

Are Expanding Pink Salmon (*Oncorhynchus gorbuscha***) Populations in the Arctic Produced from Regional Watersheds?**

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March 2024 Final Report OCS Study BOEM 2024-006 Contact Information: Email to uaf-cmi@alaska.edu CFOS Research Homepage at https://www.uaf.edu/cfos/research/cmi

This study was funded by the [University of Alaska Coastal Marine Ins](https://www.uaf.edu/cfos/research/cmi)titute and the U.S. Department of the Interior, Bureau of Ocean Energy Management Alaska OCS Region (cooperative agreement M20AC10013-00). This report, OCS Study BOEM 2024-006, is available electronically from https://www.boem.gov/akpubs

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Citation:

Gorman, K.B., M.K. Nims, T.L. Sformo, and P.S. Rand. 2024. Are Expanding Pink Salmon (*Oncorhynchus gorbuscha*) Populations in the Arctic Produced from Regional Watersheds? Final Report, OCS Study BOEM 2024-006, University of Alaska Coastal Marine Institute and USDOI, BOEM Alaska OCS Region.

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Abstract

The Arctic is experiencing rapid environmental changes, including increasing freshwater and marine temperatures and reductions in sea ice and regional snowpack. These environmental changes appear to be associated with the poleward expansion of sub-Arctic fish populations into the region. Pink salmon (*Oncorhynchus gorbuscha*), for example, are an important species in many coastal marine ecosystems of Alaska because of their abundance, influence on nutrient flux and trophic cascades, as well as their importance as a commercial and subsistence resource. The species has been increasingly observed in Alaskan Arctic watersheds although observations of the species in the Arctic date to the late 1800's. Whether Arctic pink salmon are produced locally or originate from outside the region remains an important, yet unresolved topic of debate. The objective of this project was to use otolith geochemistry to determine if adult pink salmon captured in the Arctic originated from local waters. Water and otolith samples collected in the Alaskan Arctic, northwest Alaska, and Prince William Sound in 2020 and 2021 were analyzed for ⁸⁷Sr/⁸⁶Sr isotopes, Sr/Ca and Ba/Ca ratios, as well as stable $\delta^{18}O$ and $\delta^{13}C$ isotopes. Based on water chemistry, ${}^{87}Sr/{}^{86}Sr$ was the most discriminative marker for determining the provenance of pink salmon in this study. Results indicate that the majority (65%) of adult pink salmon caught in the Alaskan Arctic likely did not originate from the region. Strontium isotopic ratios in the freshwater portion of the otolith were highly variable among individuals both within and across populations, reflecting life history diversity which is an important consideration in future geochemical studies of salmon otoliths. However, there was a small but notable portion of fish (35%) for which Arctic origin could not be ruled out. Thus, our results indicate that approximately one third of the fish sampled in this study may have originated in the Arctic, however, we were unable to definitively assign these fish to a particular watershed since the pink salmon otoliths analyzed in this study did not appear to fully equilibrate with local water. Multivariate analyses suggested that Arctic Alaska pink salmon populations were more variable in otolith core and early ocean $\delta^{18}O$ and $\delta^{13}C$ isotopic area than Prince William Sound pink salmon populations, supporting our conclusion that pink salmon collected in the Arctic are likely of multiple origins. Further, a hierarchical cluster analysis suggests some course-scale clustering of Arctic and Prince William Sound pink salmon separately, which we interpret to indicate that pink salmon sampled in Arctic watersheds are perhaps strays from some similar source population outside of the Arctic region. Refining tools to better understand the use of Arctic habitat by pink salmon is critical for developing mitigation and management measures.

1.0 Study Introduction

1.1 Background and Justification

Irvine et al. (2009) posed a series of key questions regarding responses by Arctic marine ecosystems to ongoing, rapid environmental changes - What is the potential of the Arctic Ocean, including the Chukchi and Beaufort seas for Pacific salmon? With climate change, will this area become a major salmon rearing environment? Will Arctic watersheds become important salmon producers? Answers to these questions are far from resolved, although the issues are now a focus of an increasing number of investigations and ongoing debate (Irvine et al. 2009, Moss et al. 2009, Dunmall et al. 2013, Nielsen et al. 2013, Wechter et al. 2017, Carothers et al. 2019). The Arctic has experienced increases in air temperatures on the order of 2-3 \degree C during summer, and 4 C during winter, since the 1950's (IPCC 2014) and similar warming trends are evident for marine waters (Huntington et al. 2020). Not surprisingly, increases in Arctic air and ocean temperatures, associated reductions in sea ice and regional snowpack, and increases in precipitation and the input of terrestrial organic matter into coastal ecosystems is expected to profoundly change Arctic coastal and marine pelagic ecosystems. The grand challenge for ecologists is to not only document these alterations, but also develop detailed understandings of these changing ecosystems to better predict responses by key trophic levels, in particular Pacific salmon (*Oncorhynchus* spp., hereafter salmon), under increasingly variable and complex environmental conditions. Additionally, detailed ecological knowledge of Arctic ecosystems can inform the management of human activities, and importantly help mitigate anthropogenic impacts.

Salmon are key species of interest in the context of a rapidly changing Arctic. Five species of Pacific salmon, Chinook (*O. tshawytscha*), sockeye (*O. nerka*), coho (*O. kisutch*), chum (*O. keta*), and pink salmon (*O. gorbuscha*) are the foundation of important commercial and sport fisheries, as well as subsistence harvest in lower latitude regions of Alaska and British Columbia, Canada including the Gulf of Alaska and the eastern Bering Sea (i.e., Bristol Bay, Yukon River) (Schindler et al. 2010, Murphy et al. 2017, Ruggerone and Irvine 2018) with some presence at higher latitudes in the Chukchi and Beaufort Seas (Bacon et al. 2011, Logerwell et al. 2015, Carothers et al. 2019). Thus, the economic importance of salmon at regional, national and international scales cannot be overstated, along with their cultural significance (Criddle and Shimizu 2014). Importantly, the extensive literature on salmon has shown that throughout their core range in the North Pacific Ocean, salmon populations predictably track thermal regimes and ocean productivity (Mantua 2015). Therefore, as Arctic coastal and marine habitats change in response to temperature increases, shifts in salmon species distributions and population establishment might be expected, as is generally predicted for ecological communities in response to climate change (Parmesan 2006, Pinsky et al. 2013). Irvine et al. (2009) reviewed the history of salmon presence in the Arctic, noting that although Arctic salmon abundance time series are rare, pink and chum salmon appear to have the broadest Arctic distributions having been documented from northeastern Siberia (Lena River) to the Mackenzie River (Northwest Territories, Irvine et al. 2009). In fact, small populations of chum salmon appear to be natal to the Mackenzie River, while both pink and chum salmon have been caught in rivers near Prudhoe Bay, Alaska including the Colville River (Irvine et al. 2009, see Figure 1). A recent working group at the National Center for Ecological Analysis and Synthesis (NCEAS, led by co-I Rand) focused on assembling data on the occurrence of pink salmon in the Arctic region (P. Rand,

Prince William Sound Science Center [PWSSC], unpubl. data). Two long term data sets were identified that provide reliable indicators of expansion of pink salmon in the Arctic: a time series of catches in a fyke net deployed in Prudhoe Bay (T. Sutton, University of Alaska Fairbanks [UAF], unpubl. data) and reporting from a community-based monitoring effort among subsistence harvesters in the western Canadian Arctic (K. Dunmall, Department of Fisheries and Oceans Canada, unpubl. data). Both time series indicate an increased presence of the species in Arctic waters (Figure 1), with a dominance in even years. The year 2008 (both series) and 2016 (Canadian series) were identified as years with anomalously high pink salmon presence. This NCEAS working group also identified increased presence of juvenile pink salmon in the northern Bering Sea, suggesting suitable habitat may be shifting northward (Farley et al. 2020). A recent ethnographic study in Utqiaġvik and Nuiqsut, Alaska reported that Elders and fisherman generally agreed that harvests of salmon have increased from the 1990s and 2000s, although some individuals interviewed expressed no trend, and even declining trends, in regional salmon abundance (Carothers et al. 2019). Importantly, Carothers et al. (2019) reported new streams for the presence of salmon, including spawning activity within and near the following river systems (west to east): Sagavanirtok (Sag), Itkillik, Chipp, Walakpa, Ketik, Utukok, Kokolik, Kukpowruk, Wulik, and Noatak rivers.

Figure 1: Catches of pink salmon in the Arctic region (P. Rand, unpubl. data). Prudhoe Bay data represent catches of pink salmon using a fyke net in coastal waters, data from the Canadian arctic represent pink salmon captured by subsistence harvesters.

The possible establishment of breeding populations of Pacific salmon in Arctic regions of Alaska presents new and fascinating socio-ecological issues for this rapidly changing region. In some areas, new salmon resources might bring novel economic and subsistence opportunities, while in other areas native fish species that hold important cultural and subsistence value might be outcompeted by the establishment of salmon populations. While fishing survey data and interviews of regional communities provide invaluable information on possible population expansions by salmon in the Alaskan Arctic, it is important to note that the adult salmon observed in the Arctic region may be strays that have originated from distant watersheds. Straying is an important strategy that salmon adopt to colonize new habitats (Quinn 2005), and thus their presence in Arctic waters does not necessarily indicate the existence of naturallyreproducing populations in local drainages. Determining the broad-scale origins of adult Pacific salmon observed in the Arctic is the key question addressed by this research project. We tackle this issue by taking advantage of the complete life history record captured by natural markers of geological variability and water temperature based on geochemical signatures (isotope ratios of $87\text{Sr}/86\text{Sr}$, $18\text{O}/16\text{O}$ and $13\text{C}/12\text{C}$, and element concentrations) in the otoliths of pink salmon, specifically.

1.2 Geochemical Rationale

The putative origins of pink salmon were determined by analysis of geochemical signatures in otoliths and water samples collected from rivers across broad regions including Arctic Alaska (otoliths and water samples), Northwestern Alaska (water samples), and Southcentral Alaska (otoliths and water samples). Otoliths are acellular, mineralized structures of the inner ear consisting of calcium carbonate (CaCO3) in a protein matrix. These structures grow sequentially, and are not reabsorbed, over the lifetime of a fish (Wurster et al. 2005), and therefore show predictable patterns that provide information on annual growth and age (Panella 1980). Otolith aragonite reflects elements and compounds in the ambient environment experienced by fish (Barnett-Johnson et al. 2008) and for this reason can provide potentially powerful information on the watershed origins of individuals. In addition to the structural components of calcium (Ca), carbon (C) and oxygen (O_2) , elements such as strontium (Sr) and barium (Ba) for which there is limited physiological regulation also accumulate in otoliths through branchial and dietary uptake. Because they are metabolically inert, the elemental and isotopic signatures incorporated into the structure of the otolith provide a permanent record of the environmental life history of each fish within a population (Trueman et al. 2012). Otolith geochemistry techniques can therefore offer a unique understanding of the more intractable aspects of Pacific salmon life histories. Clearly, pink and chum salmon are focal species for better understanding Arctic range expansions by salmon based on the literature (e.g., Irvine et al. 2009, Dunmall et al. 2013, Nielsen et al. 2013, Carothers et al. 2019). Importantly, geochemical gradients in the underlying regional geology (elaborated upon below) and marine ecosystems (Saupe et al. 1989, Belt et al. 2008) potentially used by Arctic-reared pink and chum salmon provide baselines to compare with otolith material. This is important given that pink and chum salmon are unique from other salmon species in that their early freshwater residency is reduced, thereby potentially limiting the otolith material useful for understanding watershed origins (Zimmerman et al. 2013). By examining the unique signatures in the otolith material formed during both the freshwater and early marine juvenile periods, our geochemical approach is important preliminary step at identifying the broad-scale region of origin (i.e., Arctic or elsewhere) for individual adult pink salmon returning to Artic coastal watersheds. Importantly, investigations such as the one described here will advance

knowledge regarding the utility of techniques such as otolith geochemistry as a potentially important tool for understanding broader issues as to whether and where salmon in the Arctic will be able to establish spawning populations. The study described here was designed as an initial assessment of the watershed origins of pink salmon appearing in the Arctic coastal freshwater environments of the US Outer Continental Shelf (OCS) in Alaska using geochemical signatures in the otoliths of pink salmon.

1.3 Relevance to BOEM

High latitude environments such as the Arctic are changing rapidly in response to climate perturbations, and these changes will have important implications for aquatic habitats and the fish species that depend on these ecosystems. Fish are an important resource in the Arctic because of their economic, cultural and ecological significance to local stakeholders, particularly for subsistence utilization. Although few fish populations in the Arctic region have been highly impacted by human activity, widespread resource development on the OCS in the Arctic has the potential to exacerbate changes in a species thermal and chemical environment.

Understanding the resilience of marine and freshwater habitats to support important fish species under climate stress and OCS development has relevance for fisheries science and management throughout the Alaskan Arctic. For example, projected losses of critical marine and freshwater habitat in the coming decades due to climate stress are expected to have profound effects on the distribution and abundance of species such as Pacific salmon, particularly in the southern part of their distribution. The present summer ocean thermal habitat of Chinook salmon is predicted to decline 40% or more by 2040 and more than double this amount by 2080 under various greenhouse gas emission scenarios (Abdul-Aziz et al. 2011). Similar temperature-driven changes have been projected for freshwater environments including more winter precipitation falling as rain than snow, earlier snowmelt runoff, and generally more extreme hydrologic episodes of drought and flooding (Crozier et al. 2008). By contrast, increasing marine and freshwater temperatures in the Arctic are facilitating movements by Pacific salmon into habitats that were previously unexploited, and this range expansion appears to be circumpolar (Carothers et al. 2019, Millane et al. 2019, Sandlund et al. 2019). The demographic consequences for Pacific salmon and native fish species potentially displaced by these changes are difficult to predict, as are the effects on subsistence harvests and the health and socioeconomic well-being of Arctic residents. This uncertainty underscores the need for studies to identify the responses of fish communities to climate variability and continued development of the OCS.

The Bureau of Ocean Energy Management (BOEM) has estimated that the Chukchi Sea and Beaufort Sea planning areas of the OCS contain nearly 25 billion barrels of recoverable oil and more than 100 trillion cubic feet of recoverable natural gas (BOEM 2017), or approximately 90% and 80% of the total oil and gas in the Alaska OCS, respectively. These planning areas border the documented spawning locations of pink and chum salmon throughout the region (see Carothers et al. 2019), creating significant potential for interactions between oil and gas development and fishery harvest by local stakeholders. Although subsistence fisheries have been traditionally dominated by native species including broad whitefish (*Coregonus nasus*), Arctic cisco (*C. autumnalis*), least cisco (*C. sardinella*), Arctic grayling (*Thymallus arcticus*), burbot (*Lota lota*), lake trout (*Salvelinus namaycush*) and dolly varden (*Salvelinus malma*), pink and chum salmon have in recent years become an increasingly important part of the total harvest, as

discussed previously (Irvine et al. 2009, Dunmall et al. 2013, Carothers et al. 2019), and will likely continue to do so along with other Pacific salmon if they establish self-sustaining populations in river systems draining into the waters of the Chukchi and Beaufort Seas. An added concern is that the range expansion of Pacific salmon into Arctic freshwater habitats may result in displacement of native fishes (see Lennox et al. 2023 for review), leading to even greater reliance on salmon species for subsistence harvest. Moreover, Pacific salmon produce large numbers of juvenile fish that are highly dependent on coastal waters to forage and grow before leaving to the open ocean to complete the marine phase of their life cycle. Environmental impacts to coastal marine habitats from oil and gas development could have significant negative consequences for early ocean survival and hence salmon population resilience. Juvenile salmon also serve as a forage base for other keystone species such as marine mammals and seabirds. Coupled with the likely northward movement of economically important groundfish (e.g. Pacific pollock *Gadus chalcogrammus*) into Arctic waters, these ecological changes will have broad and important implications for development activities in the OCS.

The research project described here focuses on pink salmon given recent observations of the presence of this species in Arctic coastal ecosystems (Figure 1), as well as populations from outside the Arctic that will serve as outgroups to resolve potentially confounding effects from the species life history. Broadly, this work addresses BOEM interests in Arctic marine biodiversity monitoring and studies of stressors that will improve understanding of cumulative effects on the Alaska OCS environment. We analyzed the geochemical signatures of otolith samples of pink salmon caught in coastal waters of the Beaufort and Chukchi Seas, and northern Gulf of Alaska (outgroup) to compare these signatures to water samples collected from these regional watersheds including the North Slope, Northwestern and Southcentral Alaska (Prince William Sound, PWS, region). Our main objective was to determine whether pink salmon harvested in OCS coastal areas of Arctic Alaska represent fish that have possibly originated from rivers along the North Slope, or from river systems elsewhere that function as potential source populations. Overall, this study is a preliminary assessment of the geochemical variation present in pink salmon harvested in coastal waters of the Alaskan Arctic. The project significantly leveraged participation of local communities in Utqiaġvik, Alaska; Alaska and Department of Fish & Game (ADF&G) field operations in Northwestern Alaska; and an industry supported field project in the PWS region to assist with otolith and water sample collections.

1.4 Study Objectives and Hypotheses

The objective of this project was to determine whether pink salmon that are captured as adults in coastal and fresh waters of the Alaskan Arctic originated from freshwater drainages of the broad Alaskan Arctic region. Although we focus here on only one species of Pacific salmon and are limited to a relatively small sample of individuals given budget constraints, this study provides an important first step in investigating whether these salmon are pioneers in an early stage of range expansion that could have broad implications for the future of this Arctic region. Our primary hypothesis is that the conditions for spawning in North Slope rivers are too cold, or are lacking in other attributes, therefore the adult pink salmon sampled in Arctic coastal waters of Alaska are likely individuals that have strayed from natural spawning populations possibly from western Alaska rivers, or elsewhere, with otolith geochemical signatures that do not closely reflect values from regional North Slope water samples. The specific hypotheses of our study include:

1. Pink salmon caught in coastal waters along the North Slope are of regional or non-regional origin.

2. Otolith core or freshwater region for ${}^{87}Sr/{}^{86}Sr$ and element/Ca ratios can be used as effective discriminatory markers to assign North Slope adult pink salmon to their region of origin. 3. δ^{18} O and δ^{13} C values can be used as discriminatory markers for assignment of North Slope adult pink salmon to their region of early marine residence.

2.0 Methods

2.1 Study Area and Sample Collection

The study area ranged from the Noatak River and Kobuk River drainages in the northwest near Kotzebue, Alaska to Kaktovik located in northeastern corner of Arctic Alaska (Figure 2), in addition to the PWS area in Southcentral Alaska that served as an outgroup for the Arctic study sites. These watershed systems were chosen as the study boundaries because they represent the nearest locations where Pacific salmon, and pink salmon specifically, are known to have naturally sustaining populations outside the Arctic. There is currently no specific evidence that the North Slope region has self-sustaining populations of pink salmon. The geology of the area is heterogeneous, which increases the likelihood that the chemical signatures present in the various river systems, and hence the fish that reside in these waters, can be distinguished from each other. For example, the Noatak and Kobuk rivers drain from sedimentary rocks that are of roughly 540-250 million years old (mya) (Paleozoic) and 250-65 mya (Mesozoic), respectively. Moving eastward, much of the North Slope is also underlain with Mesozoic sedimentary rock until reaching Prudhoe Bay and the Sag River where much younger Cenozoic (65 mya to present) sedimentary rocks appear. The Sag and other nearby rivers such as the Colville and Itkillik, however, also have their headwaters in the Paleozoic rocks of the Brooks Range, which adds another element of geologic heterogeneity. We hypothesized this geological variation would contribute to finding detectable geochemical signatures in stream water and potentially salmon reared from these river systems. This hypothesis is supported by previous investigations of the variation in water chemistry in relation to the lithology of the region. For example, Brennan et al. (2014) reported that the strontium isotopic ratio (${}^{87}Sr/{}^{86}Sr$) in rivers draining northward from the Brooks Range varied between ~0.708-0.715, whereas Sr concentrations ranged between 0.00035-0.00352 mmol. Lithology modeled estimates of $87\text{Sr}/86\text{Sr}$ from other North Slope rivers are reported by Bataille et al. (2014).

2.3 Field Collections of Water and Otolith Samples

Water samples from North Slope Alaska and Northwestern Alaska (Kotzebue) locations were collected from nine sites including Tusikvoak Lake, Camp-99, Topaguruk River, Atqasuk (Meade River), Nuiqsut/Niglig Channel (Colville River), "No Name", Kukpuk River, Kokolik River, and Kukpowruk River by co-I Sformo and others at North Slope Borough during July and August 2020 and October 2021. Two water samples were collected by ADF&G staff working on the Kobuk and Noatak Rivers in Northwestern Alaska during August 2020 and August 2021, respectively. Water samples from PWS were collected from two sites, Hartney Creek and Jackson Creek, by co-I Rand and others at Prince William Sound Science Center during July and August 2020. Two water samples were collected per site. Sample sites were selected to attain a

broad spatial representation of water chemistry throughout the North Slope, Northwestern Alaska and the PWS region (Figure 2). As the objective of this study was focused on determining the origin of pink salmon caught in the Arctic, specifically, this study focused on capturing geographic rather than temporal variation in tributary water chemistry throughout our study regions. Although Alaskan streams have been shown to demonstrate inter-annual variation in the isotope ratios of ⁸⁷Sr/⁸⁶Sr (Brennan et al. 2015), geochemical differences among streams are typically greater than seasonal differences (Barnett-Johnson et al. 2008, Hegg et al. 2013, Brennan et al. 2015).

Water samples were collected in acid-washed 60 mL perfluoroalkoxy alkane (PFA) bottles following the procedures described by Linley et al. (2016). After collection, water samples were refrigerated and shipped on cool packs to the Pacific Northwest National Laboratory (PNNL). Upon arrival at PNNL, water samples were acidified to 2% with Optima-grade nitric acid (Fisher Scientific).

Adult pink salmon from Arctic Alaska were caught by subsistence fishers during July and August of 2020 and 2021. Pink salmon were collected opportunistically in four different coastal marine locations throughout the North Slope of Alaska (Elson Lagoon, Camp-99, Kaktovik, Point Hope, see Figure 2). Elson Lagoon is a bay located on the North Slope, near Utqiaġvik (formerly Barrow), Alaska. The Elson Lagoon watershed is made up of many small streams, draining an area of approximately 580 km^2 (Rawlins 2021). The salinity of Elson Lagoon varies from 5 to 30 ppt, while water temperature ranges from -1 to 14°C (Sformo et al. 2019). These changes in temperature and salinity are driven by seasonal differences in runoff, contribution of sea ice melt, and mixing of sea water from the Beaufort and Chukchi Seas via wind-inducement movement (Sformo et al. 2019). Otoliths collected from Camp-99 were taken from fish caught in an unnamed tributary to the Inaru River, located 28 river miles upstream from Elson Lagoon. Otolith samples from Kaktovik were taken from pink salmon caught in a lagoon with similar conditions to Elson Lagoon. Otoliths from Point Hope were collected in the nearshore ocean on the southside of the city of Point Hope. Adult pink salmon from PWS were collected during July and August of 2020 from established pink salmon populations returning to Hartney and Jackson creeks (Figure 2) in PWS (Southcentral Alaska). Fish were collected at Hartney Creek using blocking seines and dipnets, while post-spawn carcasses were collected from the water or riverbank at Jackson Creek. Run size of PWS pink salmon was estimated to be between 50 – 142 million individuals between $2013 - 2015$, of which an estimated $56 - 86\%$ are of hatchery origin (Knudsen et al. 2021). Hartney Creek located on the east side of PWS is known as a primarily wild run, while pink salmon returning to Jackson Creek located in western PWS are comprised of both hatchery- and wild-origin fish due to the proximity of Jackson Creek to a number of pink salmon hatcheries in the region broadly distributed throughout western PWS (Knudsen et al. 2021). Importantly, in addition to spawning in freshwater, pink salmon in PWS are known to spawn in intertidal zones (Helle 1970). All collected otoliths were rinsed, dried, stored individually, and shipped to PNNL for geochemical analysis.

Figure 2. Otolith (orange circles, $n = 6$) and water (blue triangles, $n = 13$) sample collection locations throughout the North Slope, Northwestern, and Southcentral Alaska.

2.4 Water Sample Preparation and Analysis

Water samples were filtered (PFA, 1-2 μ m) and heated at 80°C until completely dry. To remove organic matter, samples were treated with ultra-high purity 15M nitric acid, dried completely, and then treated with 30% hydrogen peroxide. This procedure was carried out at least three times. Water samples were then re-suspended in 7M nitric acid and divided into aliquots for Sr isotopic and elemental analysis.

Cation column procedures to purify the Sr aliquot for isotopic analyses were done using Eichrom Sr Spec resin, following the procedures described by Dunnigan et al. (2023). To prevent contamination, sample preparation and column chemistry procedures were performed under a class 100 laminar flow hood.

Water samples were analyzed for ⁸⁷Sr/⁸⁶Sr using multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS, NuPlasma 3, Nu Instruments). The Sr carbonate isotopic standard reference material SRM 987 (National Institute of Standards and Technology [NIST]) was analyzed prior to and throughout the analysis to assess instrument performance. Replicate analyses of SRM 987 yielded a mean value of 0.71025+0.00001 (*n* = 8), compared to the literature reported value of ${}^{87}Sr/{}^{86}Sr = 0.71025$ (GeoRem 2022).

Water samples for elemental concentrations were diluted to appropriate concentrations for analysis using 2% nitric acid. Samples were analyzed quantitatively for ${}^{43}Ca$, ${}^{88}Sr$, and ${}^{137}Ba$ using a Thermo Scientific X-Series II quadrupole inductively couple plasma mass spectrometer (Q-ICP-MS). An internal standard solution of indium was added in-line to samples prior to introduction into the instrument to monitor instrument stability. The instrument was calibrated using an external standard calibration curve (standard concentrations ranging from 0.05 – 10 parts per billion; High-Purity Standards Corporation). A series of calibration and blank verification checks were analyzed after the initial instrument calibration and after every 10 samples. All measured calibration verifications were within +10% of their known concentrations, while all blank verifications were below calculated detection limits.

Water δ^{18} O was determined from samples run in duplicate on a Los Gatos Liquid Water Isotope Analyzer (San Jose, CA). Each set of $n = 4$ samples was bracketed by three Los Gatos Research standards (δ^{18} Ovsmow = -19.49, -16.24, and -13.39‰) calibrated to Vienna Standard Mean Ocean Water (δ^{18} Ovsmow = 0‰) and Standard Light Antarctic Precipitation (δ^{18} Ovsmow = -55.5‰) to calculate water $\delta^{18}O$. Analytical precision for all samples and standards was $\leq 0.1\%$.

Dissolved inorganic carbon in water $(\delta^{13}C_{\text{DIC}})$ was measured using the same procedure employed for otoliths (see below) except that 1.5 mL of sample water was added to each 15 mL glass vial and mixed with 0.2 mL of 85% H₃PO₄ to elute CO₂ for analysis by IRMS. Samples of $n = 10$ for δ^{13} C_{DIC} were standard corrected by bracketed analysis of two in-house sodium bicarbonate standards, which had been previously calibrated against NBS-19. Analytical precision for all samples and standards was ≤ 0.1‰.

Instrument isotope values for otolith and water δ^{18} O were measured relative to VSMOW and converted to Vienna Pee Dee Belemnite (VPDB) according to Coplen et al. (1983).

 δ^{18} Ovsmow = 1.03091 x δ^{18} Ovens + 30.91 (Equation 1)

2.5 Otolith Preparation and Analysis

2.5.1 Laser ablation

In preparation for laser ablation analysis, otoliths were first mounted on glass sides using thermoplastic glue (Crystalbond 509). Due to their small size, otoliths did not need to be sectioned. Otoliths were polished on both sides, in the sagittal plane, until the core was visible. Silicon carbide sanding paper of successively finer grit (400 – 1200 grit, Allied High-Tech Products) was used in the polishing process.

Otoliths were analyzed via laser ablation by using a NWR 213nm Nd:YAG laser (Electro Scientific Industries, Inc.) coupled to the ICP-MS instruments previously described above for their respective analyses. Prior to analysis, a low-powered laser pulse (5% power) was used as a cleaning pass across the otolith to remove potential surface contamination prior to analysis

(Gover et al. 2014). Laser analysis transects were laid from outside the freshwater zone (early ocean) of the otolith, passing directly through the core, to outside the freshwater zone on the opposite side, perpendicular to the sulcus. Parameters for laser ablation are as follows - spot size: 40 µm; power output: 50%; scan speed: 3 µm/second; repetition rate: 5 Hz. For both isotopic and elemental analysis, the ablated material was carried from the laser to the ICP-MS using a flow of argon carrier gas at 500 mL/min. Sample washout was monitored between each otolith sample to ensure that signals had returned to background levels before proceeding with subsequent analyses.

For Sr isotopic analyses, the MC-ICP-MS was tuned using a marine coral standard to ensure that the ${}^{87}Sr/{}^{86}Sr$ value of modern seawater $({}^{87}Sr/{}^{86}Sr = 0.70918)$ (Elderfield 1986) was obtained within 2 standard errors $({}^{87}Sr/{}^{86}Sr = 0.70916 + 0.00003$, $n = 21$). The same coral standard was analyzed after every 10-15 samples to monitor instrument drift. To correct for the isobaric interference of Krypton-84 (84 Rb) with Sr-84 (84 Sr), on-peak zeroes were measured and subtracted prior to each run. A 86 Rb correction was also applied to the 86 Sr signal by measuring the ${}^{85}Rb$ signal and calculating the corresponding ${}^{86}Rb$ value assuming natural Rb isotopic composition. To account for mass fractionation, an exponential correction factor (Equation 2, Russell et al. 1978) was obtained from the measured ⁸⁶Sr/⁸⁸Sr based on its variation from the accepted value of 0.1194. This correction factor was then applied to the $87\text{Sr}/86\text{Sr}$ ratio.

True Ratio = Measured Ratio *
$$
\left(\frac{Atomic Mass of X}{Atomic Mass of Y}\right)^{Fractionation Factor}
$$
 (Equation 2)

For elemental analysis, an external calibration curve was constructed using two silicate glass standard reference materials (SRM 612 and 610; NIST). Known values for reference materials were taken from (Pearce et al. 1997). Samples were analyzed quantitatively for ^{43}Ca , ^{88}Sr , and 137 Ba. All data was background subtracted by running blanks prior to analysis. The instrument was re-calibrated each time that the laser chamber was opened to change samples ($n = 15$). Otolith elemental concentration data was converted from element to calcium molar ratios for reporting.

2.5.2 Micromilling

Each otolith was visually inspected for the presence of translucent growth suggesting the potential inclusion of vaterite, and these were excluded from the analysis. The remaining otolith (after laser ablation) from each individual was polished as previously described and the core and early ocean portion was removed using a micromill (New Wave Research, Fremont, CA). The core and early ocean regions of the otoliths were determined by visual inspection as the core is discernable under a microscope and the early ocean region is that area of the otolith just outside the core. Milled material was placed into a gas-tight 15 mL glass vial with a chlorobutyl septum (Labco Exetainer, Lampeter, Wales) and flushed for 15 minutes using ultra-high purity helium at a flow rate of 100 mL min⁻¹. After flushing, 0.2 mL of 100% phosphoric acid (H₃PO₄) was added to each vial and allowed to incubate for a minimum of 3 hours at 70 °C before analysis. The resulting CO₂ was introduced into a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS; Bremen, Germany) using a Thermo Gas Bench II to determine otolith $\delta^{18}O$ and $\delta^{13}C$. Otolith samples of $n = 6$ were standard corrected by bracketed analyses of the international

carbonate standard NBS-19 (δ^{18} OvPDB = -2.20‰, δ^{13} CvPDB = +1.95‰). An in-house calcium carbonate standard was also analyzed to validate the isotopic correction using NBS-19.

All otolith and water isotope measurements were expressed in conventional δ notation as

$$
\delta = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 1000 \, (\%_0)
$$
 (Equation 3)

where R is the ratio of ${}^{18}O/{}^{16}O$ or ${}^{13}C/{}^{12}C$ in the sample or standard. For this study, both $\delta {}^{18}O$ and δ^{13} C are reported relative to the international standard Vienna Pee Dee Belemnite (VPDB).

2.6 Identifying Otolith Core and Freshwater Regions for Laser Ablation Data

Otolith data were smoothed using a 7-point moving average to reduce variation in signal due to the laser ablation sampling method. Core and freshwater regions in the otolith were first identified on ⁸⁷Sr/⁸⁶Sr transects and then the corresponding portion in the Ba/Ca and Sr/Ca transect was extracted. To identify these otolith regions, ${}^{87}Sr/{}^{86}Sr$ transects from laser ablation analyses were first plotted in relation to distance from the core. The core region in most otoliths was easily identifiable for strontium isotopic signatures due to its proximity to the global marine $87Sr/86Sr$ value (0.70918). The beginning of the freshwater transect of the otolith was identified as a significant departure (either positive or negative) from the core value. The end of the freshwater transect was identified as a return to the global marine ${}^{87}Sr/{}^{86}Sr$ value. Once the freshwater portion of the otolith was established, the maximum (or minimum) freshwater $87Sr/86Sr$ value was identified and a 10-point average around this point was taken and reported as the freshwater ${}^{87}Sr/{}^{86}Sr$ value for each individual fish.

2.7 Statistical Analyses

To describe variation in water chemistry parameters, i.e., ${}^{87}\text{Sr/}^{86}\text{Sr}$ and elemental ratios (Br/Ca and Sr/Ca) and $\delta^{18}O$ and $\delta^{13}C_{\text{DIC}}$ isotopes, across collection locations (North Slope, Northwestern Alaska including the Noatak and Kobuk rivers only, and PWS), linear regression (i.e., ANOVA) was employed to determine significant differences (α < 0.05) among locations for each water parameter, as well as a combined PC score including all significant water parameters. Analyses were conducted using the R language environment (R Core Team 2023).

To describe variation in otolith geochemistry, i.e., ⁸⁷Sr/⁸⁶Sr and elemental ratios (Br/Ca and Sr/Ca) of otolith core and freshwater (FW) regions, and $\delta^{18}O$ and $\delta^{13}C$ isotopes of otolith core and early ocean (EO) regions, two analyses were performed. Linear regression (i.e., ANOVA) was first used to determine significant differences between the otolith core and FW regions for 87 Sr/ 86 Sr and elemental ratios, and between otolith core and EO regions for δ^{18} O and δ^{13} C isotope metrics (Otolith Analysis 1). Linear regression (i.e., ANOVA) was also employed to determine significant differences (α < 0.05) for each otolith geochemical metric among all six collection locations (Otolith Analysis 2). We predicted that $\frac{87}{3}$ Sr/86Sr values of otolith core across all collection sites would reflect the global marine value of $87\text{Sr}/86\text{Sr}$ (0.70918) due to maternal influence, and therefore is unlikely to discriminate fish among collection locations. This was our general prediction as well for otolith core elemental ratios. We predicted that $87\text{Sr}/86\text{Sr}$ and

elemental ratios of the otolith FW region, and core $\delta^{18}O$ and $\delta^{13}C$ values, would likely be more discriminatory among collection locations (see also details in the Discussion) given that these metrics should be more reflective of regional geology, and the isotopic composition and temperature of stream water (see Discussion details). Differences (or lack thereof) between $87Sr/86Sr$ and elemental ratios of otolith core versus FW regions can indicate the level of departure of the core values from marine (maternal) values suggesting some level of equilibration with local water condition.

Additionally, isotopic area of $\delta^{18}O$ and $\delta^{13}C$ for core and early ocean regions of pink salmon otoliths was examined Bayesian functions within the SIBER package (Jackson et al. 2011) using the R language environment (R Core Team 2023) (Otolith Analysis 3). Within a Bayesian framework, SIBER functions model probability distributions for stable isotope ellipse area by incorporating sources of variability such as that within the originally derived means of collection area groupings. Bayesian models ran for 2 million iterations with a burn-in of 50,000 and was thinned by 15, resulting in 130,000 posterior draws. Finally, a hierarchical clustering analysis was performed using all geochemical metrics combined ($87\text{Sr}/86\text{Sr}$, Br/Ca, Sr/Ca, $\delta^{18}O$ and δ^{13} C_{DIC}) using the hclust function in R with average link and Euclidean distance methods. This approach builds a hierarchy of clusters from data more similar to each other in multivariate space, which in this case, was based on otolith geochemical signatures. For this analysis, the geochemical data from otolith FW regions for $\frac{87}{5}$ Sr/86Sr isotope and elemental ratios of Br/Ca and Sr/Ca was combined with $\delta^{18}O$ and $\delta^{13}C$ isotope values from the otolith core as these metrics all reflect aspects of the environment during freshwater residency. This approach allowed for testing the hypothesis that pink salmon collected from North Slope Alaska locations would not cluster into one specific group, in particular if North Slope pink salmon were strays originating from multiple areas outside the North Slope region (Otolith Analysis 4).

3.0 Results

3.1 Water Chemistry

Results for water values of ⁸⁷Sr/⁸⁶Sr ratios, the elemental concentrations of Br/Ca and Sr/Ca, and δ^{18} O and δ^{13} C_{DIC} isotopes at each sampling location are presented in Table 1. ANOVA models revealed that ⁸⁷Sr^{/86}Sr values were discriminatory of each region ($p = 0.0003$, Adjusted R² = 0.77) with mean ${}^{87}Sr/{}^{86}Sr$ values for each region: North Slope (0.71074), Northwestern Alaska (0.71358), and Prince William Sound (0.70722, see Figure 3). Similarly, results for Br/Ca elemental ratios discriminated the North Slope region from Northwestern Alaska and PWS, which were similar ($p = 0.03$, Adjusted R² = 0.37) with mean Br/Ca values for each region: North Slope (1.001), Northwestern Alaska (0.3015), and Prince William Sound (0.3095, Figure 3). ANOVA results for Sr/Ca, and $\delta^{18}O$ and $\delta^{13}C_{\text{DIC}}$ isotopes of water samples were not significant, indicating that these water parameters were not specific to each region considered (Figure 3). The PC1 score including both ${}^{87}Sr/{}^{86}Sr$ and Br/Ca values was also significant (*p* = 0.02, Adjusted $R^2 = 0.48$), but didn't provide any further resolution than the Br/Ca model (Figure 3). Importantly, water $87Sr/86Sr$ values from this study are in agreement with the regional trends in Sr isotopic composition for Alaskan rivers as reported by Brennan et al. (2014) (Figure 4). Overall, ⁸⁷Sr/⁸⁶Sr values of freshwater were the best predictor of each region across all Sr isotope and elemental concentration parameters. However, Ba/Ca values of North Slope water had a much broader range in comparison with Northwestern Alaska and PWS, meaning that Ba/Ca

values could potentially be used to determine if pink salmon are of North Slope origin if fish have otolith Ba/Ca signatures well outside the range of PWS water.

Region	Site	Lat	Long	${}^{87}Sr/{}^{86}Sr$ $+$	Ba/Ca	Sr/Ca	$\overline{\delta}^{18}O$ (‰,	$\delta^{13}C_{\text{DIC}}$ (‰,
				1SE	$(mmol/mol)$ ±	$(mmol/mol)$ ±	VSMOW) \pm	VPDB) \pm
					1 SD	1 SD	1 SD	1 SD
North Slope	Tusikvoak Lake	71.1255	-156.18	$0.711189 \pm$	1.64 ± 0.013	2.50 ± 0.007	-11.52 ± 0.295	-2.6 ± 0.08
				4.79E-05				
North Slope	Camp-99	70.94694	-156.6475	$0.710321 \pm$	1.43 ± 0.024	2.37 ± 0.019	-10.87 ± 0.10	-2.67 ± 0.42
				7.86E-06				
North Slope	Topaguruk	$\overline{70.756193}$	-155.92908	$0.710112 \pm$	0.935 ± 0.026	1.61 ± 0.026	-13.78 ± 0.03	-4.07 ± 0.58
	River			8.79E-06				
North Slope	Atqasuk	70.479694	-157.40593	$0.711234 \pm$	1.04 ± 0.023	1.17 ± 0.037	-17.02 ± 0.654	-9.70 ± 0.088
	(Meade River)			1.09E-05				
North Slope	Nuiqsut	70.22165	-150.99211	$0.711194 \pm$	1.08 ± 0.038	1.57 ± 0.026	-20.11 ± 0.595	-8.80 ± 0.056
	(Colville River)			9.78E-06				
North Slope	Kokolik River	69.74905	-162.93388	$0.709300 \pm$	0.526 ± 0.011	7.94 ± 0.099	-11.45 ± 0.16	-3.30 ± 0.71
				8.86E-06				
North Slope	Kukpowruk	69.615593	-163.0152	$0.709932 \pm$	0.505 ± 0.010	3.66 ± 0.039	-15.74 ± 0.19	-1.06 ± 0.59
	River			8.78E-06				
North Slope	No Name	69.514075	-154.779	$0.712851 \pm$	1.32 ± 0.003	2.50 ± 0.007	-16.96 ± 0.496	-9.2 ± 0.1
				5.30E-05				
North Slope	Kukpuk River	68.38426	-166.29838	$0.710518 \pm$	0.542 ± 0.013	3.39 ± 0.056	-14.26 ± 0.296	-9.00 ± 0.016
				9.36E-06				
Northwestern	Noatak River	67.166018	-162.58976	$0.712859 \pm$	0.398 ± 0.004	1.58 ± 0.0090	-16.60 ± 0.476	-7.5 ± 0.004
Alaska				7.40E-06				
Northwestern	Kobuk River	66.961158	-160.45951	$0.714305 \pm$	0.205 ± 0.013	2.19 ± 0.032	-16.85 ± 0.15	-3.15 ± 0.85
Alaska				8.12E-06				
Prince William	Hartney Creek	60.502129	-145.86033	$0.707002 \pm$	0.339 ± 0.002	6.18 ± 0.242	-12.70 ± 0.18	-7.26 ± 0.14
Sound				7.33E-06				
Prince William	Jackson Creek	60.324029	-148.27864	$0.707446 \pm$	0.340 ± 0.027	2.47 ± 0.120	-13.64 ± 0.03	-7.24 ± 0.14
Sound				8.27E-06				

Table 1: Location and values of 87 Sr/ 86 Sr isotopes, elemental ratios (mmol/mol), $\delta^{18}O$, and δ^{13} CDIC for water samples collected from North Slope Alaska, Northwestern Alaska (Noatak and Kobuk rivers only), and Prince William Sound.

Figure 3: Mean $(\pm 1 \text{ SE})$ water chemistry values for the North Slope, Northwestern (NW), and Prince William Sound regions of Alaska. ANOVA model results are shown. NW Alaska includes water data for the Noatak and Kobuk rivers, while PWS includes water data for Hartney and Jackson creeks.

Figure 4: The ⁸⁷Sr/⁸⁶Sr of Alaskan Rivers as reported in Brennan et al. (2014; circles) and this study (triangles). The water ${}^{87}Sr/{}^{86}Sr$ results from this study are in general agreement with the Sr isotopic data for AK rivers previously published and follow the general trends in Alaskan bedrock geology.

3.2 Otolith Chemistry: ⁸⁷Sr/⁸⁶Sr (Otolith Analyses 1 and 2)

3.2.1 ⁸⁷Sr/⁸⁶Sr Otolith Analysis 1

Otolith data are summarized in Tables 2 and 3. There were significant differences between the core and freshwater portions of pink salmon otoliths for $87Sr/\sqrt{86}Sr$ values among fish sampled throughout the North Slope, but not among pink salmon sampled in PWS (Figure 5). For North Slope pink salmon, the freshwater portion of the otolith had significantly lower ${}^{87}Sr/{}^{86}Sr$ values than the otolith core, while both otolith core and freshwater $87\text{Sr}/86\text{Sr}$ values were lower than the global marine ${}^{87}Sr/{}^{86}Sr$ value of seawater (0.70918).

Region	Site	Lat	Long	${}^{87}Sr/{}^{86}Sr$	${}^{87}Sr/{}^{86}Sr$	${}^{87}Sr/{}^{86}Sr$	Ba/Ca	Ba/Ca	Ba/Ca	Sr/Ca	Sr/Ca	Sr/Ca
				Sample	$±1$ SD	±1 SD	Sample	$(\mu$ mol/mol)	$(\mu$ mol/mol)	Sample	(mmol/mol)	(mmol/mol)
				Size	(Core)	(FW)	Size	±1 SD	±1 SD	Size	±1 SD	\pm 1 SD
								(Core)	(FW)		(Core)	(FW)
North	Elson	71.300228	-156.23717	83	0.70894	0.70874	58	8.27 ± 4.26	12.7 ± 6.91	58	2.22 ± 0.44	1.99 ± 0.43
Slope	Lagoon				± 0.0004	± 0.0007						
North	Camp-99	70.94694	-156.6475	14	0.70918	0.70910	6	6.98 ± 2.75	10.1 ± 4.44	6	1.91 ± 0.21	1.59 ± 0.21
Slope					± 0.0004	± 0.0008						
North	Kaktovik	70.127993	-143.57982	5	0.70875	0.70861	5	7.88 ± 1.59	7.51 ± 4.33	5	2.50 ± 0.93	2.35 ± 0.98
Slope					± 0.0004	± 0.0004						
North	Point	68.413884	-166.3815	14	0.70852	0.70816	14	22.5 ± 12.11	16.9 ± 15.4	14	3.97 ± 0.84	3.74 ± 0.95
Slope	Hope				± 0.0003	± 0.0007						
Prince	Hartney	60.502129	-145.86033	18	0.70900	0.70894	13	31.9 ± 18.7	35.0 ± 20.0	13	2.26 ± 0.27	2.32 ± 0.41
William	Creek				± 0.0005	± 0.0008						
Sound												
Prince	Jackson	60.324029	-148.27864	19	0.70918	0.70920	14	2.44 ± 14.0	29.1 ± 13.4	14	2.44 ± 0.41	2.30 ± 0.24
William	Creek				± 0.0006	± 0.0004						
Sound												

Table 2: Otolith core and freshwater (FW) values for ⁸⁷Sr/⁸⁶Sr isotope and elemental ratios of otoliths from adult pink salmon captured from the North Slope and Prince William Sound regions of Alaska.

Region	Site	$\delta^{18}O$	$\delta^{18}O$ (%0,	$\delta^{18}O$ (‰,	$\delta^{13}C_{\text{DIC}}$	$\delta^{13}C_{\text{DIC}}$ (‰,	$\delta^{13}C_{\text{DIC}}$ (‰,
		Sample	VSMOW)	VSMOW)	Sample	VPDB)	VPDB)
		Size	$±1$ SE	\pm 1 SE	Size	\pm 1 SE	$±1$ SE
			(Core)	(EO)		(Core)	(EO)
North	Elson	70	-7.47 ± 2.44	-2.23 ± 3.23	70	-7.57 ± 1.54	-5.65 ± 1.50
Slope	Lagoon						
North	Camp-99	10	-5.14 ± 3.27	-1.79 ± 1.47	10	-7.52 ± 1.59	-5.12 ± 1.20
Slope							
North	Kaktovik	5	-13.18 ± 2.38	-8.30 ± 4.50	5	-7.03 ± 1.47	-6.42 ± 2.80
Slope							
North	Point	13	-6.02 ± 4.43	-3.23 ± 3.76	13	-7.65 ± 2.22	-6.98 ± 2.18
Slope	Hope						
Prince	Hartney	9	-6.31 ± 1.76	-3.94 ± 1.51	9	-7.08 ± 0.70	-5.83 ± 0.50
William	Creek						
Sound							
Prince	Jackson	18	-7.27 ± 1.10	-3.13 ± 2.16	18	-6.51 ± 1.03	-5.73 ± 1.01
William	Creek						
Sound							

Table 3: Otolith core and early ocean (EO) values for $\delta^{18}O$ and $\delta^{13}C_{\text{DIC}}$ isotopes of otoliths from adult pink salmon captured from the North Slope and Prince William Sound regions of Alaska.

The significant difference between otolith core and freshwater $87Sr/86Sr$ values for North Slope pink salmon (Figure 5) was primarily driven by differences among Elson Lagoon fish, as this was the only population for which this difference was significant, all other populations (i.e., Camp-99, Kaktovik, and Point Hope) were not different in their otolith core and freshwater $87Sr/86Sr$ values ($p > 0.05$, Figure 6, see also Table 2). However, small sample sizes may have contributed to the lack of difference in ⁸⁷Sr/⁸⁶Sr values between otolith regions as the Point Hope population had a nearly significant result ($p = 0.07$) with the freshwater portion also having lower $87\text{Sr}/86\text{Sr}$ values than the otolith core, similar to Elson Lagoon fish (Figure 6).

The right panels in Figures 5 and 6 show the change in $87\text{Sr}/86\text{Sr}$ values for individual fish as the data were paired observations for each region of the otolith (core, freshwater). A majority of North Slope pink salmon tended to have lower freshwater ⁸⁷Sr/⁸⁶Sr values than the otolith core, while a smaller number of fish had higher freshwater ${}^{87}Sr/{}^{86}Sr$ values than the otolith core. The pink salmon sampled from PWS did not vary in their ⁸⁷Sr/86Sr values between the otolith core and freshwater portion (Figure 5). In fact, for both regions of the otolith for PWS fish, mean values were not significantly different from the global marine value for ${}^{87}Sr/{}^{86}Sr$ (see also Figure 7). While some individual pink salmon sampled from PWS had lower or higher ⁸⁷Sr/⁸⁶Sr values for the freshwater portion of the otolith, many individuals did not change $87\text{Sr}/86\text{Sr}$ values between the core and freshwater portion (PWS right panel, Figures 5 and 7).

Strontium isotopic analysis of pink salmon otoliths from North Slope Alaska locations demonstrated two general trends (Figure 8): fish that had $87\text{Sr}/86\text{Sr}$ otolith values higher than the global marine value (0.70918) outside of the core (i.e., in the freshwater portion of the otolith) and fish that had otolith values lower than the global marine value outside of the core. Given that the mean North Slope water ${}^{87}Sr/{}^{86}Sr$ (0.711256 \pm 1.64E-5, Table 1) is well above the global marine value, this indicates that fish from North Slope Alaska locations that did not have freshwater otolith values greater than 0.70918 likely did not originate in the Arctic. However, for fish from North Slope Alaska locations that had freshwater otolith values greater than the global marine strontium isotopic value, Arctic origin cannot be ruled out as it appears this fish may be equilibrating to ${}^{87}Sr/{}^{86}Sr$ values that approach that of the North Slope region. Thus, the majority of North Slope fish examined in this study (65%) were likely not of Arctic origin (Figure 5). Further, none of the fish captured at Kaktovik or Point Hope appear to be of Arctic origin as none of these fish had freshwater otolith ${}^{87}Sr/{}^{86}Sr$ values above the global marine value (Figure 6). However, for 35% of the fish examined in this study, Arctic origin cannot be ruled out given freshwater otolith ⁸⁷Sr/⁸⁶Sr values above the global marine value (Figure 5). Some fish captured at both Elson Lagoon and Camp-99 had freshwater otolith ⁸⁷Sr/⁸⁶Sr values that were higher than the global marine $87\text{Sr}/86\text{Sr}$ value (Figure 6).

PWS pink salmon otoliths revealed two distinct general life history trends (Figure 9). First, some individuals had otolith $87\text{Sr}/86\text{Sr}$ values that never fell below the global marine strontium isotopic value (0.70918). As the water ⁸⁷Sr/⁸⁶Sr of PWS locations in this study had a lower mean value of $0.707224 \pm 7.8E-6$ than seawater, this result suggests that these individuals may have spawned in an intertidal location (if the freshwater portion of the otolith is equal to the core values and both approximate the marine signature) or possibly an unknown hatchery location (possibly if the freshwater portion of the otolith is higher than the marine signature). However, there was another group of individuals that did demonstrate a clear downward trend in $87\text{Sr}/86\text{Sr}$ in the freshwater portion of the otolith, suggesting some equilibration with local PWS water.

Figure 5: Variation in pink salmon otolith core and freshwater (FW) ⁸⁷Sr^{/86}Sr values for adults sampled from the North Slope and Prince William Sound region (left panels show mean ± 1 SE values). The panels on the right show the individual change in ⁸⁷Sr/⁸⁶Sr values between the otolith core and freshwater region.

Figure 6: Variation in pink salmon otolith core and freshwater (FW) ⁸⁷Sr/⁸⁶Sr values for adults sampled from specific North Slope locations (left panels show mean ± 1 SE values). The panels on the right show the individual change in ${}^{87}Sr/{}^{86}Sr$ values between the otolith core and freshwater region.

Figure 7: Variation in pink salmon otolith core and freshwater (FW) ⁸⁷Sr/⁸⁶Sr values for adults sampled from specific Prince William Sound locations (left panels show mean ± 1 SE values). The panels on the right show the individual change in $87\text{Sr}/86\text{Sr}$ values between the otolith core and freshwater region.

Figure 8: The ⁸⁷Sr/⁸⁶Sr otolith transects of representative fish from Camp-99 (in blue) and Elson Lagoon (in orange). The ${}^{87}Sr/{}^{86}Sr$ of seawater (0.70918) is indicated by the black dashed line. The location of the otolith core, as well as the freshwater and marine portions, are noted in the figure. Towards the end of both transects, the ${}^{87}Sr/{}^{86}Sr$ values approach the global marine value, reflecting the migration of this fish to the ocean after emergence. The average $87\text{Sr}/86\text{Sr}$ for Northern Alaska water samples from this study was $0.711256 \pm 1.64E$ -5. Therefore, the Elson Lagoon fish is likely not of Arctic origin because the otolith $87Sr/86Sr$ signature decreases after the core. However, the otolith ⁸⁷Sr/⁸⁶Sr of the Camp-99 fish increases after the core, suggesting that Arctic origin cannot be ruled out for this individual.

Figure 9: The ⁸⁷Sr/⁸⁶Sr otolith transect of two different fish from the reference pink salmon population in Hartney Creek, Prince William Sound (PWS). The ${}^{87}Sr/{}^{86}Sr$ of seawater (0.70918) is indicated by the black dashed line. The location of the core and freshwater portion of the otolith are noted in the figure. These individual fish are representative of the two different life history trends observed in PWS pink salmon otoliths. The $87Sr/86Sr$ green transect never falls below the global marine strontium isotopic value after the core, indicating that this fish may have spawned in an intertidal location. The ${}^{87}Sr/{}^{86}Sr$ dark grey transect does fall below the global marine strontium isotopic value after the core, trending in the direction of the freshwater value of Hartney Creek (${}^{87}Sr/{}^{86}Sr = 0.707002$). Although this second fish indicates a clear departure from the core for the freshwater portion of the otolith in the downward direction, the otolith value does not reach equilibrium with water from Hartney Creek.

Although fish that displayed this second life history pattern do have a clear departure from the core towards lower values, the otolith value does not reach equilibrium with water from Hartney Creek $({}^{87}Sr/{}^{86}Sr = 0.707002$, Table 1) suggesting that while otolith microchemistry can be used successfully to examine the origins of pink salmon on a regional scale, it is unlikely that the exact rivers that fish originate from can be identified using strontium isotopes alone, at least for PWS.

3.2.2 ⁸⁷Sr/⁸⁶Sr Otolith Analysis 2

Otolith freshwater ⁸⁷Sr/⁸⁶Sr values were significantly variable across collection locations, with Point Hope and Jackson Creek having the lowest and highest mean values, respectively (Figure 10). Camp-99, along with PWS creeks all had mean values that overlapped the global marine value for $87Sr/86Sr$. Significant departures from the global marine value suggest that some equilibration with freshwater occurred, however ${}^{87}Sr/{}^{86}Sr$ values for all locations trended towards water with lower ${}^{87}Sr/{}^{86}Sr$ values such as that found in the PWS region, and not the higher ⁸⁷Sr/⁸⁶Sr water values for North Slope or northwestern Alaska.

Figure 10: Mean otolith ${}^{87}Sr/{}^{86}Sr$ values (± 1 SE) for the freshwater portion of pink salmon otoliths collected from the North Slope and Prince William Sound (PWS) regions of Alaska. For reference, the ${}^{87}Sr/{}^{86}Sr$ of seawater (0.70918) is indicated by the black line (along with ${}^{87}Sr/{}^{86}Sr$ values for North Slope (NS), PWS, and Northwestern Alaska (NW AK).

3.3 Otolith Chemistry: Ba/Ca and Sr/Ca (Otolith Analyses 1 and 2)

3.3.1 Ba/Ca and Sr/Ca Otolith Analysis 1

There were no significant differences for Ba/Ca and Sr/CA elemental concentrations between otolith core and freshwater regions of (*p* > 0.05) for both North Slope Alaska and Prince William Sound populations.

3.3.2 Ba/Ca and Sr/Ca Otolith Analysis 2

There were significant differences across collection locations for otolith freshwater values of Ba/Ca and Sr/Ca concentrations (Figure 11). Primarily, Ba/Ca values distinguished PWS fish from North Slope collected fish, while Sr/Ca values distinguished Point Hope from other locations (Figure 11). Sr/Ca and Ba/Ca are often used together in fisheries geochemistry studies to investigate origins of fish, as they co-vary (Peek and Clementz 2012). The relationship between Ba/Ca and Sr/Ca values for pink salmon in this study is shown in Figure 12. Some individual otoliths did have anomalously high Ba/Ca values (>20 µmol/mol, primarily PWS and Point Hope locations) in the freshwater portion of the otolith. Water Ba/Ca can vary widely on a seasonal basis due to differences in input and flow, thus water sampling on a finer temporal scale is required to capture seasonal variability in Ba/Ca in Alaskan rivers to use Ba/Ca as a discriminative geochemical marker of origin.

Figure 11: Variation in pink salmon otolith freshwater Ba/Ca and Sr/Ca values across collection locations. Mean values are presented $(\pm 1 \text{ SE})$.

Figure 12: Scatterplot of Sr/Ca and Ba/Ca values for the freshwater portion of pink salmon otoliths collected from the North Slope and Prince William Sound, Alaska.

3.4 *Otolith Chemistry:* $\delta^{18}O$ and $\delta^{13}C$ *(Otolith Analyses 1 and 2)*

3.4.1 δ^{18} O and δ^{13} C Otolith Analysis 1

There were significant differences ($p < 0.05$) between otolith core and early ocean regions for both $\delta^{18}O$ and $\delta^{13}C$ values. Biplots of $\delta^{18}O$ and $\delta^{13}C$ show that early ocean values for pink salmon tended to shift to higher values in comparison with otolith core values (Figure 13, left panels, see also Figure 14 both left and right panels).

 $3.4.2 \delta^{18}$ O and δ^{13} C Otolith Analysis 2

There were significant differences across collection locations for otolith core and early ocean values of $\delta^{18}O$ as otoliths of pink salmon collected from Kaktovik tended to have lower $\delta^{18}O$ values than all other locations (Figure 13). Otolith core δ^{13} C values were not different across collection locations, while early ocean δ^{13} C values were different between Point Hope and Camp-99 (Figure 14).

Figure 13: Scatterplots of $\delta^{18}O$ and $\delta^{13}C$ values of pink salmon otolith core (top left panel) and early ocean (bottom left panel) regions for all collection locations. Group ellipses showing the 95% confidence level for $\delta^{18}O$ and $\delta^{13}C$ isotope space occupied for otolith core (top right panel) and early ocean (bottom right panel) regions for each collection location.

Figure 14: Variation in $\delta^{18}O$ and $\delta^{13}C$ values of pink salmon otolith core and early ocean regions across collection locations. Mean values are presented (±1 SE).

3.4.3 δ^{18} O and δ^{13} C Otolith Analysis 3

Ellipses (95% confidence levels of isotopic space) of $\delta^{18}O$ and $\delta^{13}C$ data suggest that PWS pink salmon show less variation in the isotopic space of both otolith core and early ocean regions than North Slope Alaska pink salmon (Figure 13, right panels). These differences in ellipse areas were formalized using Bayesian functions. Results suggest that for otolith core, PWS populations had lower mean ellipse areas than North Slope pink salmon populations (except for Kaktovik given 95% credible intervals) (Figure 15, left panel). For early ocean, $\delta^{18}O$ and $\delta^{13}C$ isotopic area was also low for PWS populations, but Elson Lagoon fish also had a low isotopic space, while other North Slope Alaska populations had larger $\delta^{18}O$ and $\delta^{13}C$ isotopic areas (Figure 15, right panel).

Figure 15: Mean Bayesian standard ellipse $\delta^{18}O$ and $\delta^{13}C$ isotopic areas (%²) for otolith core (left panel) and early ocean (right panel) regions of pink salmon otoliths. Error bars are 95% credible intervals for the mean estimate for each collection location.

3.4.3 δ^{18} O and δ^{13} C Otolith Analysis 4

A hierarchical cluster analysis indicated that while some North Slope collected pink salmon were clustered with fish collected from southcentral Alaska at fine scales, there did appear to be some course-scale structure in the geochemical data suggesting that North Slope Alaska and PWS fish tended to cluster separately (Figure 16). The two deep, course-scale nodes in Figure 16 suggest some splitting between PWS fish in purple and blue versus North Slope Alaska fish (yellow to green) although there is clearly some finer-scale grouping of North Slope Alaska fish with PWS fish (far left clusters in Figure 16).

Otolith FW Clustering Analysis

Figure 16: Hierarchical cluster analysis of pink salmon collected throughout Arctic and southcentral Alaska based on all geochemical metrics for the freshwater (FW) region of the otolith.

4.0 Discussion

The shift of sub-Arctic populations of pink salmon northward has the potential to drastically impact the Arctic ecosystem. Using a variety of otolith geochemical markers, data from this study suggests that the majority (65%) of adult pink salmon caught in Arctic Alaska were likely not of local origin, given ⁸⁷Sr/⁸⁶Sr values that trended lower than the global marine value and away from our calculated average North Slope water value. However, for a smaller proportion of pink salmon (35%), an Arctic origin could not be conclusively ruled out. If pink salmon are in fact reproducing in the Arctic this could have profound consequences for native subsistence fisheries in the Arctic and for the ecosystem as a whole (Dunmall et al. 2013, Carothers et al. 2019). We note that researchers working in the North Slope of Alaska during summer 2023 observed chum salmon actively spawning (P. Westley, pers. obs, WIRED science article, October 18, 2023) (see also Dunmall et al. 2022). Thus, similar studies to the one reported here conducted on chum salmon would be a worthwhile effort, and our research group has recently received funding to conduct a similar pilot study to compare with results from those reported here (National Petroleum Reserve – Alaska Impact Mitigation Program funds to T. Sformo, North Slope Borough). Thus, the early investment by BOEM for this study is now developing into further related studies that will continue to explore the field of geochemical-based provenance studies for Arctic salmon.

Recent reports indicate that pink salmon are increasing in abundance in the Arctic (Dunmall et al. 2013, Eisner et al. 2013, Dunmall et al. 2018, Carothers et al. 2019, Chila et al. 2022). It is not surprising that pink salmon may be attempting to colonize newly accessible habitat, given that they have the highest stray rate among Pacific salmon (Hendry et al. 2004, Quinn 2005, Pess et al. 2012, Keefer and Caudill 2014). The stray rate of pink salmon has been estimated to be between 2.5 to >10%, depending on the population (Pess et al. 2012, Keefer and Caudill 2014). This high stray rate provides the potential for pink salmon to colonize newly available habitat in the Arctic. Pink salmon have previously been shown to rapidly colonize areas when new habitat becomes available. For example, after a passage barrier at Hell's Gate was removed in the Fraser River, pink salmon established spawning populations quickly and became abundant within decades (Pess et al. 2012). The Arctic's dynamic conditions may pose a challenge in finding suitable habitats for egg rearing and juvenile survival, despite the potential opportunities for colonization by pink salmon strays (Dunmall et al. 2016). For example, life cycle models examining the response of pink salmon to warming conditions in the northern Bering Sea suggest that the majority of variability in survival observed for juvenile pink salmon in the Bering Sea occurs during early life history stages (Farley et al. 2020).

Pink salmon have been observed spawning in several streams in Northern AK and the Canadian Arctic (Stephenson 2006, Carothers et al. 2019), although the production of viable offspring from these individuals has not been confirmed. However, the successful spawning and early marine survival of chum salmon has been established via the presence of a juvenile chum salmon in Arctic waters (Dunmall et al. 2022), indicating that the North Slope of AK does have viable salmon spawning habitat. Notably, the juvenile chum salmon reported by Dunmall et al. (2022) was captured near Kaktovik, one of the locations where adult pink salmon were captured in fresh water in the present study. Previous research has indicated that physical conditions could provide an opportunity for pink salmon to successfully reproduce in the Arctic (Irvine et al. 2009, Dunmall et al. 2016, Farley et al. 2020).

For example, thermal tolerance limits of pink salmon and the locations of perennial Arctic groundwater streams suggests that there are several tributaries that might support the establishment of pink salmon in Alaska and the Yukon Territory of Canada (Dunmall et al. 2016). Although none of the streams examined by Dunmall et al. (2016) overlap with the sites examined in the present study, previous research indicates that groundwater streams can provide sufficient thermal refugia for incubation of pink salmon. Additionally, the Bering Sea offers an opportunity for juvenile salmon to overwinter in an area with higher temperatures that could allow for enhanced marine survival (Irvine et al. 2009). Overall, pink salmon possess a tremendous capacity for the straying and colonization of newly available habitat in the Arctic due to their high stray rate, limited rearing time in freshwater, overall population abundance, short 2-year lifecycle, and high population productivity (Quinn 2005, Pess et al. 2012, Dunmall et al. 2016, Farley et al. 2020).

Although the majority of pink salmon examined in this study appear to not be of local Arctic Alaska origin, there was a proportion of fish (35%) for which Arctic origin could not be ruled out. Although this result could indicate that some fish were returning to their stream of origin as adults, it is interesting that this these fish were only observed from Elson Lagoon and Camp-99 and not from sampling locations east (Kaktovik) and west (Point Hope). This suggests that if this proportion of fish are from an establishing Arctic Alaska population, similar dynamics do not appear to be occurring consistently throughout the North Slope of Alaska given that no fish sampled from Kaktovik or Point Hope showed an increase trend in $87\text{Sr}/86\text{Sr}$ values between otolith core and freshwater regions. This underscores the importance of local scale processes likely responsible for determining outcomes for salmon populations expanding into Arctic regions.

Strontium isotopic compositions have been widely used to identify the natal origins of fishes (Kennedy et al. 2000, Miller and Kent 2009, Bourret and Clancy 2018). In this study, otolith $87Sr/86Sr$ was the most discriminative geochemical marker to determine pink salmon provenance. Although Sr/Ca and Ba/Ca values have been used to identify the origin and migration patterns of fish (Miller 2011, Linley et al. 2016, Chen et al. 2017), in this study Ba/Ca and Sr/Ca ratios overlapped between PWS and Northern AK study locations, resulting in limited utility for the purposes of this study. However, due to the wide range in Ba/Ca water concentrations, there is potential for this marker to be used for future applications in geochemistry studies of fisheries in the Arctic. The ${}^{87}Sr/{}^{86}Sr$ of water in this study was consistent with data previously reported for Northern Alaska by Brennan et al. (2014). There is a distinct trend in geochemical markers north of the Alaska Range (Figure 4) which allows for the conclusion that adult salmon captured on the North Slope with otolith ${}^{87}Sr/{}^{86}Sr$ above the global marine value (0.70918) are likely not originating from southern AK populations.

This study used reference populations from PWS pink salmon to assess freshwater signatures of otoliths from an established population. However, no individuals from PWS had an otolith $87Sr/86Sr$ signature that matched water from the PWS region (0.707224). As otolith and water $87Sr/86Sr$ typically exhibit a 1:1 relationship (Kennedy et al. 2000), we hypothesize that many of the PWS pink salmon sampled in this study were intertidal spawners, which would account for the fact that no differences were detected between otolith core and the freshwater portion for

 $87Sr/86Sr$ values of PWS pink salmon. Therefore, it is notable that data from PWS demonstrate one of the key difficulties in applying the geochemical technique to pink salmon given population-specific life history diversity. Although the freshwater portion of the otolith was discernible for many of the pink salmon sampled in this study (i.e., divergent from the seawater signature), particularly those from Arctic Alaska, it was difficult to assign a discriminative origin for these fish. We attribute this to the fact that these fish are likely strays from areas outside Arctic Alaska. However, previous research has demonstrated that it takes approximately 3-4 weeks for otolith chemistry to reach equilibrium with the water (Doubleday et al., 2013), although changes can be seen in as little as 2-3 days (Chen et al., 2015). In Pacific salmon, the equilibration time for ${}^{87}Sr/{}^{86}Sr$ during the transition from yolk to exogenous feeding can be over 30 days, which can be influenced by the magnitude of difference of isotopic sources to the otolith and the equilibration time (Janak et al., 2021). Another factor that can confound interpretation is the influence of maternal signatures in the otolith. Pacific salmon otoliths have been shown to have a significant influence from the signature of the mother in the core (Hegg et al., 2019; Janak et al., 2021; Miller and Kent, 2009). Maternal influence on offspring otoliths occurs because the geochemical signature of the egg yolk will often be reflective of the maturation environment, which can be significantly different from the spawning environment in the case of Pacific salmon (Quinn 2005, Miller and Kent 2009, Hegg et al. 2019). Given that otoliths begin to develop prior to hatch and exogenous feeding, the isotopic signature in the core of the otolith will reflect a heavy influence of the maternal contribution to the yolk, with maternal contributions of greater than 90% in some salmonids (Hegg et al. 2019, Janak et al. 2021). Although the attenuation of the marine maternal influence in the otolith core is useful in identifying the freshwater portion of the otolith, it can confound interpretation of the freshwater location where fish reared, especially when freshwater rearing is limited as is the case with pink salmon. However, as shown in this study, differences between core and freshwater geochemical values, can offer a first approximation of the likely source of freshwater origin. In the case of this study, we show that a majority of pink salmon sampled from Arctic Alaska had freshwater values that were trending towards water sources that were unlikely to be from the Arctic given water chemistry end points.

Although Sr isotopic ratios were the most discriminative marker in this study, the combination of geochemical markers can be especially useful because changes in otolith ⁸⁷Sr/⁸⁶Sr and elemental ratios have been shown to equilibrate on slightly different timescales. For example, Hegg et al. (2019) found that ${}^{87}Sr/{}^{86}Sr$ equilibrated more gradually, while elemental ratios of manganese, barium, and strontium had more rapid equilibrations. While markers such as ${}^{87}Sr/{}^{86}Sr$ and elemental ratios have a longer equilibration time, δ^{18} O could prove valuable due to its relatively faster equilibration time provided sufficient geochemical variation exists in the Arctic. Thus, the multivariate analyses we present in this report perhaps offer some of our more robust conclusions. First, there is some indication from our Bayesian ellipse models for isotopic area that PWS populations do in fact have less variation in their $\delta^{18}O$ and $\delta^{13}C$ values, which would likely reflect the fact that these are more established pink salmon populations. Second, our hierarchical cluster analysis suggests some course-scale grouping between pink salmon sampled from Arctic Alaska regions in comparison with fish sampled from Prince William Sound. Given our earlier conclusion that 65% of Arctic sampled pink salmon are likely not of local origin, we interpret our cluster analysis to suggest that Arctic sampled pink salmon may be individuals

straying from a similar source population, which would account for the course-scale clustering of Artic salmon in our analysis.

Despite the fact that pink salmon have been reported in increasing abundance in recent years (Carothers et al., 2019; Chila et al., 2022; Dunmall et al., 2018, 2013; Eisner et al., 2013), the distribution and abundance of pink salmon in the Arctic is still not well understood. Although this study indicates that most of the pink salmon observed are likely not produced in Arctic watersheds, it also suggests that there may be establishing populations reproducing successfully in the Arctic. This provides further support to the growing body of evidence indicating that pink salmon have a high potential to invade the Arctic and take advantage of previously inaccessible habitat. Decreases in sea ice, coupled with increase temperatures have already led to an observed increase in primary productivity in the Arctic (Ardyna and Arrigo 2020, Rawlins 2021). These changes in habitat can potentially result in higher survival of juvenile salmonids, leading to the establishment of self-sustaining Arctic populations of pink salmon (Moss et al. 2009, Lewis et al. 2020, Dunmall et al. 2022).

Developing tools to better understand the use of Arctic habitat by pink salmon is critical for developing mitigation and management measures for a dynamic region. With continued climate warming, there will be increased opportunities for pink salmon in the Arctic (Dunmall et al. 2013, Dunmall et al. 2016, Farley et al. 2020, Rawlins 2021). Pink salmon are known for their high population productivity (Quinn 2005), meaning that if they were to establish successful spawning populations in the Arctic, it could have significant impacts on native subsistence fish species (Carothers et al. 2019, Pecuchet et al. 2020). Well-documented impacts of invasive species on native ecosystems in aquatic environments include predation, disease, as well as pressure from resource competition (as reviewed by Gallardo et al. 2016). Additionally, as global climate change continues to drive increased physical changes in the Arctic, the region may become further exploited for resources such as oil and natural gas (Southcott et al. 2018, Romasheva and Dmitrieva 2021). If pink salmon do establish sustaining populations in the region, it will be important to balance the conservation of these populations with the need to develop resources in the Arctic. Therefore, it is important to continue to improve our understanding of how these fish are utilizing newly accessible habitat and how this will impact native fish species and the entire ecosystem.

5.0 Acknowledgements

Funding for this project was provided by the U.S. Department of the Interior, Bureau of Ocean Energy Management through a grant to the University of Alaska-Fairbanks Coastal and Marine Institute. The research team sincerely thanks all the individuals who offered their subsistence pink salmon to be processed for this study. Kimberly Pikok and Ana Stringer, interns with the North Slope Borough, were instrumental in collecting pink salmon otoliths for this project. Jim Menard and Brendan Scanlon (ADF&G biologists) kindly collected water samples for this project from Northwestern Alaska. Timothy Linley (now retired), Jill Janak, and Lisa Kant (PNNL) all assisted with various phases of the project including proposal preparation, water sample and otolith analysis, and data organization. Strontium isotopic samples were analyzed in the Environmental Signatures Lab at PNNL (Chief Scientist: Eirik Krogstad, Analyst: Steve Shen).

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