FIFTY YEARS OF COOK INLET BELUGA WHALE ECOLOGY RECORDED AS ISOTOPES IN BONE AND TEETH

By

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Abstract

Beluga whales (*Delphinapterus leucas*) are found across the Arctic and Subarctic in seasonally ice covered waters. Five stocks of beluga whales are associated with the waters near Alaska for at least part of the year and four of those five stocks are abundant and commonly hunted by Alaskan Natives. The belugas resident in Cook Inlet are also an important cultural and subsistence resource to Alaskan Natives in the area, but a ~50% decline in abundance in the 1990's led to the stock being designated as depleted under the Marine Mammal Protection Act in 2000 and listed as endangered under the Endangered Species Act in 2008. Numerous studies of beluga whales in relation to stranding events, predation (killer whales), parasitism, disease, contaminants, and other potential population threats have not identified the reason for their inability to recover. Changes in diet have been considered, but are difficult to study because observations of feeding in muddy water and beluga stomachs are difficult to obtain. To investigate the past feeding ecology of beluga whales from Cook Inlet I sampled bone and teeth for isotopic analyses. I sampled bone from 20 individuals that died between 1964 and 2007 for stable carbon and nitrogen isotope analysis (values expressed as $\delta^{13}C$ and $\delta^{15}N$ values). I also micro-sampled annual growth layer groups in the teeth of 26 individuals representing the years from 1962 to 2007. Bone and tooth data showed a general decrease in $\delta^{13}C$ and $\delta^{15}N$ values over time. The $\delta^{13}C$ values from analyses of growth layer groups declined from -13.4‰ to -16.2‰ and $\delta^{15}N$ values declined from 17.2‰ to 15.4‰. Although these values are consistent with a change in feeding ecology over time, the magnitude of the decrease in $\delta^{15}N$ values (~2‰) is insufficient for a full trophic level shift (~3‰).The relatively large decrease in the $\delta^{13}C$ values over the same time period (~3‰), however, is much greater than a full trophic level shift (~1‰) and suggests an increase in prey associated with freshwater, which typically have lower $\delta^{13}C$

values than prey associated with marine water. To test this hypothesis I analyzed the strontium isotope composition ($^{87}Sr/^{86}Sr$ ratios) of growth layer groups in teeth from a sub-set of individuals. The resulting $^{87}Sr/^{86}Sr$ ratios trended away from the global marine signature (0.70918) over time and toward the more freshwater signatures measured in rivers flowing into the upper reaches of Cook Inlet. These results indicate that the diet of Cook Inlet beluga whales has changed over time. This could be from feeding on different, more freshwater derived prey species, or from feeding on the same species, but on individuals from locations with a more freshwater influence. Both of these interpretations are consistent with population survey data indicating a retraction in beluga range into the upper reaches of Cook Inlet. This study presents the first evidence of a long term (~50 years) change in Cook Inlet beluga whale feeding ecology. The consequences of this change toward more freshwater-influenced prey, and how this change relates to Cook Inlet beluga whales' decline or recovery remains unknown. However, to better examine this change in feeding ecology a follow-up study will; 1) develop a strontium isoscape for the Cook Inlet watershed; 2) analyze more teeth to better analyze changes in feeding ecology by demographic group (sex, age); and 3) analyze growth layer groups from Bristol Bay beluga teeth for a comparison with Cook Inlet belugas to determine if the changes represent an ecosystem change within Cook Inlet or a broader scale change affecting another region. This study builds towards a better understanding of the changes in Cook Inlet beluga feeding ecology and will help to determine if changes in diet could be a factor in their recovery.

Dedication

This thesis is dedicated to my parents, Robert and Jane Nelson. Thank you for your unwavering support and for always encouraging me to look over the next ridge.

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Chapter 1: General Introduction

Beluga whales (*Delphinapterus leucas*; Pallas, 1776) are medium-sized, toothed whales that share the family Monodontidae with one other species; the narwhal (*Monodon monoceros*). Belugas are found in the seasonally ice covered waters of the Arctic and Subarctic and are recognized by their white color, large melon (a structure on forehead composed of fat and connective tissue used by toothed whales for echolocation; McKenna et al. 2012), and the ability to turn their heads due to unfused neck vertebrae. At least 19 distinct stocks of beluga whales are found in the waters of Canada, Greenland, Norway, Russia, and the United States (five stocks occur in Alaska; Fig. 1.1; Smith et al. 1990, Laidre et al. 2015).

These five stocks of beluga whales are commonly associated with waters in Alaska for at least part of the year (Fig. 1.2). The five stocks are named by the general geographic location where they spend time during the summer months (i.e., Eastern Beaufort Sea, Eastern Chukchi Sea, Eastern Bering Sea, Bristol Bay, and Cook Inlet; Fig. 1.2), and all stocks, except the Cook Inlet beluga whale stock (CIBW), spend the winter months in the Bering Sea (Laidre et al. 2015, Citta et al. 2016a). The Eastern Beaufort Sea, Eastern Chukchi Sea, and Eastern Bering Sea stocks undertake a seasonal migration and spend the winter relatively far from their summer ranges (Citta et al. 2016a). The other two stocks in Alaska, Bristol Bay and Cook Inlet, are less migratory and spend the winter relatively close to their summer ranges (Hobbs et al. 2005, Rugh et al. 2010, Citta et al. 2016a, b). With the exception of CIBW, all stocks of beluga whales in Alaska are healthy, abundant, and hunted by Alaska Natives when available.

CIBW are the only stock of beluga whales in Alaska that have a documented decline in abundance from an estimate of 1,300 in 1979 (Calkins 1989) to 340 in 2014 (Shelden et al. 2015a). National Marine Fisheries Service (NMFS) began annual aerial surveys to estimate abundance in 1994, which revealed a 50% decline (1,300 to 650 whales) from 1979 (Shelden et al. 2015a). Subsequent surveys from 1994 to 1998 detected another 50% decline (650 to 347 whales). Declining abundance was attributed to an increase in subsistence hunting mainly by hunters who traveled to Anchorage from other communities (Mahoney & Shelden 2000). The success of the harvests in the 1990s was evident by the sale of beluga muktuk (beluga skin and blubber eaten together, a common food item from beluga) in Anchorage.

Once it was realized the population was declining (Mahoney and Shelden 2000), beluga hunters voluntarily stopped hunting in Cook Inlet in 1999. Soon after, NMFS proposed harvest reporting regulations, and in 2000 designated CIBW as depleted under the Marine Mammal Protection Act (MMPA; Mahoney & Shelden 2000). Because the decline was attributed to an increased subsistence harvest (approximately 30 whales taken per year during 1995-1998), restricting the harvest would stop the decline and the population would begin to recover, instead, a 10-year declining trend continued from 2004 to 2014 at -0.4% $year^{-1}$ (SE = 1.3%, Fig. 1.3.; Hobbs et al. 2015, Shelden et al. 2015b, NMFS 2016). In 2008, CIBW were listed as endangered under the Endangered Species Act (ESA; NOAA 2008). As part of the listing process a recovery plan was completed in 2016 that outlined potential threats to CIBW recovery (NMFS 2016). Threats were rated as low (pollution, predation, subsistence hunting), medium (disease, habitat loss, reduction in prey, unauthorized take), and high (catastrophic events, cumulative effects of stressors, noise) relative concern (NMFS 2016). Concurrent with the decline in abundance, CIBW range has contracted into the upper Cook Inlet (Rugh et al. 2010). While the reason for the range contraction is unknown, possible explanations include avoidance of killer whales (*Orcinus orca*; Shelden et al. 2003), reduced intra-specific competition (fewer individuals require less space; Goetz et al. 2007), and a reduction in prey availability (Moore et al. 2000).

Studying the effects of killer whale predation and intra-specific competition are difficult tasks, but studying diet or prey is possible through stomach content analysis and with isotope analysis.

Stomach contents from dead CIBW have provided substantial diet information, including prey items identified to species (Quakenbush et al. 2015). Although this method allows for a detailed (often to species) analysis of prey items, it is limited to when (season) and which (sex and age) belugas die. Additionally, stomachs from known healthy belugas have become less available since the harvest was curtailed and limited samples in some years mean this method is not well suited to determine how diet has changed over time.

Isotope analysis is another approach for examining the feeding ecology of animals, which uses the chemical makeup of tissues to make predictions about what prey items were eaten, digested, and used to form those tissues. Each tissue within an animal forms and regenerates at different rates, some regenerate quickly (e.g., muscle) and represent more recent diet items whereas other tissues turn over very slowly (e.g. bone) and may represent an average of years or a lifetime of feeding (Fry 2008). This thesis uses carbon, nitrogen, and strontium isotope analysis of bone and teeth to describe CIBW feeding ecology over the last half century.

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1.2 Figures

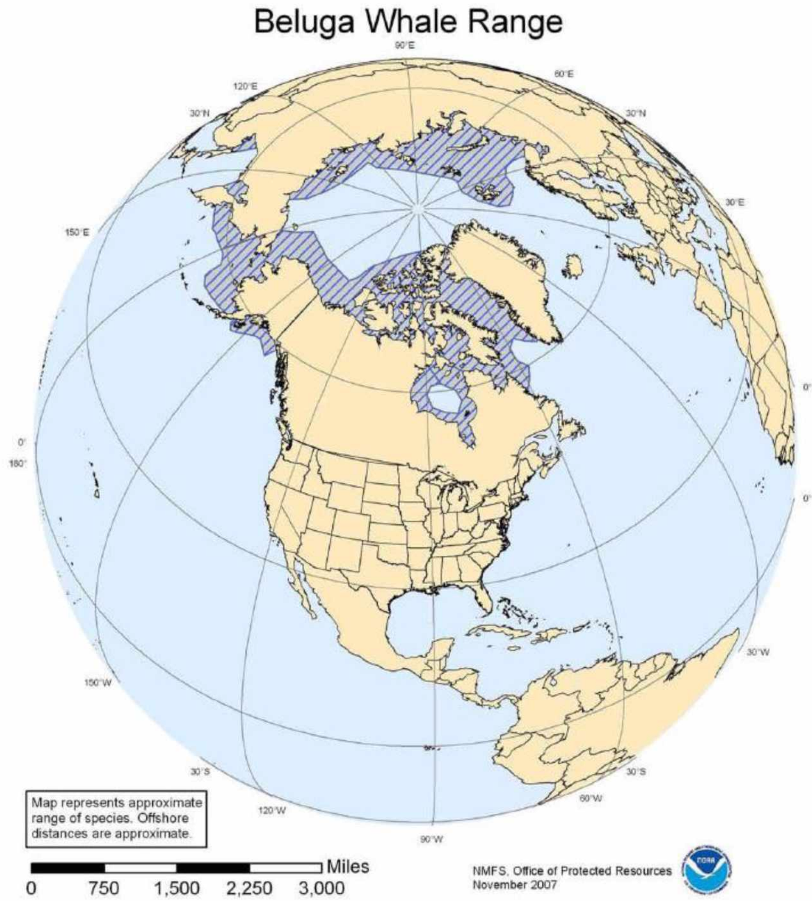

Figure 0.1: Worldwide beluga whale range map. Source (NOAA 2007).

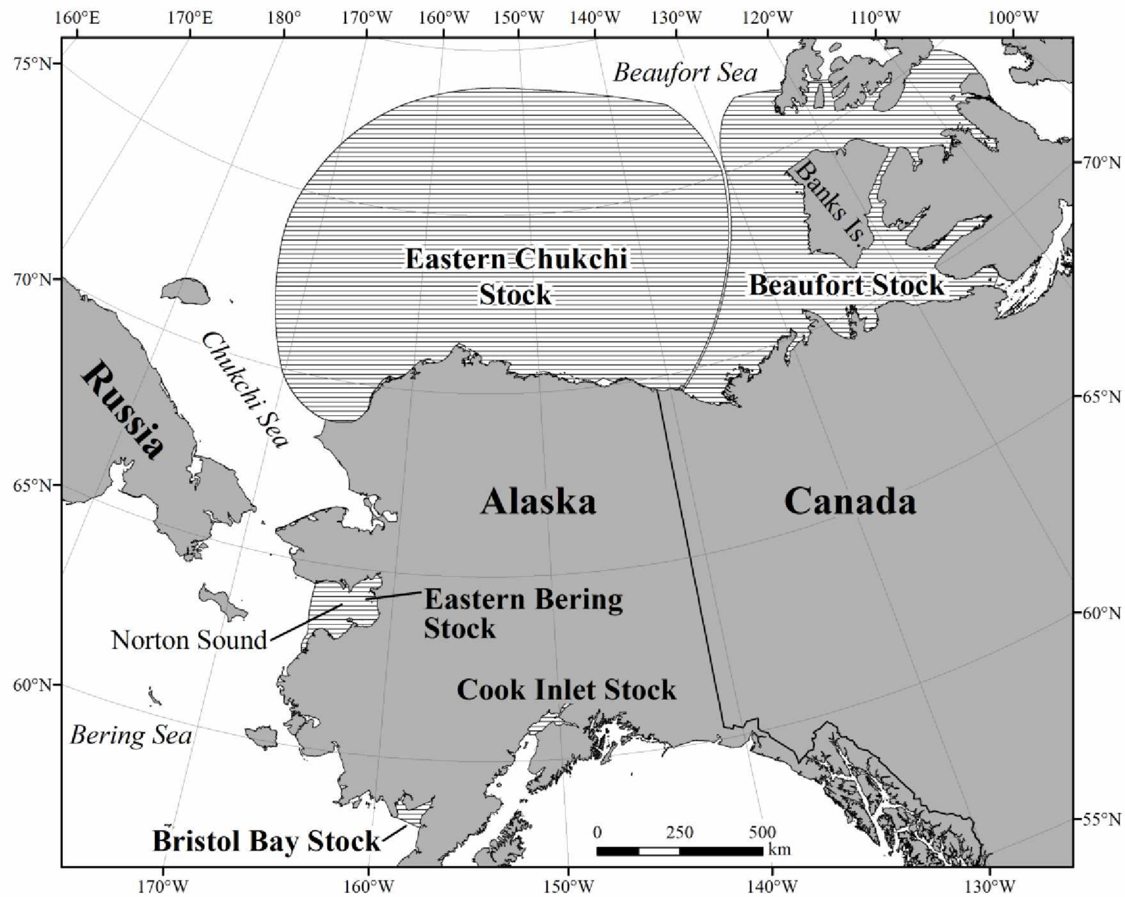

Figure 0.2: Summer distribution of five beluga whale stocks commonly associated with waters in Alaska. Prepared by Justin Crawford (ADFG).

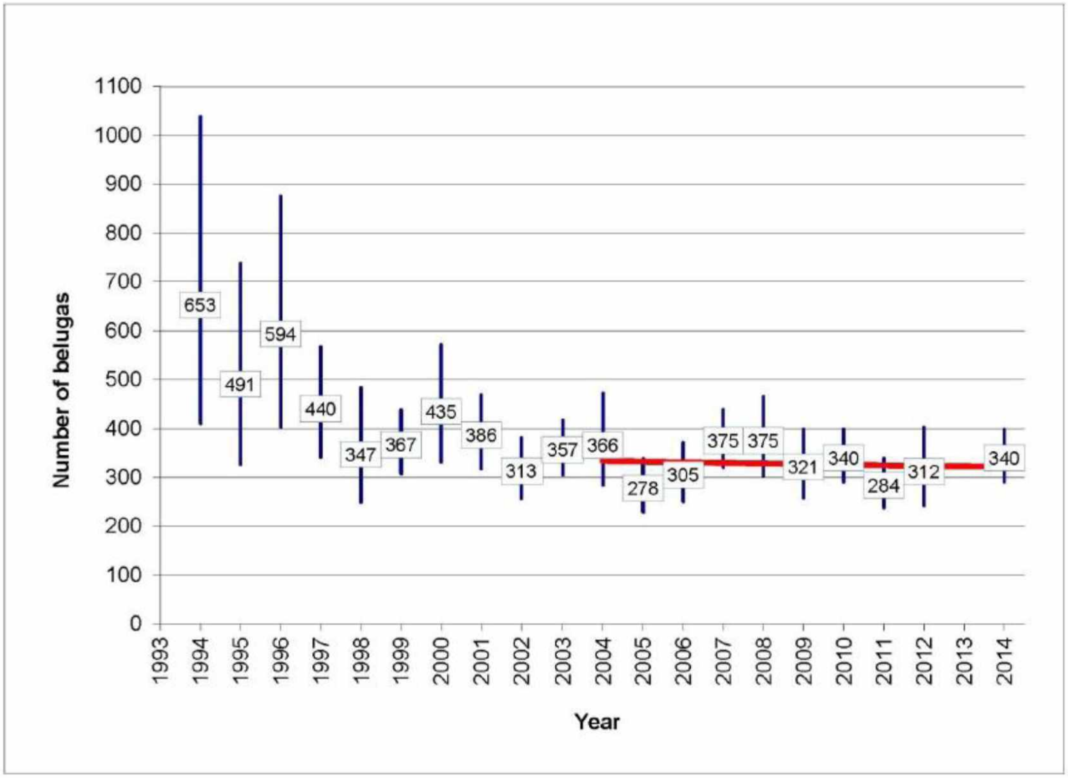

Figure 0.3: Estimated abundance of Cook Inlet beluga whales from 1994 to 2014. Blue bars represent 95% confidence intervals for each estimate (Shelden et al. 2015b)[1]. Red line indicates the 10-year declining trend of -0.4% (SE = 1.3%).

[1] Shelden et al. 2015b; Figure notes: Abundance estimates for belugas in Cook Inlet with 95% confidence intervals for revised coefficients of variation (CVs) (vertical bars). From 1994 to 1998, when the harvest was unrestricted, the annual rate of decline was -13.7% (SE = 0.045) per year. In the years since a hunting quota was in place (1999–2014), the rate of decline was -1.3% (SE = 0.7%) per year. The 10-year trend (2004–2014) was -0.4% (SE = 1.3%) per year.

Chapter 2: Fifty years of Cook Inlet beluga whale feeding ecology from isotopes in bone and teeth[1]

2.1 Abstract

Beluga whales (*Delphinapterus leucas*) that reside in Cook Inlet (CIBW) are important to coastal Alaska Native culture and subsistence, tourism, and ecologically as a top level predator. Due to a ~50% population decline in the 1990's, the distinct population segment in Cook Inlet was designated depleted under the Marine Mammal Protection Act in 2000 and listed as endangered under the Endangered Species Act in 2008. Diet changes are a concern in CIBW lack of recovery, but their feeding ecology is difficult to study. Skulls from 20 CIBW and tooth growth layer groups (GLGs) from 26 individual CIBW showed decreasing trends for both nitrogen and carbon stable isotope ratios (expressed as $\delta^{15}N$ and $\delta^{13}C$ values) during 1962 to 2007. The decline in $\delta^{15}N$ values (~1 to 2‰) is less than expected for a trophic level shift, but the magnitude of decline in $\delta^{13}C$ values (~3‰) is much greater (>5 times greater) than expected for a trophic level shift. A decline in $\delta^{13}C$ values could be explained by an increase in freshwater influenced prey. We investigated this possibility by analyzing the strontium isotope composition ($^{87}Sr/^{86}Sr$ ratios) of GLGs and compared them to rivers that flow into Cook Inlet. Over time the $^{87}Sr/^{86}Sr$ ratios trended away from the global marine signature and towards values from rivers flowing into the upper reaches of Cook Inlet. This study presents the first evidence for a long-term (~50 years) change in CIBW feeding ecology.

[1] Submitted to Endangered Species Research as Nelson MA, Quakenbush LT, Mahoney BA, Wooller MJ "Fifty years of Cook Inlet beluga whale feeding ecology from isotopes in bone and teeth"

2.2 Introduction

The beluga whales (*Delphinapterus leucas*) in Cook Inlet (CIBW), Alaska (Fig. 1) are isolated genetically (O'Corry-Crowe et al. 2002) and geographically (Hobbs et al. 2005, Hobbs et al. 2008, Rugh et al. 2010, Goetz et al. 2012, Shelden et al. 2015) from the other four beluga whale stocks in Alaska (Allen and Angliss 2015). Aerial surveys, including those conducted by the National Marine Fisheries Service (NMFS), revealed a decline in the CIBW population by ~50% between 1994 (653 belugas) and 1998 (347 belugas; Hobbs et al. 2015). This decline was attributed to an unsustainable subsistence harvest estimated between 287 and 406 for those five years (Mahoney and Shelden 2000). In 1999, the harvest was greatly reduced; first by a temporary voluntary hunting moratorium and soon after by harvest regulations. CIBW were designated as depleted under the Marine Mammal Protection Act in 2000 (NOAA 2000), listed as endangered under the Endangered Species Act in 2008 (NOAA 2008), critical habitat was designated in 2011 (NOAA 2011), and a recovery plan was published in 2016 (NMFS 2016). CIBW were also classified as critically endangered under the IUCN Red List of Threatened Species in 2012 (Lowry et al. 2012).

CIBW have been hunted throughout recorded history by coastal Alaska Natives for food and cultural purposes (Huntington 2000, Mahoney and Shelden 2000), and intermittently during the 20th century by non-Natives for commercial and sporting purposes. Marine mammals were protected in 1972 when the Marine Mammal Protection Act was passed by Congress; however, an exemption allows the taking of marine mammals by coastal Alaska Natives, provided such taking is for subsistence purposes and conducted in a non-wasteful manner.

Five CIBW have been harvested since 1999; one in 2001, 2002, and 2003, and two in 2005 (Mahoney and Shelden 2000). With the reduction in harvest the CIBW population was predicted to increase, but instead a slow (-0.4% $year^{-1}$, standard error (SE) = 1.3%) 10-year decline occurred from 2004 to 2014 (NMFS 2016). Concurrent with the population decline, the summer range of CIBW contracted into the upper reaches of Cook Inlet (Rugh et al. 2010, Shelden et al. 2015). Although a range contraction may be the direct result of fewer animals requiring a smaller area, how a contracted range influences beluga feeding ecology is unknown. Increased concerns about their susceptibility to potential threats (NMFS 2016) were summarized in the 2008 status review, which included catastrophic events, disease, predation, small population effects, noise, ship strikes, and decreased prey availability (Hobbs 2008). The recovery plan examined these threats further and found that while the threat of a reduction in prey was of medium concern, little was known about prey availability and how availability has changed over time (NMFS 2016).

The diet of CIBW is mostly known from stomach contents of stranded and harvested animals (Quakenbush et al. 2015) and from subistence hunter knowledge (Huntington 2000). CIBW summer prey are known to be mainly seasonally available fish (e.g., eulachon, *Thaleichthys pacificus*; Chinook salmon, *Onchorhynchus tshawytscha*; chum salmon, *O. keta*; and coho salmon, *O. kisutch*) that pass through Cook Inlet to spawn. Other fish prey available year-around include saffron cod, *Eleginus gracilis*; walleye pollock, *Theragra chalcogramma*; Pacific cod, *Gadus macrochephalus*; starry flounder, *Platichthys stellatus*; and yellowfin sole, *Limanda aspera.* Invertebrates are also eaten, primarily shrimp from the families Caridea and Crangonidae; however, species of Polychaeta, Amphipoda, and Oregoniidae crabs are also eaten (Huntington 2000, Hobbs et al. 2008, Quakenbush et al. 2015). Stomach contents for CIBW provide prey identifiable to species, but content analysis is limited to whales that die shortly after eating that are available for sampling. Because of these limitations, there were not enough CIBW stomachs available throughout the year or annually to provide information about whether CIBW diet has changed over time.

Because studying the diet of CIBW directly is difficult, examining changes in prey availability over time may also be a better indicator of changes in diet. Prey assemblages in the Gulf of Alaska have changed among warm (1947 to 1976), cold (1977 to 1997) and warm regimes (1998 to present) at a multi-decadal scale (Anderson and Piatt 1999, Overland et al. 2008). Cold regimes produce more high quality forage fish for birds and marine mammals than warm regimes (Anderson and Piatt 1999). Little is known about how Cook Inlet prey assemblages are influenced by regime shifts in the Gulf of Alaska, but closures of commercial shrimp, crab, and herring (*Clupea pallasii*) fisheries in lower Cook Inlet have occurred (Moore et al. 2000, Hollowell et al. 2016, Shields and Dupuis 2017). Between the 1980s and 1990s the coho salmon escapement increased, while Chinook, chum, and pink (*O. gorbuscha*) salmon declined, and sockeye salmon (*O. nerka*) remained fairly stable in the Susitna River drainage (Moore et al. 2000). However, total salmon escapement may not be an appropriate estimator to determine the number of salmon available for CIBW because escapement in a particular river does not equate to fish available as CIBW prey (Moore et al. 2000, Citta et al. 2016).

Isotopic analyses of hard tissues from belugas can provide general diet information integrated over long periods (e.g., bone integrates an average diet of ≥10 years) or short periods (e.g., a growth layer group from a tooth integrates an average diet of one year), depending on how the tissue was formed and how it is maintained (turnover rate; Tieszen et al. 1983, Peterson and Fry 1987, Newsome et al. 2010, Rioux et al. 2012, Witteveen et al. 2012). We examined changes in CIBW feeding ecology and foraging location using nitrogen, carbon, and strontium isotope ratio data (expressed as $\delta^{15}N$ and $\delta^{13}C$ values, and $^{87}Sr/^{86}Sr$ ratios respectively) from bone ($\delta^{15}N$ and

$\delta^{13}C$ values only) and annual growth layer groups (GLGs) in teeth ($\delta^{15}N$ and $\delta^{13}C$ values, and $^{87}Sr/^{86}Sr$ ratios) to determine if changes in CIBW feeding ecology occurred during the last 50 years. $\delta^{15}N$ and $\delta^{13}C$ values can be used to detect changes in trophic level because when a whale feeds a full trophic level higher, the isotopic value of their tissues increase for both $\delta^{15}N$ and $\delta^{13}C$ by ~3 and 1‰, respectively (e.g., Peterson and Fry 1987, Hobson et al. 1996, Kelly 2000, Newsome et al. 2009). $\delta^{13}C$ values can also be used to determine if the carbon source of prey is marine or freshwater. For example, marine prey items often have higher $\delta^{13}C$ values than freshwater prey (e.g., Tieszen et al. 1983, Peterson and Fry 1987, Bentzen et al. 2007). $^{87}Sr/^{86}Sr$ ratios differ by geologic formation and influence the water flowing through them (e.g., Brennan et al. 2014, Brennan et al. 2015, Padilla et al. 2015), while marine waters are relatively uniform (Veizer 1989, Brennan et al. 2015), thus $^{87}Sr/^{86}Sr$ ratios can be used to determine the likely location within an estuarine system where prey or predators spent time.

2.3 Materials and Methods

Bone collagen extraction and preparation:

Bone was sampled, cleaned, demineralized, and collagen extracted from the skulls of 20 CIBW (8 males, 6 females, and 6 unknown) that died during 1964 and 2007 (Fig. 2a), and were archived at the University of Alaska, Museum of the North (UAMN) in Fairbanks, Alaska. Skulls from immature belugas, defined as having unfused sutures between skull plates, were not used in this analysis because their bone would be primarily influenced by suckling, which could be as much as a full trophic level higher than their mothers (Newsome et al. 2010).

A small piece (1 cm x 5 cm x 5 cm) of bone from the zygomatic arch was cut out with a rotary tool, sanded to remove the outer cortex, and the clean solid bone placed into a labeled glass culture tube. Each sample was then cleaned by: 1) rinsing twice with deionized water, 2) bathing for one minute in a sonication bath, 3) soaking for eight hours in ethanol, 4) soaking for eight hours in methanol, 5) rinsing with deionized water, 6) soaking for eight hours in acetone, 7) rinsing with deionized water, 8) soaking twice for eight hours in chloroform and air drying under a fume hood, and 9) rinsing with deionized water (entire process modified from Matheus 1997). The cleaned bone was air-dried under a fume hood overnight and weighed. Demineralization occurred by covering the bone with water, adding 1.0 to 2.0 ml of 6N hydrochloric acid (HCl), and refrigerating at 3 °C for 12 to 24 hours. Once the solution stopped bubbling, it was decanted and rinsed with fresh deionized water, then recharged with fresh HCl and repeated until bubbling ceased. Typically this step took 7–10 days to completely demineralize the bone samples. Once demineralized, the sample was gelatinized with HCl at a pH of 3 to 4 and capped with helium to displace the air. The samples were then placed in a heating block that was kept at 65 °C until the collagen dissolved, at which point the samples were then centrifuged for 3 to 6 minutes at 2000 rpm. The supernatant was passed through a filter, 50 mm in diameter with a pore size of 0.45 μm, and the filtrate collected in a scintillation vial, which was covered with a glass filter disk and freeze-dried (lyophilized) until only dry collagen remained. A 0.2 to 0.5 mg sub-sample of each dried collagen sample was sealed in a tin capsule for stable carbon and nitrogen isotope analysis (described below).

Tooth collagen extraction and preparation:

Beluga teeth (n = 26 teeth from individual whales; 14 males, 10 females, and 2 unknown; Fig. 2a), previously used to age the whales, were also analyzed for stable isotope composition. A thin longitudinal section was cut from the center of each tooth, leaving two halves (Fig. 2b; Vos 2003). A micromill (ESI® New Wave™ Research) was used to remove dentin material along the selected GLG from one of the halves. A GLG was defined as a light and a dark layer of dentin (Vos 2003; Fig. 2b) representing one year of growth (Stewart et al. 2006). GLGs from whales determined to be three years of age and younger were not used in this analysis to avoid diet signals associated with suckling (Newsome et al. 2010). Mathews and Ferguson (2015) showed that 17 of 18 (94%) belugas had weaned during or before their third year.

The drilled powder from each GLG was collected with a small paintbrush into a microcentrifuge vial and demineralized by adding 0.25 N HCl to cover the powder, which was then left overnight in a refrigerator. Samples were centrifuged at 5000 rpm for five minutes to concentrate the remaining powder at the bottom of the vial and the liquid was pipetted off and discarded (Newsome et al. 2009). This was repeated until the white powder turned translucent indicating demineralization was complete. The samples were then rinsed with deionized water to remove HCl, frozen, and freeze-dried until dry fluffy collagen remained (~10 hours). A 0.2 to 0.5 mg sub-sample of the isolated collagen was sealed into a tin cup for isotope analysis.

Stable nitrogen and carbon isotope analysis:

The $\delta^{15}N$ (n = 343 GLGs) and $\delta^{13}C$ (n = 296 GLGs) values for bone collagen and tooth dentin samples were produced using a Costech® Elemental Analyzer coupled to a ThermoFisher Scientific™ Delta V™ Isotope Ratio Mass Spectrometer. Stable isotope ratios are presented in delta (δ) notation:

$$\delta X = (R_{sample}/R_{standard} - 1) * 1000$$

where R_{sample} is the ratio of the heavy to light isotopes of the sample and $R_{standard}$ is the ratio of the heavy to light isotopes of the standards (i.e. atmospheric N_2, for nitrogen and Vienna Pee Dee Belemnite for carbon). Analytical precision was validated by running a laboratory standard (peptone) after every ten samples, and standard deviation (of 71 analyses) was ≤ 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. Increased burning of fossil fuels since the industrial revolution continues to alter the isotopic composition of CO_2 in the atmosphere and the ocean, a phenomenon known as the Suess effect, which gradually lowers the background $\delta^{13}C$ value over time (Francey et al. 1999). The background $\delta^{13}C$ values decreased by ~0.8‰ from 1960 to 2010 and all $\delta^{13}C$ values in this study were corrected for this effect following the approach described by Misarti et al. (2009).

Collagen quality:

The quality of collagen from bone and teeth was evaluated by atomic carbon to nitrogen ratio (C:N) calculated by the formula:

$$C{:}N = \left(\frac{14}{12}\right) \times \left(\frac{Concentration\ (\%)\ Carbon}{Concentration\ (\%) Nitrogen}\right)$$

All C:N ratios for bone were between 3.1 and 3.5 (Appendix 1), indicating the collagen was of good quality (i.e., between 2.9 and 3.6; DeNiro et al. 1985, Ambrose 1990, Tatsch et al. 2016). C:N ratios from CIBW tooth dentin GLGs were between 3.1 and 5.7 (Appendix 2). When a sample had a C:N ratio ≥3.6, only its $\delta^{15}N$ data were used in further data analyses because C:N ratios higher than 3.6 indicate possibly compromised $\delta^{13}C$ values (DeNiro et al. 1985, Ambrose 1990, Tatsch et al. 2016).

Tooth dentin preparation:

A subset of GLGs previously analyzed for $\delta^{15}N$ and $\delta^{13}C$ values were selected for strontium isotope ratio ($^{87}Sr/^{86}Sr$) analyses using approximately 20 mg of powdered dentin. GLGs that spanned 1968 to 2005 from three males and two females were analyzed (Appendix 4). The GLGs (n = 44) were chosen to match the years analyzed for carbon and nitrogen to better interpret those results.

Dentin strontium isotope analysis:

A ThermoFisher Scientific™, High Resolution Neptune™ Multicollector-Inductively Coupled Plasma Mass Spectrometer was used to analyze strontium samples, which had been purified for the $^{87}Sr/^{86}Sr$ analysis via an introduction system of aqueous solution using an inline chromatographic column (Brennan et al. 2014, Mackey and Fernandez 2011). This solution method was used to measure the $^{87}Sr/^{86}Sr$ ratios of water samples collected from the Cook Inlet region. Blanks or NIST standard reference material SRM987 were run between samples and the mean 1 SE was $\leq$0.00001 for all samples.

Water collection and strontium isotope analysis:

Water samples were collected (in triplicate) from eight freshwater rivers and one marine location (Turnagain Arm) in upper Cook Inlet and one marine location (Kachemak Bay) in the lower inlet during 27 May to 5 June 2016. Freshwater river samples were either collected above tidal influence (Susitna River, Yentna River, and Eagle River), or at low tide when access to areas above tidal influence was more difficult (Bird Creek, 20 Mile River, Portage Creek, and

Placer River). Samples were collected for strontium isotope analysis following field collection methods described by Brennan et al. (2014), and sent via 2-day FedEx® to the Geochemistry Laboratory at the University of Utah, Salt Lake.

Mean $^{87}Sr/^{86}Sr$ ratios from water samples collected in triplicate were calculated by weighting each individual mean by their respective SE.

Weighted triplicate mean (TM) = $\left(\left(\frac{1}{SE_1^2}\right)(Sr_1) + \left(\frac{1}{SE_2^2}\right)(Sr_2) + \left(\frac{1}{SE_3^2}\right)(Sr_3)\right) / \left(\left(\frac{1}{SE_1^2}\right) + \left(\frac{1}{SE_2^2}\right) + \left(\frac{1}{SE_3^2}\right)\right)$

Sr_1 is the $^{87}Sr/^{86}Sr$ ratio from sample 1 and SE_1 is the analytical measurement error from sample analysis from sample 1.

Weighted SE of the triplicate was calculated as:

$$(SE) = \left(\sqrt{\frac{\left(\left(\frac{1}{SE_1^2}\right)((Sr_1-TM)^2)+\left(\frac{1}{SE_2^2}\right)((Sr_2-TM)^2)+\left(\frac{1}{SE_3^2}\right)((Sr_3-TM)^2)\right)}{\left(\frac{2\left(\left(\frac{1}{SE_1^2}\right)+\left(\frac{1}{SE_2^2}\right)+\left(\frac{1}{SE_3^2}\right)\right)}{3}\right)}}\right) / (\sqrt{3})$$

$^{87}Sr/^{86}Sr$ ratios are presented as triplicate mean ±2SE, where SE is calculated as above for samples collected in triplicate. Samples not collected in triplicate are presented as $^{87}Sr/^{86}Sr$ ratios $\pm 2SE^a$, where SE^a is the analytical measurement error calculated when the samples were analyzed.

2.4 Results

The $\delta^{15}N$ and $\delta^{13}C$ values of CIBW bone collagen generally declined over time (Fig. 3, Appendix 1). The mean $\delta^{15}N$ value for belugas that died before the documented population decline in 1995, (16.4‰ ±0.2, n = 7) was significantly higher than the mean value after 1995 (15.6‰ ±0.1, n = 11, $p < 0.01$). Similarly, the mean $\delta^{13}C$ value for belugas that died before 1995 (-12.6‰ ±0.7, n = 7) was also significantly higher than for those that died after 1995 (-14.0‰ ±0.2, n = 11, $p < 0.01$, two sample t-test). A decline in both $\delta^{15}N$ and $\delta^{13}C$ values across the entire study period was also significant (R = 0.81, $p < 0.01$, and R = 0.83, $p < 0.01$; for $\delta^{15}N$ and $\delta^{13}C$ values, respectively; Fig. 3).

Annual GLGs from CIBW teeth showed significant declines in $\delta^{15}N$ and $\delta^{13}C$ values over time ($p < 0.01$; Figs. 4a and b, and Appendix 2). Although the decline in $\delta^{13}C$ values (n = 296 GLGs) was well explained by a linear regression (R = 0.76, $p < 0.01$; Fig. 4b and Appendix 2), the relationship for $\delta^{15}N$ values (n = 343 GLGs) was not (R = 0.27, $p < 0.01$; Fig. 4a and Appendix 2). The relationship for $\delta^{15}N$ values was more variable and possibly cyclic in nature (~10 years between highs and lows).

$^{87}Sr/^{86}Sr$ (±2SE) ratios varied across the sample locations in the Cook Inlet watershed (Fig. 1, Table 1, Appendix 3). The highest $^{87}Sr/^{86}Sr$ ratio was found in Kachemak Bay (near Homer Spit), in lower Cook Inlet (#15, Fig. 1, Table 1, and Appendix 3) and was equivalent to the global marine ratio (0.70918 ± 0.00006; Brennan et al. 2015). The second highest $^{87}Sr/^{86}Sr$ ratio was also from marine waters in Turnagain Arm in upper Cook Inlet (#10, Fig. 1, Table 1, and Appendix 3). The $^{87}Sr/^{86}Sr$ ratios of all rivers tested were lower than the marine values; Bird Creek (#9, Fig. 1) was highest, followed by Susitna River below the Yentna River (#5, Fig. 1), Yentna River (#4, Fig. 1), Susitna River above the Yentna River (#3, Fig. 1), Twenty Mile River (#11, Fig. 1), Placer River (#13, Fig. 1), Portage Creek (#12, Fig. 1), and Eagle River (#8, Fig. 1, Table 1, and Appendix 3).

We found a significant declining trend in annual GLG (n = 44) $^{87}Sr/^{86}Sr$ ratios (Fig. 5, Appendix 4) from 1968 to 2005 ($R = 0.51$, $p < 0.01$) when all GLGs from all whales were combined. We also compared the $^{87}Sr/^{86}Sr$ ratios with paired $\delta^{15}N$ and $\delta^{13}C$ values (same GLG on the same tooth; n = 34 GLGs) and found a significant positive correlation ($R = 0.48$, $p < 0.01$) with $\delta^{13}C$ values but not with $\delta^{15}N$ values.

2.5 Discussion

Stable isotope ratios preserved in CIBW bone and GLGs from teeth integrate the isotopic composition of beluga diet in Cook Inlet and this study analyzed them from the 1950s to 2007. Bone continuously replaces old material (made with diet items of the past) with new material (made with diet items available currently) at a very slow rate (2 to 3% year^{-1}; Clarke 2008), such that bone integrates dietary isotopes for at least 10 years and possibly for the lifetime of a beluga whale. In contrast, GLGs remain biochemically unchanged after they are formed and, because belugas form one GLG each year, the isotopes in each GLG integrated diet for one year of a beluga's life (Stewart et al. 2006, Luque et al. 2007). Both $\delta^{15}N$ and $\delta^{13}C$ values from CIBW declined during our study period (1950s to 2007) in both the bone and the teeth, providing definitive evidence that a change in CIBW feeding ecology occurred. For a full decrease in trophic level we would expect $\delta^{15}N$ values to decline by ~3‰ and $\delta^{13}C$ values to decline by ~1‰ (Peterson and Fry 1987). However, our data showed less than a full trophic level decline for $\delta^{15}N$ (~1 to 2‰) and more than a trophic level decline for $\delta^{13}C$ (~3‰; Fig. 4).

Declining $\delta^{15}N$ and $\delta^{13}C$ values could represent a change in prey source (Peterson and Fry 1987), however a change in the $\delta^{15}N$ and $\delta^{13}C$ baseline values at the level of primary production in the environment could appear to be a change in prey when in fact the prey species are the same but their isotopic signature has changed over time (Schell 2000, Post 2002, Christensen and

Richardson 2008, Casey and Post 2011, Marcoux et al. 2012). Because of the documented range contraction from more marine areas, middle and lower Cook Inlet, to more freshwater influenced areas in upper Cook Inlet (Rugh et al. 2010), we suspected the decline in $\delta^{13}C$ values indicated a change from a marine prey base to a more freshwater influenced prey base.

To test if the declining $\delta^{13}C$ values indicated a change to more freshwater prey we used isotopes of a third element, strontium. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) can be used to discern the role of freshwater-influenced prey because they are taken up by organisms via the water in which they reside. Beluga whales are not known to drink water, but rather receive their metabolic water, and thus strontium, from their prey. Fish exchange water passively over gills and their internal $^{87}Sr/^{86}Sr$ ratio reflects their surroundings (Brennan et al. 2014, Padilla et al. 2015). When prey is consumed, strontium is incorporated into hard structures, such as bone and teeth, because of its similarity to calcium (Britton et al. 2009). The ratio of $^{87}Sr/^{86}Sr$ in a river's water remains relatively unchanged at a specific site, but varies along the length of the river as a result of the geologic formation that river flows through. The world's oceans are mixed and essentially homogenous throughout. Therefore, $^{87}Sr/^{86}Sr$ ratios measured in the hard structures of an organism can be compared to the $^{87}Sr/^{86}Sr$ ratios of various estuarine and freshwater sources, and used to determine when an organism switches from a marine influenced prey to a more freshwater influenced prey. In some cases, the $^{87}Sr/^{86}Sr$ ratio can identify the source (river) of freshwater prey.

We compared $^{87}Sr/^{86}Sr$ ratios and $\delta^{13}C$ values from the same GLG on the same tooth for five whales (representing diet during 1968 to 2005) to determine whether a change in CIBW diet to a more freshwater influenced prey was indicated. We found that the recent $\delta^{13}C$ values were lower and correlated with lower $^{87}Sr/^{86}Sr$ ratios ($R = 0.48$, $p < 0.01$), suggesting that the recent lower

$\delta^{13}C$ values were associated with more freshwater influenced prey. To determine which freshwater input was most influential, we compared the $^{87}Sr/^{86}Sr$ ratio from GLGs to known ratios in river systems flowing into Cook Inlet available from Brennan et al. (2014) and from this study (Fig. 1). Figure 5 shows that $^{87}Sr/^{86}Sr$ ratios from beluga GLGs were relatively steady between the late 1960's and the late 1980s followed by a declining trend beginning about 1990. This shift most likely represents a dietary change from marine to more freshwater prey that started in ~1990, which is when CIBW were declining. Furthermore, this dietary change is concurrent with the contraction of CIBW into the upper reaches of Cook Inlet (Rugh et al. 2010), where freshwater prey items are more available. Although it was not possible to identify which river(s) influence CIBW the most, the recent lower $^{87}Sr/^{86}Sr$ ratios indicate that CIBW are now feeding on prey that is more influenced by freshwater.

Our carbon and nitrogen isotope data indicate a change in CIBW feeding ecology and the strontium data indicate a change towards a more freshwater influenced diet, however, whether these changes could also be explained by an environmental change in baseline was unknown. Therefore, we analyzed the $\delta^{15}N$ values of the amino acid phenylalanine from five bone collagen samples from CIBW that died during 1964 (2 whales) and after 2000 (3 whales). Phenylalanine is an essential amino acid (i.e., not manufactured or altered by digestion) and the $\delta^{15}N$ values of this amino acid have been used to identify the $\delta^{15}N$ value at the base of food chains (e.g., Chikaraishi et al. 2014; see Appendix 5 for methodology). We found the mean $\delta^{15}N$ value of phenylalanine from beluga whales that died more recently (after 2000) was lower than those that died at the beginning of our study period (during 1964; Appendix 6). Therefore the $\delta^{15}N$ value of the base of the food chain sustaining CIBW has changed over the duration of this record, although further analyses would be beneficial to strengthen this compound specific data set and approach as part of future research directions. However, a change in the isotopic (N) composition of the base of the food chain could result from two scenarios: 1) CIBW have foraged in a similar location through time and the $\delta^{15}N$ value of the base of the food chain changed at that location or, 2) CIBW now forage in a different location with a different $\delta^{15}N$ value at the base of the food chain than in the past.

A change in CIBW distribution to greater use of the upper inlet was documented over three periods; 1978–1979, 1993–1997, and 1998–2008 (Rugh et al. 2010). This change in location puts CIBW in closer proximity to freshwater and likely to prey influenced by freshwater. Indeed, we suspect the decline in $\delta^{13}C$ values indicate a change from marine prey base to more freshwater influenced prey base. The correlation between $^{87}Sr/^{86}Sr$ ratios and $\delta^{13}C$ values also supports greater freshwater influence in foraging ecology as does the change in isotopic (N) composition of the amino acid phenylalanine in the CIBW. Based on our complete data set taken as a whole (including strontium isotope data that indicates a move in CIBW towards a more freshwater influenced diet) and the previous survey findings (Rugh et al. 2010) we consider the most likely explanation to be scenario 2 from above, that CIBW now forage in a different location (upper Cook Inlet) with a different $\delta^{15}N$ value at the base of the food chain than in the past. Although, our isotope data are consistent with more freshwater influenced prey through time the data also indicate that the change in foraging behavior began in the 1950s (Fig. 4), long before the documented population decline in the 1990s. Therefore, if this change in habitat and prey is related to the decline in CIBW abundance, then we must look farther back in time (i.e., before the documented decline in the 1990s) to determine what was responsible for the greater freshwater influence in CIBW feeding ecology.

Unfortunately, useful prey data for most of Cook Inlet is limited, however, decreases in shrimp, crab, and some fish including herring and salmon (chinook, chum, and pink) over the last few decades have been documented (Moore et al. 2000, Hollowell et al. 2016, Shields and Dupuis 2017). Determining whether declines in prey populations correspond to declining CIBW foraging opportunities is difficult (Moore et al. 2000). The change in $\delta^{15}N$ values, while generally declining through the study period, appeared to show periodic oscillations (Fig. 4a). These oscillations could be related to long-term climate and regime shifts in the North Pacific (e.g., Pacific Decadal Oscillation) that influence primary productivity, as suggested for $\delta^{15}N$ values in Northern fur seal (*Callorhinus ursinus*) teeth (Newsome et al. 2007), but more evidence is necessary to determine the cause of the oscillations in CIBW.

We are pursuing additional research using strontium (e.g., more water sample locations, GLGs representing more recent years), carbon and nitrogen, and phenylalanine as these appear to be important to better understand the changes we identified in this study. Our data provides evidence of a change to a more freshwater influenced feeding ecology that supports a long-term shift into more freshwater influenced habitats. How this behavior relates to the CIBW continued decline or their possible recovery, however, remains unknown.

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2.8 Figures

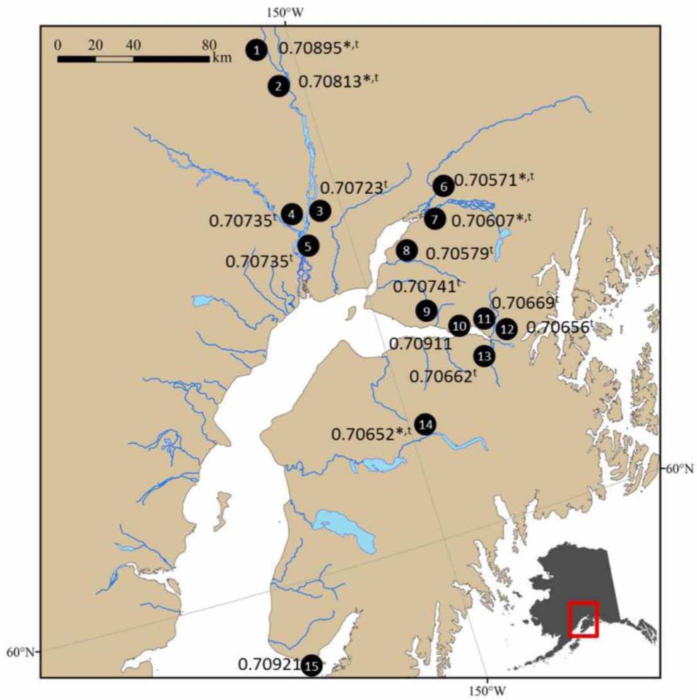

Figure 0.1: Map of Cook Inlet with $^{87}Sr/^{86}Sr$ ratios marked from this study, numbers in black circles correspond to the ID in Table 1. Inset shows location of Cook Inlet in Alaska. (*denotes ratios from Brennan et al. (2014) and ^{t}denotes weighted average ratio derived from samples taken in triplicate)

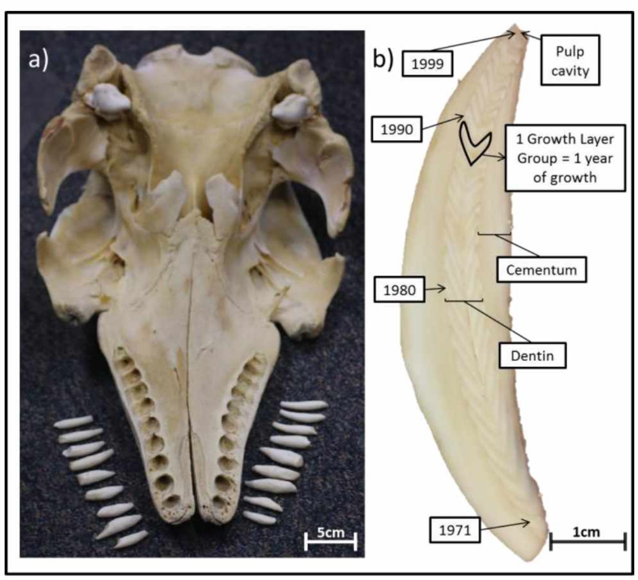

Figure 0.2: (a) A skull of a Cook Inlet beluga whale (CIBW) and (b) a CIBW tooth cut longitudinally to expose annual growth layer groups.

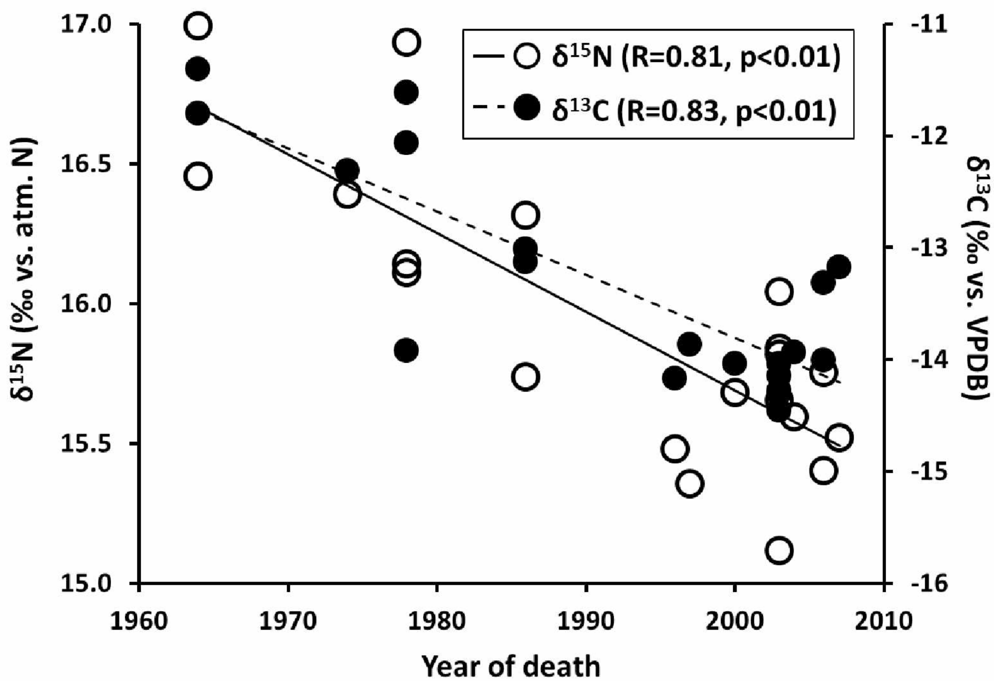

Figure 0.3: $\delta^{15}N$ and $\delta^{13}C$ values from Cook Inlet beluga bone collagen (skulls) plotted against year and regression (linear) lines for both.

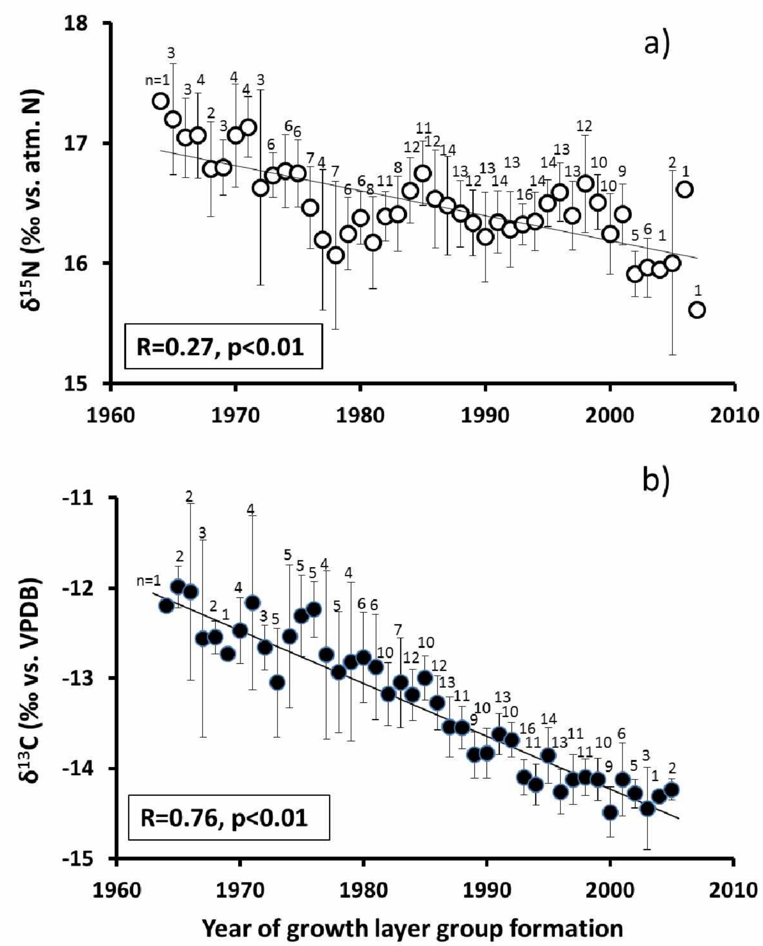

Figure 0.4: $\delta^{15}N$ (a) and $\delta^{13}C$ (b) values (± 2 standard error) from Cook Inlet beluga tooth growth layer groups (GLGs) plotted by year of formation. The number above each mean value represents the number of GLGs that make up that mean value.

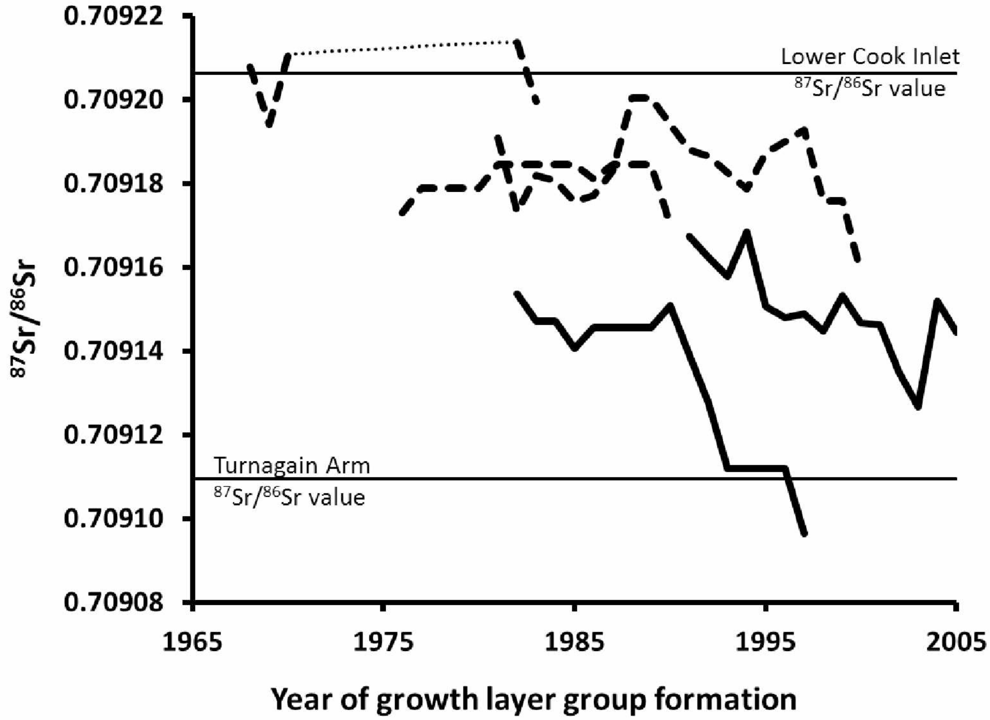

Figure 0.5: $^{87}Sr/^{86}Sr$ ratios from Cook Inlet beluga growth layer groups (GLGs) by year of formation. Solid line = female, large dashed line = male, and small dashed line = period with no data.

2.9 Table

Table 2.1: Summary of $^{87}Sr/^{86}Sr$ ratios from Cook Inlet rivers and marine waters, including date and location of sampling sites. ID matches the ID used in Fig. 2.1. Data from Brennan et al. (2014) are indicated by an *, average ratios and the standard error (SE) from triplicate samples, indicated by superscript ^{t}, are weighted by the SE from analysis. SE of single samples (i.e., not collected in triplicate) represent the analytical measurement error (SE^{a}).

ID	Date Collected	Waterbody	Latitude	Longitude	$^{87}Sr/^{86}Sr$	$\pm 2SE^{(a)}$
$1^{*,t}$	11-Sep-10	Chulitna R.	62.568	-150.236	0.708948	0.000042
$2^{*,t}$	11-Sep-10	Susistna R.	62.178	-150.172	0.708127	0.000057
3^{t}	27-May-16	Susitna R.	61.588	-150.410	0.707232	0.000039
4^{t}	27-May-16	Yentna R.	61.608	-150.508	0.707349	0.000011
5^{t}	27-May-16	Susitna R.	61.533	-150.545	0.707353	0.000074
$6^{*,t}$	14-Sep-10	Matanuska R.	61.734	-148.765	0.705711	0.000013
$7^{*,t}$	14-Sep-10	Knik R.	61.476	-148.876	0.706074	0.000075
8^{t}	2-Jun-16	Eagle R.	61.309	-149.574	0.705793	0.000020
9^{t}	2-Jun-16	Bird Cr.	60.973	-149.467	0.707406	0.000050
10	5-Jun-16	Turnagain Arm	60.937	-149.265	0.709110	0.000021
11^{t}	2-Jun-16	20 Mile R.	60.845	-148.989	0.706692	0.000014
12^{t}	2-Jun-16	Portage Cr.	60.827	-148.977	0.706558	0.000041
13^{t}	2-Jun-16	Placer R.	60.817	-148.988	0.706623	0.000005
$14^{*,t}$	12-Sep-10	Kenai R.	60.487	-149.935	0.706517	0.000012
15	2-Jun-16	Lower Cook Inlet	59.606	-151.436	0.709206	0.000028

2.10 Appendices

Appendix 2.1: $\delta^{15}N$ and $\delta^{13}C$ values and their atomic C:N ratios from Cook Inlet beluga whale bone. Identifiers are included for both National Marine Fisheries Service (NMFS ID) and University of Alaska, Museum of the North (UAMN ID).

NMFS ID	UAMN ID	Sex	Year beluga died	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Atomic C:N ratio
	11642	unknown	1964	16.5	-11.4	3.1
	11643	unknown	1964	17.0	-11.8	3.2
	16009	male	1974	16.4	-12.3	3.2
	16006	unknown	1978	16.9	-11.6	3.1
	16008	unknown	1978	16.1	-13.9	3.5
	16010	male	1978	16.1	-12.1	3.3
	16013	female	1986	16.3	-13.0	3.2
	16014	unknown	1986	15.7	-13.1	3.4
28-Aug-96 #2	48680	male	1996	15.5	-14.2	3.3
27-May-97	48678	male	1997	15.4	-13.9	3.3
19-Jun-00	97401	male	2000	15.7	-14.0	3.3
13-Oct-03	91731	male	2003	15.8	-14.3	3.3
	91732	male	2003	16.0	-14.0	3.3
16-Sep-03	91769	female	2003	15.7	-14.2	3.2
17-Sep-03	91771	male	2003	15.8	-14.5	3.3
31-Mar-03	92569	female	2003	15.1	-14.3	3.4
	94549	female	2004	15.6	-13.9	3.2
10-Oct-06	86886	female	2006	15.8	-13.3	3.2
30-Sep-06	87332	female	2006	15.4	-14.0	3.3
29-Jan-07	87979	unknown	2007	15.5	-13.2	3.1

Appendix 2.2: Mean $\delta^{15}N$ and $\delta^{13}C$ values (2 standard error) and atomic C:N ratios (±2 standard error) from each Cook Inlet beluga whale tooth growth layer groups (GLGs). Identifiers are included for both National Marine Fisheries Service (NMFS ID) and University of Alaska, Museum of the North (UAMN ID).

NMFS ID	UAMN ID	Sex	Tooth side	Tooth number	Years of growth	$\delta^{15}N$			$\delta^{13}C$		
						Number GLGs	Mean ‰ (2SE)	Mean atomic C:N (2SE)	Number GLGs	Mean ‰ (2SE)	Mean atomic C:N (2SE)
25-Sep-92		male	N/A	N/A	1963-1991	31	16.5 (0.2)	3.5 (0.1)	26	-13.1 (0.3)	3.4 (0.1)
30-Jun-93		male	N/A	N/A	1962-1988	19	16.9 (0.2)	3.7 (0.2)	12	-12.2 (0.4)	3.4 (0.1)
5-Jun-95	36565	female	left	5	1968-1994	20	16.5 (0.2)	3.6 (0.1)	12	-13.1 (0.5)	3.5 (0.1)
13-Jul-96		unknown	right	1	1988-1991	4	16.4 (1.2)	3.3 (0.1)	4	-13.3 (0.3)	3.3 (0.1)
28-Aug-96 #2	48680	male	left	5	1962-1989	15	16.5 (0.2)	3.6 (0.1)	8	-13.5 (0.4)	3.5 (0.1)
7-Oct-96	67159	male	left	5	1969-1995	18	16.3 (0.4)	3.5 (0.1)	14	-12.8 (0.4)	3.4 (0.1)
27-May-97	48678	male	left	5	1979-1996	17	16.6 (0.1)	3.5 (0.2)	16	-13.5 (0.3)	3.4 (0.1)
28-Jul-98		male	N/A	1	1989-1998	9	16.8 (0.2)	3.4 (0.1)	9	-13.8 (0.4)	3.4 (0.1)
11-Aug-98		male	left	6	1987-1997	11	16.6 (0.4)	3.4 (0.1)	11	-14.0 (0.2)	3.4 (0.1)
1-Sep-99 #1		female	right	4	1978-1999	20	16.2 (0.2)	3.3 (0.1)	19	-13.1 (0.3)	3.3 (0.1)
12-Jun-00		male	left	8	1968-1993	19	16.4 (0.3)	3.5 (0.1)	17	-13.0 (0.4)	3.5 (0.1)
19-Jun-00	97401	male	N/A	N/A	1996-2000	5	17.4 (0.8)	3.4 (0.1)	5	-14.1 (0.4)	3.4 (0.1)
24-Jun-00		male	left	7	1992-2000	8	17.0 (0.3)	3.4 (0.3)	7	-13.7 (0.2)	3.3 (0.1)
25-Sep-00		female	left	4	1978-1998	20	16.3 (0.2)	3.3 (0.1)	18	-13.1 (0.3)	3.3 (0.0)
26-Sep-00 #1		female	N/A	5	1987-1997	2	14.9 (1.7)	4.1 (1.3)	1	-14.4 (-)	3.4 (-)
21-Jul-01	63041	female	left	6	1988-2001	13	16.9 (0.1)	3.3 (0.1)	13	-13.7 (0.2)	3.3 (0.1)
23-Sep-01		male	N/A	2	1982-2001	13	15.8 (0.4)	3.7 (0.3)	10	-13.9 (0.5)	3.5 (0.1)
4-Oct-01		female	left	7	1975-2001	24	16.3 (0.1)	3.3 (0.1)	24	-13.1 (0.3)	3.3 (0.1)
10-Oct-01		male	right	6	1994-2001	7	16.8 (0.2)	3.6 (0.3)	4	-14.0 (0.5)	3.3 (0.1)
13-Oct-01		female	right	6	1980-2001	18	17.1 (0.2)	3.6 (0.1)	12	-14.1 (0.3)	3.4 (0.1)
31-Mar-03	92569	female	N/A	N/A	1984-2003	20	16.5 (0.1)	3.4 (0.1)	18	-13.9 (0.3)	3.3 (0.1)
16-Sep-03	91769	female	N/A	N/A	1984-2003	11	16.2 (0.4)	3.4 (0.1)	9	-14.1 (0.4)	3.3 (0.1)
17-Sep-03	91771	male	N/A	N/A	1989-2003	15	16.4 (0.1)	3.4 (0.1)	14	-14.4 (0.3)	3.3 (0.1)
13-Oct-03	91731	male	left	6	1977-2003	27	16.9 (0.1)	3.4 (0.1)	25	-13.6 (0.3)	3.3 (0.1)
10-Oct-06	86886	female	N/A	N/A	1983-2006	18	16.5 (0.2)	3.4 (0.1)	16	-14.1 (0.2)	3.4 (0.1)
29-Jan-07	87979	unknown	N/A	N/A	1981-2007	19	16.5 (0.3)	3.4 (0.1)	18	-13.6 (0.3)	3.4 (0.1)

Appendix 2.3: $^{87}Sr/^{86}Sr$ ratios (±2 standard error) and concentration of strontium, calcium, magnesium, sodium, and potassium from all water samples collected. The ID matches Fig. 1 sampling locations.

Figure 1. ID	Date collected	Waterbody	Latitude	Longitude	$^{87}Sr/^{86}Sr$	±2SE	Sr (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)
3	27-May-16	Susitna River	61.588	-150.41	0.707208	0.000028	0.090	13.66	2.533	2.782	1.094
3	27-May-16	Susitna River	61.588	-150.41	0.707266	0.000025	0.091	13.94	2.537	2.389	1.199
3	27-May-16	Susitna River	61.588	-150.41	0.707210	0.000028	0.089	13.36	2.484	2.313	1.130
4	27-May-16	Yentna River	61.608	-150.508	0.707335	0.000029	0.135	18.41	4.371	1.805	0.994
4	27-May-16	Yentna River	61.608	-150.508	0.707354	0.000023	0.136	17.92	4.386	1.733	0.954
4	27-May-16	Yentna River	61.608	-150.508	0.707352	0.000022	0.136	18.14	4.395	1.768	0.969
5	27-May-16	Susitna River	61.533	-150.545	0.707361	0.000035	0.134	17.08	4.305	1.668	0.907
5	27-May-16	Susitna River	61.533	-150.545	0.707411	0.000032	0.123	16.07	3.964	1.582	0.906
5	27-May-16	Susitna River	61.533	-150.545	0.707287	0.000032	0.091	13.76	2.541	2.327	1.173
8	2-Jun-16	Eagle River	61.309	-149.574	0.705768	0.000027	0.216	18.48	3.851	1.531	0.239
8	2-Jun-16	Eagle River	61.309	-149.574	0.705794	0.000020	0.233	20.29	4.164	1.933	0.225
8	2-Jun-16	Eagle River	61.309	-149.574	0.705806	0.000022	0.225	22.54	4.045	2.181	0.239
9	2-Jun-16	Bird Creek	60.973	-149.467	0.707434	0.000025	0.143	13.57	9.108	64.43	2.599
9	2-Jun-16	Bird Creek	60.973	-149.467	0.707418	0.000029	0.141	13.51	8.828	62.25	2.396
9	2-Jun-16	Bird Creek	60.973	-149.467	0.707349	0.000031	0.138	13.47	8.331	58.27	2.273
10	5-Jun-16	Turnagain Arm	60.937	-149.265	0.709110	0.000021	2.387	150.2	395.2	3,729	184.1
11	2-Jun-16	20 Mile River	60.845	-148.989	0.706677	0.000024	0.111	10.60	1.603	1.442	0.267
11	2-Jun-16	20 Mile River	60.845	-148.989	0.706702	0.000024	0.106	10.52	1.536	1.651	0.177
11	2-Jun-16	20 Mile River	60.845	-148.989	0.706695	0.000019	0.111	10.66	1.607	1.738	0.194
12	2-Jun-16	Portage Creek	60.827	-148.977	0.706517	0.000036	0.040	6.785	0.440	0.634	0.245
12	2-Jun-16	Portage Creek	60.827	-148.977	0.706552	0.000035	0.042	7.072	0.481	0.995	0.263
12	2-Jun-16	Portage Creek	60.827	-148.977	0.706586	0.000027	0.040	6.796	0.455	1.125	0.070
13	2-Jun-16	Placer River	60.817	-148.988	0.706629	0.000031	0.072	9.055	0.624	0.788	0.215
13	2-Jun-16	Placer River	60.817	-148.988	0.706621	0.000028	0.075	9.468	0.667	0.798	0.249
13	2-Jun-16	Placer River	60.817	-148.988	0.706621	0.000025	0.075	9.349	0.677	1.208	0.310
15	2-Jun-16	Lower Cook Inlet	59.606	-151.436	0.709206	0.000028	6.884	515.4	>1,000	11,492	563.1

Appendix 2.4: Strontium isotope ratios ($^{87}Sr/^{86}Sr$; ±2 standard error) from individual growth layer groups. Identifiers are included for both National Marine Fisheries Service (NMFS ID) and University of Alaska, Museum of the North (UAMN ID).

NMFS ID	UAMN ID	Year of growth	Sex	$^{87}Sr/^{86}Sr$	±2SE
25-Sep-92		1968	male	0.70921	0.00002
25-Sep-92		1969	male	0.70919	0.00003
25-Sep-92		1970	male	0.70921	0.00003
25-Sep-92		1982	male	0.70921	0.00003
25-Sep-92		1983	male	0.70920	0.00006
7-Oct-96	67159	1976	male	0.70917	0.00001
7-Oct-96	67159	1981	male	0.70918	0.00001
7-Oct-96	67159	1986	male	0.70918	0.00002
7-Oct-96	67159	1990	male	0.70917	0.00002
13-Oct-01		1982	female	0.70915	0.00002
13-Oct-01		1985	female	0.70914	0.00002
13-Oct-01		1990	female	0.70915	0.00002
13-Oct-01		1992	female	0.70913	0.00002
13-Oct-01		1997	female	0.70910	0.00002
10-Oct-96	86886	1991	female	0.70917	0.00001
10-Oct-96	86886	1992	female	0.70916	0.00001
10-Oct-96	86886	1993	female	0.70916	0.00001
10-Oct-96	86886	1994	female	0.70917	0.00001
10-Oct-96	86886	1995	female	0.70915	0.00001
10-Oct-96	86886	1996	female	0.70915	0.00001
10-Oct-96	86886	1997	female	0.70915	0.00001
10-Oct-96	86886	1998	female	0.70914	0.00001
10-Oct-96	86886	1999	female	0.70915	0.00001
10-Oct-96	86886	2000	female	0.70915	0.00001
10-Oct-96	86886	2001	female	0.70915	0.00001
10-Oct-96	86886	2002	female	0.70914	0.00001
10-Oct-96	86886	2003	female	0.70913	0.00001
10-Oct-96	86886	2004	female	0.70915	0.00001
10-Oct-96	86886	2005	female	0.70914	0.00001
13-Oct-03	91731	1981	male	0.70919	0.00005
13-Oct-03	91731	1982	male	0.70917	0.00003
13-Oct-03	91731	1983	male	0.70918	0.00001
13-Oct-03	91731	1984	male	0.70918	0.00003
13-Oct-03	91731	1985	male	0.70918	0.00001
13-Oct-03	91731	1986	male	0.70918	0.00002
13-Oct-03	91731	1987	male	0.70918	0.00002
13-Oct-03	91731	1988	male	0.70920	0.00003
13-Oct-03	91731	1989	male	0.70920	0.00004
13-Oct-03	91731	1991	male	0.70919	0.00002
13-Oct-03	91731	1992	male	0.70919	0.00002

13-Oct-03	91731	1994	male	0.70918	0.00002
13-Oct-03	91731	1995	male	0.70919	0.00001
13-Oct-03	91731	1997	male	0.70919	0.00001
13-Oct-03	91731	2000	male	0.70916	0.00001

Appendix 2.5: Amino acid (phenylalanine) isotope analysis

To examine whether the stable nitrogen isotopic composition of the baseline has changed between the 1960s and 2000s we analyzed the stable nitrogen isotope composition of phenylalanine preserved in the collagen from beluga whale bone. Previous research has shown that the $\delta^{15}N$ value of phenylalanine retains a record of the $\delta^{15}N$ value at the base of the food chain with little fractionation (Chikaraishi et al. 2014, Yamaguchi and McCarthy 2017). In a few cases we had some collagen remaining from the bulk $\delta^{15}N$ analyses we had previously reported on during this research. We selected two individuals from early in the record (both died in 1964) and three individuals from late in the record (all died after 2000; Appendix 6). We prepared and analyzed the $\delta^{15}N$ of phenylalanine following previously published protocols (Yamaguchi and McCarthy 2017). We found that the mean $\delta^{15}N$ value of phenylalanine from beluga whales that died after 2000 (8.4‰, SE = 1.5) were lower than those that died during 1964 (13.6‰, SE = 0.3). Therefore, the $\delta^{15}N$ value of the base of the food chain appears to have changed over the duration of this record.

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Appendix 2.6: University of Alaska Museum of the North identifier, year beluga died, and $\delta^{15}N$ values of phenylalanine from bone collagen. Mean values for the two time periods are included with their associated standard error (SE).

UAMN ID	Year beluga died	Phenylalanine (‰)
11643	1964	13.8
11642	1964	13.3
	Mean (SE)	13.6 (0.3)
92569	2003	6.6
86886	2006	11.5
87979	2007	7.2
	Mean (SE)	8.4 (1.5)

Chapter 3: General Conclusions

Beluga whales (*Delphinapterus leucas*) in Cook Inlet (CIBW) have long been important to the region for subsistence, as a top level predator, and more recently, for tourism. While historically there were well over 1,000 individuals, recently the population has declined to around 300 individuals (NMFS 2016). The reason for the initial decline during the 1990s was identified as excessive subsistence harvest, mostly by hunters that traveled to Anchorage from other parts of Alaska (Mahoney and Sheldon 2000). CIBW numbers were expected to increase following a reduction in harvest, but the population continued to decline, was declared depleted in 2000 (NOAA 2000), listed as endangered under the Endangered Species Act in 2008 (NOAA 2008), and a recovery plan was finalized in 2016 (NMFS 2016). The continued decline in abundance following harvest reductions sparked concerns that other parameters might be preventing the population from recovering. The recovery plan identified potential threats to CIBW including a change in habitat or available prey (NMFS 2016). The largest change in CIBW ecology has been the contraction of their range into the upper reaches of Cook Inlet during the summer months (Rugh et al. 2010). Although the range contraction has been well documented, the reasons CIBW are using only a fraction of their historic summer range has not (NMFS 2016). Furthermore, understanding how the contracted range influences prey availability is unknown.

In Chapter 2 I used naturally occurring isotope ratios of carbon, nitrogen, and strontium to study the feeding ecology of CIBW over the last 50 years. Carbon and nitrogen stable isotope values (expressed as $\delta^{13}C$ and $\delta^{15}N$ respectively) of bone collagen from 20 individual CIBWs indicated that diet from the 1950s to 2007 changed as both $\delta^{13}C$ and $\delta^{15}N$ values declined. Because bone is continuously regenerating itself, the material that makes up bone reflects an average of diet over a long period of time (10 years to life; Clarke 2008). In order to analyze the changes over specific and known periods of time I used collagen from the dentin layers in teeth from 26 whales. Beluga teeth grow continually throughout life, laying down one light and one dark layer annually. These growth layer groups (GLGs) remain biochemically unchanged once formed and each GLG represents the diet for one year (Stewart et al. 2006, Luque et al. 2007); the specific year can be determined because the year of death is known. $\delta^{13}C$ and $\delta^{15}N$ values from the GLGs also indicated that beluga diet had changed over time, but the magnitude of change in $\delta^{13}C$ (3‰) was much greater (~7 times) than would be expected for a trophic level only change in $\delta^{15}N$ (~1 to 2‰; Fry 2008). Because $\delta^{13}C$ values are better at indicating changes in prey source or location, I wondered if the documented range contraction (Rugh et al. 2010) was influencing their feeding ecology (e.g., available prey, foraging location). CIBW in the upper Cook Inlet tend to concentrate in areas with substantial river discharge (e.g., Knik Arm, Susitna flats, Turnagain Arm) and by using strontium isotope ratios from tooth dentin I showed that the lower values of $\delta^{13}C$ were correlated with strontium ($^{87}Sr/^{86}Sr$ ratios). This indicates that CIBW feeding ecology has shifted towards a diet of prey with a greater freshwater influence. I then collected water samples from rivers and marine areas in the Cook Inlet watershed and attempted to determine which river(s) were the most important. Due to limited funding I was only able to show that declining $^{87}Sr/^{86}Sr$ ratios are consistent with a change to more freshwater influenced prey.

To further study these trends a more complete strontium isoscape (map of $^{87}Sr/^{86}Sr$ ratio changes across the waterscape) of the Cook Inlet watershed is necessary to compare $^{87}Sr/^{86}Sr$ ratios to the tooth dentin samples. For Chapter 2 I was only able to analyze GLGs from five CIBW teeth due to funding constraints, but sampling more teeth by demographic group (age, sex) may identify segments of the CIBW population that are using specific regions of Cook Inlet and which rivers are associated with the changes in CIBW feeding ecology. In order to continue this research I co-wrote a proposal that was funded as a three-year award (2017-2020) by the National Oceanic and Atmospheric Administration (NOAA) ESA Section 6 program. The research objectives are: 1) to develop a strontium isoscape for the Cook Inlet watershed; 2) strategically analyze GLGs from more CIBW for carbon, nitrogen, and strontium to allow for analysis by demographic group (sex, age); and 3) to analyze GLGs from beluga whales in Bristol Bay (a healthy, similarly non-migratory, stock of beluga whales) for comparison to the CIBW stock. Analyzing changes in feeding ecology (via changes in isotope ratios) by demographic group is critical to identifying which segments of the population have changed the most and when those changes occurred. This information must also be compared to a similar stock of beluga whales, like those found in Bristol Bay, to determine if these changes are unique to CIBW or if there have been widespread ecological changes in the Northern Pacific Ocean.

Although I have not identified what is preventing CIBW from recovering, I have documented, for the first time, a change in their feeding ecology over time and have ensured that additional research will continue to determine what changed, when the change occurred, and if the changes are related to CIBWs failure to recover.

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