen of walruses in the Chukchi Sea from 4100 BP and 2016, with the mean difference being less than 1‰. In contrast, SBS polar bear $\delta^{13}C$ significantly declined from 1954–2019 by 2‰ (discussed in detail in Chapter 3), consistent with patterns observed in $\delta^{13}C$ values of SBS ringed seal claws during this time period (Boucher et al. 2020). The Beaufort Sea ecosystem, and therefore SBS polar bears, could be subjected to an increase of terrestrial carbon to the environment due to melting permafrost ($\delta^{13}C$: -25.8‰, Mu et al. 2015), in particular, because the Beaufort Sea has been more affected than the Chukchi Sea by the warming Arctic (Frey et al. 2015; Onarheim et al. 2018). As the Arctic continues to change, this decline in $\delta^{13}C$ values will likely continue, making the SBS subpopulation even more distinct from the CS subpopulation. Similarly, $\delta^{15}N$ values declined in SBS over the same time period, 1954–2019, by ~1‰ (Figure 2.3). Comparable declines in $\delta^{15}N$ values in SBS bears have been noted by Bentzen et al. (2007) and have been attributed to an increased proportion of bowhead whale carcasses to SBS polar bear diets (Rogers et al. 2015). Thus, the differences we observed here between subpopulations are likely to persist.

Several stable isotope studies have focused on the SBS subpopulation, which would allow for a large dataset, including a wide variety of tissues and sampling locations. In 2007, $\delta^{13}C$ and $\delta^{15}N$ values of polar bear red blood cells were used to assess diet composition of SBS polar bears (Bentzen et al. 2007). These authors were able to estimate the proportion of bowhead whales in the polar bear diets. Another study in 2015 used several different tissues from polar bears, including blood serum, red blood cells, adipose tissue, hair, and breath for stable carbon and nitrogen isotope analyses (Rogers et al. 2015). The amount of stable isotope data presented regarding the diets of the SBS subpopulation is substantially more than data available for CS polar bears and demonstrates the need for future work involving stable isotopes and the CS polar bear subpopulation.

We also examined polar bear sex differences for their contribution to the separation of the SBS and CS subpopulations. The only sex differences were for $\delta^{13}C$ and $\delta^{15}N$ values in SBS polar bears with higher $\delta^{13}C$ and $\delta^{15}N$ values in females. Higher $\delta^{15}N$ values in female SBS polar bears could occur due to hibernation, pregnancy, and/or lactation (Polischuk et al. 2001). More importantly however, there were sex differences for $\delta^{13}C$ values in SBS polar bears, and this could be the driving force for the variability in $\delta^{13}C$ values in SBS bears over time. The carbon difference between sexes is small but could be due to the uneven sample size (43 males and 22 females) or male polar bears consuming more bowhead whale muscle (−20.7‰±0.82‰; Dehn et al. 2006) than females (Herreman and Peacock 2013). The small sample size for females (n=10) in the CS subpopulation is likely the reason why no sex differences were observed.

The large range in $\delta^{13}C$ values for the SBS polar bear subpopulation could further be explained by the potential harvest of polar bears from the NBS subpopulation. Currently, there are several studies on NBS polar bears, but there is a critical knowledge gap regarding stable isotopes in this subpopulation. Non-isotope related studies have focused on body condition assessments (Amstrup et al. 2006), population estimates (Stirling et al. 2007, 2011), and genetics (Paetkau et al. 1995). A recent study using stable isotopes of hair and claws from polar bears in Canada focused on the SBS subpopulation (directly to the left of the geographical border with the NBS subpopulation) and determined the foraging range in that area (Boucher et al. 2019). In addition, the geographical boundary separating the NBS and SBS subpopulations has also changed over time, with the most recent change recorded in 2014 (IUCN/SSC Polar Bear Specialist Group 2019).

The differences in diet and available prey between the SBS and CS polar bears could also be driving the isotopic separation shown. These SBS bears have regular access to bowhead whale carcasses from Native subsistence harvests in the fall (Miller et al. 2006), while the CS subpopulation is known to consume more gray whales and walruses (Laidre et al. 2018; U.S. Fish and Wildlife Service 2019). These prey items have similar $\delta^{15}N$ values (all muscle tissue; bowhead whales: 13.2‰±0.7‰; gray whale: 12.93‰±1.01‰; 12.6‰±0.5‰), but different $\delta^{13}C$ values (all muscle tissue; bowhead whales: -20.7‰±0.56‰; gray whales: -16.6‰±0.9‰; walruses: -16.8‰±0.2‰), which could further contribute to the isotopic distinction between the bone collagen from these two subpopulations (Lee et al. 2005; Horstmann-Dehn et al. 2012; Clark et al. 2019b).

The stable isotopic profiles of these Alaskan polar bears allowed their placement into their respective subpopulations. The LR had a 2% stronger prediction rate with the current geographical border, which separates the two subpopulations, at Utqiaġvik rather than at Icy Cape (Amstrup et al. 2005). With the geographical border at Utqiaġvik, we were able to test how many samples from Wainwright, AK, the geographical area between the two proposed borders (Figure 2.1), are still showing an SBS stable isotope signature. Wainwright polar bears consisted of about 11% of our samples, and they were predicted to fall almost 50/50 between SBS and CS subpopulations. This could mean that this area is a mixing ground for the two subpopulations, which is supported by telemetry data (Scharf et al. 2018). Our predictions and overall accuracy of about 82%-84% from the LR is analogous to that of a 2019 study on nine polar bear subpopulations in Canada, which included the SBS subpopulation, but not the NBS subpopulation (Koehler et al. 2019). These authors used stable isotopes in polar bear hair to identify which subpopulation Canadian polar bears likely belonged to. The model the authors used was able to assign the polar bears to defined management areas with about 80% accuracy (Koehler et al. 2019). Boucher et al. (2019) also used bulk $\delta^{13}C$ and $\delta^{15}N$ values from hair and claws from polar bears in the Beaufort Sea. These authors found evidence of spatial fidelity either on- or off-shore based mainly on $\delta^{15}N$ values (Boucher et al. 2019). These findings indicate that other matrices (i.e., hair, claws, muscle) with faster turnover rates (Tieszen et al. 1983), can also be used for stable isotope analysis to assign Alaska polar bears to their respective subpopulations, in addition to bone collagen.

The geographical boundary separating the CS and SBS subpopulations is and has been changing between Icy Cape and Utqiaġvik. While the CS and SBS polar bear populations have been difficult to distinguish using genetics and/or spatial data (Kutschera et al. 2016; Scharf et al. 2018), our stable isotope approach combined with the LR can help differentiate the two subpopulations (Figure 2.2). Therefore, our data provides a refined understanding about polar bear population structure in Alaska, and how similar and/or distinct they are from one another. The current study provides an important spatial context for subsistence hunting quotas as determined by the U.S.-Russia Bilateral Agreement and will aid in determining which subpopulation a harvested bear belongs to for quota purposes, especially considering the almost 50/50 split for the Wainwright area.

Future studies should validate if other tissues, such as muscle, skin, claws, or fur can also be used effectively for stable isotope analyses to discriminate CS and SBS polar bear subpopulations. Using bone collagen for stable isotope analysis, although useful for long-term studies (Clark et al. 2019a), is very time consuming and logistically more challenging to process for stable isotope analysis than other tissues. For example, muscle can be processed quickly with results available in a matter of days. If results similar to our data can be produced using more accessible tissues (i.e., muscle biopsy) with a faster turn-around at near-real time, it would allow for a more recent representation of SBS and CS habitat distribution and be therefore a useful tool for stock management.

2.6 References

Amirkhanov A, Kavry S, Siekaniec G, Brower C (2018) Report of the proceedings of the 10th meeting of the Russian-American Commission on polar bears. Egvekinot, Russian Federation.

Amstrup S, Stirling I, Lentfer J (1986) Past and present status of polar bears in Alaska. Wildl Soc Bull 14: 241–254.

Amstrup S, DeMaster D (1988) Polar bear–*Ursus maritimus*. In: Lentfer J (eds) Selected marine mammals of Alaska: Species accounts with research and management recommendations. Marine Mammal Commission, Washington, DC, USA, pp 39–56.

Amstrup S, Durner G, Mulcahy D, Garner G (2001) Comparing movement patterns of satellite-tagged male and female polar bears. Can J Zool 79: 2147–2158. https://doi.org/10.1139/z01-174

Amstrup SC, Durner GM, Stirling I, Mcdonald TL (2005) Allocating harvests among polar bear stocks in the Beaufort Sea. Arctic 58: 247–259. https://doi.org/10.14430/arctic426

Amstrup S, Stirling I, Smith T, Perham C, Thiemann G (2006) Recent observations of intraspecific predation and cannibalism among polar bears in the southern Beaufort Sea. Polar Biol 29: 997–1002. https://doi.org/10.1007/s00300-006-0142-5

Arrigo K, Perovich D, Pickart R, Brown Z, van Dijken G, Lowry K, Mills M, Palmer M, Balch W, Bahr F, Bates N, Benitez-Nelson C, Bowler B, Brownlee E, Ehn J, Frey K, Garley R, Laney S, Lubelczyk L, Mathis J, Matsuoka A, Mitchell B, Moore G, Ortega-Retuerta E, Pal S, Polashenski C, Reynolds R, Schieber B, Sosik H, Stephens M, Swift J (2012) Massive phytoplankton blooms under Arctic sea ice. Science 336: 1408. https://doi.org/10.1126/science.1215065

Arrigo K, Perovich D, Pickart R, Brown Z, van Dijken G, Lowry K, Mills M, Palmer M, Balch W, Bates N, Benitez-Nelson C, Brownlee E, Frey K, Laney S, Mathis J, Matsuoka A, Mitchell B, Moore G, Reynolds R, Sosik H, Swift J (2014) Phytoplankton blooms beneath the sea ice in the Chukchi Sea. Deep Res Part II Top Stud Oceanogr 105: 1–16. https://doi.org/10.1016/j.dsr2.2014.03.018

Atwood T, Bromaghin J, Patel V, Durner G, Douglas D, Simac K (2020) Analyses on the subpopulation abundance and annual number of maternal dens for the U.S. Fish and Wildlife Service on polar bears (*Ursus maritimus*) in the southern Beaufort Sea. U.S. Geological Survey Open File Report: in prep.

Bas M, García N, Crespo E, Cardona L (2020) Intraskeletal variability in stable isotope ratios of C and N among pinnipeds and cetaceans. Mar Mammal Sci 36: 375–385. https://doi.org/10.1111/mms.12644

Bentzen T, Follmann E, Amstrup S, York G, Wooller M, O'Hara T (2007) Variation in winter diet of southern Beaufort Sea polar bears inferred from stable isotope analysis. Can J Zool 85: 596–608. https://doi.org/10.1139/Z07-036

Boucher N, Derocher A, Richardson E (2019) Space use patterns affect stable isotopes of polar bears (*Ursus maritimus*) in the Beaufort Sea. Polar Biol 42: 1581–1593. https://doi.org/10.1007/s00300-019-02546-9

Boucher NP, Derocher AE, Richardson ES (2020) Spatial and temporal variability in ringed seal (*Pusa hispida*) stable isotopes in the Beaufort Sea. Ecol Evol 10: 4178–92. https://doi.org/10.1002/ece3.6186

Boveng P, Cameron M, Conn P, Moreland E (2017) Abundance estimates of ice-associated seals: Bering Sea populations that inhabit the Chukchi Sea during the open-water period. Final Report. BOEM Report 2016-077. Bureau of Ocean Energy Management, Alaska Outer Continental Shelf Region, Anchorage, Alaska, USA.

Braund S, Lawrence P, Sears E, Schraer R, Regehr E, Adams B, Hepa R, George J, Von Duyke A (2018) Polar bear TEK: A pilot study to inform polar bear management models. North Slope Borough Department of Wildlife Management, Research Report NSB.DWM.RR.2018-01. Utqiaġvik, Alaska USA.

Bromaghin J, Mcdonald T, Stirling I, Derocher A, Richardson E, Regehr E, Douglas D, Durner G, Atwood T, Amstrup S (2015) Polar bear population dynamics in the Southern Beaufort Sea during a period of sea ice decline. Ecol Appl 25: 634–651. https://doi.org/10.1890/14-1129.1

Clark C, Horstmann L, Misarti N (2017) Quantifying variability in stable carbon and nitrogen isotope ratios within the skeletons of marine mammals of the suborder Caniformia. J Archaeol Sci Reports 15: 393–400. https://doi.org/10.1016/j.jasrep.2017.09.007

Clark C, Horstmann L, de Vernal A, Jensen A, Misarti N (2019a) Pacific walrus diet across 4000 years of changing sea ice conditions. Quat Res 1–17. https://doi.org/10.1017/qua.2018.140

Clark C, Horstmann L, Misarti N (2019b) Lipid normalization and stable isotope discrimination in Pacific walrus tissues. Sci Rep 9: 1–13. https://doi.org/10.1038/s41598-019-42095-z

Collins M, Nielsen-Marsh C, Hiller J, Smith C, Roberts J, Prigodich R, Wess T, Csapò J, Millard A, Turner-Walker G (2002) The survival of organic matter in bone: A review. Archaeometry 44: 383–394. https://doi.org/10.1111/1475-4754.t01-1-00071

Dehn LA, Follmann EH, Rosa C, Duffy LK, Thomas DL, Bratton GR, Taylor RJ, O'Hara TM (2006) Stable isotope and trace element status of subsistence-hunted bowhead and beluga whales in Alaska and gray whales in Chukotka. Mar Pollut Bull 52:301–19. http://doi.org/10.1016/j.marpolbul.2005.09.001

Dehn L, Sheffield G, Follmann E, Duffy L, Thomas D, O'Hara T (2007) Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biol 30: 167–181. https://doi.org/10.1007/s00300-006-0171-0

DeNiro M (1985) Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. Nature 317: 806–809. https://doi.org/10.1038/317806a0

DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42: 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

Derocher A, Stirling I (1990) Distribution of polar bears (*Ursus maritimus*) during the ice-free period in western Hudson Bay. Can J Zool 68: 1395–1403. https://doi.org/10.1139/z90-208

Derocher A, Stirling I (1995) Estimation of polar bear population size and survival in western Hudson Bay. J Wildl Manage 59: 215–221.

Divine L, Bluhm B, Mueter F, Iken K (2017) Diet analysis of Alaska Arctic snow crabs (*Chionoecetes opilio*) using stomach contents and δ^{13}C and δ^{15}N stable isotopes. Deep Res Part II Top Stud Oceanogr 135: 124–136. https://doi.org/10.1016/j.dsr2.2015.11.009

Dunton K, Weingartner T, Carmack E (2006) The nearshore western Beaufort Sea ecosystem: Circulation and importance of terrestrial carbon in arctic coastal food webs. Prog Oceanogr 71: 362–378. https://doi.org/10.1016/j.pocean.2006.09.011

Durner G, Amstrup S (1995) Movements of a polar bear from northern Alaska to northern Greenland. Arctic 48: 338–341. https://doi.org/10.14430/arctic1257

Durner G, Douglas D, Nielson R, Amstrup S, McDonald T, Stirling I, Mauritzen M, Born E, Wiig Ø, DeWeaver E, Serreze M, Belikov S, Holland M, Maslanik J, Aars J, Bailey D, Derocher A (2009) Predicting 21st-century polar bear habitat distribution from global climate models. Ecol Monogr 79: 25–58. https://doi.org/10.1890/07-2089.1

Fischbach A, Monson D, Jay C (2009) Enumeration of Pacific walrus carcasses on beaches of the Chukchi Sea in Alaska following a mortality event, September 2009: U.S. Geological Survey Open-File Report 2009- 1291.

Folch J, Lees M, Sloane Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509.

Fox J, Weisberg S (2019) An {R} Companion to Applied Regression, Third. ed. Thousand Oaks CA: Sage.

Frey K, Moore G, Cooper L, Grebmeier J (2015) Divergent patterns of recent sea ice cover across the Bering, Chukchi, and Beaufort seas of the Pacific Arctic Region. Prog Oceanogr 36: 32–49. https://doi.org/10.1016/j.pocean.2015.05.009

Fry B (2006) Stable isotope ecology. Springer Science+Business Media,LLC, New York, NY. https://doi.org/10.1007-0-387-33745-8

Grebmeier J, Cooper L, Feder H, Sirenko B (2006) Ecosystem dynamics of the Pacific-influenced Northern Bering and Chukchi Seas in the Amerasian Arctic. Prog Oceanogr 71: 331–361. https://doi.org/10.1016/j.pocean.2006.10.001

Hare E, Fogel M, Stafford Jr T, Mitchell A, Hoering T (1991) The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18: 277–292. https://doi.org/10.1016/0305-4403(91)90066-X

Herreman J, Peacock E (2013) Polar bear use of a persistent food subsidy: Insights from non-invasive genetic sampling in Alaska. Ursus 24: 148–163. https://doi.org/10.2192/URSUS-D-12-00030.1

Hobson K, Piatt J, Pitocchelli J (1994) Using stable isotopes to determine seabird trophic relationships. J Anim Ecol 63: 786–798. https://doi.org/10.2307/5256

Horstmann-Dehn L, Follmann E, Rosa C, Zelensky G, George C (2012) Stable carbon and nitrogen isotope ratios in muscle and epidermis of arctic whales. Mar Mammal Sci 28: E173–E190. https://doi.org/10.1111/j.1748-7692.2011.00503.x

Hunter C, Caswell H, Runge M, Regehr E, Amstrup S, Stirling I (2010) Climate change threatens polar bear populations: A stochastic demographic analysis. Ecology 91: 2883–2897. https://doi.org/10.1890/09-1641

IUCN/SSC Polar Bear Specialist Group (2019) Status report on the world's polar bear subpopulations. 2019 Status Report, pp 1–31. http://pbsg.npolar.no/export/sites/pbsg/en/docs/2019-StatusReport.pdf

Jay C, Fischbach A, Kochnev A (2012) Walrus areas of use in the Chukchi Sea during sparse sea ice cover. Mar Ecol Prog Ser 468: 1–13. https://doi.org/10.3354/meps10057

Koehler G, Kardynal K, Hobson K (2019) Geographical assignment of polar bears using multi-element isoscapes. Sci Rep 9: 1–9. https://doi.org/10.1038/s41598-019-45874-w

Kutschera VE, Frosch C, Janke A, Skírnisson K, Bidon T, Lecomte N, Fain SR, Eiken HG, Hagen SB, Arnason U, Laidre KL, Nowak C, Hailer F (2016) High genetic variability of vagrant polar bears illustrates importance of population connectivity in fragmented sea ice habitats. Anim Conserv 19: 337–349. https://doi.org/10.1111/acv.12250

Laidre KL, Stirling I, Estes JA, Kochnev A, Roberts J (2018) Historical and potential future importance of large whales as food for polar bears. Front Ecol Environ 16: 515–24. http://dx.doi.org/10.1002/fee.1963

Lee S, Schell D, McDonald T, Richardson W (2005) Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. Mar Ecol Prog Ser 285: 271–287. https://doi.org/10.3354/meps285271

Lunn N, Andriashek D, Kolenosky G (1997) Re-estimating the size of the polar bear population in western Hudson Bay. Arctic 50: 234–240. https://doi.org/10.14430/arctic1105

Miller S, Schliebe S, Proffitt K (2006) Demographics and behavior of polar bears feeding on bowhead whale carcasses at Barter and Cross Islands, Alaska, 2002-2004. Final Report. Alaska. https://doi.org/10.16258/j.cnki.1674-5906.2006.01.022

Misarti N, Finney B, Maschner H, Wooller M (2009) Changes in northeast Pacific marine ecosystems over the last 4500 years: Evidence from stable isotope analysis of bone collagen from archeological middens. The Holocene 19: 1139–1151. https://doi.org/10.1177/0959683609345075

Monson D, Udevitz M, Jay C (2013) Estimating age ratios and size of Pacific walrus herds on coastal haulouts using video imaging. PLoS One 8: 1–12. https://doi.org/10.1371/journal.pone.0069806

Mu C, Zhang T, Wu Q, Cao B, Zhang X, Peng X, Wan X, Zheng L, Wang Q, Cheng G (2015) Carbon and nitrogen properties of permafrost over the Eboling Mountain in the upper reach of Heihe River basin, Northwestern China. Arctic Antarct Alp Res 47: 203–211. https://doi.org/10.1657/AAAR00C-13-095

Newsome S, Clementz M, Koch P (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mammal Sci 26: 509–572. https://doi.org/10.1111/j.1748-7692.2009.00354.x

Obbard ME, Thiemann GW, Peacock E, Debruyn TD (2010) Proceedings of the 15th working meeting of the IUCN/SSC Polar Bear Specialist Group, 29 June-3 July 2009, Copenhagen, Denmark. Gland, Switzerland and Cambridge, UK: IUCN.

Onarheim I, Eldevik T, Smedsrud L, Stroeve J (2018) Seasonal and regional manifestation of Arctic sea ice loss. J Clim 31: 4917–4932. https://doi.org/10.1175/JCLI-D-17-0427.1

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4: 347–354. https://doi.org/10.1111/j.1365-294X.1995.tb00227.x

Paetkau D, Amstrup C, Born E, Calvert W, Derocher A, Garner G, Messier F, Stirling I, Taylor M, Wiig Ø, Strobeck C (1999) Genetic structure of the world's polar bear populations. Mol Ecol 8: 1571–1584. https://doi.org/10.1046/j.1365-294x.1999.00733.x

Polischuk S, Hobson K, Ramsay M (2001) Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Can J Zool 79: 499–511. https://doi.org/10.1139/cjz-79-3-499

R Core Team (2020) R: A language and environment for statistical computing, 2020. R Foundation for Statistical Computing, Vienna, Austria.

Regehr E, Hunter C, Caswell H, Amstrup S, Stirling I (2010) Survival and breeding of polar bears in the Southern Beaufort Sea in relation to sea ice. J Anim Ecol 79: 117–127. https://doi.org/10.1111/j.1365-2656.2009.01603.x

Regehr E, Hostetter N, Wilson R, Rode K, St. Martin M, Converse S (2018) Integrated population modeling provides the first empirical estimates of vital rates and abundance for polar bears in the Chukchi Sea. Sci Rep 8: 1–12. https://doi.org/10.1038/s41598-018-34824-7

Rode K, Regehr E, Douglas D, Durner G, Derocher A, Thiemann G, Budge S (2014) Variation in the response of an Arctic top predator experiencing habitat loss: feeding and reproductive ecology of two polar bear populations. Glob Chang Biol 20: 76–88. https://doi.org/10.1111/gcb.12339

Rogers M, Peacock E, Simac K, O'Dell M, Welker J (2015) Diet of female polar bears in the southern Beaufort Sea of Alaska: Evidence for an emerging alternative foraging strategy in response to environmental change. Polar Biol 38: 1035–1047. https://doi.org/10.1007/s00300-015-1665-4

Saupe S, Schell D, Griffiths W (1989) Carbon-isotope ratio gradients in western arctic zooplankton. Mar Biol 103: 427–432. https://doi.org/10.1038/164914a0

Scharf HR, Hooten MB, Wilson RR, Durner GM, Atwood TC (2018) Accounting for phenology in the analysis of animal movement. arXiv Prepr. arXiv1806.09473. https://doi.org/10.1111/biom.13052

Schell DM, Saupe SM, Haubenstock N (1989) Natural isotope abundances in bowhead whale (*Balaena mysticetus*) baleen: Markers of aging and habitat usage. In: Rundel PW, Ehleringer JR, Nagy KA (eds), Stable Isotopes in Ecological Research. Ecological Studies (Analysis and Synthesis). Springer, New York, NY, pp 260–269. https://doi.org/10.1007/978-1-4612-3498-2_15

Schell D, Barnett B, Vinette K (1998) Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi and Beaufort seas. Mar Ecol Prog Ser 162: 11–23. https://doi.org/10.3354/meps162011

Starkweather J, Moske A (2011) Multinomial logistic regression. https://doi.org/10.1097/00006199-200211000-00009

Stirling I, Archibald W, DeMaster D (1977a) Distribution and abundance of seals in the eastern Beaufort Sea. J Fish Res Board Canada 34: 976–988. https://doi.org/10.1139/f77-150

Stirling I, Jonkel C, Smith P, Robertson R, Cross D (1977b) The ecology of the polar bear (*Ursus maritimus*) along the western coast of Hudson Bay. Can Wildl Serv 33: 1–64.

Stirling I, Andriashek D, Spencer C, Derocher A (1988) Assessment of the polar bear population in the eastern Beaufort Sea. Final report to the Northern Oil and Gas Assessment Program. Edmonton, Alberta, Canada.

Stirling I, Mcdonald T, Richardson E, Regehr E (2007) Polar bear population status in the Northern Beaufort Sea, USGS Alaska Science Center, Anchorage, Administrative Report. U.S. Geological Survey, Reston, Virginia.

Stirling I, McDonald T, Richardson E, Regehr E, Amstrup S (2011) Polar bear population status in the northern Beaufort Sea, Canada, 1971-2006. Ecol Appl 21: 859–876. https://doi.org/10.1890/10-0849.1

Taylor M, Lee J (1995) Distribution and abundance of Canadian polar bear populations: A management perspective. Arctic 48: 147–154. https://doi.org/10.14430/arctic1236

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57: 32–37. https://doi.org/10.1007/BF00379558

U.S. Fish and Wildlife Service (2019) Pacific walrus: Use of coastal haulouts along the Chukchi Sea coast.

Voorhees H, Sparks R, Huntington H, Rode KD (2014) Traditional knowledge about polar bears (*Ursus maritimus*) in northwestern Alaska. Arctic 67: 523–536. https://doi.org/10.14430/arctic4425

West JB, Bowen GJ, Cerling TE, Ehleringer JR (2006) Stable isotopes as one of nature's ecological recorders. TRENDS Ecol Evol 21: 408–414. https://doi.org/10.1016/j.tree.2006.04.002

Wickham H, François R, Henry L, Müller K (2020) dplyr: A Grammar of Data Manipulation.

Wilson R, Horne J, Rode K, Regehr E, Durner G (2014) Identifying polar bear resource selection patterns to inform offshore development in a dynamic and changing Arctic. Ecosphere 5: 1–24. https://doi.org/10.1890/ES14-00193.1

2.7 Figures and Tables

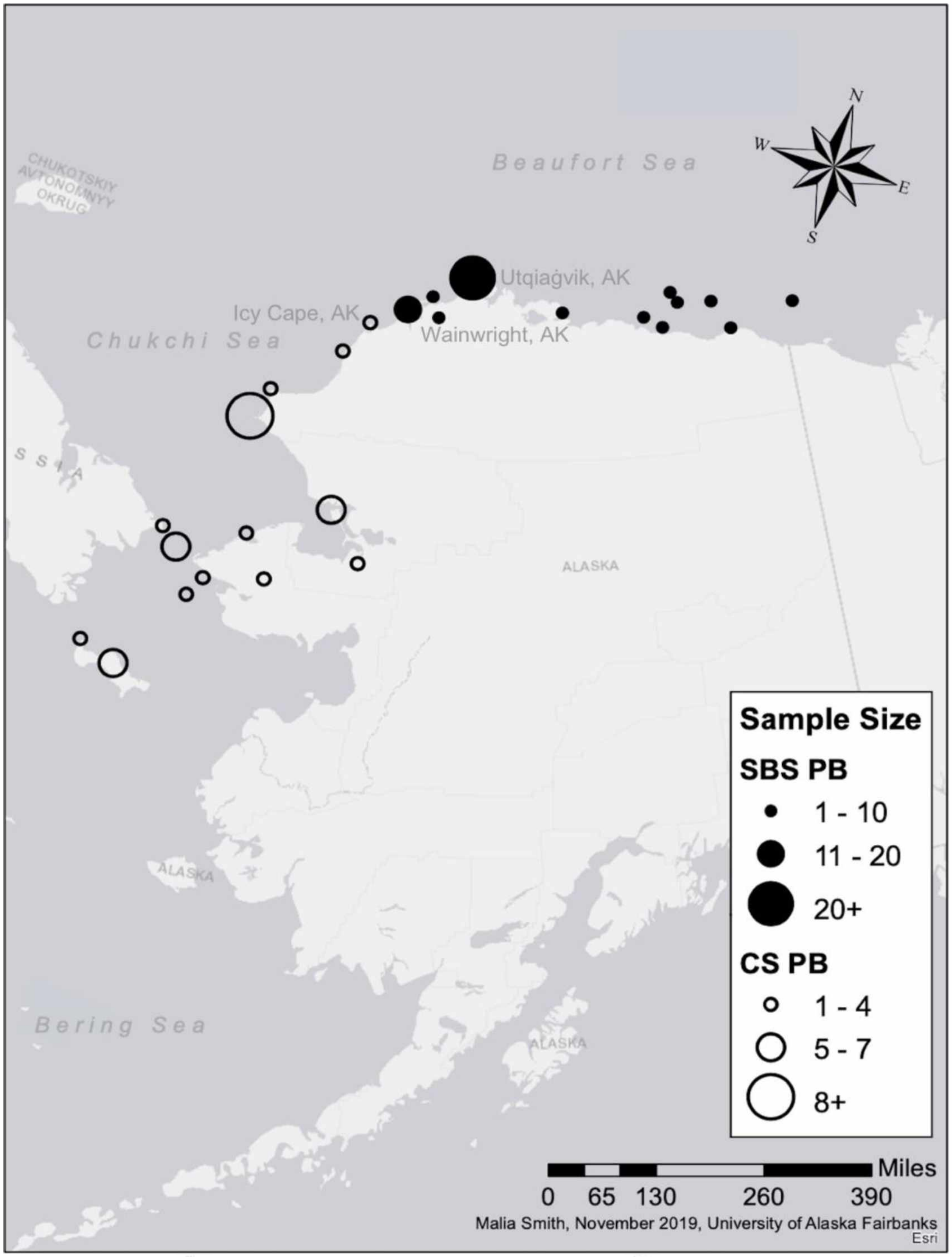

Figure 2.1 Map of polar bear bone collage collections. Size of symbols reflects sample size from that specific location. Bone samples from the Southern Beaufort Sea (SBS PB) subpopulation are shown as solid circles, specimens from the Chukchi Sea (CS PB) subpopulations are shown as open circles. Division of the polar bear subpopulations was presumed to be at Icy Cape (Amstrup et al. 2005).

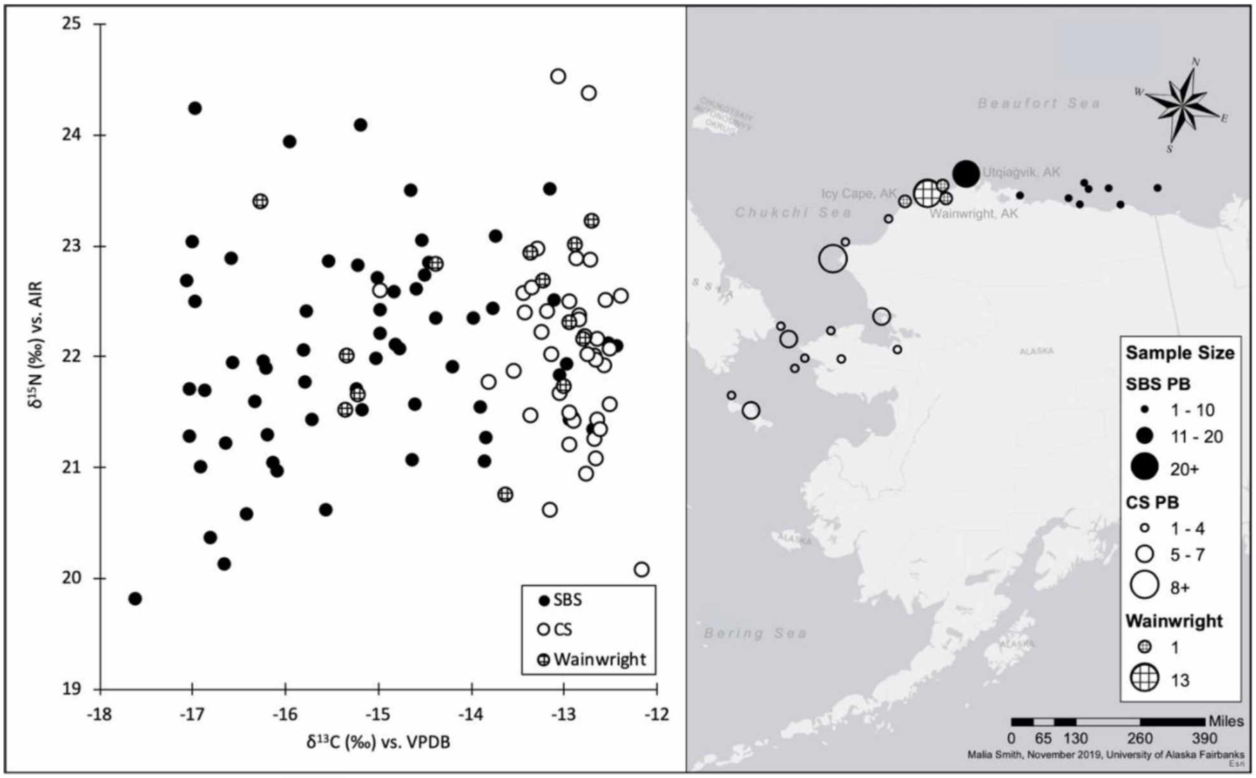

Figure 2.2 Comparison of bulk stable carbon and nitrogen isotopes in polar bear bone collagen (left). The spatial distribution of these individuals is shown on the right (shading is coordinated between left and right panels as Southern Beaufort Sea (SBS PB, solid), Chukchi Sea (CS PB, open), and a mixing area around Wainwright (grid)). Icy Cape is the presumed boundary between the two subpopulations (Amstrup et al. 2005).

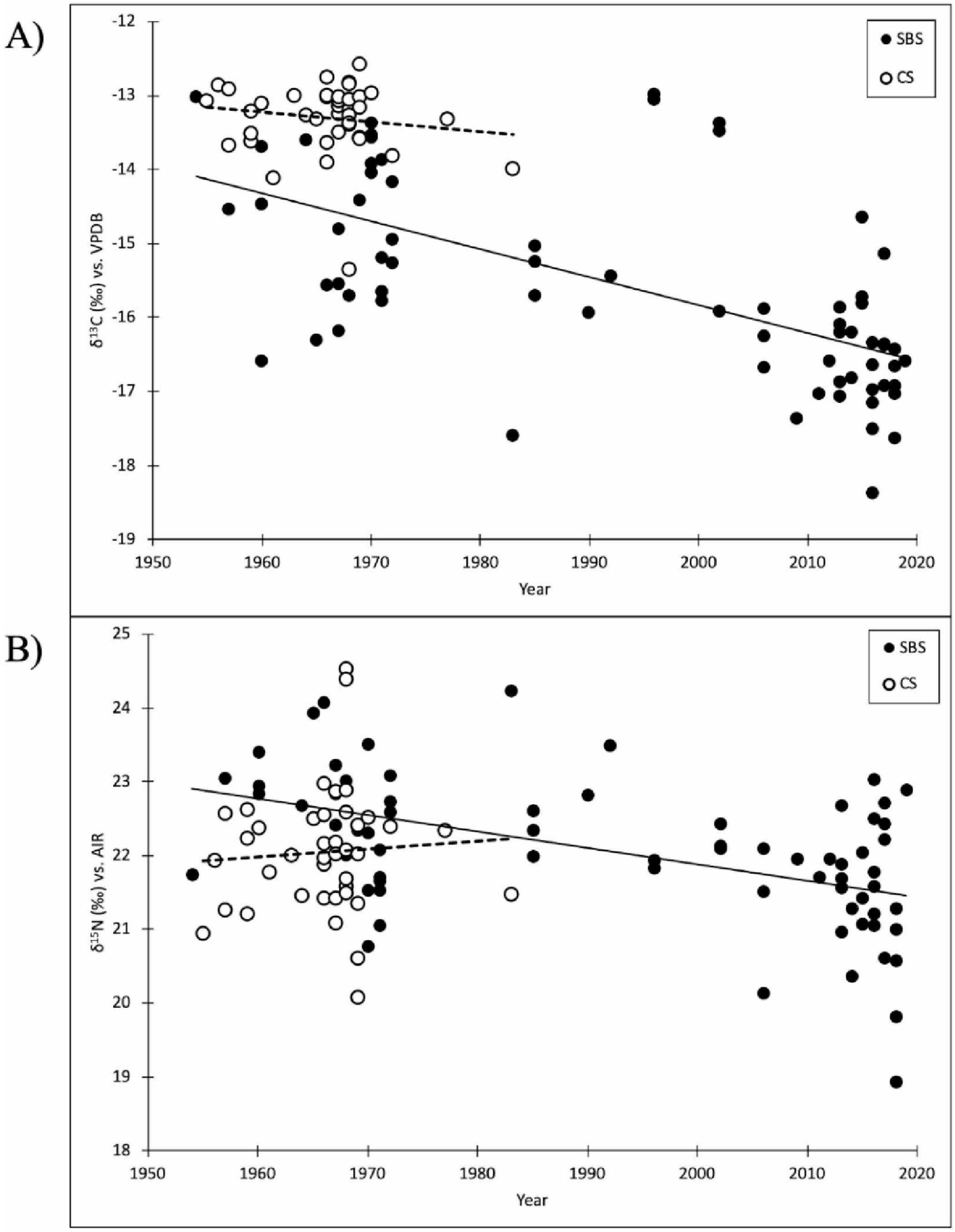

Figure 2.3 A) Measured bulk δ^{13}C values (‰) vs. Vienna Pee Dee Belemnite (VPDB) of Southern Beaufort Sea (SBS; solid circles) and Chukchi Sea (CS; open circles) polar bear bone collagen from 1954–2019. All bulk δ^{13}C values have been Suess corrected following Misarti et al. (2009). Black (SBS) and dashed (CS) lines represent the best linear fit ($y=-0.04x+59.41$, $R^2=0.38$, $P<0.001$; and $y=-0.01x+12.26$, $R^2=0.02$, $P=0.368$, respectively) with decline of about 2‰ from 1954–2019 for the SBS polar bears. B) Measured bulk δ^{15}N values (‰) vs. atmospheric nitrogen gas (AIR) of SBS and CS polar bear bone collagen from 1954–2019. Black (SBS) and dashed (CS) lines represent the best linear fit ($y=-0.02x+66.47$, $R^2=0.26$, $P<0.001$; and $y=0.01x+2.04$, $R^2=0.00$, $P=0.677$, respectively) with a decline of about 1‰ from 1954–2019 for the SBS polar bears.

Table 2.1 Polar bear bone collagen samples of the Chukchi Sea and Southern Beaufort Sea subpopulations, and the “Potential Mixing Zone” of Wainwright and Icy Cape, AK by decade.

Year range	**Chukchi Sea**	**Southern Beaufort Sea**	**Potential Mixing Zone**
1950-1959	7	1	-
1960-1969	30	7	6
1970-1979	3	8	6
1980-1989	1	4	-
1990-1999	-	4	-
2000-2009	-	7	-
2010-2019	-	28	-
Total	**41**	**59**	**12**

Table 2.2 Complete list of polar bear samples used in this study including either original sample ID or University of Alaska Museum/Arctos Database ID, year collected, stock, sex, and age class. $\delta^{15}N$ values (‰), $\delta^{13}C$ values (‰), percent collagen extracted, and calculated atomic C:N ratios of bone collagen for all Southern Beaufort Sea (SBS) and Chukchi Sea (CS) polar bears are provided. The outlier CS sample, which was removed from all analyses, is highlighted in bold.

Sample ID	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
UAM:Mamm:3399	1955	20.9	-12.8	24.7	3.4	CS	male	unknown
UAM:Mamm:3400	1956	21.9	-12.6	21.7	3.2	CS	female	adult
UAM:Mamm:3406	1957	22.6	-13.4	30.5	3.3	CS	male	unknown
UAM:Mamm:3398	1957	21.3	-12.7	23.4	3.2	CS	male	unknown
UAM:Mamm:4510	1959	22.6	-13.4	22.8	3.4	CS	female	unknown
UAM:Mamm:4248	1959	21.2	-12.9	27.0	3.1	CS	male	unknown
UAM:Mamm:4247	1959	22.2	-13.2	23.4	3.3	CS	male	adult
UAM:Mamm:5234	1960	22.4	-12.8	18.9	3.2	CS	unknown	unknown
UAM:Mamm:4814	1961	21.8	-13.8	24.0	3.6	CS	unknown	unknown
UAM:Mamm:16516	1963	22.0	-12.7	25.5	3.1	CS	male	unknown
UAM:Mamm:36590	1964	21.5	-12.9	23.5	3.4	CS	female	unknown
UAM:Mamm:16348	1965	22.5	-12.9	24.2	3.4	CS	male	unknown

Table 2.2 Cont.

Sample ID	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
UAM:Mamm:16533	1966	22.2	-12.7	22.1	3.3	CS	female	unknown
UAM:Mamm:16465	1966	22.0	-12.7	22.8	3.3	CS	female	unknown
UAM:Mamm:16525	1966	21.9	-13.6	22.9	3.3	CS	male	unknown
UAM:Mamm:16425	1966	22.5	-12.4	23.2	3.0	CS	male	unknown
UAM:Mamm:16352	1966	23.0	-13.3	25.4	3.2	CS	male	unknown
UAM:Mamm:16536	1966	21.4	-12.7	23.7	3.2	CS	male	unknown
UAM:Mamm:18466	1967	22.2	-12.8	21.2	3.1	CS	female	adult
UAM:Mamm:87069	1967	21.4	-12.9	22.8	3.3	CS	male	unknown
UAM:Mamm:16478	1967	22.9	-12.7	24.4	3.2	CS	male	unknown
UAM:Mamm:16446	1967	21.1	-12.7	26.5	3.3	CS	male	unknown
UAM:Mamm:87070	1967	22.0	-13.2	21.7	3.2	CS	male	unknown
UAM:Mamm:16426	1968	22.1	-12.5	22.9	3.2	CS	female	unknown
UAM:Mamm:87072	1968	22.9	-12.9	23.7	3.2	CS	male	unknown
UAM:Mamm:16458	1968	21.6	-12.5	24.7	3.3	CS	male	unknown

Table 2.2 Cont.

Sample ID	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
UAM:Mamm:16447	1968	24.5	-13.1	20.2	3.2	CS	male	unknown
UAM:Mamm:16434	1968	21.5	-13.0	23.6	3.3	CS	male	unknown
UAM:Mamm:16427	1968	24.4	-12.7	18.9	3.1	CS	male	unknown
UAM:Mamm:16381	1968	21.7	-13.1	32.1	3.3	CS	male	unknown
UAM:Mamm:16481	1968	22.6	-15.0	19.4	3.2	CS	male	unknown
UAM:Mamm:16488	**1969**	**3.9**	**-19.7**	**18.6**	**3.2**	**CS**	**female**	**unknown**
UAM:Mamm:16459	1969	22.4	-13.2	23.1	3.2	CS	female	unknown
UAM:Mamm:18449	1969	21.4	-12.6	31.0	3.3	CS	male	adult
UAM:Mamm:18448	1969	22.0	-12.8	28.4	3.3	CS	male	adult
UAM:Mamm:18447	1969	20.6	-13.2	26.1	3.2	CS	male	adult
UAM:Mamm:16441	1969	20.1	-12.2	21.0	3.1	CS	male	unknown
UAM:Mamm:87066	1970	22.5	-12.6	19.8	3.2	CS	female	unknown
UAM:Mamm:16421	1972	22.4	-13.4	19.9	3.2	CS	male	unknown
UAM:Mamm:41926	1977	22.3	-12.9	22.0	3.3	CS	female	unknown

Table 2.2 Cont.

Sample ID	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
PB Pt Lay 1983	1983	21.5	-13.4	31.8	3.3	CS	unknown	unknown
UAM:Mamm:4729	1954	21.7	-13.0	24.3	3.2	SBS	male	unknown
UAM:Mamm:3424	1957	23.0	-14.5	23.9	3.3	SBS	unknown	unknown
UAM:Mamm:4726	1960	22.9	-13.4	18.9	3.2	SBS	female	unknown
UAM:Mamm:4727	1960	23.4	-16.3	26.7	3.5	SBS	male	juvenile
UAM:Mamm:4946	1960	22.8	-14.5	22.6	3.2	SBS	male	unknown
UAM:Mamm:16414	1964	22.7	-13.2	23.9	3.4	SBS	female	unknown
UAM:Mamm:7276	1965	23.9	-16.0	35.4	3.4	SBS	female	juvenile
UAM:Mamm:87064	1966	24.1	-15.2	25.4	3.5	SBS	female	unknown
UAM:Mamm:16469	1967	23.2	-12.7	23.7	3.3	SBS	female	unknown
UAM:Mamm:16486	1967	22.4	-15.8	20.7	3.3	SBS	male	unknown
UAM:Mamm:16450	1967	22.8	-14.4	27.2	3.5	SBS	female	unknown
UAM:Mamm:87059	1967	22.9	-15.5	24.9	3.4	SBS	unknown	unknown
UAM:Mamm:16492	1968	22.0	-15.3	22.4	3.3	SBS	male	unknown

Table 2.2 Cont.

Sample ID	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
UAM:Mamm:16376	1968	23.0	-12.9	23.4	3.3	SBS	female	unknown
UAM:Mamm:16436	1969	22.4	-14.0	23.5	3.3	SBS	male	unknown
UAM:Mamm:16456	1970	23.5	-13.2	19.5	3.2	SBS	female	unknown
UAM:Mamm:18459	1970	22.3	-13.0	19.3	3.2	SBS	female	adult
UAM:Mamm:16472	1970	22.5	-13.1	21.8	3.2	SBS	female	unknown
UAM:Mamm:16423	1970	20.8	-13.6	24.2	3.2	SBS	male	unknown
UAM:Mamm:16419	1970	21.5	-13.9	26.2	3.3	SBS	male	unknown
UAM:Mamm:16382	1971	21.5	-15.4	22.4	3.3	SBS	female	unknown
UAM:Mamm:87082	1971	21.7	-15.2	18.0	3.3	SBS	female	unknown
UAM:Mamm:87080	1971	22.1	-14.8	28.7	3.4	SBS	male	unknown
UAM:Mamm:18460	1971	21.7	-15.2	26.4	3.0	SBS	male	subadult
UAM:Mamm:87081	1971	21.1	-13.9	25.5	3.2	SBS	male	unknown
UAM:Mamm:16403	1972	22.7	-14.5	23.8	3.2	SBS	female	unknown
UAM:Mamm:16538	1972	23.1	-13.7	18.7	3.1	SBS	female	unknown

Table 2.2 Cont.

Sample ID	Year	δ^{15}N (‰)	δ^{13}C (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
UAM:Mamm:16514	1972	22.6	-14.8	23.7	3.3	SBS	male	unknown
UAM:Mamm:16544	1983	24.2	-17.0	29.3	3.2	SBS	female	juvenile
UAM:Mamm:16541	1985	22.6	-14.6	22.4	3.2	SBS	female	unknown
UAM:Mamm:16540	1985	22.3	-14.4	26.2	3.2	SBS	female	adult
UAM:Mamm:16545	1985	22.0	-15.0	21.7	3.2	SBS	male	adult
6375PB 1990	1990	22.8	-15.2	33.1	3.4	SBS	unknown	unknown
UAM:Mamm:24875	1992	23.5	-14.7	20.4	3.2	SBS	female	unknown
FWS PBR 960106	1996	21.9	-13.0	28.1	3.2	SBS	unknown	cub
FWS PBR 960103	1996	21.8	-13.0	22.6	3.3	SBS	unknown	cub
UAM:Mamm:123094	2002	22.1	-12.4	24.5	3.3	SBS	male	adult
UAM:Mamm:123095	2002	22.1	-12.5	22.2	3.1	SBS	male	adult
UAM:Mamm:111725	2002	22.4	-15.0	17.1	3.1	SBS	female	adult
UAM:Mamm:87964	2006	21.5	-15.2	22.5	3.2	SBS	unknown	unknown
UAM:Mamm:87963	2006	22.1	-14.8	22.5	3.1	SBS	male	unknown

Table 2.2 Cont.

Sample ID	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
USIK 01-10-06	2006	20.1	-16.7	29.5	3.3	SBS	male	unknown
UAM:Mamm:138279	2009	22.0	-16.2	22.9	3.5	SBS	female	adult
UN2011BW04	2011	21.7	-17.0	38.6	3.3	SBS	male	unknown
KAK001-12	2012	21.9	-16.6	28.6	3.2	SBS	male	unknown
N01-2013	2013	21.0	-16.1	29.7	3.3	SBS	male	unknown
N12-2013PB	2013	22.7	-17.1	28.7	3.2	SBS	male	unknown
N13-2013PB	2013	21.7	-16.9	27.5	3.2	SBS	male	unknown
N16-2013PB	2013	21.9	-16.2	14.1	3.2	SBS	male	unknown
N80B 2013	2013	21.6	-14.6	28.6	3.3	SBS	male	unknown
2014PB04	2014	21.3	-16.2	29.1	3.2	SBS	male	unknown
2014PB05	2014	20.4	-16.8	30.0	3.2	SBS	male	unknown
15HPB1	2015	21.1	-14.6	26.6	3.2	SBS	male	unknown
2015PB07	2015	22.1	-15.8	25.8	3.2	SBS	male	unknown
2015PB08	2015	21.4	-15.7	26.9	3.2	SBS	male	unknown
2016PB0111-5	2016	22.5	-17.0	10.2	3.3	SBS	male	unknown
2016PB0505-5	2016	21.2	-16.6	33.3	3.4	SBS	male	unknown

Table 2.2 Cont.

Sample ID	Year	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	% Collagen Yield	Atomic C:N Ratio	Stock	Sex	Age Class
2016PB0512-5	2016	21.6	-16.3	28.7	3.2	SBS	male	unknown
2016USFWS N0060	2016	21.0	-16.1	28.4	3.3	SBS	male	unknown
2016PB0111a	2016	21.8	-15.8	24.9	3.6	SBS	female	unknown
2016PB0111b	2016	23.0	-17.0	44.3	4.0	SBS	female	unknown
2017PB0903-s	2017	20.6	-15.6	29.0	3.4	SBS	male	unknown
2017PB0424-s/hc	2017	22.7	-15.0	27.7	3.3	SBS	male	unknown
2017PB0222-s	2017	22.2	-15.0	28.2	3.3	SBS	male	unknown
2017PB0214-s	2017	22.4	-13.8	29.0	3.4	SBS	male	unknown
2018PB0304-s	2018	18.9	-16.7	28.0	3.3	SBS	male	unknown
2018PB0205-s	2018	19.8	-17.6	27.7	3.6	SBS	male	unknown
2018PB0204-s	2018	20.6	-16.4	26.7	3.3	SBS	male	unknown
2018 PB XXXX S	2018	21.3	-17.0	21.9	3.2	SBS	male	unknown
2018 PB 1213 FD KAK	2018	21.0	-16.9	23.6	3.2	SBS	male	unknown
2019 PB 0409	2019	22.9	-16.6	24.4	3.3	SBS	male	unknown

Chapter 3: Compound-specific stable isotope analyses of amino acids provide evidence of two distinct ecotypes of Southern Beaufort Sea polar bears[2]

3.1 Abstract

The subpopulation of polar bears (*Ursus maritimus*) in the Southern Beaufort Sea (SBS) exhibited a substantial population decline in recent years concurrent with increases in the proportion of bears coming on shore, and the duration they spend there. Both of these changes have been associated with the loss of access to their primary ice habitat, which is mainly utilized to hunt seals. The purpose of this study was to examine long-term patterns in polar bear dietary trophic level and reliance on terrestrially derived carbon sources in the SBS subpopulation over a 65-year period, as polar bears have increasingly used coastal environments. A total of 77 polar bear samples dating from 1954–2019 were analyzed for bulk stable carbon and nitrogen isotope ratios (expressed as $\delta^{13}C$ and $\delta^{15}N$ values) and compound-specific stable carbon isotopes of amino acids ($\delta^{13}C_{AA}$ values). An additional 50 bone collagen samples from terrestrial mammals and pinnipeds from northern Alaska were analyzed to provide a regional comparative dataset. In polar bears, bulk $\delta^{13}C$ and $\delta^{15}N$ values decreased by 2‰ and 1‰, respectively, over the 65-year period. After the beginning of several record sea ice minimums starting in 2007, the $\delta^{13}C$ values of isoleucine (-17.9‰±2.2‰), serine (-12.6‰±3.0‰), and glycine (-23.2‰±1.2‰) in polar bears approached values of Arctic brown bears (*Ursus arctos*) (isoleucine: -24.2‰±1.7‰; serine: -7.1‰±2.0‰; glycine: -26.0‰±1.7‰) suggesting that some polar bears are incorporating terrestrially derived carbon into these amino acids at levels similar to those observed in Arctic

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[2] Prepared for submission to Oikos as Smith MEK, Stimmelmayr R, Rode K, Wooller MJ, Horstmann L "Compound-specific stable isotope analyses of amino acids provide evidence of two distinct ecotypes of southern Beaufort Sea polar bears"

brown bears. However, the small change in $\delta^{15}N$ values of the polar bears indicates that they were not switching to a different trophic level. Over the 65-year study period, two distinct groups of polar bears were identified based on their $\delta^{13}C$ values of proline: a high $\delta^{13}C_{Pro}$ group (1.8‰±2.3‰, n=45) and a low $\delta^{13}C_{Pro}$ group (-15.7‰±1.9‰, n=26). The high group had $\delta^{13}C_{Pro}$ values similar to those of Arctic brown bears (0.4‰±1.6‰), and the low group had $\delta^{13}C_{Pro}$ values similar to ice seals (-15.3‰±1.2‰). These high and low groups suggest a dichotomy in the habitats used by polar bears in the SBS consistent with previous studies that have identified bears that range farther offshore in more pelagic environments and others that remain more coastal. This study provides evidence that two distinct ecotypes in SBS polar bears, pelagic and coastal, have existed since at least the 1950's using bulk and compound-specific stable isotope analyses.

3.2 Introduction

Polar bears (*Ursus maritimus*) are charismatic apex predators of the circumpolar Arctic. These marine bears are uniquely adapted to hunt from sea ice in the Arctic Ocean in pursuit of ice seals, their preferred prey (Best 1985; Stirling 2002). Reduced availability of sea ice has forced polar bears to spend more time on land (Stirling et al. 1999; Atwood et al. 2016). With less seasonal sea-ice habitat available as a hunting platform, it is more difficult for polar bears to capture their preferred ice seal prey, and several studies attest to prey shifts (Mckinney et al. 2009, 2013, 2017; Stirling and Derocher 2012) and changes in body condition (Stirling et al. 1999; Rode et al. 2014, 2010; Obbard et al. 2016). Polar bears have become the internationally recognized face of climate change in the Arctic due to their reliance on sea ice habitat for most aspects of their life history (Comiso 2002). There have been several recent major sea ice minima, e.g., 2007, 2012, and 2019 (Fetterer et al. 2017). In 2008, Alaska polar bear stocks were listed as threatened under the Endangered Species Act (U.S. Fish and Wildlife Service 2008). Projections for circumpolar polar bear abundance are worsening, with anticipated summer habitat loss of almost 70% by the end of the 21st century, and projected extirpation of about one-third of the world's polar bear population (Durner et al. 2009; Hunter et al. 2010; Regehr et al. 2010).

There are 19 polar bear subpopulations recognized worldwide, with the Southern Beaufort Sea (SBS) and Chukchi Sea (CS) subpopulations occurring in Alaska (Figure 3.1; Obbard et al. 2010). The SBS and CS regions are experiencing some of the highest rates of sea ice loss in the Arctic (Onarheim et al. 2018). Population trajectories are different for the SBS and CS polar bears, even though they are neighboring subpopulations. The SBS subpopulation has decreased by 40% since 2001 with an estimated population abundance of fewer than 1,000 total animals as of 2010 (Bromaghin et al. 2015; IUCN/SSC Polar Bear Specialist Group 2019). The CS subpopulation is currently believed to be stable (IUCN/SSC Polar Bear Specialist Group 2019) and has a population size of approximately 3,000 bears (Regehr et al. 2018). CS bears are in better body condition, are of larger body size, and exhibit higher reproductive success, in comparison to SBS bears (Rode et al. 2014), despite 40% of adult females summering on shore in the CS compared (Rode et al. 2015) to 25-30% of adult females in the SBS subpopulation (Atwood et al. 2016; Pongracz and Derocher 2017).

Notable differences in ecosystem productivity could be driving the observed differences in body condition of Alaska polar bear stocks. The Beaufort Sea is less productive than the Chukchi Sea, which supports large ice seal populations and Pacific walrus haul-outs (*Odobenus rosmarus divergens*; Frost et al. 2002; Sakshaug 2004). Greater prey availability allows CS bears to maintain better body condition than their SBS counterparts (Gittleman 1985; Rode et al. 2010 & 2014). The SBS subpopulation also tends to separate into two cohorts during the spring ice melt, referred to as the pelagic group and the coastal group (Ferguson et al. 1999; Mauritzen et al. 2001; Rogers et al. 2015). The pelagic polar bears tend to stay out farther from the coast on the sea ice hunting ice seals, while the coastal group are known to utilize bowhead whale (*Balaena mysticetes*) carcasses originating from Alaska Native subsistence harvests along the coastline (Schliebe et al. 2008; Herreman and Peacock 2013; Rogers et al. 2015).

Bulk stable isotope analysis (BSIA) of carbon and nitrogen is a well-established tool to study feeding ecology of marine and terrestrial mammals (West et al. 2006). The method is based on the premise that the ratio of stable isotopes (expressed in δ notation) in an animal's tissue is derived from the animal's average food source with a predictable offset, shown in units of ‰ (Hobson et al. 1994). A common indicator of trophic position is a tissue's $\delta^{15}N$ value (Fry 2006), while $\delta^{13}C$ values can trace carbon sources, e.g., terrestrial or aquatic (DeNiro and Epstein 1978). When moving higher up a food web, it is expected that as $\delta^{13}C$ values increase by ~1‰, $\delta^{15}N$ values will increase by about 3‰ (DeNiro and Epstein 1981). This stepwise increase in $\delta^{15}N$ values is viewed as a change to a higher trophic level (McCutchan et al. 2003). BSIA has advantages over traditional stomach contents analysis, as it is not biased towards hard parts and provides an integrated and assimilated diet rather than a snapshot into recently consumed prey (Bowen and Iverson 2013). However, interpreting BSIA results can sometimes be difficult, because tissue turnover time is variable, ranging from days in blood, to years or even a lifetime in bone collagen (Hobson and Clark 1992). This can lead to different results and interpretations of an animal's diet (Tieszen et al. 1983; Hare et al. 1991).

The long turnover time of carbon and nitrogen in bone collagen is advantageous to investigate long-term diet selection that is not influenced by opportunistic dietary forays. In addition, bone typically remains in archaeological excavations and can be archived in museum collections for centuries without advanced preservation methods (e.g., liquid nitrogen). This opens up archives of animal collections, such as polar bears, spanning decades, centuries, and even millennia, particularly because there is no appreciable variation in stable isotope signatures of the skeleton, except distal limb bones (Clark et al. 2017; Bas et al. 2020). Furthermore, the stable isotopic signatures of bone collagen are not influenced by external degradation and contamination due to protection from the bone's structure (Collins et al. 2002).

An animals' feeding ecology determined from bone collagen can be refined by compound-specific stable isotope analysis (CSIA) of carbon and nitrogen of individual amino acids (AA). CSIA-AA can disentangle shifts of carbon and nitrogen at the base of food webs (McMahon and McCarthy 2016) and more readily distinguish between food sources, e.g., terrestrial or marine (Bowes and Thorp 2015). This can be helpful in determining potential shifting baselines in changing ecosystems. AAs are defined as either "source" AAs or "trophic" AAs (O'Connell 2017). Source, or essential AAs, include lysine (Lys), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), threonine (Thr), valine (Val), and conditionally glycine (Gly), and they have to be taken up in the diet (McMahon et al. 2013). Source AA fractionate, or alter, their relative abundance only minimally during trophic transfer, which provides a virtually unchanged isotopic signature of the AAs from the primary producer to the apex predator (McMahon and McCarthy 2016). This allows source AAs to be excellent indicators of the regional isotopic baseline from primary producers of the originating ecosystem (Whiteman et al. 2019). Comparatively, trophic or nonessential AA fractionate heavily during trophic transfer (McMahon and McCarthy 2016) and can be synthesized in the body (McMahon et al. 2013). Trophic AAs include alanine (Ala), asparagine/aspartate (Asp), glutamine/glutamate (Glu), Gly, serine (Ser), and proline (Pro) and are used to build proteins and are utilized for energy metabolism (McMahon and McCarthy 2016). Pro and Gly, specifically, are used to synthesize bone collagen (Li and Wu 2018). Gly is a trophic AA, while it is also conditionally a source AA (Jackson 1991). This means that additional Gly is needed for better health and growth of an animal; the amount synthesized in the body as a trophic AA is not sufficient for an animal (Lewis et al. 2005; Wu 2010).

In this study, we used polar bear bone collagen extracted from samples collected within the boundaries of the SBS subpopulation to examine long-term patterns in bulk and compound-specific stable isotopes over a 65-year period from 1954–2019. Specifically, we sought to determine if there were changes in dietary trophic level and in the degree of incorporation of terrestrially derived carbon after a sea ice minimum in September 2007. We used bulk $\delta^{15}N$ and $\delta^{13}C$ values to identify changes in dietary trophic level, and $\delta^{13}C$ values in AA to identify marine versus terrestrially derived carbon by comparing polar bear collagen values with those of a variety of marine and terrestrial species. Given the lifetime isotopic signature from bone collagen, the use of polar bear bone archives allows the study of past and present of this subpopulation. There have been several major sea ice minimum years (Fetterer et al. 2017) in the Arctic, which are likely to have potentially lasting effects on polar bear ecology. Looking into several years of changing habitat in the Southern Beaufort Sea, and how polar bears diets may have changed, allows for a better understanding of their resiliency and future prospects. This study includes a comprehensive look at SBS polar bears before and after 2007 to provide evidence of the changing feeding ecology of these bears with the loss of Arctic sea ice.

3.3 Materials and Methods

We used 71 polar bear bone samples from the SBS subpopulation, spanning 65 years from 1954–2019, to quantify changes in carbon sources through time (Figure 3.2). Samples from 1954–2009 were obtained from the Mammalogy Collection at the University of Alaska Museum of the North, and those from 2006–2019 were provided by Alaska Native subsistence harvests from Utqiaġvik, AK (formerly Barrow). The sex was known for 65 samples (22 females and 43 males), but few have tooth ages associated. Subsistence hunted polar bear samples were categorized into age classes (young of the year, subadult, adult, etc.) based on body size and hunter assessment. Polar bear samples were analyzed under authority of permit number MA80164B-0 issued to the North Slope Borough Department of Wildlife Management (Appendix B). To compare bulk ($\delta^{13}C$ and $\delta^{15}N$) and CSIA-AA ($\delta^{13}C$) values of the polar bears to those of prey and other species in this ecosystem, we analyzed bone collagen from subsistence harvested animals from Utqiaġvik, AK and the Mammalogy Collection at the University of Alaska Museum of the North, including 13 Arctic brown bears (*Ursus arctos*; 1960–2005), two caribou (*Rangifer tarandus*; 2003, 2017), one lynx (*Lynx canadensis*; 2017), 15 wolverines (*Gulo gulo*; 2014–2019), eight ice seals (two bearded, *Erignathus barbatus*; five ringed, *Pusa hispida*; and one spotted, *Phoca largha*; 2002–2017), and six walruses (*Odobenus rosmarus divergens*; 2016; Table 3.1). This variety of animal samples allowed comparison of polar bear bone collagen isotopic values with those of some their primary marine prey (i.e., bearded and ringed seals), other marine mammals that may be consumed to a lesser degree (walruses and spotted seal), terrestrial carnivores (lynx and wolverines), terrestrial omnivores (brown bears), and terrestrial herbivores (caribou). All ice seals were collected and analyzed under the authority of permit number 17350-02 NMFS issued to the North Slope Borough Department of Wildlife Management (Appendix C). Coastal brown bears in Arctic Alaska do not feed on Pacific salmon (*Oncorhynchus* spp.) like many other Alaskan brown bears, but rather consume diets that are 100% derived from terrestrial meat and plants (Phillips 1987; MacHutchon and Wellwood 2003). We used the stable carbon isotope signatures from these animals to develop a regional comparative baseline for marine and terrestrial sources to compare them with our polar bear samples.

To prepare bone collagen, we removed approximately 5 g of cortical bone from each individual animal (lower mandibles, tarsals, or bacula, depending on availability) using a Dremel® with a diamond blade attachment. Bone was polished with a Dremel® sand drum attachment to remove outside contamination and cleaned in an Elmasonic P 300 H sonicator from Elma Schmidbauer GmbH for several minutes. All samples were prepared following bone collagen extraction procedures described by Clark et al. (2019) with lipids removed using 2:1 chloroform/methanol (Folch et al. 1957) and freeze-dried for 48 hours. Following the extraction and preparation of bone collagen, BSIA and CSIA-AA were performed. All bulk stable isotope samples were analyzed using a Costech ECS4010 elemental analyzer coupled to a Finnegan DeltaPlus XP to determine $\delta^{13}C$ and $\delta^{15}N$ values. A peptone standard (No. P-7750 bovine-based protein, Sigma Chemical Company, lot #76f-0300 [$\delta^{13}C$, -15.8‰; $\delta^{15}N$, 7.0‰]) was analyzed after every 10 bulk samples. Quality-control analysis from all peptone standard runs (n=26) provided an instrument error of ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. Following analysis, bulk $\delta^{13}C$ values were Suess corrected following Misarti et al. (2009) to account for anthropogenic CO_2 in the atmosphere since the Industrial Revolution. The C:N ratios of all samples were calculated to assess if bone collagen quality declined with increased archival time (Table 3.1).

The atomic C:N ratio is calculated using the formula $\left(\frac{14}{12} \times \frac{\%C}{\%N}\right)$, which considers the atomic mass of each element. The expected atomic C:N ratio value for bone collagen with no organic carbon contamination is 2.9–3.6 (DeNiro 1985). Only one sample fell outside of this range and did not influence the results whether included or not (Table 3.1).

For CSIA-AA, only the $\delta^{13}C$ values of individual amino acids were determined following procedures outlined in Larsen et al. (2013). Bone collagen proteins were hydrolyzed with 1 mL of 6N HCl and heated at 110°C on a heating block for 20 hours. An internal standard of norleucine (Nor) was added before samples were evaporated to dryness using N_2 gas, while being heated in a water bath at 60°C. Samples were then esterified with acetyl chloride and 2-propanol and heated again at 110°C on a heating block for 60 minutes. Samples were cooled and dried down using N_2 gas in a 60°C water bath before washing with dichloromethane (DCM) and evaporated again to dryness. The samples were then derivatized with trifluoroacetic anhydride and DCM and were placed on a heating block at 100°C for 10 minutes. Next, all samples were cooled to room temperature and dried down with N_2 gas, washed again with DCM, dried with N_2 gas, and reconstituted with ethyl acetate. Finally, samples were analyzed using a Thermo Scientific Trace Gas Chromatograph Ultra linked to an isotope ratio mass spectrometer (IRMS - Thermo Finnigan Delta V) through a combustion interface (IsoLink). All CSIA-AA bone collagen samples were corrected for fractionation and carbon added through chemicals in the derivatization procedure by also analyzing an AA standard sample with known $\delta^{13}C$ values with each batch (O'Brien et al. 2002). The internal Nor standard (50 µl of 0.1 mM) added to each sample was used to correct for instrument error. The amino acids that could be reliably identified in every sample were Ala, Gly, Thr, Ser, Val, Leu, Ile, Nor (internal standard), Pro, Asp, Glu, and Phe. All analyses were performed at the Alaska Stable Isotope Facility at the University of

Alaska Fairbanks. All samples were run in duplicates, unless there were differences greater than the instrument error, determined by standard samples, between the duplicates, in which case a triplicate sample was also analyzed. Instrument error for each AA was based on the standard samples' standard deviation for each batch of samples (Table 3.2). Following analyses, AA peaks for each sample, including every duplicate and potential triplicate, were manually integrated to account for errors with computer integration.

Statistical analyses were performed on both the bulk and CSIA-AA data using R version 1.2.5019 (alpha=0.05). Polar bear samples were tested for normality using a Shapiro-Wilk's test from the package *stats* version 3.6.1 (R Core Team 2019) in R. Based on non-normality of our data, a Kruskal-Wallis rank sum test from the same R package was used. We performed linear regression analyses to test for correlations of bulk or CSIA-AA data over time. Bulk $\delta^{13}C$ and $\delta^{15}N$ values were tested for sex differences using the Kruskal-Wallis test. Similarly, the same statistical test was used to test for differences in CSIA-AA and bulk $\delta^{13}C$ and $\delta^{15}N$ values before and after 2007. To test if any AA were driving the bulk stable isotope composition, $\delta^{13}C$ values were calculated and compared to the measured bulk values. This was done by multiplying the percent contribution of each AA in the sample by the $\delta^{13}C$ value of each AA and summing all AA values. Not all AAs were used in calculating the expected $\delta^{13}C$ values due to AAs that had co-eluted, or did not fully separate. This exclusion of some AAs caused an offset between the calculated and measured $\delta^{13}C$ values. Therefore, creating a 1:1 line for the calculated and measured $\delta^{13}C$ values was done by using the means of the x- and y-axis values to calculate the intercepts and form the equation of the line. All data are reported as mean ± 1 standard deviation (SD) unless otherwise noted.

3.4 Results

Through 1954–2019, bulk $\delta^{15}N$ and $\delta^{13}C$ values in polar bear bone collagen declined on average by 1‰ and 2‰, respectively (Figure 3.4; Table 3.2). The bulk Suess corrected $\delta^{13}C$ values for SBS polar bears ranged from -13.0‰ in 1954 to -18.4‰ in 2016, while the $\delta^{15}N$ values ranged from 24.2‰ in 1983 to 18.9‰ in 2018. The mean ± 1SD of $\delta^{13}C$ and $\delta^{15}N$ values from before 2007 were -14.8‰±1.2‰ and 22.4‰±0.9‰, respectively. After 2007, the mean ± 1SD of $\delta^{13}C$ and $\delta^{15}N$ values were -16.4‰±0.7‰ and 21.6‰±0.9‰, respectively (Table 3.2). There was a significant difference between time periods, i.e., before and after 2007, in both bulk $\delta^{13}C$ (df=68, t-statistic=-7.849, $p<0.001$) and $\delta^{15}N$ values (df=57, t-statistic=-3.971, $p<0.001$). Females (n=22) had higher $\delta^{15}N$ values than males (n=43, df=2, chi-squared=22.135, $p<0.001$), and there was a significant difference for $\delta^{13}C$ values (df=2, chi-squared=7.208, p=0.027). The bulk $\delta^{13}C$ and $\delta^{15}N$ values of Arctic brown bears, walruses, caribou, lynx, ice seals, and wolverines are represented in Figure 3.2. The atomic C:N ratios and collagen yield (percentage of dry bone weight) for polar bear (3.3±0.1; 25.4%±5.1%), ice seal (3.2±0.0; 24.7%±4.1%), walrus (3.3±0.1; 24.2%±2.6%), Arctic brown bear (3.3±0.2; 20.4%±3.3%), wolverine (3.2±0.0; 24.6%±1.7%), caribou (3.3±0.1; 23.0%±3.6%), and lynx (3.3; 6.2%) are given in Table 3.1.

The CSIA-AA analysis showed a significant decline in $\delta^{13}C$ values over time for Gly ($y=-0.14x+267.23$, $R^2=0.30$, $p<0.001$), Ser ($y=-0.14x+274.69$, $R^2=0.18$, $p<0.001$), and Ile ($y=-0.07x+125.96$, $R^2=0.34$, $p<0.001$) in SBS polar bears from 1954–2020 (Table 3.2). After 2007, Gly in particular declined sharply with polar bear values (-23.6‰±1.4‰) approaching those for Arctic brown bears (-27.9‰±3.5‰; Figure 2.5). We compared the measured bulk $\delta^{13}C$ values to calculated $\delta^{13}C$ values based on proportional contribution and the sum of $\delta^{13}C$ values of individual AAs. Two groups of polar bears emerged (Figure 3.6). The expected relationship would be 1:1 between the measured bulk $\delta^{13}C$ values and calculated $\delta^{13}C$ values, instead there was a mean squared error of 1.41 overall, 1.64 for the lower group, and 1.27 for the higher group, respectively (Figure 3.6). After further investigation, the $\delta^{13}C$ values of Pro separated into the same two polar bear groups, one with a high $\delta^{13}C_{Pro}$ mean (±1SD) value of 1.8‰±2.3‰ (n=45) and one with a low $\delta^{13}C_{Pro}$ mean (±1SD) value of -15.7‰±1.9‰ (n=26; Figures 3.7 and 3.8). These two groups were also compared to the $\delta^{13}C_{Pro}$ values of Arctic brown bears and ice seals for a coastal and pelagic comparison (Figure 3.7) resulting in no significant differences between the high $\delta^{13}C_{Pro}$ group and the Arctic brown bears (chi-squared=3.516, df=1, p=0.061) or the low $\delta^{13}C_{Pro}$ group and the ice seals (chi-squared=0.182, df=1, p=0.670).

3.5 Discussion

Bulk $\delta^{13}C$ and $\delta^{15}N$ values of polar bear bone collagen and $\delta^{13}C$ values of several AAs declined over time. While bulk $\delta^{13}C$ values varied widely over time, $\delta^{15}N$ values changed very little. Similarly, several $\delta^{13}C_{AA}$ values mirrored the observed trend in bulk $\delta^{13}C$ values and provided further insight into changes in carbon sources at the base of the polar bear's food web. The $\delta^{13}C$ values of Pro in bone collagen did not change over time but were responsible for completely separating polar bears into two distinct groups.

The dichotomous pattern observed in $\delta^{13}C_{Pro}$, supports the idea that two distinct ecotypes, one coastal and one pelagic occur in the SBS polar bear subpopulation. The tissue we used for this study, bone collagen, has a slow turnover and therefore provides a long-term average signature representative of the prey an animal consumed over its entire lifetime (Manolagas

2000). Pro, making up a greater proportion of bone collagen than most AAs (Li and Wu 2018), plays a strong role in synthesizing bone collagen (Li and Wu 2018). Several studies have shown that consuming higher concentrations of this AA can also cause differences in the size or body condition of an animal (Wu et al. 2011; Kang et al. 2014; Middleton et al. 2017). A larger body size has been linked to higher Pro concentrations, and vice versa (Middleton et al. 2017). However, the body condition and size of these two separate groups, specifically, have not been studied.

We observed substantial variation in $\delta^{13}C$ values in SBS polar bear bone collagen, which can be a gauge of the proportional contribution of terrestrial vs. marine sources (DeNiro and Epstein 1978). This rather large spread of $\delta^{13}C$ values could be due to changes in distribution and habitat use and incorporation of terrestrial derived material into their diet, perhaps even as runoff from permafrost melt. Typical $\delta^{13}C$ values from permafrost runoff are relatively low (-25.8‰, Mu et al. 2015), which could explain the lower $\delta^{13}C$ values after the 2007 sea ice minimum (before 2007: -14.8‰±1.4‰; after 2007: -16.6‰±0.6‰). However, it is not likely that this variation in $\delta^{13}C$ values is due to a change in trophic level, sex differences, or Suess effect. In our polar bear samples, bulk $\delta^{15}N$ values only declined by about 1‰ from 1954–2019. A change in trophic level can only be considered when $\delta^{15}N$ values change by at least 3‰ (McCutchan et al. 2003). It is therefore unlikely that polar bears are switching to terrestrial protein sources, e.g., caribou. While this scenario would explain a substantial change in $\delta^{13}C$ values, it does not support the relatively unchanged trophic position of polar bears over time. Caribou bone collagen has a $\delta^{15}N$ value of 5.1‰, and a polar bear exclusively consuming terrestrial ungulates would show a $\delta^{15}N$ value of ~8‰ (assuming a 3‰ increase from prey to predator), a value not supported by our data. However, polar bears have been observed to feed on Arctic char

(*Salvelinus alpinus*), which have reported $\delta^{13}C$ and $\delta^{15}N$ values of -22.3‰ and 16.4‰, respectively (Dyck and Romberg 2007; Swanson et al. 2010). A switch to char could therefore explain our data, although we were not able to test Arctic char bone collagen. It is important to keep in mind that bone collagen represents a long-term diet average, and for our polar bear values to implicate Arctic char, the bears would have to consume a diet of almost exclusively fish. While not impossible, it is unlikely, and warrants further study.

Our observed decline in bulk $\delta^{13}C$ values in polar bear bone collagen over time could be due to the Suess Effect, the change in $\delta^{13}C$ values from anthropogenic CO_2 in the atmosphere since the Industrial Revolution (Suess 1955). However, as our bulk data were already corrected for the Suess Effect (Misarti et al. 2009), this explanation does not hold. In addition, there is evidence that the Arctic is only minimally affected by the Suess Effect compared to lower latitudes, therefore, if anything, our data might be over corrected, i.e., too positive (Tagliabue and Bopp 2008; de la Vega et al. 2019).

There were significant differences in bulk $\delta^{13}C$ and $\delta^{15}N$ values between male and female polar bears. While the difference in $\delta^{13}C$ values between sexes is small, it could be due to uneven sample sizes (22 females and 43 males) or different prey preferences as male polar bears have been observed to consume more bowhead whale muscle (−20.7‰±0.82‰; Dehn et al. 2006) than females (Herreman and Peacock 2013). Previous studies have also shown that juveniles and female polar bears typically hunt small ringed seals, while males will hunt larger bearded seals (Derocher and Stirling 1990; Thiemann et al. 2008). $\delta^{15}N$ values for bearded and ringed seal muscle tissue are similar, although their $\delta^{13}C$ values differ (Dehn et al. 2007), suggesting that the carbon and nitrogen pattern we detected could be due to minor differences in foraging. The stable isotope values in bone collagen represent a lifetime average, so it is likely that the observed differences in male and female $\delta^{15}N$ values can also be explained by denning/hibernation and fasting of pregnant or lactating females (Polischuk et al. 2001; Bentzen et al. 2007).

Similar to bulk $\delta^{13}C$ values, $\delta^{13}C$ values of the AAs Gly, Ser, and Ile gradually declined over the past 65 years in polar bear bone collagen, approaching isotopic values similar to Arctic brown bears (Table 3.2). These AAs support that the decline in $\delta^{13}C$ values is likely occurring at the base of the food web, which is affecting all trophic levels leading up to polar bears. While Ile is an essential AA, Ser is nonessential, and Gly is somewhat essential. Both Gly and Ser can be synthesized in the body, while Ile can only be synthesized in primary producers, i.e., the base of the food web (McMahon et al. 2013). However, Gly is also considered to be conditionally essential, as it is needed in large amounts by the body for proper bone growth (Lewis et al. 2005; Wu 2010). Ser is also synthesized from Gly, which is used in collagen synthesis (Thureen et al. 1995; Li and Wu 2018). Approaching Arctic brown bear $\delta^{13}C_{AA}$ values, the decline observed in these three AAs in polar bears speaks to a changing carbon source (likely terrestrial) as a building block of body proteins (Lemon 1995). Arctic brown bears are completely terrestrial in their life histories (Phillips 1987), and comparable $\delta^{13}C$ values suggest that a similar ecological niche and/or similar carbon sources could be utilized by SBS polar bears.

Potential carbon sources being incorporated into polar bear tissues could include increased run off (Stuefer et al. 2017) or melting tundra permafrost (Mu et al. 2015). The amount of river run off from several locations along the Alaskan Beaufort Sea coastline has increased from 1970–2015 (Stuefer et al. 2017). Vertebrates and invertebrates living in the Beaufort Sea have about 4-5‰ lower $\delta^{13}C$ values when moving eastward along the coast (Dunton et al. 2006). Similarly, soil in permafrost has relatively negative $\delta^{13}C$ values (-25‰), which could be carried to surrounding areas or water masses as the permafrost melts (Mu et al. 2015). The SBS polar bears could be using these two carbon sources from their ecosystem when building new proteins.

Both essential and non-essential AA are used to create proteins in the body. If a large amount of blubber is available and consumed as part of polar bear diet, the AAs from the blubber will be routed for protein synthesis. Lipids are known to have lower $\delta^{13}C$ values (DeNiro and Epstein 1977), leading to an expected depleted value in the proteins formed from the AAs routed from blubber (Newsome et al. 2014). Blubber and bone collagen contain high amounts of Pro and Gly (Lockyer et al. 1984; Li and Wu 2018), and it is possible that the pelagic/marine polar bears are eating more blubber from ice seals (Rogers et al. 2015; Boucher et al. 2019). Higher amounts of blubber in the diet could explain the observed difference in $\delta^{13}C_{Pro}$ values among bears.

Bulk $\delta^{15}N$ values of polar bears and Arctic brown bears were substantially different, but the bulk $\delta^{13}C$ values are becoming more similar over time, indicating that polar bears might be using a similar food source, but a different trophic niche. An alternative explanation could be that bulk stable isotopes of polar bears are a result of extended fasting, which has become more common in recent years (Pagano et al. 2018; Whiteman et al. 2019). As reported in previous studies, a change in $\delta^{13}C$ and $\delta^{15}N$ values can occur in an animal that is experiencing starvation (Hobson et al. 1993; Cherel et al. 2005). The majority of polar bears included in this study were harvested by Native subsistence hunters and assessed as healthy and in good condition by hunters. Findings from post-mortem examination (wildlife veterinarian) corroborate hunter assessment, so it is unlikely that any of the isotopic values were influenced by phase three starvation.

When exploring variability in $\delta^{13}C$ values, the quality, i.e., composition and stability of the bone collagen used in this study (especially from the earliest time periods) has to be considered. Previous archaeological studies have shown a decline in collagen quality over long time periods (Tuross 2002; Harbeck and Grupe 2009; Brock et al. 2012), but quality can be evaluated by assessing the atomic carbon to nitrogen (C:N) ratio of any bone collagen sample. In our study, the mean atomic C:N ratio of 3.3±0.2 for the polar bear samples is within an acceptable range for bone collagen (van Klinken 1999) and indicates that the collagen quality did not change over time or with external handling of the bones (DeNiro 1985).

Finally, the collagen composition was assessed to determine if there were any AAs driving the bulk $\delta^{13}C$ values. In theory, the measured $\delta^{13}C$ value from the instrument should be tightly correlated to a calculated $\delta^{13}C$ value using the sum of all measured AA carbon signatures (Jim et al. 2006). However, when we plotted these data of measured and calculated bulk $\delta^{13}C$ values, there was a split into two polar bear groups (Figure 3.6). This split into these two groups was also seen in $\delta^{13}C_{Pro}$ values when plotted over time and against bulk $\delta^{13}C$ values. Several studies in the Beaufort Sea have shown that SBS polar bears are separating into pelagic and coastal ecotypes (Ferguson et al. 1999; Mauritzen et al. 2001; Rogers et al. 2015). It is possible that the two polar bear groups identified by values of $\delta^{13}C_{Pro}$, an AA that helps create bone collagen, could be related to the pelagic and coastal groups recognized in previous studies. This is further supported when comparing high and low $\delta^{13}C_{Pro}$ values in SBS polar bears to Arctic brown bears and ice seals. The high $\delta^{13}C_{Pro}$ value of polar bears is very similar to Arctic brown bears, while the low $\delta^{13}C_{Pro}$ value of polar bears is comparable to ice seals (Figure 3.7). This suggests that polar bears with higher $\delta^{13}C_{Pro}$ values could be coastal polar bears and the lower $\delta^{13}C_{Pro}$ value group may be the pelagic cohort. A recent study focusing on fatty acid signatures of adipose tissue from SBS polar bears captured between Utqiaġvik, AK and Yukon, Canada showed that ice seals still make up the highest proportion of polar bear diets (Bourque et al. 2020). Further, Boucher et al. (2019) estimated similar diets for southern Beaufort Sea polar bears that had distributed pelagically in comparison to those that distributed coastally throughout the year based on bulk $\delta^{13}C$ and $\delta^{15}N$ in claws and hair. Our results may have been influenced by an uneven number of pelagic (n=26) and coastal (n=45) polar bears based on Native hunting preferences and small sample sizes within specific time periods.

The $\delta^{13}C_{Pro}$ values, along with previous reports (Rogers et al. 2015; Boucher et al. 2019), support two different ecotypes of SBS polar bears; a coastal and a pelagic group. Polar bears are reliant on a lipid-rich diet (Rode et al. 2015); the coastal polar bears are more likely to summer on shore, where they feed mainly on bowhead whale carcasses, which consist mainly of bones, organs, and skeletal muscle (Rogers et al. 2015), while the pelagic polar bears remain with the sea ice throughout the year, feeding primarily on ice seals. A more lipid-rich diet would cause the AAs, specifically Pro, in the blubber to be routed into protein synthesis. However, the coastal/terrestrial group may consume less blubber, particularly since the bowhead whales they consume are stripped off blubber by Native hunters. This could lead to a lipid- and proline-limited, protein-rich diet. Without this direct dietary source of Pro, coastal ecotype polar bears would have to synthesize Pro from other AAs and protein from their diet (Newsome et al. 2014), causing Pro to become enriched as it becomes modified, as we see with the $\delta^{13}C_{Pro}$ values in the coastal group.

Previous studies have shown that Pro can be influential on animal growth and size (Wu et al. 2000, 2005, 2011; Kang et al. 2014; Middleton et al. 2017). Thus, lower Pro concentrations may result in smaller body size. A recent study showed that domestic dogs (*Canis lupus* *familiaris*) with a smaller body size also had lower concentrations of Pro than larger dogs (Middleton et al. 2017) supporting the idea that Pro has an effect on growing conditions of animals and might in fact be considered essential under certain conditions as suggested by Newsome et al. (2014). This is further supported by a review of pigs (*Sus scrofa domesticus*), and other animals, and their need for Pro at an early age (Wu et al. 2000, 2005, 2011). Furthermore, direct daily Pro injections will increase the body size of piglets and improve overall growth performance (Kang et al. 2014). This difference in body size could be a factor separating the two groups of polar bears identified in this study. A decline in polar bear skull and body size has been observed in SBS polar bears (Regehr et al. 2006; Rode et al. 2010). There are also body size differences between Arctic brown bears and those in other areas of Alaska (McDonough and Christ 2012), with the coastal southern brown bears, which consume lipid-rich salmon, being larger (Rausch 1963; Hilderbrand et al. 1999).

The results from this study provide compelling isotopic evidence that the SBS polar bears have two ecotypes. Future studies should consider incorporating these data with genomic data and other health indices, e.g., body condition and morphometric measurements. A comparison of our data with samples from the Chukchi Sea subpopulation of polar bears would allow for a better understanding of the response of Alaska polar bears to climate change, habitat loss, and prey switching. The CS subpopulation inhabits a more productive ecosystem (Grebmeier et al. 2006) and has three times the number of polar bears (IUCN/SSC Polar Bear Specialist Group 2019), that are in better body condition (Rode et al. 2015). Additional stable isotope work should also be considered for Arctic char, and its potential role in the diet of polar bears. Finally, further studies should focus on blubber AA composition and values in polar bears and their prey items to allow for a better understanding of both the trophic and source AA's roles in polar bear metabolism.

This study supports the idea that two ecotypes (pelagic and coastal) are present among SBS polar bears, while also incorporating additional terrestrial carbon into their diet. Our data report isotopic evidence over the time period of 1954–2019 and provide evidence that these two ecotypes have existed over this entire time period. It is possible that a continued decrease in sea ice extent could further increase the pelagic/marine group, as they continue to follow the sea ice offshore through time.

3.6 References

Atwood T, Peacock E, McKinney M, Lillie K, Wilson R, Douglas D, Miller S, Terletzky P (2016) Rapid environmental change drives increased land use by an Arctic marine predator. PLoS One 11: 1–18. https://doi.org/10.1371/journal.pone.0155932

Bas M, García N, Crespo E, Cardona L (2020) Intraskeletal variability in stable isotope ratios of C and N among pinnipeds and cetaceans. Mar Mammal Sci 36: 375–385. https://doi.org/10.1111/mms.12644

Bentzen T, Follmann E, Amstrup S, York G, Wooller M, O'Hara T (2007) Variation in winter diet of southern Beaufort Sea polar bears inferred from stable isotope analysis. Can J Zool 85: 596–608. https://doi.org/10.1139/Z07-036

Best R (1985) Digestibility of ringed seals by the polar bear. Can J Zool 63: 1033–1036. https://doi.org/10.1139/z85-155

Boucher N, Derocher A, Richardson E (2019) Space use patterns affect stable isotopes of polar bears (*Ursus maritimus*) in the Beaufort Sea. Polar Biol 42: 1581–1593. https://doi.org/10.1007/s00300-019-02546-9

Bourque J, Atwood T, Divoky G, Stewart C, McKinney M (2020) Fatty acid-based diet estimates suggest ringed seal remain the main prey of southern Beaufort Sea polar bears despite recent use of onshore food resources. Ecol Evol 10: 2093–2103. https://doi.org/10.1002/ece3.6043

Bowen W, Iverson S (2013) Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Mar Mammal Sci 29: 719–754. https://doi.org/10.1111/j.1748-7692.2012.00604.x

Bowes R, Thorp J (2015) Consequences of employing amino acid vs. bulk-tissue, stable isotope analysis: A laboratory trophic position experiment. Ecosphere 6: 1–12. https://doi.org/10.1890/ES14-00423.1

Brock F, Wood R, Higham T, Ditchfield P, Bayliss A, Ramsey C (2012) Reliability of nitrogen content (%N) and carbon:nitrogen atomic ratios (C:N) as indicators of collagen preservation suitable for radiocarbon dating. Radiocarbon 54: 879–886. https://doi.org/10.1017/CBO9781107415324.004

Bromaghin J, Mcdonald T, Stirling I, Derocher A, Richardson E, Regehr E, Douglas D, Durner G, Atwood T, Amstrup S (2015) Polar bear population dynamics in the Southern Beaufort Sea during a period of sea ice decline. Ecol Appl 25: 634–651. https://doi.org/10.1890/14-1129.1

Cherel Y, Hobson K, Bailleul F, Groscolas R (2005) Nutrition, physiology, and stable isotopes: New information from fasting and molting penguins. Ecology 86: 2881–2888. https://doi.org/10.1890/05-0562

Clark C, Horstmann L, Misarti N (2017) Quantifying variability in stable carbon and nitrogen isotope ratios within the skeletons of marine mammals of the suborder Caniformia. J Archaeol Sci Reports 15: 393–400. https://doi.org/10.1016/j.jasrep.2017.09.007

Clark C, Horstmann L, de Vernal A, Jensen A, Misarti N (2019) Pacific walrus diet across 4000 years of changing sea ice conditions. Quat Res 1–17. https://doi.org/10.1017/qua.2018.140

Collins M, Nielsen-Marsh C, Hiller J, Smith C, Roberts J, Prigodich R, Wess T, Csapò J, Millard A, Turner-Walker G (2002) The survival of organic matter in bone: A review. Archaeometry 44: 383–394. https://doi.org/10.1111/1475-4754.t01-1-00071

Comiso J (2002) A rapidly declining perennial sea ice cover in the Arctic. Geophys Res Lett 29: 1–4. https://doi.org/10.1029/2002GL015650

de la Vega C, Jeffreys R, Tuerena R, Ganeshram R, Mahaffey C (2019) Temporal and spatial trends in marine carbon isotopes in the Arctic Ocean and implications for food web studies. Glob Chang Biol 25: 4116–4130. https://doi.org/10.1111/gcb.14832

Dehn LA, Follmann EH, Rosa C, Duffy LK, Thomas DL, Bratton GR, Taylor RJ, O'Hara TM (2006) Stable isotope and trace element status of subsistence-hunted bowhead and beluga whales in Alaska and gray whales in Chukotka. Mar Pollut Bull 52:301–19. http://doi.org/10.1016/j.marpolbul.2005.09.001

Dehn L, Sheffield G, Follmann E, Duffy L, Thomas D, O'Hara T (2007) Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biol 30: 167–181. https://doi.org/10.1007/s00300-006-0171-0

DeNiro M (1985) Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. Nature 317: 806–809. https://doi.org/10.1038/317806a0

DeNiro M, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197: 261–263. https://doi.org/10.1126/science.327543

DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42: 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

DeNiro M, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45: 341–351. https://doi.org/10.1016/0016-7037(81)90244-1

Derocher A, Stirling I (1990) Distribution of polar bears (*Ursus maritimus*) during the ice-free period in western Hudson Bay. Can J Zool 68:1395–1403. https://doi-org/10.1139/z90-208

Dunton K, Weingartner T, Carmack E (2006) The nearshore western Beaufort Sea ecosystem: Circulation and importance of terrestrial carbon in arctic coastal food webs. Prog Oceanogr 71: 362–378. https://doi.org/10.1016/j.pocean.2006.09.011

Durner G, Douglas D, Nielson R, Amstrup S, McDonald T, Stirling I, Mauritzen M, Born E, Wiig Ø, DeWeaver E, Serreze M, Belikov S, Holland M, Maslanik J, Aars J, Bailey D, Derocher A (2009) Predicting 21st-century polar bear habitat distribution from global climate models. Ecol Monogr 79: 25–58. https://doi.org/10.1890/07-2089.1

Dyck M, Romberg S (2007) Observations of a wild polar bear (*Ursus maritimus*) successfully fishing Arctic char (*Salvelinus alpinus*) and fourhorn sculpin (*Myoxocephalus quadricornis*). Polar Biol 30: 1625–1628. https://doi.org/10.1007/s00300-007-0338-3

Ferguson S, Taylor M, Born E, Rosing-Asvid A, Messier F (1999) Determinants of home range size for polar bears (*Ursus maritimus*). Ecol Lett 2: 311–318. https://doi.org/10.1046/j.1461-0248.1999.00090.x

Fetterer F, Knowles K, Meier WN, Savoie M, Windnagel AK (2017) updated daily, Sea Ice Index, Version 3. [Monthly]. Boulder, Colorado USA. NSIDC: National Snow and Ice Data Center. https://doi.org/10.7265/N5K072F8. [November 03, 2020].

Folch J, Lees M, Sloane Stanley G (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226: 497–509.

Frost K, Lowry L, Pendleton G, Nute H (2002) Monitoring distribution and abundance of ringed seals in northern Alaska, Final report from the Alaska Department of Fish and Game, Juneau, AK, for U.S. Minerals Management Service, Anchorage, AK. https://doi.org/10.1007/978-1-4614-7990-1

Fry B (2006) Stable isotope ecology. Springer Science+Business Media,LLC, New York, NY. https://doi.org/10.1007-0-387-33745-8

Gittleman J (1985) Carnivore body size: Ecological and taxonomic correlates. Oecologia 67: 540–554. https://doi.org/10.1007/BF00790026

Grebmeier J, Cooper L, Feder H, Sirenko B (2006) Ecosystem dynamics of the Pacific-influenced Northern Bering and Chukchi Seas in the Amerasian Arctic. Prog Oceanogr 71: 331–361. https://doi.org/10.1016/j.pocean.2006.10.001

Harbeck M, Grupe G (2009) Experimental chemical degradation compared to natural diagenetic alteration of collagen: Implications for collagen quality indicators for stable isotope analysis. Archaeol Anthropol Sci 1: 43–57. https://doi.org/10.1007/s12520-009-0004-5

Hare E, Fogel M, Stafford Jr T, Mitchell A, Hoering T (1991) The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18: 277–292. https://doi.org/10.1016/0305-4403(91)90066-X

Herreman J, Peacock E (2013) Polar bear use of a persistent food subsidy: Insights from non-invasive genetic sampling in Alaska. Ursus 24: 148–163. https://doi.org/10.2192/URSUS-D-12-00030.1

Hilderbrand G, Schwartz C, Robbins C, Jacoby M, Hanley T, Arthur S, Servheen C (1999) The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. Can J Zool 77: 132–138. https://doi.org/10.1139/z98-195

Hobson K, Clark R (1992) Assessing avian diets using stable isotopes I: Turnover of ^{13}C in tissues. Condor 94: 181–188. https://doi-org/10.2307/1368807

Hobson K, Alisauskas R, Clark R (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. Condor 95: 388–394. https://doi-org/10.2307/1369361

Hobson K, Piatt J, Pitocchelli J (1994) Using stable isotopes to determine seabird trophic relationships. J Anim Ecol 63: 786–798. https://doi.org/10.2307/5256

Hunter C, Caswell H, Runge M, Regehr E, Amstrup S, Stirling I (2010) Climate change threatens polar bear populations: A stochastic demographic analysis. Ecology 91: 2883–2897. https://doi.org/10.1890/09-1641

IUCN/SSC Polar Bear Specialist Group (2019) Status report on the world's polar bear subpopulations. 2019 Status Report, pp 1–31. http://pbsg.npolar.no/export/sites/pbsg/en/docs/2019-StatusReport.pdf

Jackson A (1991) The glycine story. Eur J Clin Nutr 45: 59–65.

Jim S, Jones V, Ambrose S, Evershed R (2006) Quantifying dietary macronutrient sources of carbon for bone collagen biosynthesis using natural abundance stable carbon isotope analysis. Br J Nutr 95: 1055–1062. https://doi.org/10.1079/bjn20051685

Kang P, Zhang L, Hou Y, Ding B, Yi D, Wang L, Zhu H, Liu Y, Yin Y, Wu G (2014) Effects of l-proline on the growth performance, and blood parameters in weaned lipopolysaccharide (LPS)-challenged pigs. Asian-Australasian J Anim Sci 27: 1150–1156. https://doi.org/10.5713/ajas.2013.13828

Larsen T, Ventura M, Andersen N, O'Brien D, Piatkowski U, McCarthy M (2013) Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. PLoS One 8: 1–9. https://doi.org/10.1371/journal.pone.0073441

Lemon P (1995) Do athletes need more dietary protein and amino acids? Int J Sport Nutr 5: s39–s61. https://doi.org/10.1123/ijsn.5.s1.s39

Lewis R, Godfrey K, Jackson A, Cameron I, Hanson M (2005) Low serine hydroxymethyltransferase activity in the human placenta has important implications for fetal glycine supply. J Clin Endocrinol Metab 90: 1594–1598. https://doi.org/10.1210/jc.2004-0317

Li P, Wu G (2018) Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino Acids 50: 29–38. https://doi.org/10.1007/s00726-017-2490-6

Lockyer C, McConnel L, Waters T (1984) The biochemical composition of fin whale blubber. Can J Zool 62: 2553–2562. https://doi-org/10.1139/z84-373

MacHutchon A, Wellwood D (2003) Grizzly bear food habits in the Northern Yukon, Canada. Ursus 14: 225–235.

Manolagas S (2000) Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21: 115–137. https://doi.org/10.1210/er.21.2.115

Mauritzen M, Derocher A, Wiig Ø (2001) Space-use strategies of female polar bears in a dynamic sea ice habitat. Can J Zool 79: 1704–1713. https://doi.org/10.1139/cjz-79-9-1704

McCutchan J, Lewis W, Kendall C, McGrath C (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102: 378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

McDonough T, Christ A (2012) Geographic variation in size, growth, and sexual dimorphism of Alaska brown bears, *Ursus arctos*. J Mammal 93: 686–697. https://doi.org/10.1644/11-mamm-a-010.1

McKinney M, Peacock E, Letcher R (2009) Sea Ice-associated diet change increases the levels of chlorinated and brominated contaminants in polar bears. Environ Sci Technol 43: 4334–4339. https://doi.org/10.1021/es900471g

McKinney M, Iverson S, Fisk A, Sonne C (2013) Global change effects on the long-term feeding ecology and contaminant exposures of East Greenland polar bears. Glob Chang Biol 19: 2360–2372. https://doi.org/10.1111/gcb.12241

McKinney M, Atwood T, Iverson S, Peacock E (2017) Temporal complexity of southern Beaufort Sea polar bear diets during a period of increasing land use. Ecosphere 8: 1–14. https://doi.org/10.1002/ecs2.1633

McMahon K, Hamady L, Thorrold S (2013) Ocean ecogeochemistry: A review. Oceanogr Mar Biol An Annu Rev 51: 327–374. https://doi.org/10.1201/b15406

McMahon K, McCarthy M (2016) Embracing variability in amino acid $\delta^{15}N$ fractionation: Mechanisms, implications, and applications for trophic ecology. Ecosphere 7: 1–26. https://doi.org/10.1002/ecs2.1511

Middleton R, Lacroix S, Scott-Boyer M, Dordevic N, Kennedy A, Slusky A, Carayol J, Petzinger-Germain C, Beloshapka A, Kaput J (2017) Metabolic differences between dogs of different body sizes. J Nutr Metab 2017: 1–11. https://doi.org/10.1155/2017/4535710

Misarti N, Finney B, Maschner H, Wooller M (2009) Changes in northeast Pacific marine ecosystems over the last 4500 years: Evidence from stable isotope analysis of bone collagen from archeological middens. The Holocene 19: 1139–1151. https://doi.org/10.1177/0959683609345075

Mu C, Zhang T, Wu Q, Cao B, Zhang X, Peng X, Wan X, Zheng L, Wang Q, Cheng G (2015) Carbon and nitrogen properties of permafrost over the Eboling Mountain in the upper reach of Heihe River basin, Northwestern China. Arctic Antarct Alp Res 47: 203–211. https://doi.org/10.1657/AAAR00C-13-095

Newsome S, Wolf N, Peters J, Fogel M (2014) Amino acid $\delta^{13}C$ analysis shows flexibility in the routing of dietary protein and lipids to the tissue of an omnivore. Integr Comp Biol 54: 890–902. https://doi.org/10.1093/icb/icu106

O'Brien D, Fogel M, Boggs C (2002) Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera. Proc Natl Acad Sci USA 99: 4413–4418. https://doi.org/10.1073/pnas.072346699

O'Connell T (2017) 'Trophic' and 'source' amino acids in trophic estimation: A likely metabolic explanation. Oecologia 184: 317–326. https://doi.org/10.1007/s00442-017-3881-9

Obbard ME, Thiemann GW, Peacock E, Debruyn TD (2010) Proceedings of the 15th working meeting of the IUCN/SSC Polar Bear Specialist Group, 29 June-3 July 2009, Copenhagen, Denmark. Gland, Switzerland and Cambridge, UK: IUCN.

Obbard M, Cattet M, Howe E, Middel K, Newton E, Kolenosky G, Abraham K, Greenwood C (2016) Trends in body condition in polar bears (*Ursus maritimus*) from the Southern Hudson Bay subpopulation in relation to changes in sea ice. Arct Sci 2: 15–32. https://doi-org/10.1139/as-2015-0027

Onarheim I, Eldevik T, Smedsrud L, Stroeve J (2018) Seasonal and regional manifestation of Arctic sea ice loss. J Clim 31: 4917–4932. https://doi.org/10.1175/JCLI-D-17-0427.1

Pagano A, Durner G, Rode K, Atwood T, Atkinson S, Peacock E, Costa D, Owen M (2018) High-energy, high-fat lifestyle challenges an Arctic apex predator, the polar bear. Science 359: 568–572. https://doi.org/http://dx.doi.org/10.1126/science.aan8677

Phillips M (1987) Behavior and habitat use of grizzly bears in northeastern Alaska. Int Conf Bear Res Manag 7: 159–167.

Polischuk S, Hobson K, Ramsay M (2001) Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Can J Zool 79: 499–511. https://doi.org/10.1139/cjz-79-3-499

Pongracz J, Derocher A (2017) Summer refugia of polar bears (*Ursus maritimus*) in the southern Beaufort Sea. Polar Biol. 40: 753–763. https://doi.org/10.1007/s00300-016-1997-8

Rausch R (1963) Geographic variation in size in North American brown bears, *Ursus arctos l.*, as indicated by condylobasal length. Can J Zool 41: 33–45. https://doi.org/10.1139/z63-005

Regehr E, Amstrup S, Stirling I (2006) Polar bear population status in the Southern Beaufort Sea, Open-File Report. https://doi.org/10.3133/ofr20061337

Regehr E, Hunter C, Caswell H, Amstrup S, Stirling I (2010) Survival and breeding of polar bears in the Southern Beaufort Sea in relation to sea ice. J Anim Ecol 79: 117–127. https://doi.org/10.1111/j.1365-2656.2009.01603.x

Regehr E, Hostetter N, Wilson R, Rode K, St. Martin M, Converse S (2018) Integrated population modeling provides the first empirical estimates of vital rates and abundance for polar bears in the Chukchi Sea. Sci Rep 8: 1–12. https://doi.org/10.1038/s41598-018-34824-7

Rode K, Amstrup S, Regehr E (2010) Reduced body size and cub recruitment in polar bears associated with sea ice decline. Ecol Appl 20: 768–782. https://doi.org/10.1890/08-1036.1

Rode K, Regehr E, Douglas D, Durner G, Derocher A, Thiemann G, Budge S (2014) Variation in the response of an Arctic top predator experiencing habitat loss: feeding and reproductive ecology of two polar bear populations. Glob Chang Biol 20: 76–88. https://doi.org/10.1111/gcb.12339

Rode K, Robbins C, Nelson L, Amstrup S (2015) Can polar bears use terrestrial foods to offset lost ice-based hunting opportunities? Front Ecol Environ 13: 138–145. https://doi.org/10.1890/140202

Rogers M, Peacock E, Simac K, O'Dell M, Welker J (2015) Diet of female polar bears in the southern Beaufort Sea of Alaska: Evidence for an emerging alternative foraging strategy in response to environmental change. Polar Biol 38: 1035–1047. https://doi.org/10.1007/s00300-015-1665-4

Sakshaug E (2004) Primary and Secondary Production in the Arctic Seas. In: Stein R, MacDonald R (eds), The Organic Carbon Cycle in the Arctic Ocean. Springer, Berlin, Heidelberg, pp 57–81. https://doi-org/10.1007/978-3-642-18912-8_3

Schliebe S, Rode K, Gleason J, Wilder J, Proffitt K, Evans T, Miller S (2008) Effects of sea ice extent and food availability on spatial and temporal distribution of polar bears during the fall open-water period in the Southern Beaufort Sea. Polar Biol 31: 999–1010. https://doi.org/10.1007/s00300-008-0439-7

Stirling I (2002) Polar bears and seals in the eastern Beaufort Sea and Amundsen Gulf: A synthesis of population trends and ecological relationships over three decades. Arctic 55: 59–76. https://doi.org/10.14430/arctic735

Stirling I, Lunn N, Iacozza J (1999) Long-term trends in the population ecology of polar bears in Western Hudson Bay in relation to climatic change. Arctic 52: 294–306.

Stirling I, Derocher A (2012) Effects of climate warming on polar bears: a review of the evidence. Glob Chang Biol 18: 2694–2706. https://doi.org/10.1111/j.1365-2486.2012.02753.x

Stuefer S, Arp C, Kane D, Liljedahl A (2017) Recent extreme runoff observations from coastal arctic watersheds in Alaska. Water Resour Res 53: 9145–9163. https://doi.org/10.1002/2017WR020567

Suess H (1955) Radiocarbon concentration in modern wood. Science 122: 415–417. https://doi.org/10.1017/CBO9781107415324.004

Swanson H, Kidd K, Babaluk J, Wastle R, Yang P, Halden N, Reist J (2010) Anadromy in Arctic populations of lake trout (*Salvelinus namaycush*): Otolith microchemistry, stable isotopes, and comparisons with Arctic char (*Salvelinus alpinus*). Can J Fish Aquat Sci 67: 842–853. https://doi.org/10.1139/F10-022

Tagliabue A, Bopp L (2008) Towards understanding global variability in ocean carbon-13. Global Biogeochem Cycles 22: 1–13. https://doi.org/10.1029/2007GB003037

Thiemann G, Iverson S, Stirling I (2008) Polar bear diets and Arctic marine food webs: Insights from fatty acid analysis. Ecol Monogr 78: 591–613. https://doi.org/10.1890/07-1050.1

Thureen P, Narkewicz M, Battaglia F, Tjoa S, Fennessey P (1995) Pathways of serine and glycine metabolism in primary culture of ovine fetal hepatocytes. Pediatr Res 38: 775–782. https://doi.org/10.1203/00006450-199511000-00023

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57: 32–37. https://doi.org/10.1007/BF00379558

Tuross N (2002) Alterations in fossil collagen. Archaeometry 44: 427–434. https://doi.org/10.1111/1475-4754.00075

U.S. Fish and Wildlife Service (2008) Endangered and threatened wildlife and plants: Determination of threatened status for the polar bear (*Ursus maritimus*) throughout its range. Fed Regist 73: 28211-28303.

van Klinken G (1999) Bone collagen quality indicators for palaeodietary and radiocarbon measurements. J Archaeol Sci 26: 687–695. https://doi.org/10.1006/jasc.1998.0385

West JB, Bowen GJ, Cerling TE, Ehleringer JR (2006) Stable isotopes as one of nature's ecological recorders. TRENDS Ecol Evol 21: 408–414. https://doi.org/10.1016/j.tree.2006.04.002

Whiteman J, Smith E, Besser A, Newsome S (2019) A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. Diversity 11: 1–18. https://doi.org/10.3390/d11010008

Wu G (2010) Functional amino acids in growth, reproduction, and health. Adv Nutr 1: 31–37. https://doi.org/10.3945/an.110.1008.1

Wu G, Flynn N, Knabe D (2000) Enhanced intestinal synthesis of polyamines from proline in cortisol-treated piglets. Am J Physiol Endocrinol Metab 279: E395–E402. https://doi.org/10.1152/ajpendo.2000.279.2.e395

Wu G, Bazer F, Hu J, Johnson G, Spencer T (2005) Polyamine synthesis from proline in the developing porcine placenta. Biol Reprod 72: 842–850. https://doi.org/10.1095/biolreprod.104.036293

Wu G, Bazer F, Burghardt R, Johnson G, Kim S, Knabe D, Li P, Li X, McKnight J, Satterfield M, Spencer T (2011) Proline and hydroxyproline metabolism: Implications for animal and human nutrition. Amino Acids 40: 1053–1063. https://doi.org/10.1007/s00726-010-0715-z.Proline

3.7 Figures and Tables

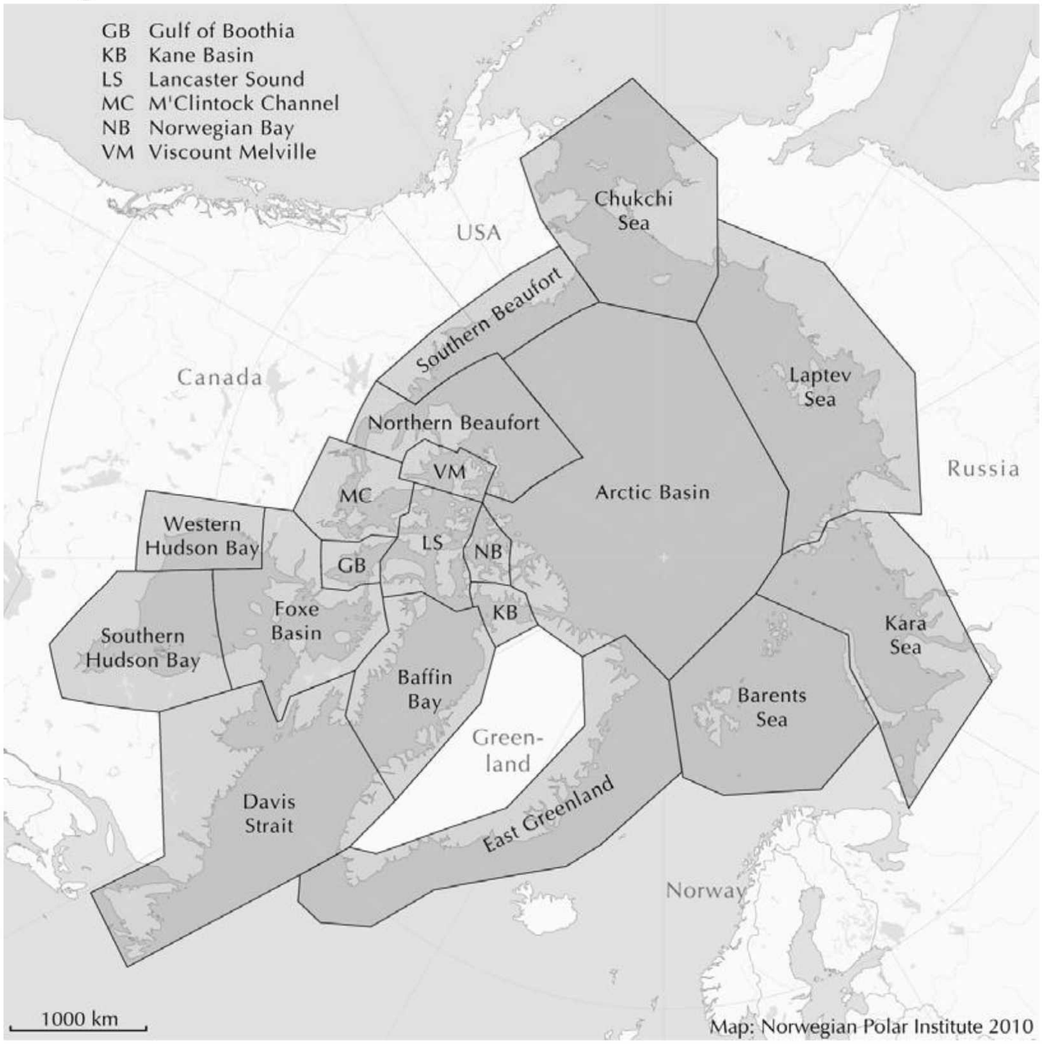


Figure 3.1 Map showing the distribution of the world's polar bear subpopulations (Obbard et al. 2010).

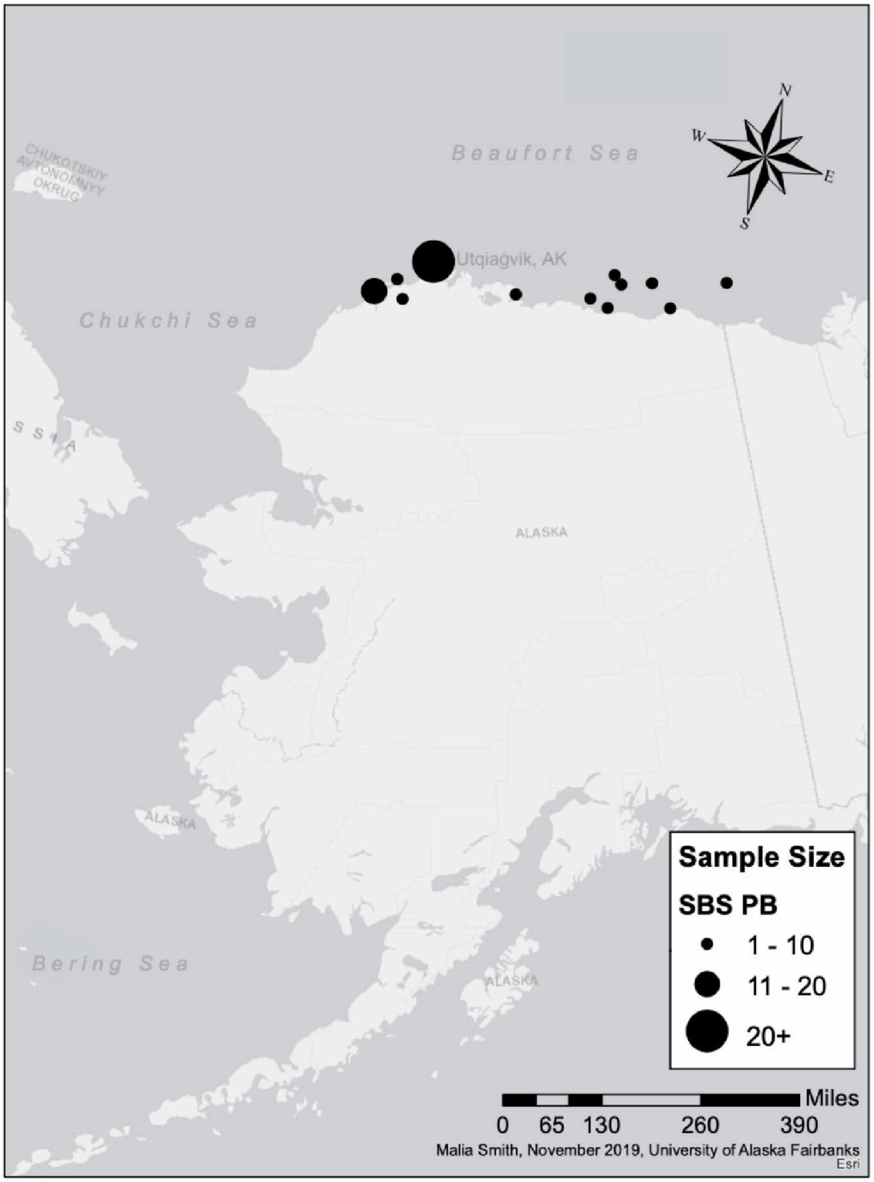


Figure 3.2 Map of Southern Beaufort Sea polar bear (SBS PB) bone samples used for stable isotope analyses from 1954–2019. All other animals used in this study (brown bear, wolverine, caribou, lynx, ice seal, and walrus) were collected from the Utqiaġvik, AK area. Size of symbols reflects their relative sample size from a specific location.

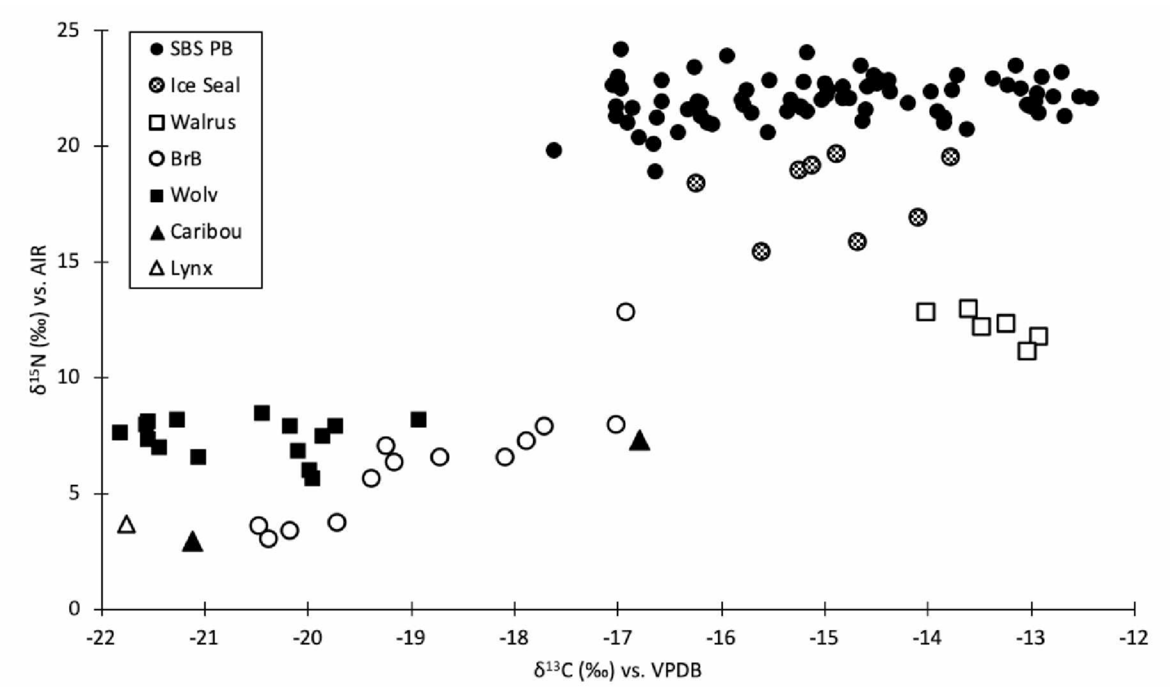


Figure 3.3 Measured bulk $\delta^{15}N$ values (‰) vs. atmospheric nitrogen gas (AIR) and $\delta^{13}C$ values (‰) vs. Vienna Pee Dee Belemnite (VPDB) in bone collagen of Southern Beaufort Sea polar bears (SBS PB; solid circles), ice seals (patterned circles), walruses (open squares), Arctic brown bears (BrB; open circles), wolverines (Wolv; solid squares), caribou (solid triangles), and lynx (open triangle). All $\delta^{13}C$ values are Suess corrected, and all samples were collected from the Alaska North Slope region, near Utqiaġvik, AK.

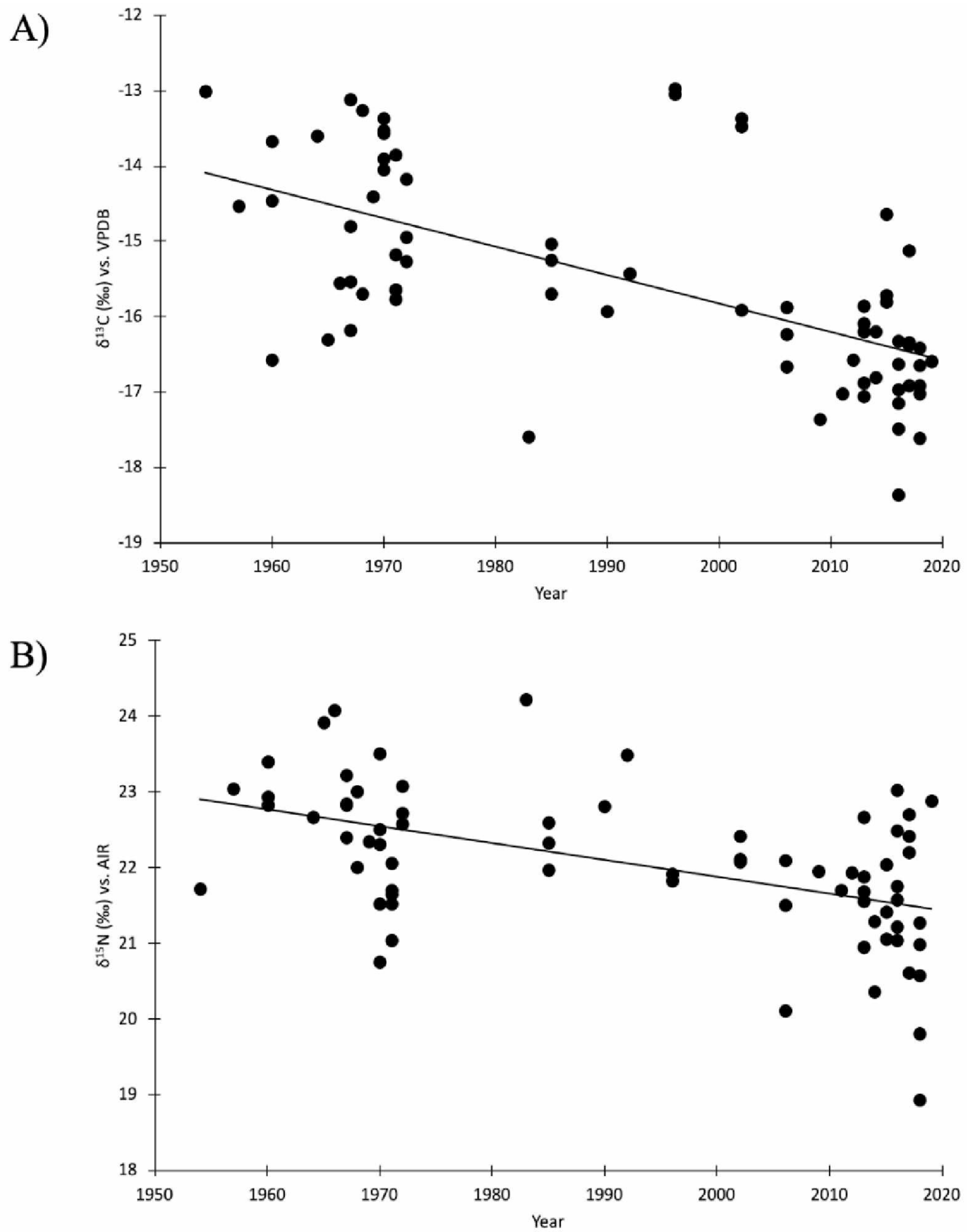


Figure 3.4 A) Measured bulk $\delta^{13}C$ (‰) vs. Vienna Pee Dee Belemnite (VPDB) of Southern Beaufort Sea polar bear bone collagen from 1954–2019. All bulk $\delta^{13}C$ values have been Suess corrected following Misarti et al. (2009). Black line represents the best linear fit (y=-0.04x+60.13; $R^2=0.38$; $p<0.001$). B) Measured bulk $\delta^{15}N$ (‰) vs. atmospheric nitrogen gas (AIR) of Southern Beaufort Sea polar bear bone collagen from 1954–2019. Black line represents the best linear fit (y=-0.02x+65.94; $R^2=0.25$; $p<0.001$).

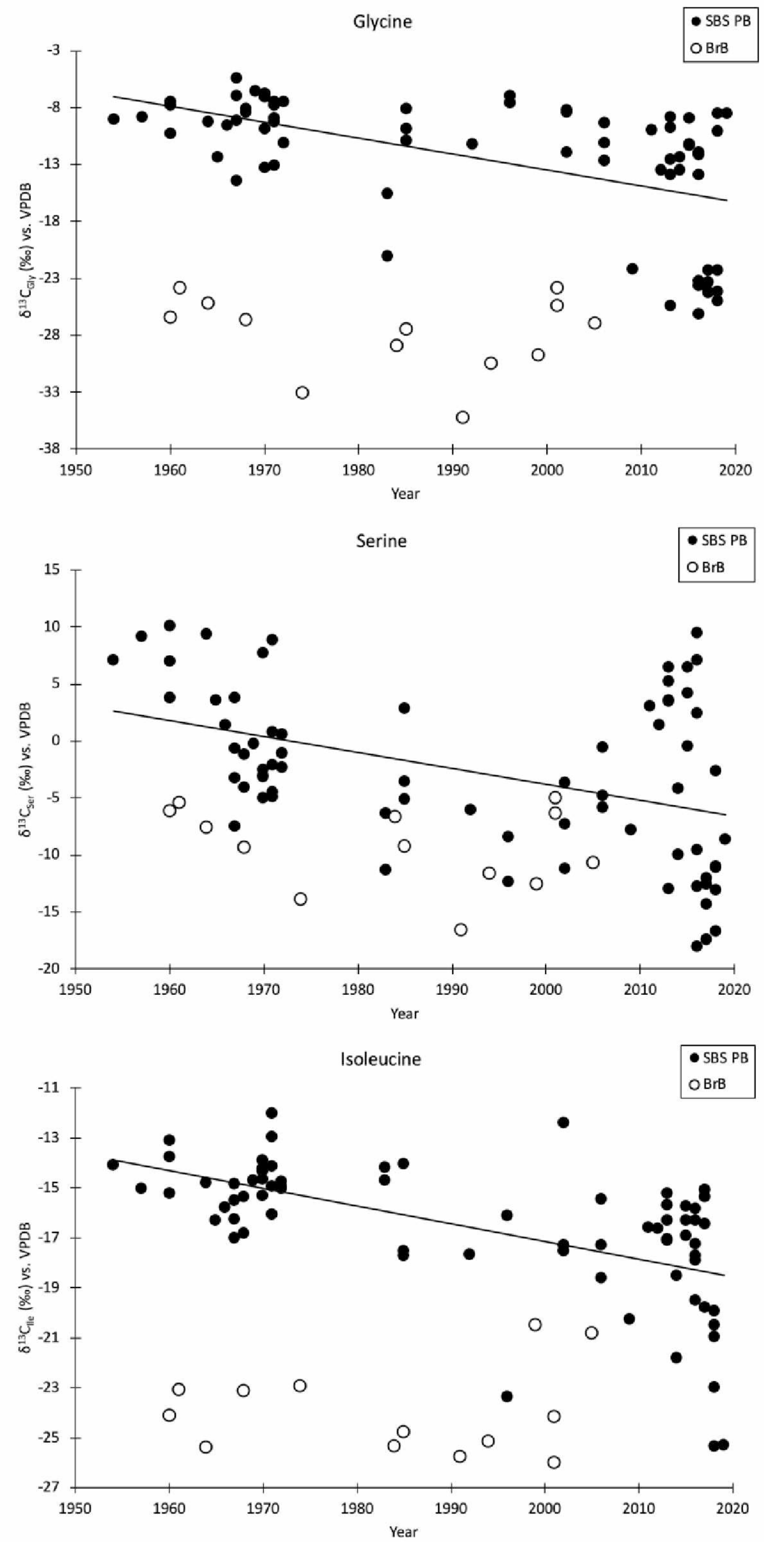


Figure 3.5 Compound-specific stable isotopes of amino acids of $\delta^{13}C$ (‰) vs. Vienna Pee Dee Belemnite (VPDB) in bone collagen for glycine (Gly), serine (Ser), and isoleucine (Iso) from 1954–2019. All linear regressions are significant ($p<0.05$), and all equations are given in Table 2.2. Southern Beaufort Sea polar bears (SBS PB) are shown as solid black circles, while Arctic brown bears (BrB) are shown as open circles.

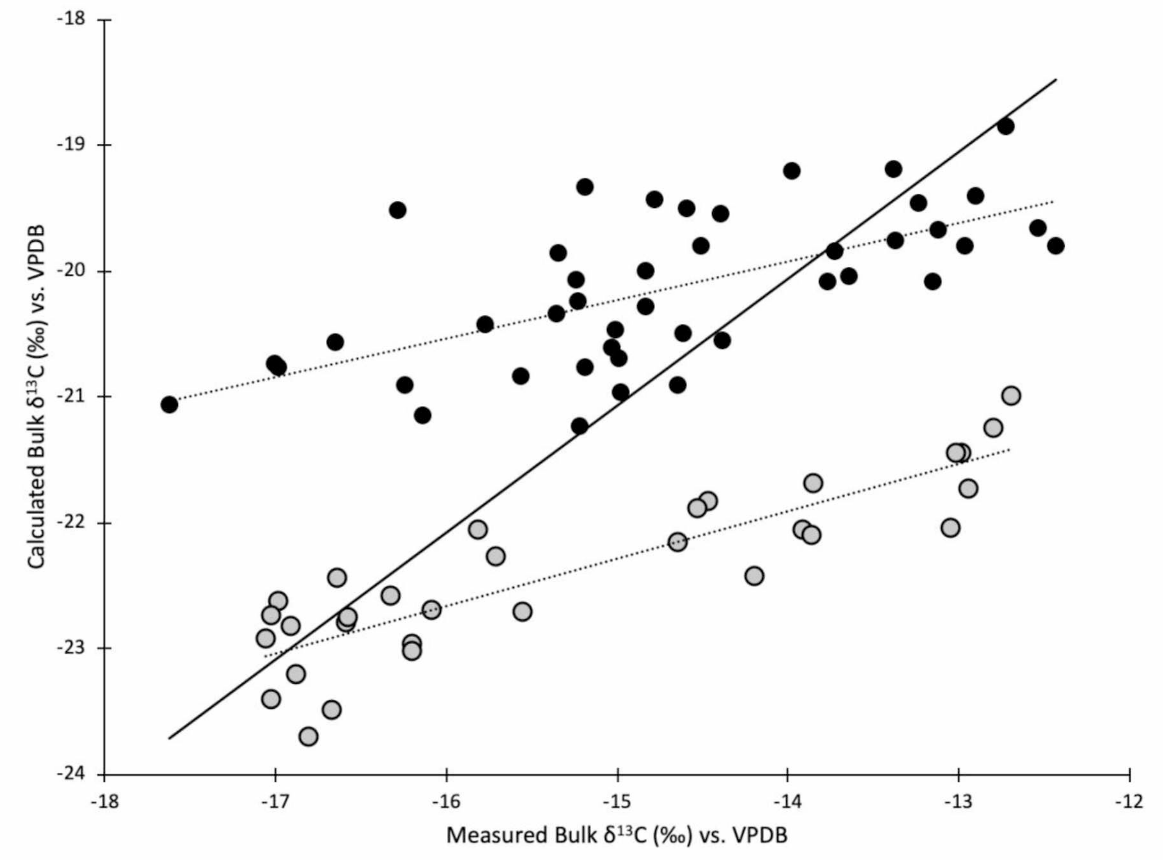


Figure 3.6 Measured bulk δ^{13}C values (‰) vs. Vienna Pee Dee Belemnite (VPDB) compared to calculated δ^{13}C values (‰) vs. VPDB for all amino acids reliably identified in chromatograms of Southern Beaufort Sea polar bear bone collagen. Solid black line represents the expected 1:1 relationship ($y=x-5.98$; $R^2=0.30$), dotted lines represent the best linear fit for the higher (solid black circles; $y=0.31x-15.64$; $R^2=0.41$; $p<0.001$) and lower (gray circles; $y=0.38x-16.61$; $R^2=0.77$; $p<0.001$) groups. Deviation from the expected 1:1 line resulted in a mean squared error of 1.41 overall, with 1.64 and 1.27 for the lower and higher groups, respectively.

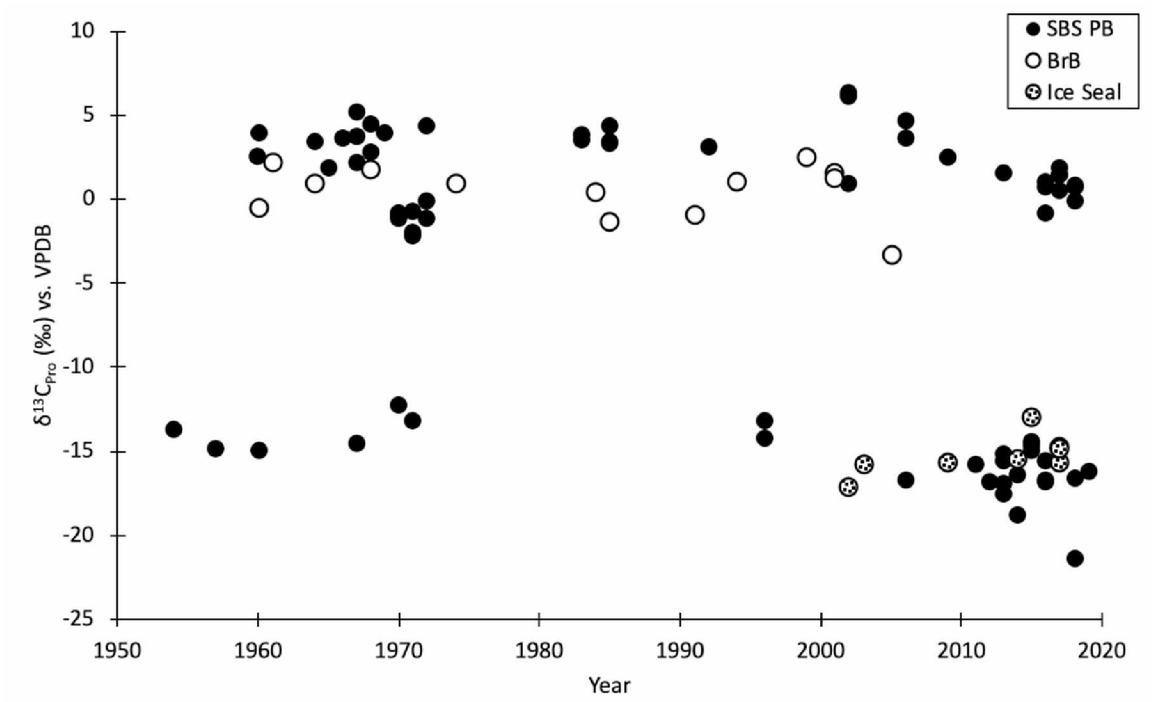


Figure 3.7 Measured $\delta^{13}C_{Pro}$ (proline) values (‰) vs. Vienna Pee Dee Belemnite (VPDB) in bone collagen of Southern Beaufort Sea polar bears (SBS PB; solid black circles), Arctic brown bears (BrB; open circles), and ice seals (patterned circles) from 1954–2019. SBS polar bears are falling into two groups; one with higher $\delta^{13}C_{Pro}$ values and one with lower $\delta^{13}C_{Pro}$ values. The higher $\delta^{13}C_{Pro}$ value group has similar values to Arctic brown bears, while the lower $\delta^{13}C_{Pro}$ value group is comparable to ice seals. The emerging two groups shown here are mirroring those in Figure 3.8.

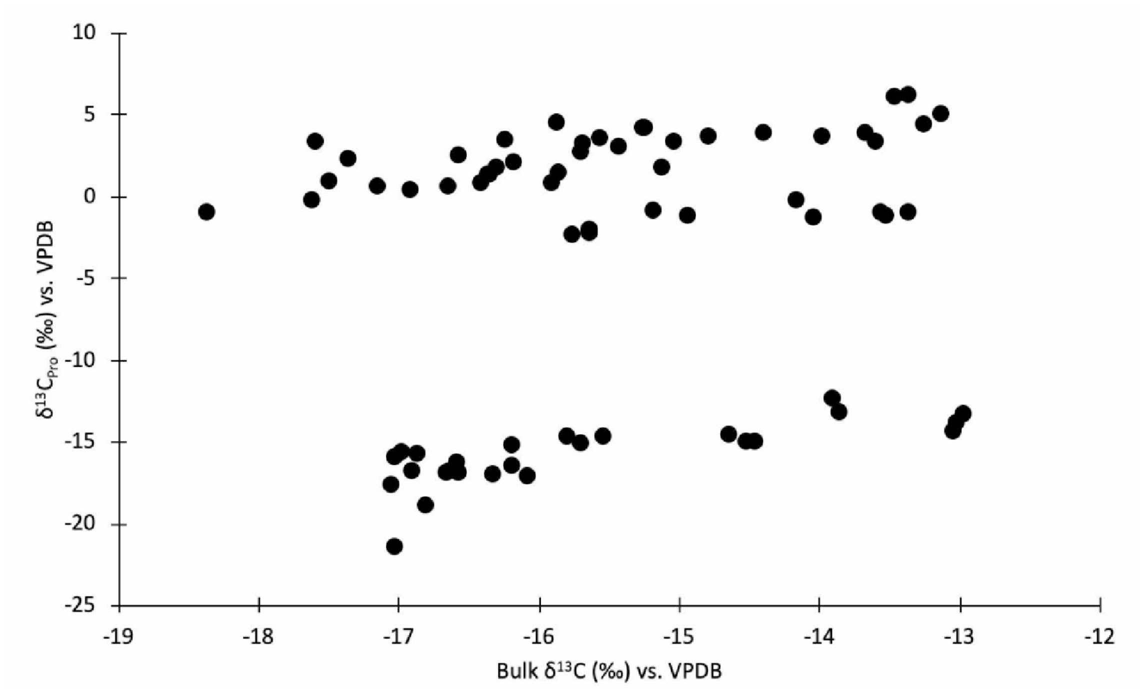


Figure 3.8 Measured $\delta^{13}C_{Pro}$ (proline) values (‰) vs. Vienna Pee Dee Belemnite (VPDB) compared to bulk $\delta^{13}C$ values (‰) vs. VPDB of Southern Beaufort Sea polar bear bone collagen. Polar bears are falling into two groups; one with higher $\delta^{13}C_{Pro}$ values and one with lower $\delta^{13}C_{Pro}$ values. The emerging two groups shown here are mirroring those in Figure 3.7.

Table 3.1 Calculated atomic C:N ratios (mean ± 1 SD) and percent collagen extracted (mean ± 1 SD) of bone collagen for all Southern Beaufort Sea (SBS) polar bears (3.3±0.1%; 25.4±5.1%), ice seals (3.2±0.0%; 24.7%±4.1%), walruses (3.3±0.1%; 24.2%±2.6%), Arctic brown bears (3.3±0.2%; 20.4%±3.3%), wolverines (3.2±0.0%; 24.6%±1.7%), caribou (3.3±0.1%; 23.0%±3.6%), and lynx (3.3%; 6.2%) collected from the Utqiaġvik area. Sample ID's are given from a museum or Utqiaġvik veterinarian.

Sample ID	Species	Year of Collection	% Collagen Extracted	Atomic C:N Ratio
UAM:Mamm:4729	*Ursus maritimus*	1954	24.3	3.2
UAM:Mamm:3424	*Ursus maritimus*	1957	23.9	3.3
UAM:Mamm:4726	*Ursus maritimus*	1960	18.9	3.2
UAM:Mamm:4727	*Ursus maritimus*	1960	26.7	3.5
UAM:Mamm:4946	*Ursus maritimus*	1960	22.6	3.2
UAM:Mamm:16414	*Ursus maritimus*	1964	23.9	3.4
UAM:Mamm:7276	*Ursus maritimus*	1965	35.4	3.4
UAM:Mamm:87064	*Ursus maritimus*	1966	25.4	3.5
UAM:Mamm:16469	*Ursus maritimus*	1967	23.7	3.3
UAM:Mamm:16486	*Ursus maritimus*	1967	20.7	3.3
UAM:Mamm:16450	*Ursus maritimus*	1967	27.2	3.5
UAM:Mamm:87059	*Ursus maritimus*	1967	24.9	3.4
UAM:Mamm:16492	*Ursus maritimus*	1968	22.4	3.3
UAM:Mamm:16376	*Ursus maritimus*	1968	23.4	3.3
UAM:Mamm:16436	*Ursus maritimus*	1969	23.5	3.3
UAM:Mamm:16456	*Ursus maritimus*	1970	19.5	3.2
UAM:Mamm:18459	*Ursus maritimus*	1970	19.3	3.2
UAM:Mamm:16472	*Ursus maritimus*	1970	21.8	3.2
UAM:Mamm:16423	*Ursus maritimus*	1970	24.2	3.2
UAM:Mamm:16419	*Ursus maritimus*	1970	26.2	3.3

Table 3.1 Cont.

Sample ID	Species	Year of Collection	% Collagen Extracted	Atomic C:N Ratio
UAM:Mamm:16382	*Ursus maritimus*	1971	22.4	3.3
UAM:Mamm:87082	*Ursus maritimus*	1971	18.0	3.3
UAM:Mamm:87080	*Ursus maritimus*	1971	28.7	3.4
UAM:Mamm:18460	*Ursus maritimus*	1971	26.4	3.0
UAM:Mamm:87081	*Ursus maritimus*	1971	25.5	3.2
UAM:Mamm:16403	*Ursus maritimus*	1972	23.8	3.2
UAM:Mamm:16538	*Ursus maritimus*	1972	18.7	3.1
UAM:Mamm:16514	*Ursus maritimus*	1972	23.7	3.3
UAM:Mamm:16544	*Ursus maritimus*	1983	29.3	3.2
UAM:Mamm:16541	*Ursus maritimus*	1985	22.4	3.2
UAM:Mamm:16540	*Ursus maritimus*	1985	26.2	3.2
UAM:Mamm:16545	*Ursus maritimus*	1985	21.7	3.2
6375PB 1990	*Ursus maritimus*	1990	33.1	3.4
UAM:Mamm:24875	*Ursus maritimus*	1992	20.4	3.2
FWS PBR 960106	*Ursus maritimus*	1996	28.1	3.2
FWS PBR 960103	*Ursus maritimus*	1996	22.6	3.3
UAM:Mamm:123094	*Ursus maritimus*	2002	24.5	3.3
UAM:Mamm:123095	*Ursus maritimus*	2002	22.2	3.1
UAM:Mamm:111725	*Ursus maritimus*	2002	17.1	3.1
UAM:Mamm:87964	*Ursus maritimus*	2006	22.5	3.2
UAM:Mamm:87963	*Ursus maritimus*	2006	22.5	3.1
USIK 01-10-06	*Ursus maritimus*	2006	29.5	3.3
UAM:Mamm:138279	*Ursus maritimus*	2009	22.9	3.5

Table 3.1 Cont.

Sample ID	Species	Year of Collection	% Collagen Extracted	Atomic C:N Ratio
UN2011BW04	*Ursus maritimus*	2011	38.6	3.3
KAK001-12	*Ursus maritimus*	2012	28.6	3.2
N01-2013	*Ursus maritimus*	2013	29.7	3.3
N12-2013PB	*Ursus maritimus*	2013	28.7	3.2
N13-2013PB	*Ursus maritimus*	2013	27.5	3.2
N16-2013PB	*Ursus maritimus*	2013	14.1	3.2
N80B 2013	*Ursus maritimus*	2013	28.6	3.3
2014PB04	*Ursus maritimus*	2014	29.1	3.2
2014PB05	*Ursus maritimus*	2014	30.0	3.2
15HPB1	*Ursus maritimus*	2015	26.6	3.2
2015PB07	*Ursus maritimus*	2015	25.8	3.2
2015PB08	*Ursus maritimus*	2015	26.9	3.2
2016PB0111-5	*Ursus maritimus*	2016	10.2	3.3
2016PB0505-5	*Ursus maritimus*	2016	33.3	3.4
2016PB0512-5	*Ursus maritimus*	2016	28.7	3.2
2016USFWSN0060	*Ursus maritimus*	2016	28.4	3.3
2016PB0111a	*Ursus maritimus*	2016	24.9	3.6
2016PB0111b	*Ursus maritimus*	2016	44.3	4.0
2017PB0903-s	*Ursus maritimus*	2017	29.0	3.4
2017PB0424-s/hc	*Ursus maritimus*	2017	27.7	3.3
2017PB0222-s	*Ursus maritimus*	2017	28.2	3.3
2017PB0214-s	*Ursus maritimus*	2017	29.0	3.4
2018PB0304-s	*Ursus maritimus*	2018	28.0	3.3

Table 3.1 Cont.

Sample ID	Species	Year of Collection	% Collagen Extracted	Atomic C:N Ratio
2018PB0205-s	*Ursus maritimus*	2018	27.7	3.6
2018PB0204-s	*Ursus maritimus*	2018	26.7	3.3
2018 PB XXXX S	*Ursus maritimus*	2018	21.9	3.2
2018 PB 1213 FD KAK	*Ursus maritimus*	2018	23.6	3.2
2019 PB 0409	*Ursus maritimus*	2019	24.4	3.3
Ebar-1-12-03	*Erignathus barbatus*	2003	27.3	3.3
2017-ss-0802 S	*Phoca largha*	2017	27.5	3.3
DWM 10-02 fetus	*Pusa hispida*	2002	25.2	3.3
09Rs14	*Pusa hispida*	2009	19.0	3.2
2014 Rs29	*Pusa hispida*	2014	21.3	3.2
STR-080-15	*Pusa hispida*	2015	23.3	3.2
2017 RS 0725 FD	*Pusa hispida*	2017	22.3	3.2
2017 RS 1003 FD	*Pusa hispida*	2017	31.7	3.2
G16-0006	*Odobenus rosmarus divergens*	2016	24.3	3.2
G16-0034	*Odobenus rosmarus divergens*	2016	25.9	3.2
S16-0003	*Odobenus rosmarus divergens*	2016	23.6	3.4
S16-0028	*Odobenus rosmarus divergens*	2016	23.9	3.2
S16-0034	*Odobenus rosmarus divergens*	2016	19.8	3.3
S16-0038	*Odobenus rosmarus divergens*	2016	27.5	3.4
UAM:Mamm:4719	*Ursus arctos*	1960	17.8	3.2
UAM:Mamm:4776	*Ursus arctos*	1961	19.2	3.2
UAM:Mamm:14324	*Ursus arctos*	1964	15.1	3.2
UAM:Mamm:37550	*Ursus arctos*	1968	18.4	3.2

Table 3.1 Cont.

Sample ID	Species	Year of Collection	% Collagen Extracted	Atomic C:N Ratio
UAM:Mamm:14320	*Ursus arctos*	1974	22.4	3.6
UAM:Mamm:15986	*Ursus arctos*	1984	25.6	3.2
UAM:Mamm:134670	*Ursus arctos*	1985	14.1	3.2
UAM:Mamm:64232	*Ursus arctos*	1991	21.2	3.2
UAM:Mamm:134085	*Ursus arctos*	1994	22.6	3.2
UAM:Mamm:73290	*Ursus arctos*	1999	23.6	3.4
UAM:Mamm:66484	*Ursus arctos*	2001	22.2	3.5
UAM:Mamm:66483	*Ursus arctos*	2001	21.5	3.5
UAM:Mamm:114266	*Ursus arctos*	2005	21.6	3.1
2014 02 wolv	*Gulo gulo*	2014	27.3	3.3
2015 wolv 03	*Gulo gulo*	2015	25.2	3.2
2015 wolv 04	*Gulo gulo*	2015	26.8	3.2
2017 wolv 0108 S	*Gulo gulo*	2017	24.0	3.2
2017 wolv 0127	*Gulo gulo*	2017	25.4	3.2
2017 wolv 0129_A	*Gulo gulo*	2017	24.2	3.2
2017 wolv 0418 S	*Gulo gulo*	2017	23.6	3.2
2018 wolv 0102 Sa	*Gulo gulo*	2018	26.1	3.2
2018 wolv 0102 Sb	*Gulo gulo*	2018	23.1	3.2
2018 wolv 0102 Sc	*Gulo gulo*	2018	20.4	3.3
2018 wolv 01xx Sa	*Gulo gulo*	2018	24.7	3.2
2018 wolv 01xx Sb	*Gulo gulo*	2018	26.1	3.2
2018 wolv 0507 S	*Gulo gulo*	2018	24.1	3.2
101ST 2019 M 040	*Gulo gulo*	2019	23.6	3.2

Table 3.1 Cont.

Sample ID	Species	Year of Collection	% Collagen Extracted	Atomic C:N Ratio
2019 wolv 0407 S	*Gulo gulo*	2019	25.2	3.2
CB 4-8-03	*Rangifer tarandus*	2003	20.4	3.2
TCH-2017-0302	*Rangifer tarandus*	2017	25.6	3.3
2017 Lynx 0418	*Lynx canadensis*	2017	6.2	3.3

Table 3.2 Compound-specific stable carbon isotopes on individual amino acids and bulk stable carbon and nitrogen isotope data for all Southern Beaufort Sea polar bear bone collagen samples. Data are reported as trophic or source amino acids, overall mean±1SD, mean±1SD 'Before 2007', mean±1SD 'After 2007', p-value for the significance of differences between 'Before 2007' and 'After 2007', the regression equation over time from 1954–2019, the associated R^2 value, and the p-value for the significance of the regression line against the isotope data and time. Amino acids are listed in order of elution from column. Significant p-values (<0.05) are bolded.

	Source or Trophic	Mean ±1SD (‰)	Before 2007 (‰)	After 2007 (‰)	p-value	Regression Equation	R^2	p-value
Bulk $\delta^{13}C$	-	-15.5 ±1.4	-14.8 ±1.4	-16.6 ±0.6	**<0.001**	y=-0.04x+60.13	0.38	**<0.001**
Bulk $\delta^{15}N$	-	22.0 ±1.0	22.4 ±0.7	21.5 ±0.9	**<0.001**	y=-0.02x+65.94	0.25	**<0.001**
$\delta^{13}C_{Ala}$	Trophic	-22.9 ±1.5	-22.9 ±1.5	-23.0 ±1.5	0.623	y=-0.00x-16.83	0.00	0.707
$\delta^{13}C_{Gly}$	Trophic	-12.4 ±5.8	-9.7 ±2.9	-16.4 ±6.5	**<0.001**	y=-0.14x+267.23	0.30	**<0.001**
$\delta^{13}C_{Thr}$	Source	-4.7 ±3.1	-4.3 ±2.5	-5.3 ±3.8	0.800	y=-0.01x+6.55	0.00	0.734
$\delta^{13}C_{Ser}$	Trophic	-2.7 ±7.3	-1.2 ±5.8	-4.8 ±8.7	0.075	y=-0.14x+274.69	0.18	**<0.001**
$\delta^{13}C_{Val}$	Source	-19.0 ±2.5	-18.7 ±1.9	-19.3 ±3.2	0.991	y=-0.02x+14.90	0.02	0.199
$\delta^{13}C_{Leu}$	Source	-26.4 ±1.7	-26.2 ±1.5	-26.7 ±2.0	0.475	y=-0.01x-5.06	0.02	0.237
$\delta^{13}C_{Ile}$	Source	-16.6 ±2.7	-15.4 ±2.0	-18.3 ±2.9	**<0.001**	y=-0.07x+125.96	0.34	**<0.001**
$\delta^{13}C_{Pro}$	Trophic	-4.6 ±8.8	-1.3 ±7.2	-9.3 ±8.9	**<0.001**	y=-0.13x+261.06	0.12	**0.004**
$\delta^{13}C_{Asp}$	Trophic	-19.4 ±2.6	-19.4 ±2.4	-19.5 ±2.9	0.783	y=-0.01x+10.32	0.02	0.283

Table 3.2 Cont.

	Source or Trophic	Mean ±1SD (‰)	Before 2007 (‰)	After 2007 (‰)	p-value	Regression Equation	R^2	p-value
$\delta^{13}C_{Glu}$	Trophic	-16.0 ±3.0	-14.9 ±2.7	-17.6 ±2.6	**<0.001**	y=-0.09x+157.28	0.43	**<0.001**
$\delta^{13}C_{Phe}$	Source	-25.7 ±1.5	-25.3 ±1.3	-26.2 ±1.6	**0.012**	y=-0.02x+18.98	0.11	**0.004**

Chapter 4: General Conclusion

The current rate of melting sea ice in the Arctic has caused a decline in the availability of habitat and prey for polar bears (*Ursus maritimus*) (Mckinney et al. 2013, 2017; Stirling and Derocher 2012). This change in sea ice extent has forced polar bears to move further on land in recent years (Stirling et al. 1999; Atwood et al. 2016). Some polar bear subpopulations are declining in size as the Arctic warms (IUCN/SSC Polar Bear Specialist Group 2019). The two subpopulations of polar bears in Alaska are the Southern Beaufort Sea (SBS) and Chukchi Sea (CS) subpopulations (Obbard et al. 2010). They are currently categorized as "declining" and "stable", respectively (IUCN/SSC Polar Bear Specialist Group 2019). The overall objectives of this thesis were to distinguish between the SBS and CS subpopulation using bulk stable isotope analysis, and to assess the decline $\delta^{13}C$ values in SBS polar bear bone collagen over time. We created an isotopic timeline and baseline for Alaska polar bears from 1954–2019. This allowed for an exploration into the inter- and intra-differences of the SBS and CS subpopulations.

Polar bear bone collagen was the tissue used for this project. Collagen has a lifetime average isotopic signature, allowing for a better understanding of the animal, and how it incorporates diet into its body (Manolagas 2000). In contrast to other shorter turnover tissues, bone collagen is not influenced by seasonal or short-term changes to the overall diet. We used archived bones from the University of Alaska Museum of the North, as well as samples from Native subsistence hunters. By using archived bones, we were able to create a 65-year isotopic timeline of Alaska polar bears.

Chapter 2 provides a refined understanding about Alaska polar bear population structure, and how similar and/or distinct they are from one another. We used polar bear bone collagen from the SBS and CS subpopulations. The geographical boundary, which separates the CS and SBS subpopulations has been relocated several times between Icy Cape and Utqiaġvik, AK. The polar bear subpopulations have little genetic differences, and spatial data can be confusing (Kutschera et al. 2016; Scharf et al. 2018). However, we show a significant difference in bulk $\delta^{13}C$ values between the SBS and CS subpopulations that was distinguished using logistic regression. We demonstrate an almost equal support for either geographical border location, Utqiagvik or Icy Cape, as a point of separation for bears in the two subpopulations. This study provides a new tool for managing subsistence hunting quotas as determined by the U.S.-Russia Bilateral Agreement by allowing harvested bears to be assigned to their respective subpopulations via stable isotopes in bone collagen.

Chapter 3 provides support that SBS polar bears exhibit distribution patterns that are indicative of two ecotypes (pelagic and coastal) and are incorporating additional terrestrial carbon into their diet. We used bone collagen from the SBS polar bear subpopulation, as well as several other mammals from the same geographical area, including brown bears (*Ursus arctos*), wolverines (*Gulo gulo*), caribou (*Rangifer tarandus*), lynx (*Lynx canadensis*), ice seals (*Erignathus barbatus*, *Phoca largha*, and *Pusa hispida*), and walruses (*Odobenus rosmarus divergens*). These samples were analyzed using bulk ($\delta^{13}C$ and $\delta^{15}N$) and compound-specific ($\delta^{13}C_{AA}$) stable isotope analyses. The bulk $\delta^{13}C$ values in polar bear bone collagen were wide-spread and generally decreased over time. This decline could be a result of an increase in terrestrial carbon within the Beaufort Sea, in part a result of increased run off from melting permafrost (Mu et al. 2015; Stuefer et al. 2017). There was also a separation of two polar bear groups by $\delta^{13}C_{Pro}$ (proline) values. The higher $\delta^{13}C_{Pro}$ group had values similar to Arctic brown bears, while the lower $\delta^{13}C_{Pro}$ group lined up with values from ice seals. It is likely that these two polar bear groups represent the coastal and pelagic ecotypes reported in previous research on

SBS polar bears (Rogers et al. 2015; Boucher et al. 2019). This study showed that the separation in two distinct groups existed from at least the 1950's to present, indicating that the polar bear ecotypes have not likely evolved in response to climate change or melting sea ice. It is possible that a continued decrease in sea ice extent could increase numbers in the pelagic/marine polar bear group, as they follow the sea ice offshore.

We did not use any CS polar bear samples for Chapter 3 due to availability. We were unable to attain any CS polar bear bone collagen beyond the 1980's from archived collections. These samples would be useful in comparing how the two subpopulations changed over time and with declining sea ice. The Chukchi Sea has also been experiencing sea ice decline (Onarheim et al. 2018), yet, the CS subpopulation remains categorized as "stable" with ~3,000 polar bears (Regehr et al. 2018). By using more recent CS samples, especially when applying CSIA-AA, we could learn more about the decline in $\delta^{13}C$ values over time in the SBS subpopulation. If both subpopulations are experiencing a similar decline over time, it could prompt new avenues for research, such as an increase in terrestrial carbon in either ecosystems, or even a new Arctic regime shift.

Future studies employing stable isotope analyses in CS polar bears should consider the use of other tissues, such as muscle, skin, claws, or fur to test if the differences in stable isotope signatures, between the CS and SBS subpopulations, that we show here, persist. Bone collagen is suitable for long-term studies using stable isotope analyses (Clark et al. 2019), but extracting bone collagen is time consuming and logistically more challenging than processing several other tissues for stable isotope analysis. For example, muscle tissue can be obtained relatively minimally invasively (especially when compared to bone) and can be processed quickly, with bulk stable isotope values available in a matter of days. Additional stable isotope data could be useful, if they can also show a separation of the two polar bear subpopulations using more accessible tissues (i.e., muscle biopsy). These less labor-intensive tissues have a faster lab turn-around at near-real time. However, bone collagen has a lifetime average signature, while these other tissues have turnover rates ranging from days to months, which may not reflect the overall site fidelity of polar bears. Stable isotope analysis of these tissues would allow for a more current representation of polar bear subpopulation fidelity and could therefore be a useful tool for stock and quota management.

Future studies should also consider incorporating bulk and CSIA-AA SBS polar bear data with genomic data and other health indices, e.g., body condition, stress and reproductive hormones, and morphometric measurements. A comparison of our stable isotope data with bone collagen samples from the CS subpopulation from recent decades would allow for a better understanding of the response of Alaskan polar bears to climate change, habitat loss, and prey switching. These more recent decades, 2000 and onwards, have had multiple sea ice minima, altering the main habitat for polar bears. The CS subpopulation inhabits a more productive ecosystem (Grebmeier et al. 2006) and has three times the number of polar bears (IUCN/SSC Polar Bear Specialist Group 2019), that are in better body condition (Rode et al. 2015) than the SBS subpopulation. Additional stable isotope work should also be considered for potentially new prey items, such as the Arctic char (*Salvelinus alpinus*), and any role this prey species could have in affecting the stable carbon and nitrogen isotope values of polar bears. Polar bears have been observed catching these fish (Dyck and Romberg 2007), and this potential food source has stable carbon and nitrogen values that could reasonably explain the stable isotope values (high trophic level with terrestrial rather than marine carbon signature) observed in our polar bear samples (Swanson et al. 2010). Finally, further studies should focus on blubber AA composition and values in polar bears and their prey items to allow for a better understanding of both the trophic and source AA's roles in polar bear metabolism.

This study has shown that stable isotope analyses of carbon and nitrogen can help to distinguish Alaska polar bear subpopulations and ecotypes. Specifically, this study demonstrated a separation between the CS and SBS subpopulations based on $\delta^{13}C$ values. This difference can be beneficial for understanding residence times and consequently ecosystem dependence of polar bears over their lifetimes. Additionally, we reveal the existence of two polar bear ecotypes in SBS bears that have been present for at least the past 65 years. Further research is needed on these two ecotypes, their health and physiological responses, to understand polar bear resiliency in the rapidly changing Arctic.

4.1 References

Atwood T, Peacock E, McKinney M, Lillie K, Wilson R, Douglas D, Miller S, Terletzky P (2016) Rapid environmental change drives increased land use by an Arctic marine predator. PLoS One 11: 1–18. https://doi.org/10.1371/journal.pone.0155932

Boucher N, Derocher A, Richardson E (2019) Space use patterns affect stable isotopes of polar bears (*Ursus maritimus*) in the Beaufort Sea. Polar Biol 42: 1581–1593. https://doi.org/10.1007/s00300-019-02546-9

Clark C, Horstmann L, de Vernal A, Jensen A, Misarti N (2019) Pacific walrus diet across 4000 years of changing sea ice conditions. Quat Res 1–17. https://doi.org/10.1017/qua.2018.140

Dyck M, Romberg S (2007) Observations of a wild polar bear (*Ursus maritimus*) successfully fishing Arctic char (*Salvelinus alpinus*) and fourhorn sculpin (*Myoxocephalus quadricornis*). Polar Biol 30: 1625–1628. https://doi.org/10.1007/s00300-007-0338-3

Grebmeier J, Cooper L, Feder H, Sirenko B (2006) Ecosystem dynamics of the Pacific-influenced Northern Bering and Chukchi seas in the Amerasian Arctic. Prog Oceanogr 71: 331–361. https://doi.org/10.1016/j.pocean.2006.10.001

IUCN/SSC Polar Bear Specialist Group (2019) Status report on the world’s polar bear subpopulations. 2019 Status Report, pp 1–31. http://pbsg.npolar.no/export/sites/pbsg/en/docs/2019-StatusReport.pdf

Kutschera VE, Frosch C, Janke A, Skírnisson K, Bidon T, Lecomte N, Fain SR, Eiken HG, Hagen SB, Arnason U, Laidre KL, Nowak C, Hailer F (2016) High genetic variability of vagrant polar bears illustrates importance of population connectivity in fragmented sea ice habitats. Anim Conserv 19: 337–349. https://doi.org/10.1111/acv.12250

Manolagas S (2000) Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21: 115–137. https://doi.org/10.1210/er.21.2.115

McKinney M, Iverson S, Fisk A, Sonne C (2013) Global change effects on the long-term feeding ecology and contaminant exposures of East Greenland polar bears. Glob Chang Biol 19: 2360–2372. https://doi.org/10.1111/gcb.12241

McKinney M, Atwood T, Iverson S, Peacock E (2017) Temporal complexity of southern Beaufort Sea polar bear diets during a period of increasing land use. Ecosphere 8: 1–14. https://doi.org/10.1002/ecs2.1633

Mu C, Zhang T, Wu Q, Cao B, Zhang X, Peng X, Wan X, Zheng L, Wang Q, Cheng G (2015) Carbon and nitrogen properties of permafrost over the Eboling Mountain in the upper reach of Heihe River basin, Northwestern China. Arctic Antarct Alp Res 47: 203–211. https://doi.org/10.1657/AAAR00C-13-095

Obbard ME, Thiemann GW, Peacock E, Debruyn TD (2010) Proceedings of the 15th working meeting of the IUCN/SSC Polar Bear Specialist Group, 29 June-3 July 2009, Copenhagen, Denmark. Gland, Switzerland and Cambridge, UK: IUCN.

Onarheim I, Eldevik T, Smedsrud L, Stroeve J (2018) Seasonal and regional manifestation of Arctic sea ice loss. J Clim 31: 4917–4932. https://doi.org/10.1175/JCLI-D-17-0427.1

Regehr E, Hostetter N, Wilson R, Rode K, St. Martin M, Converse S (2018) Integrated population modeling provides the first empirical estimates of vital rates and abundance for polar bears in the Chukchi Sea. Sci Rep 8: 1–12. https://doi.org/10.1038/s41598-018-34824-7

Rode K, Robbins C, Nelson L, Amstrup S (2015) Can polar bears use terrestrial foods to offset lost ice-based hunting opportunities? Front Ecol Environ 13: 138–145. https://doi.org/10.1890/140202

Rogers M, Peacock E, Simac K, O'Dell M, Welker J (2015) Diet of female polar bears in the southern Beaufort Sea of Alaska: Evidence for an emerging alternative foraging strategy in response to environmental change. Polar Biol 38: 1035–1047. https://doi.org/10.1007/s00300-015-1665-4

Scharf HR, Hooten MB, Wilson RR, Durner GM, Atwood TC (2018) Accounting for phenology in the analysis of animal movement. arXiv Prepr. arXiv1806.09473. https://doi.org/10.1111/biom.13052

Stirling I, Lunn N, Iacozza J (1999) Long-term trends in the population ecology of polar bears in Western Hudson Bay in relation to climatic change. Arctic 52: 294–306.

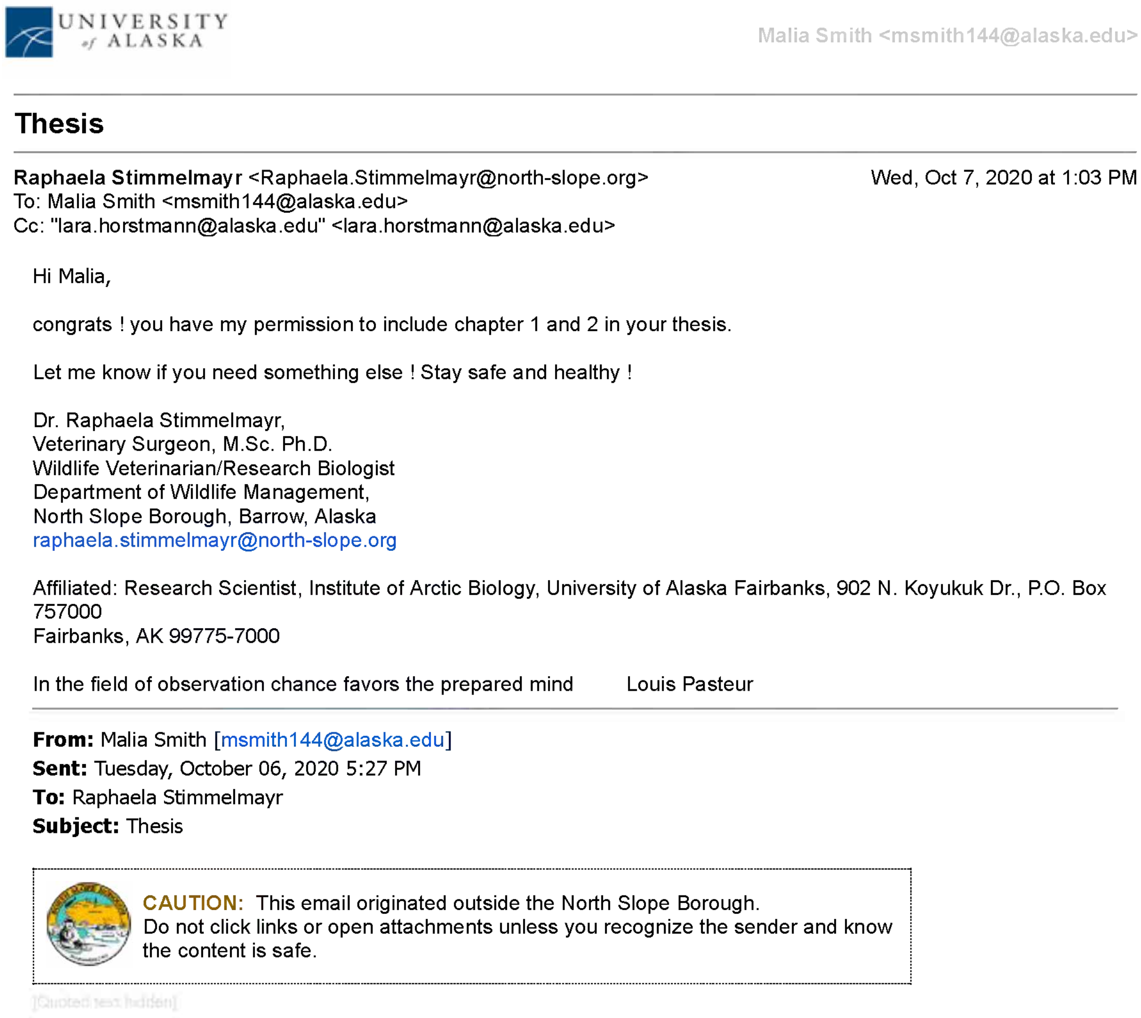
Stirling I, Derocher A (2012) Effects of climate warming on polar bears: a review of the evidence. Glob Chang Biol 18: 2694–2706. https://doi.org/10.1111/j.1365-2486.2012.02753.x

Stuefer S, Arp C, Kane D, Liljedahl A (2017) Recent extreme runoff observations from coastal arctic watersheds in Alaska. Water Resour Res 53: 9145–9163. https://doi.org/10.1002/2017WR020567

Swanson H, Kidd K, Babaluk J, Wastle R, Yang P, Halden N, Reist J (2010) Anadromy in Arctic populations of lake trout (*Salvelinus namaycush*): Otolith microchemistry, stable isotopes, and comparisons with Arctic char (*Salvelinus alpinus*). Can J Fish Aquat Sci 67: 842–853. https://doi.org/10.1139/F10-022

Appendices

Appendix A. Email from co-author Dr. Raphaela Stimmelmayr confirming the use of Chapters 1 and 2 in this thesis.



UNIVERSITY of ALASKA

Malia Smith <msmith144@alaska.edu>

Thesis

Raphaela Stimmelmayr <Raphaela.Stimmelmayr@north-slope.org>
Wed, Oct 7, 2020 at 1:03 PM
To: Malia Smith <msmith144@alaska.edu>
Cc: "lara.horstmann@alaska.edu" <lara.horstmann@alaska.edu>

Hi Malia,

congrats ! you have my permission to include chapter 1 and 2 in your thesis.

Let me know if you need something else ! Stay safe and healthy !

Dr. Raphaela Stimmelmayr,
Veterinary Surgeon, M.Sc. Ph.D.
Wildlife Veterinarian/Research Biologist
Department of Wildlife Management,
North Slope Borough, Barrow, Alaska
raphaela.stimmelmayr@north-slope.org

Affiliated: Research Scientist, Institute of Arctic Biology, University of Alaska Fairbanks, 902 N. Koyukuk Dr., P.O. Box 757000
Fairbanks, AK 99775-7000

In the field of observation chance favors the prepared mind   Louis Pasteur

From: Malia Smith [msmith144@alaska.edu]
Sent: Tuesday, October 06, 2020 5:27 PM
To: Raphaela Stimmelmayr
Subject: Thesis

CAUTION: This email originated outside the North Slope Borough.
Do not click links or open attachments unless you recognize the sender and know the content is safe.

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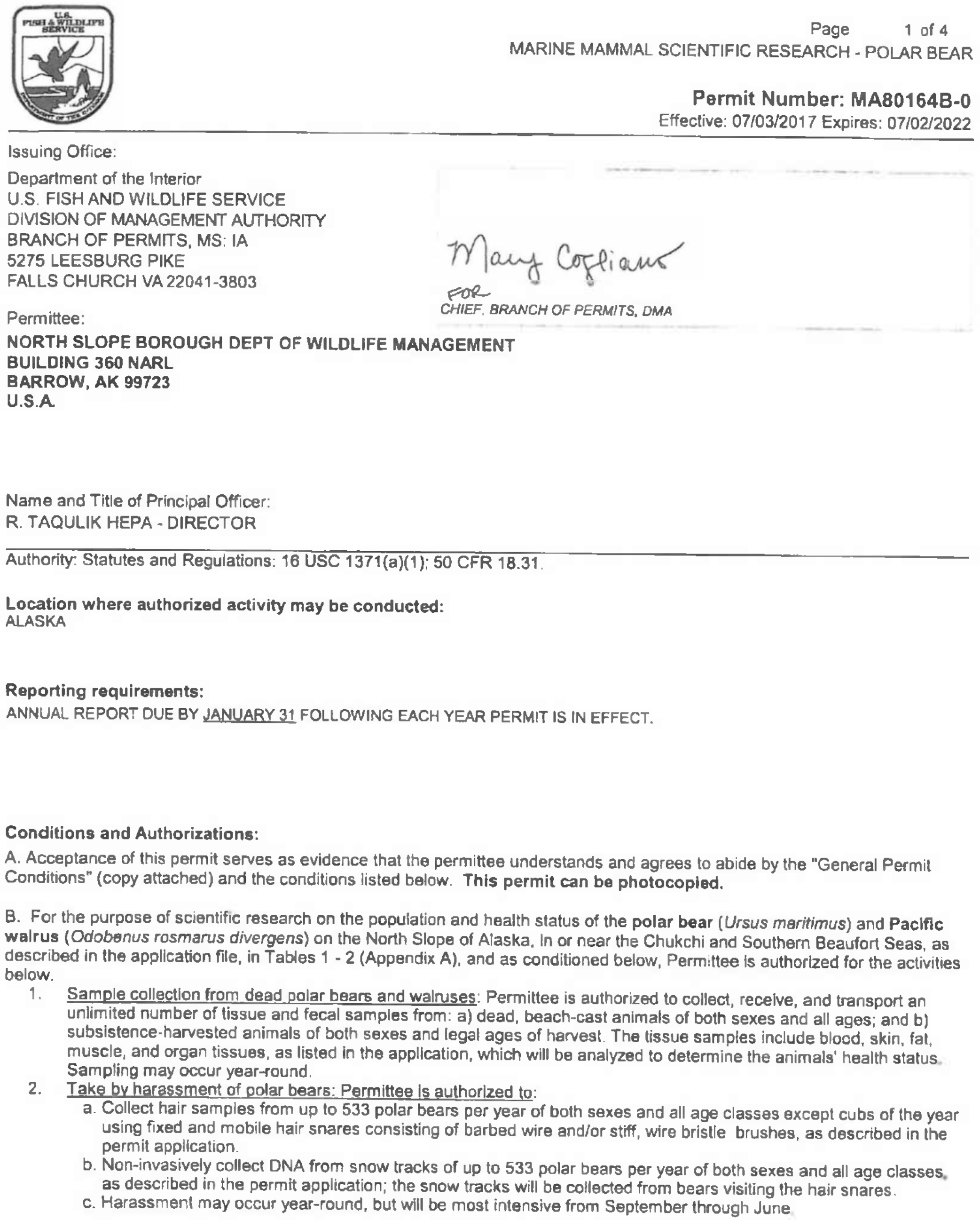
Appendix B. Permit number MA80164B-0 issued to the North Slope Borough Department of Wildlife Management to allow for collecting and analyzing polar bear samples.



Page 1 of 4

MARINE MAMMAL SCIENTIFIC RESEARCH - POLAR BEAR

Permit Number: MA80164B-0

Effective: 07/03/2017 Expires: 07/02/2022

Issuing Office:

Department of the Interior
U.S. FISH AND WILDLIFE SERVICE
DIVISION OF MANAGEMENT AUTHORITY
BRANCH OF PERMITS, MS: IA
5275 LEESBURG PIKE
FALLS CHURCH VA 22041-3803

Mary Cogliano
FOR
CHIEF, BRANCH OF PERMITS, DMA

Permittee:

NORTH SLOPE BOROUGH DEPT OF WILDLIFE MANAGEMENT
BUILDING 360 NARL
BARROW, AK 99723
U.S.A.

Name and Title of Principal Officer:
R. TAQULIK HEPA - DIRECTOR

Authority: Statutes and Regulations: 16 USC 1371(a)(1); 50 CFR 18.31.

Location where authorized activity may be conducted:
ALASKA

Reporting requirements:
ANNUAL REPORT DUE BY JANUARY 31 FOLLOWING EACH YEAR PERMIT IS IN EFFECT.

Conditions and Authorizations:

A. Acceptance of this permit serves as evidence that the permittee understands and agrees to abide by the "General Permit Conditions" (copy attached) and the conditions listed below. **This permit can be photocopied.**

B. For the purpose of scientific research on the population and health status of the **polar bear** (*Ursus maritimus*) and **Pacific walrus** (*Odobenus rosmarus divergens*) on the North Slope of Alaska, in or near the Chukchi and Southern Beaufort Seas, as described in the application file, in Tables 1 - 2 (Appendix A), and as conditioned below, Permittee is authorized for the activities below.

1. Sample collection from dead polar bears and walruses: Permittee is authorized to collect, receive, and transport an unlimited number of tissue and fecal samples from: a) dead, beach-cast animals of both sexes and all ages; and b) subsistence-harvested animals of both sexes and legal ages of harvest. The tissue samples include blood, skin, fat, muscle, and organ tissues, as listed in the application, which will be analyzed to determine the animals' health status. Sampling may occur year-round.
2. Take by harassment of polar bears: Permittee is authorized to:
    - a. Collect hair samples from up to 533 polar bears per year of both sexes and all age classes except cubs of the year using fixed and mobile hair snares consisting of barbed wire and/or stiff, wire bristle brushes, as described in the permit application.
    - b. Non-invasively collect DNA from snow tracks of up to 533 polar bears per year of both sexes and all age classes, as described in the permit application; the snow tracks will be collected from bears visiting the hair snares.
    - c. Harassment may occur year-round, but will be most intensive from September through June.

3. Incidental harassment of polar bears: Permittee is authorized to incidentally harass up to 120 polar bears per year of both sexes and all age classes during retrieval of hair samples from snares and DNA samples from snow tracks, and during the setting up and mending of fences, as described in the permit application.

4. Export of polar bear hair and DNA/snow track samples: Permittee is authorized to export hair and DNA samples to the laboratories listed in Permittee's application file for the purpose of molecular and genetic analyses. **All exports must be accompanied by the appropriate permits issued under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).**

C. **Tissue samples** may be collected from dead stranded, beach-cast carcasses or carcasses of legally subsistence-harvested bears or walruses, but may not be purchased or acquired by offering remuneration to the collector because such remuneration might encourage collection of additional animals from the wild.

D. **Coordination**: Upon receipt of permit and prior to the initiation of each field season's activities, Permittee must contact the U.S. Fish and Wildlife Service, Marine Mammals Management (MMM) at **907-786-3800** to ensure that there is effective coordination between the activities authorized under this permit and other research activities being conducted in or near the study areas, to minimize duplicative research and to avoid unnecessary disturbance of animals.

E. **Todd Sformo is hereby designated as principal investigator (PI) under this permit, and Andrew L. Von Duyke and Raphaela Stimmelmayr are designated as Co-investigators.** Activities under this permit may only be conducted under the on-site supervision of the PI or Co-investigators. Samples may be transferred to and analyzed by one of the laboratories listed in the application file. The PI may designate additional Co-investigator(s) in writing, provided the individuals have received appropriate training and possess adequate proficiency to conduct the research activities in accordance with the permit conditions. Upon designation of Co-investigator(s), the Permittee must submit the individuals' CVs to the Division of Management Authority (DMA). The names of designated Co-investigator(s) and a list of other authorized personnel must be maintained in writing by the PI for a period of at least 5 years and provided to DMA and MMM upon request.

F. **Permittee and all authorized personnel (see Condition E) must have a copy of this permit and, if applicable, all other written approvals in possession while conducting all authorized activities.** All participants in the authorized research activities must be aware of the permit restrictions and reporting requirements. Permittee must monitor each activity conducted under this permit to ensure that authorized collections and disturbances are not exceeded and that authorized and highly skilled personnel perform permitted activities.

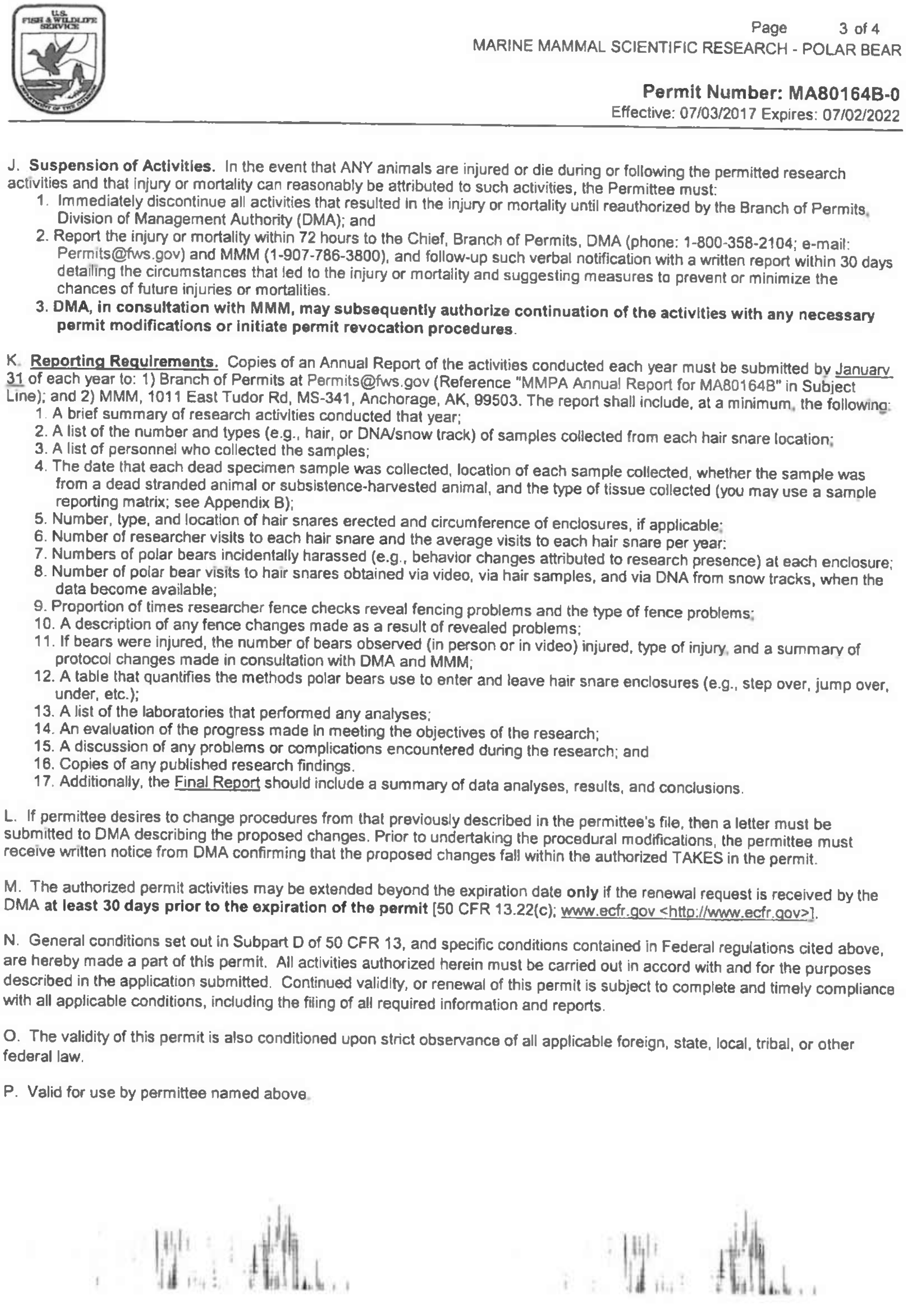
G. **Use of hair snares:**
  1. To prevent human-bear interactions with the public, **Permittee must**:
     a. Conduct outreach or post signage to notify local residents of hair snare locations that are placed near communities;
     b. Avoid placing hair snares near human use areas, such as snow machine trails on ice or along the coast; and
     c. If working near military sites or industry areas, notify the military or companies of the timing and location of hair snares in order to avoid unexpected encounters with bears.

  2. When using barbed wire hair snares, in order to monitor the safety of the bears, the **Permittee should check barbed wire fence enclosures**:
     a. Once per day for periods of high use (i.e., multiple bears using the bone piles) and at least once per week during periods of expected low use; and
     b. By conducting continuous video observations during the first 24 hours after the fences are erected when bears are expected to be present and during three 4-hour observations during the initial days when bear density is high.

H. The Permittee is NOT authorized to incidentally harass other marine mammal species. Every effort should be made to ensure that other marine mammal species are not observed in the immediate area prior to commencing authorized activities. Should any marine mammals other than polar bear be encountered during the authorized activities, the Permittee should immediately stop the activities, allow the marine mammal to leave the site or, if the marine mammal does not leave, the Permittee should slowly leave the site, detour around the animals, and resume activities after these animals are no longer present in the study area.

I. **Photographs/Videotape/Film**: Permittee may obtain photographs, video, or film if such activities are essential to achieving the research objectives (*e.g.*, documentation of research activities). However, researchers must obtain prior approval from DMA to use photographs, video, or film for non-research related purposes.

Appendix B. Cont.



J. **Suspension of Activities.** In the event that ANY animals are injured or die during or following the permitted research activities and that injury or mortality can reasonably be attributed to such activities, the Permittee must:

1. Immediately discontinue all activities that resulted in the injury or mortality until reauthorized by the Branch of Permits, Division of Management Authority (DMA); and
2. Report the injury or mortality within 72 hours to the Chief, Branch of Permits, DMA (phone: 1-800-358-2104; e-mail: Permits@fws.gov) and MMM (1-907-786-3800), and follow-up such verbal notification with a written report within 30 days detailing the circumstances that led to the injury or mortality and suggesting measures to prevent or minimize the chances of future injuries or mortalities.
3. **DMA, in consultation with MMM, may subsequently authorize continuation of the activities with any necessary permit modifications or initiate permit revocation procedures.**

K. **Reporting Requirements.** Copies of an Annual Report of the activities conducted each year must be submitted by January 31 of each year to: 1) Branch of Permits at Permits@fws.gov (Reference "MMPA Annual Report for MA80164B" in Subject Line); and 2) MMM, 1011 East Tudor Rd, MS-341, Anchorage, AK, 99503. The report shall include, at a minimum, the following:

1. A brief summary of research activities conducted that year;
2. A list of the number and types (e.g., hair, or DNA/snow track) of samples collected from each hair snare location;
3. A list of personnel who collected the samples;
4. The date that each dead specimen sample was collected, location of each sample collected, whether the sample was from a dead stranded animal or subsistence-harvested animal, and the type of tissue collected (you may use a sample reporting matrix; see Appendix B);
5. Number, type, and location of hair snares erected and circumference of enclosures, if applicable;
6. Number of researcher visits to each hair snare and the average visits to each hair snare per year;
7. Numbers of polar bears incidentally harassed (e.g., behavior changes attributed to research presence) at each enclosure;
8. Number of polar bear visits to hair snares obtained via video, via hair samples, and via DNA from snow tracks, when the data become available;
9. Proportion of times researcher fence checks reveal fencing problems and the type of fence problems;
10. A description of any fence changes made as a result of revealed problems;
11. If bears were injured, the number of bears observed (in person or in video) injured, type of injury, and a summary of protocol changes made in consultation with DMA and MMM;
12. A table that quantifies the methods polar bears use to enter and leave hair snare enclosures (e.g., step over, jump over, under, etc.);
13. A list of the laboratories that performed any analyses;
14. An evaluation of the progress made in meeting the objectives of the research;
15. A discussion of any problems or complications encountered during the research; and
16. Copies of any published research findings.
17. Additionally, the Final Report should include a summary of data analyses, results, and conclusions.

L. If permittee desires to change procedures from that previously described in the permittee's file, then a letter must be submitted to DMA describing the proposed changes. Prior to undertaking the procedural modifications, the permittee must receive written notice from DMA confirming that the proposed changes fall within the authorized TAKES in the permit.

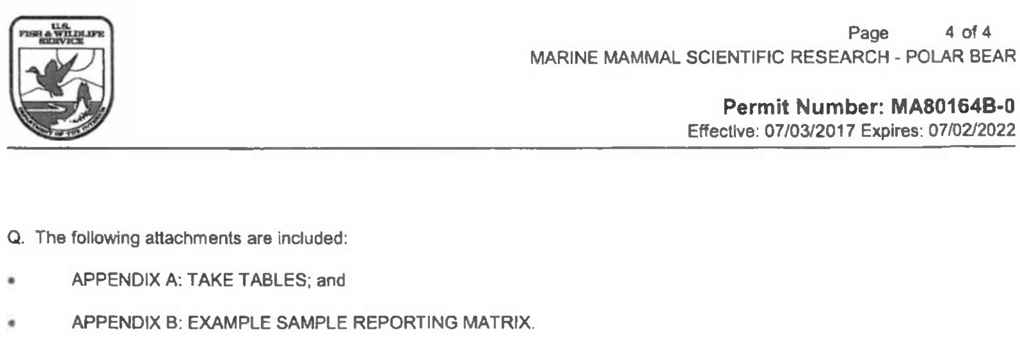
M. The authorized permit activities may be extended beyond the expiration date **only** if the renewal request is received by the DMA **at least 30 days prior to the expiration of the permit** [50 CFR 13.22(c); www.ecfr.gov <http://www.ecfr.gov>].

N. General conditions set out in Subpart D of 50 CFR 13, and specific conditions contained in Federal regulations cited above, are hereby made a part of this permit. All activities authorized herein must be carried out in accord with and for the purposes described in the application submitted. Continued validity, or renewal of this permit is subject to complete and timely compliance with all applicable conditions, including the filing of all required information and reports.

O. The validity of this permit is also conditioned upon strict observance of all applicable foreign, state, local, tribal, or other federal law.

P. Valid for use by permittee named above.

Appendix B. Cont.



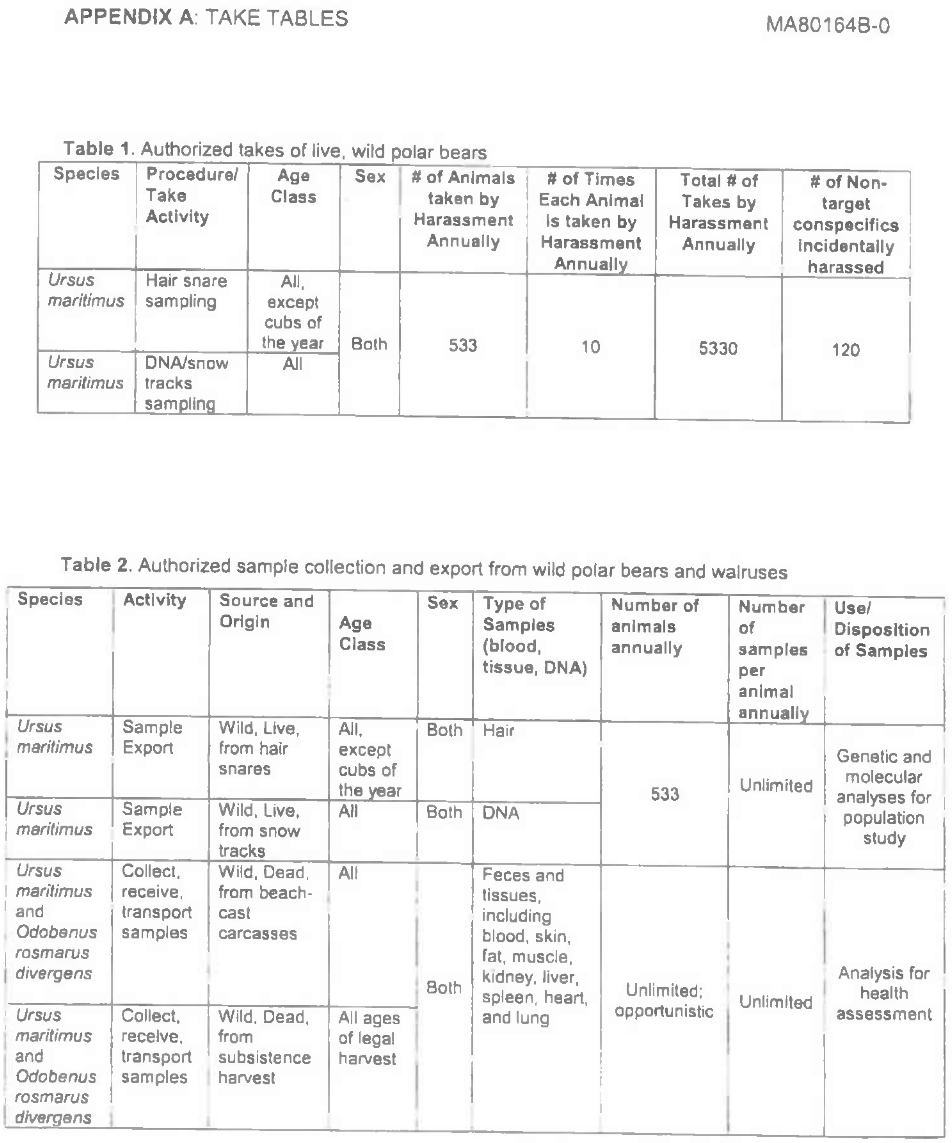
Page 4 of 4
MARINE MAMMAL SCIENTIFIC RESEARCH - POLAR BEAR

Permit Number: MA80164B-0
Effective: 07/03/2017 Expires: 07/02/2022

Q. The following attachments are included:

- APPENDIX A: TAKE TABLES; and
- APPENDIX B: EXAMPLE SAMPLE REPORTING MATRIX.

Appendix B. Cont.



APPENDIX A: TAKE TABLES MA80164B-0

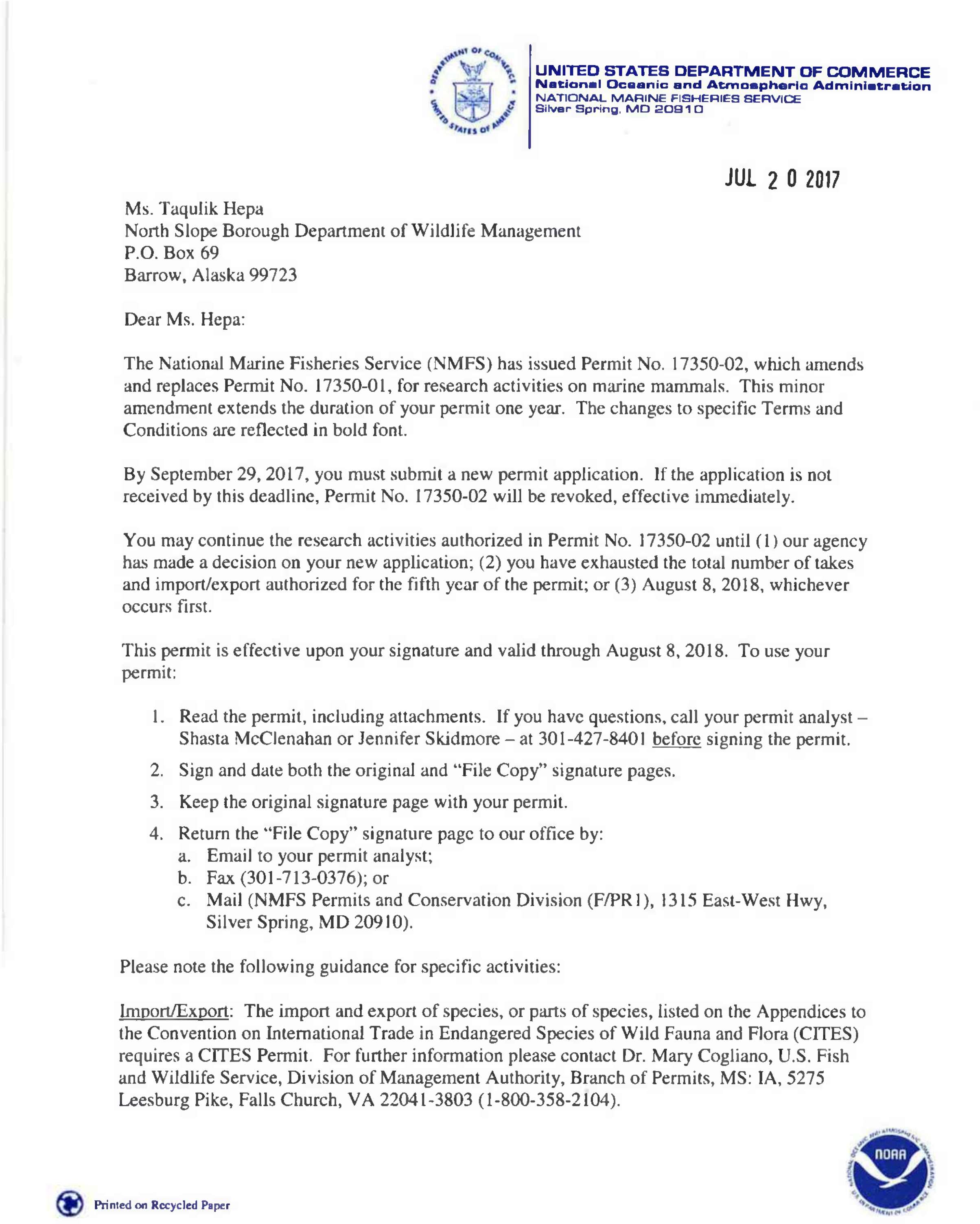
Table 1. Authorized takes of live, wild polar bears

Species	Procedure/ Take Activity	Age Class	Sex	# of Animals taken by Harassment Annually	# of Times Each Animal is taken by Harassment Annually	Total # of Takes by Harassment Annually	# of Non-target conspecifics incidentally harassed
Ursus maritimus	Hair snare sampling	All, except cubs of the year	Both	533	10	5330	120
Ursus maritimus	DNA/snow tracks sampling	All					

Table 2. Authorized sample collection and export from wild polar bears and walruses

Species	Activity	Source and Origin	Age Class	Sex	Type of Samples (blood, tissue, DNA)	Number of animals annually	Number of samples per animal annually	Use/ Disposition of Samples
Ursus maritimus	Sample Export	Wild, Live, from hair snares	All, except cubs of the year	Both	Hair	533	Unlimited	Genetic and molecular analyses for population study
Ursus maritimus	Sample Export	Wild, Live, from snow tracks	All	Both	DNA			
Ursus maritimus and *Odobenus rosmarus divergens*	Collect, receive, transport samples	Wild, Dead, from beach-cast carcasses	All	Both	Feces and tissues, including blood, skin, fat, muscle, kidney, liver, spleen, heart, and lung	Unlimited; opportunistic	Unlimited	Analysis for health assessment
Ursus maritimus and *Odobenus rosmarus divergens*	Collect, receive, transport samples	Wild, Dead, from subsistence harvest	All ages of legal harvest					

Appendix C. Permit number 17350-02 NMFS issued to the North Slope Borough Department of Wildlife Management to allow for collecting and analyzing seal samples.



UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Silver Spring, MD 20910

JUL 20 2017

Ms. Taqulik Hepa
North Slope Borough Department of Wildlife Management
P.O. Box 69
Barrow, Alaska 99723

Dear Ms. Hepa:

The National Marine Fisheries Service (NMFS) has issued Permit No. 17350-02, which amends and replaces Permit No. 17350-01, for research activities on marine mammals. This minor amendment extends the duration of your permit one year. The changes to specific Terms and Conditions are reflected in bold font.

By September 29, 2017, you must submit a new permit application. If the application is not received by this deadline, Permit No. 17350-02 will be revoked, effective immediately.

You may continue the research activities authorized in Permit No. 17350-02 until (1) our agency has made a decision on your new application; (2) you have exhausted the total number of takes and import/export authorized for the fifth year of the permit; or (3) August 8, 2018, whichever occurs first.

This permit is effective upon your signature and valid through August 8, 2018. To use your permit:

1. Read the permit, including attachments. If you have questions, call your permit analyst – Shasta McClenahan or Jennifer Skidmore – at 301-427-8401 before signing the permit.
2. Sign and date both the original and "File Copy" signature pages.
3. Keep the original signature page with your permit.
4. Return the "File Copy" signature page to our office by:
   a. Email to your permit analyst;
   b. Fax (301-713-0376); or
   c. Mail (NMFS Permits and Conservation Division (F/PR1), 1315 East-West Hwy, Silver Spring, MD 20910).

Please note the following guidance for specific activities:

Import/Export: The import and export of species, or parts of species, listed on the Appendices to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) requires a CITES Permit. For further information please contact Dr. Mary Cogliano, U.S. Fish and Wildlife Service, Division of Management Authority, Branch of Permits, MS: IA, 5275 Leesburg Pike, Falls Church, VA 22041-3803 (1-800-358-2104).

Salvage: This permit does not authorize collection or receipt of specimens from marine mammals stranded dead or alive in the United States. To obtain samples from U.S. stranded animals, contact the appropriate NMFS Regional Stranding Network Coordinators (http://www.nmfs.noaa.gov/pr/health/coordinators.htm). To receive marine mammal carcasses or parts from animals killed incidental to commercial fisheries in the United States contact the appropriate NMFS Science Centers (http://www.nmfs.noaa.gov/science.htm).

Please keep your email contact information current in our online database (https://apps.nmfs.noaa.gov/). You will receive automated email reminders of due dates for annual and final reports, and a notice prior to expiration of your permit.

Sincerely,

Jolie Harrison
Chief, Permits and Conservation Division
Office of Protected Resources

Enclosure