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ORIGINAL ARTICLE

Integrating Sr isotopes, microchemistry, and genetics to reconstruct *Salmonidae* species and life history

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Abstract

Recent approaches to fisheries research emphasize the importance of the coproduction of knowledge in building resilient and culturally mindful fisheries management frameworks. Despite widespread recognition of the need for Indigenous knowledge and historical reference points as baseline data, archaeological data are rarely included in conservation biology research designs. Here we propose a novel multiproxy method to learn from former fisheries stewards by generating archaeological data on past salmonid population parameters. We used a newly developed, high throughput qPCR (HT-qPCR) chip, originally designed for environmental DNA (eDNA), for species identification of archaeological salmonid vertebrae. We combine this with the laser ablation split-stream (LASS) approach to identify ocean-migration versus freshwater residency. We test this multidisciplinary approach using both contemporary and archaeological salmonid samples and new radiocarbon dates from the Tronsdal Site on the Skagit River, Washington State, USA. This is a useful approach for extracting information about *Salmonidae* species and life history diversity from archaeological remains to reconstruct historic baselines for several population parameters in anadromous species with long periods of freshwater residency. The approach outlined in this paper may be particularly useful for

research investigating past fisheries dynamics, offering hundreds to thousands of years of temporal depth for modern fisheries management, harvest policies, restoration ecology, and conservation biology.

KEYWORDS
ancient DNA, biodiversity, genetics, laser ablation, microchemistry, Salmonidae, strontium isotopes

INTRODUCTION

In the Pacific Northwest of North America, many fish species are considered to be economic staples and feature prominently within cultural traditions such as First Food ceremonies, oral histories and myths, and local cuisines (Gunther, 1926; Suttles, 1990). Salmonids (*Salmonidae*) are perhaps the most iconic of all Northwest aquatic fish families, as these trout, charr, and salmon species are considered both ecological and cultural keystones for the communities who have lived in the region since time immemorial (Collins, 1974; Smith, 1987; Thornton et al., 2015). The portfolio of life histories exhibited by *Salmonidae* is among the most varied of any vertebrate throughout the planet (Birnie-Gauvin et al., 2021; Schindler et al., 2010; Willson, 1997). There is significant variance in migratory patterns within individual *Salmonidae* species leading to diverse life history forms that have variable development, growth, maturation timing, reproductive strategies, and habitat (Groot & Margolis, 1991). These life history types are often defined by the timing and location to which fish return to their natal stream to spawn. Northwest peoples recognized the timing of these migratory runs to harvest and smoke or dry salmon and trout for winter storage, which formed the basis of many Indigenous economies for millennia (Cannon & Yang, 2006). Today, Salmonids continue to be coveted within regional cuisine and are targeted by sport, commercial, and Indigenous fishers, but many populations of salmonids are threatened and at the center of numerous restoration efforts.

Salmonid fisheries have dramatically declined since postcolonial settlement and with the introduction of industrial harvesting practices and broad habitat destruction in the Northwest (Gustafson et al., 2007; Thompson et al., 2019). The Skagit River basin in northern Washington State and southern British Columbia is one system wherein Salmonid populations, migrations, and abundance have been profoundly impacted by contemporary anthropogenic activities (Bettles et al., 2005; Docker et al., 2003; Muhlfeld et al., 2014; Waples et al., 2009). For Northwest Indigenous communities who continue to rely on salmonids, these declines threaten to upend traditional lifeways and connections to culture. In the past years, federal, state, tribal, and cooperative agencies and individuals throughout the Skagit River basin have worked together toward recovering these salmonid species (Morlock, 2005; Pomeroy, 1994). However, written records documenting historical ocean migration patterns and salmonid runs in the Salish Sea only extend 100 years (Gayeski et al., 2011; Quinn & Losee, 2022), and archaeological studies are often limited by the high costs of radiocarbon dates and difficulty in identifying archaeological salmonid remains using vertebral morphometrics (Huber et al., 2011; Moss et al., 2014). Understanding those past population and migration dynamics is critical to establishing baselines for modern restoration efforts. Almost 30 years ago, Pauly (1995) coined the phrase "shifting baseline syndrome" to describe the gradual creep of restoration and rehabilitation reference points used as benchmarks in fisheries management, where reduced population parameters are increasingly accepted as the natural baseline. Archaeological data provide an opportunity to add significant time depth to that baseline and expand our knowledge of past salmonid runs and life histories.

In this paper we take a novel approach in integrating microchemical and genetic data from archaeological salmonid vertebrae to reconstruct the portfolio of past salmonid life history strategies. Specifically, we draw on curated vertebrae, common in archaeological collections, from the previously excavated Tronsdal Site (45SK37) housed at the University of Washington Burke Museum. For this pilot study, we use the laser ablation split-stream (LASS) technique to determine the strontium (Sr) isotope and trace element compositions of otoliths, biomineralized ear stones responsible for hearing and balance, and vertebrae from modern samples and compare them with the compositions of archaeological vertebrae. Microchemistry results from modern samples indicate vertebrae can be used to study ocean-migration behaviors. We successfully used HT-qPCR analysis to assign archaeological salmonid vertebrae to species with minimally destructive sampling. For archaeological vertebrae, integration of microchemical and genetic data proves to be a powerful tool for identifying species and migratory life histories. In the following sections we review the cultural and ecological histories of the Northwest region and the use of genetics and microchemistry in *Salmonidae* diversity and provenance studies before introducing the environmental and cultural context of the Tronsdal Site. We conclude with a discussion on the utility of this multidisciplinary approach for understanding past population composition and anadromy in mitigating the shifting baseline syndrome. We consider how this approach may be further developed to inform contemporary fisheries management plans that foreground Indigenous practices and values.

BACKGROUND

Salmonidae diversity in the Skagit River basin

Throughout the Northwest, Pacific salmonids can migrate extensively throughout their life cycles. They exhibit strategies that vary widely between and within salmonid species including presence and degree of anadromy, out and return migration timing, and time spent in fresh water versus marine environment (e.g., lakes, rivers, estuaries, ocean) (Quinn, 2018). The migratory tactics these fish employ have significant trade-offs, which impact their adaptation suitability to various environments, risks of mortality, and food availability (Stearns, 1992). The expression of distinct migratory forms or life histories in various river systems ultimately determines whether a fish can successfully reproduce or not, thus impacting fitness. Numerous rivers often sympatrically host diverse assemblages of species and migration life history forms. In the Skagit River basin, five salmon: Chinook (*Oncorhynchus tshawytscha*), Coho (*Oncorhynchus kisutch*), Pink (*Oncorhynchus gorbuscha*), Chum (*Oncorhynchus keta*), and Sockeye (*Oncorhynchus nerka*); two trout: Steelhead (*Oncorhynchus mykiss*), and Coastal Cutthroat Trout (*Oncorhynchus clarkii*); and two charr species: Bull Trout (*Salvelinus confluentus*) and Dolly Varden (*Salvelinus malma lordi*), exhibit ocean migratory life history forms that are spawned into freshwater, migrate to the ocean for several years, and then return to freshwater as adults to spawn. These *Salmonidae* species also harbor variation with ocean-migrating, or anadromous, life histories that describe the seasonal and annual timing of their immigrations and emigrations, spawning habitats, and distributions. Sockeye (Kokanee variant), Steelhead (Rainbow variant), Coastal Cutthroat Trout, Dolly Varden, and Bull Trout also exhibit forms that can remain in freshwater throughout their entire life cycle and are often referred to as resident fish. However, even these "resident" populations vary in their propensity to migrate within rivers (fluvial) or lakes (adfluvial) (Austin et al., 2019; Chapman et al., 2012). The variance in the capacity for these species to express these diverse life history forms can be attributed to evolutionary and ecological processes that shaped species diversification (Alexandrou et al., 2013; Chapman et al., 2011; Macqueen & Johnston, 2014). Greater life history diversity in salmonids has been proposed to reduce competition and risk of mortality, ultimately reducing the

likelihood of species' population collapse, and increasing the probability of persisting against environmental fluctuation (Birnie-Gauvin et al., 2021; Kendall et al., 2014; Moore et al., 2014; Schindler et al., 2010; Waples et al., 2008). Today, anthropogenic impacts such as harvest, construction of in-stream barriers, habitat degradation, logging, and development have resulted in significant loss of these life history variants both within and across species (Ford et al., 2020; Nehlsen et al., 1991; Stouder et al., 2012; Thurow et al., 2020; Waples et al., 2009).

Genetics to identify *Salmonidae* species in contemporary and ancient riverscapes

Genetics is a powerful tool for assessing past biodiversity because species can be distinguished from archaeological materials. The morphological identification of salmonid skeletal elements to species is notoriously difficult and most vertebrae can only be reliably identified to family (Huber et al., 2011; Moss et al., 2014). Previous studies using genetic tools to identify archaeological samples to species have provided valuable insights into historical trends and changes in biodiversity (Cannon & Yang, 2006; Grier et al., 2013; Johnson et al., 2018; Morin, Zhang, et al., 2021; Moss et al., 2014; Thompson et al., 2019). However, due to degradation, historic or archaeological DNA (aDNA) is often fragmented and in low quantity making it prone to contamination from more intact and abundant modern DNA (Pääbo, 1989). This has been observed across studies trying to retrieve archaeological DNA from archaeological sites to characterize community composition (e.g., Cannon & Yang, 2006; Grier et al., 2013). Degraded DNA can be difficult to enrich for polymerase chain reaction (PCR) amplification because many common primers target amplicons that are larger than the average archaeological DNA fragment length of historic samples.

Fortunately, tools to address these issues have expanded substantially in recent years. Just like ancient DNA, environmental DNA – genetic material in the environment, such as in soil or water – must contend with cross-contamination risk and low-abundance, degraded templates. Although multiple molecular methods have been applied for the analysis of eDNA, taxon-specific quantitative polymerase chain reaction (qPCR) with hydrolysis probes has been found to be the most sensitive for rare species detection (Harper et al., 2018; Wilcox et al., 2020). Further advancements in the field have led to the development of high throughput methodology that allows for parallel targeted analysis of samples via high throughput quantitative PCR (HT-qPCR). This method splits each sample across many nano-size reactions, enabling parallel amplifications and tests of multiple taxa simultaneously; this is ideal for resource-conscious assessments of species diversity (Elmore et al., 2024; Kronenberger et al., 2022).

Otolith and vertebral provenance studies to determine *Salmonidae* migratory forms

The variations in $^{87}Sr/^{86}Sr$, Sr/Ca, and Ba/Ca between marine and freshwater environments reflect the geochemical processes operating in these environments. Elemental Sr has a long residence time in seawater, leading the global ocean to have a uniform Sr isotope value of 0.70923 (Burke et al., 1982; DePaolo & Ingram, 1985). Conversely, the Sr isotope composition of fresh water varies greatly as a function of the age and composition of watershed geology (e.g., Goldstein & Jacobsen, 1988). Because Sr has a long residence time in the oceans, seawater has a higher Sr content relative to most terrestrial waters such as rivers and lakes (Faure, 1998). The opposite is true for Ba, which has a very short residence time in the oceans. Barium ions (Ba^{2+}) readily react with seawater sulfate (SO_4^{2-}) upon flux into the ocean with continental runoff, producing authigenic barite ($BaSO_4$) (Monnin et al., 1999). Therefore, the marine stage of life in fish hard parts manifests as $^{87}Sr/^{86}Sr$ ratio of ~0.7092, high Sr/Ca, and low

Ba/Ca. Conversely, evidence of highly variable $^{87}Sr/^{86}Sr$ values, low Sr/Ca, and high Ba/Ca is more consistent with residence in freshwater (Campana, 1999).

Contemporary salmonid conservation efforts rely on otoliths for recent records of an individual fish's migration history. These otoliths grow incrementally on a daily basis as an individual fish uptakes strontium and other trace elements given their geospatial position in the river, providing a fine-grained record of the individual's age and migration history (Campana, 1999). Unfortunately, salmonid otoliths are rare in archaeological sites excavated prior to the 1980s, which used mesh screens too large to capture otoliths (<2 mm) (Butler & Campbell, 2004; Kopperl, 2001), and because past peoples often targeted salmonids for long-term storage, usually removing the heads prior to smoking or other processing activities (Butler & Chatters, 1994; Hoffman et al., 2000). Aside from otoliths, other calcified fish body parts such as scales, spines, eye lenses, and vertebrae have proven useful for studying life history patterns and aging fish (Tzadik et al., 2017). Vertebrae tend to be the largest of these body parts (>5 mm) and are therefore often recovered during archaeological excavations (Casteel, 1976). In teleosts, vertebrae grow continually during life, which is manifested by the sequential banding visible on the centra (Figure 1) (Hofkamp & Butler, 2017).

Previous studies have explored the use of the microchemistry of vertebrae growth bands in salmonids to reconstruct life histories. Early work by Koch et al. (1992) measured the Sr isotope compositions of growth bands in Atlantic Salmon (*Salmo salar*) and Sockeye to understand if the freshwater and marine phases of life were preserved in bone material. Their study documented that the centermost (youngest) growth bands had $^{87}Sr/^{86}Sr$ values consistent with freshwater residence. However, they found that these "freshwater" $^{87}Sr/^{86}Sr$ values did not agree with the $^{87}Sr/^{86}Sr$ composition of the freshwater where these fish were raised. The authors attributed this mismatch to bone resorption processes, which augmented the primary $^{87}Sr/^{86}Sr$ of the early growth bands when phosphate, and thus Sr, were remobilized during later phases of life in the open ocean. Later work also found challenges to using vertebrae to evaluate ocean migration timing due to latent ossification in Sockeye and Chinook (Zazzo et al., 2006). However, Matsubayashi et al. (2017) found that sulfur isotopes ($\delta^{34}S$) reflected migration patterns of Masu (*O. masu*). Although these previous studies demonstrate that vertebrae microchemistry is an imperfect tool for studying fish migrations, all note that vertebrae can record evidence of anadromous behavior. Ossification and chemical remobilization can alter the primary compositions of juvenile growth bands, but the microchemistry data may still reflect freshwater signals. Although vertebrae may not provide a detailed record of fish migration like otoliths, they still can be used to assess ocean-migrating behavior.

Cultural and environmental context

The Tronsdal site (45SK37) is situated along the Skagit River Delta in Washington State within the ancestral homelands of several northwestern Indigenous Communities (Figure 2). The Skagit River flows south through the rugged North Cascade Mountains from British Columbia, Canada, into Washington State, USA, where the river opens into a broad floodplain before draining into Skagit Bay and the northern end of Puget Sound. This river basin is part of the Salish Sea region within the greater Northwest ecocultural region.

This site, which has a varied history of archaeological inquiry, is described in archived notes as two mounded shell middens extending 1 m above the surface and 1.5 m below surface with extensive faunal materials. It was first recorded by A. L. Bryan and Roy Carlson in 1954. Mound B was later tested some time prior to 1960 by Robert Kidd and Doug Anderson. University of Washington Anthropology students, under the direction of Robert Greengo, extensively excavated Mound A across spring field seasons between 1960 and 1962. At that time, students excavated a series of 2 × 2-m units and one trench, alternating between 20-cm

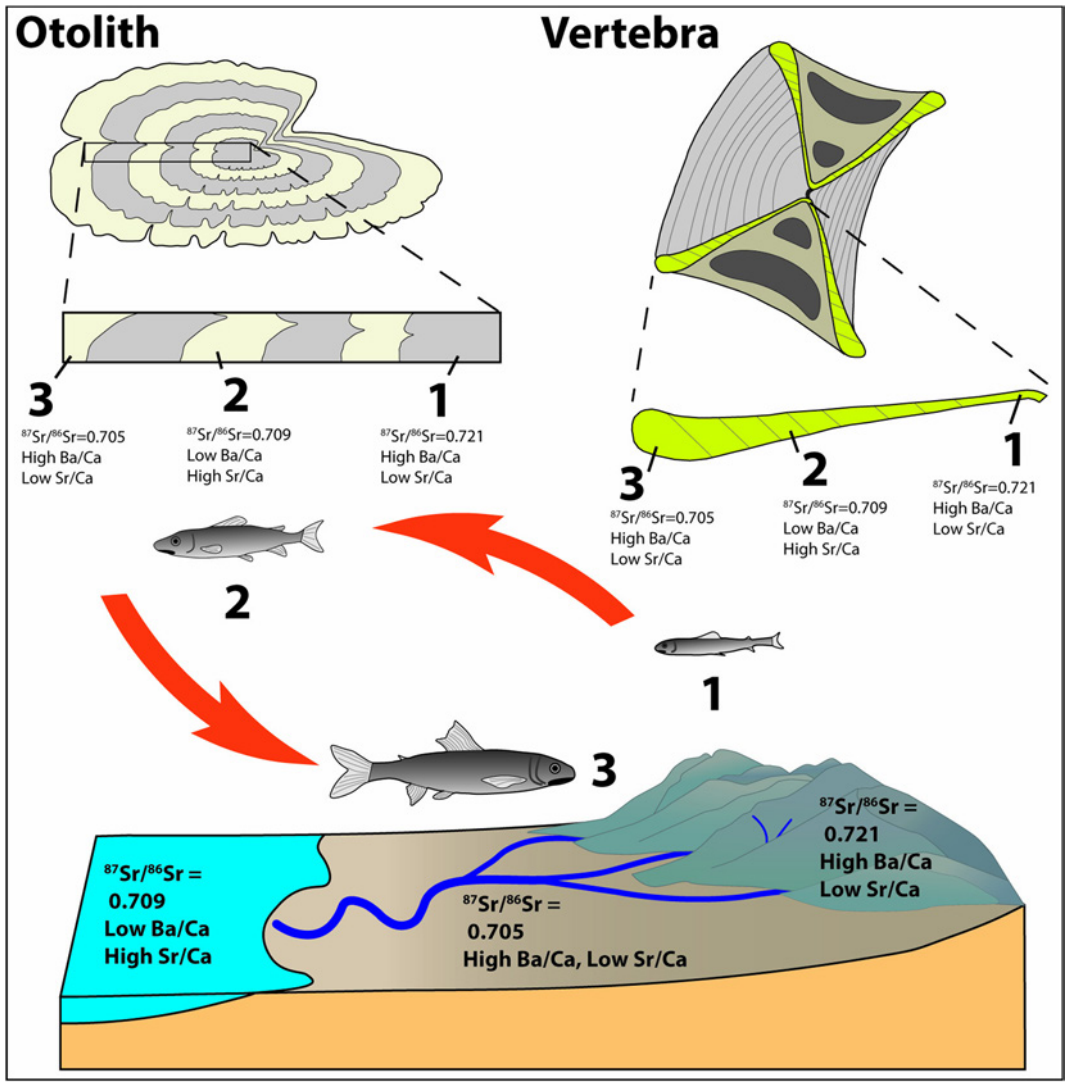

FIGURE 1 Explanation of how strontium (Sr), barium (Ba), and calcium (ca) content and Sr isotope ($^{87}Sr/^{86}Sr$) microchemistry in a vertebra or otolith may vary as a fish emigrates from freshwater early in life to the ocean and later returns to freshwater to spawn.

arbitrary levels and stratigraphic levels. All artifacts, charcoal, and zooarchaeological materials are curated with the University of Washington Burke Museum.

A proposal for this collection-based research was submitted to and approved by the Burke Museum in 2022. Radiocarbon dates from hand-picked wood charcoal indicate the mound was built relatively rapidly from about 1000–390 calibrated years before present (cal BP) (Table 1; Appendix S1). Syntheses by Matson and Coupland (1995) and Moss (2011) indicate that the ethnographically documented patterns of Northwest Coast life were in place prior to that 1000 cal BP period, wherein growing populations owned resources and stored large quantities of food, living in large plank houses within politically autonomous, semi-sedentary settlements. Recent Salish Sea zooarchaeological syntheses indicate past peoples relied on a variety of locations to harvest diverse marine resources, with salmonids among the most abundant and

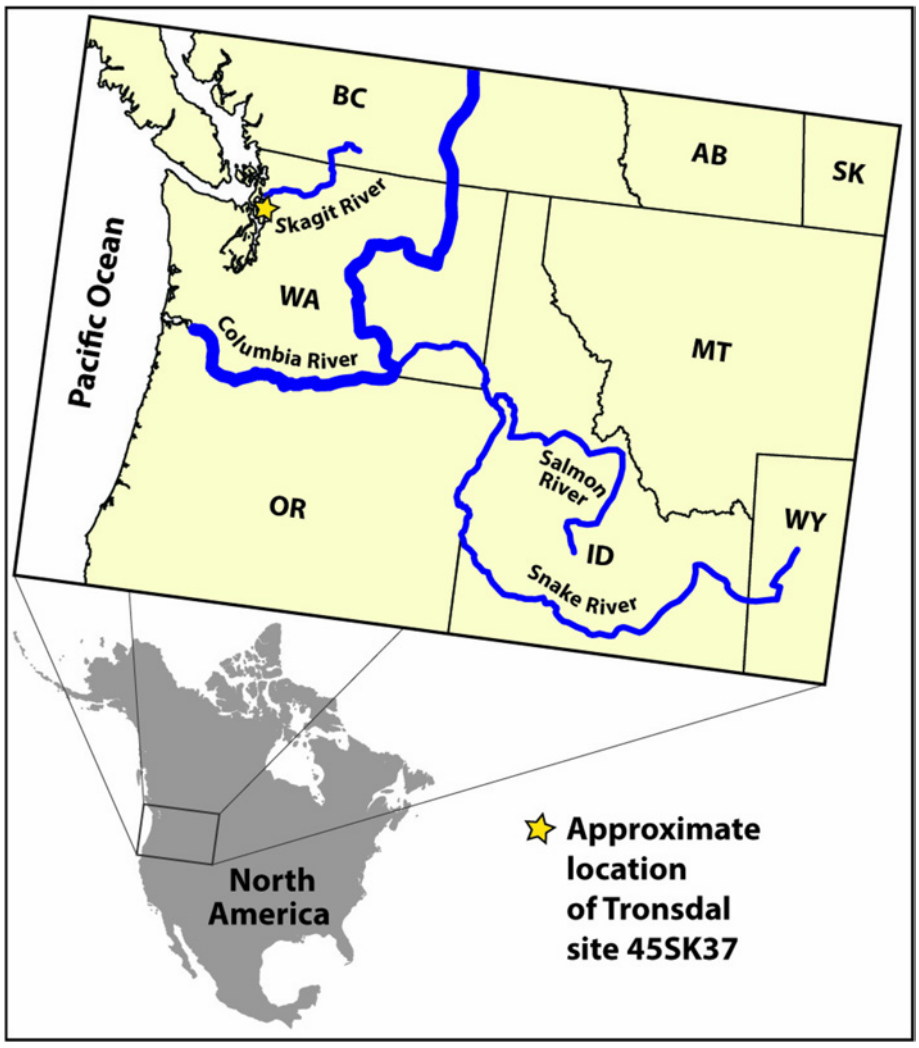

FIGURE 2 Maps showing the location of the study sites. The inset on map of North America shows the focus area in the Pacific northwest and locations of the Snake, Columbia, and Skagit Rivers. The yellow star represents the approximate location of the Tronsdal site near the mouth of the Skagit River on the Salish Sea.

ubiquitous taxa (McKechnie & Moss, 2016; Morin et al., 2023). Human use of salmonids appears to be stable throughout the Holocene until Euroamerican contact (Butler & Campbell, 2004; Campbell & Butler, 2010; Efford et al., 2023).

MATERIALS AND METHODS

Archaeological samples were obtained from the Burke Museum to attempt to identify species and microchemical signatures of individual fish migrations. Museum curators randomly selected nine unburned thoracic vertebrae from discrete contexts (units and levels) out of the 2959 total identified at the Tronsdal site. As this was a proof-of-concept study, no attempt was made to sample systematically across horizontal or vertical space or vertebrae size classes. To validate our approach, we also tested pairs of otoliths and vertebrae from contemporary fish:

TABLE 1 Radiocarbon dates were selected from hand-picked charcoal from the collections. Samples were processed at DirectAMS, calibrated using OxCal 4.4.1 with the IntCal 20 curve (Ramsey, 2009; Reimer et al., 2020), and are rounded to 10 years. N.D. indicates no available charcoal for dating from this level. Lab codes and additional information are provided in supplemental Table 1. All modern samples were harvested in 2023 or 2024.

Sample ID	Sample type	Species	DNA concentration (ng/μL)	Mean PCR ct value	Sampling location	Median date (cal BP)
45SK37–104	Archaeological	Pink	0.08	24.04	Tronsdal	390
45SK37–186	Archaeological	Pink	0.14	20.28	Tronsdal	520
45SK37–301	Archaeological	Pink	0.09	22.96	Tronsdal	490
45SK37–74	Archaeological	Chum	0.21	21.59	Tronsdal	920
45SK37–387	Archaeological	Chum	0.07	22.92	Tronsdal	390
45SK37–313	Archaeological	Chum	0.30	22.36	Tronsdal	470
45SK37–98	Archaeological	Chinook	0.24	26.60	Tronsdal	490
45SK37–221	Archaeological	Chinook	0.18	24.42	Tronsdal	500
45SK37–353	Archaeological	Chinook	0.08	29.02	Tronsdal	n.d.
STHD4	Modern	Steelhead	46.5	14.68	Snake River	N/A
STHD5	Modern	Steelhead	56.1	14.12	Snake River	N/A
SOCK1	Modern	Sockeye	0.44	16.03	Skagit River	N/A
SOCK3	Modern	Sockeye	1.73	14.9	Skagit River	N/A
CHIN1	Modern	Chinook	0.41	14.76	Skagit River	N/A
CHIN2	Modern	Chinook	0.79	18.89	Skagit River	N/A

wild Chinook and Sockeye caught by gillnet near the mouth of the Skagit River, and hatchery-reared Steelhead Trout caught by anglers in the Snake River.

Sample preparation

Detailed description of the vertebrae and otolith sample preparation methods is in Appendix S2. Archaeological vertebrae were cut and sampled for DNA extraction in a clean environment to minimize contamination from environmental DNA. All vertebrae were embedded in epoxy and sectioned through the center to expose the entire life history of the fish (Figure 3; Koch & Quist, 2007). Otoliths were prepared using the method of Heckel et al. (2020).

Species identification using archaeological DNA with high throughput quantitative polymerase chain reaction (HT-qPCR).

We extracted DNA from decontaminated archaeological vertebrae using standard sterile ancient DNA practices (Dabney & Meyer, 2019; Fulton & Shapiro, 2019), which were processed along with negative controls (Appendix S1). The known modern samples (n = 2 Sockeye, n = 2 Chinook, n = 2 Steelhead) were extracted in a separate modern lab using a standard Qiagen DNeasy extraction protocol. DNA quantity was quantified using 2 μl extracted DNA on a Qubit with High Sensitivity reagents. The HT-qPCR chip used here is described in detail in Elmore et al. (2024). The biochip contains primers and probes for 10 species including Chinook, Coho, Chum, Pink, Sockeye, Coastal Cutthroat Trout, Rainbow Trout, Bull Trout, Brook Trout (*Salvelinus fontinalis*), and Pacific Lamprey (*Entosphenus tridentatus*). In brief, all PCR setup occurred in a laboratory where no high-concentration DNA sources are handled. Potential target loci were enriched with a 12-cycle pre-amplification step. Contamination, amplification, and inhibition were monitored by the inclusion of two positive controls

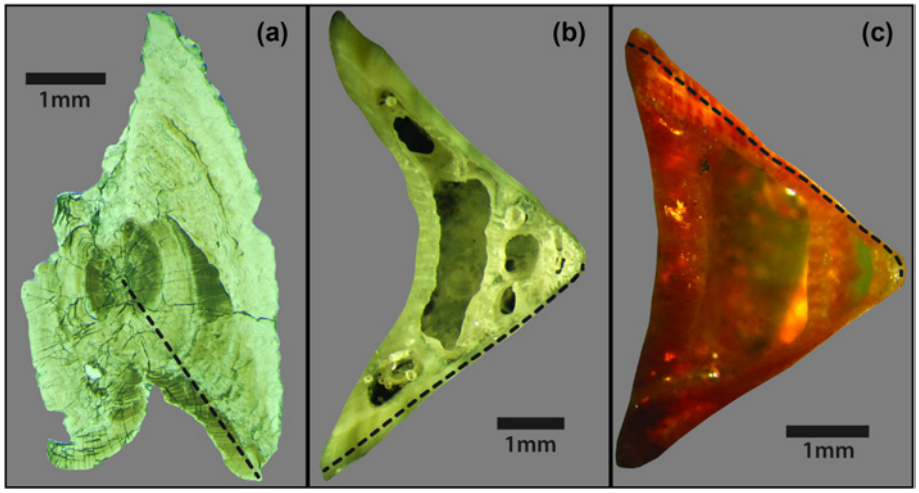

FIGURE 3 Examples of the sectioned otoliths and vertebrae with locations of the laser tracks (dashed gray lines, width not to scale). (A) Sockeye 3 otolith, (B) Sockeye 3 vertebra, C) Pink vertebra from sample 45SK37–104.

containing DNA from all 10 target taxa, seven no-template controls, negative extraction controls, and a tiled pattern of paired synthetic internal positive control (IPC) templates (refer to Elmore et al. (2024). After pre-amplification, products were exonuclease cleaned and plated in quadruplicate on a 384-well plate. Sample and assay source plates were shipped frozen to the Iowa State Veterinary Diagnostics Laboratory where the SmartChip was robotically loaded.

For eDNA purposes, each sample is usually a filter from a water source that may contain more than one target species. Because each of our samples was extracted from a single vertebra, we expected only one positive species identification. As in Elmore et al. (2024), we required that there was amplification in >1 replicate to consider a sample positive for given taxon on the chip. Even with stringent cleaning protocols, it is very difficult to avoid low-level carryover of DNA across tissue samples. In some cases, although we know that a given vertebra can only originate from one species, there may be very low-level amplification from other taxa introduced in the environment or during sample processing. However, we can filter out these low-level contaminants based on the qPCR cycle threshold (Ct), the number of PCR cycles needed to detect DNA. Earlier Ct values are associated with higher DNA concentrations. Each Ct relates to approximately one halving of initial template concentration, such that a Ct difference between two assays indicates a > 50 × difference in DNA concentration.

Life history identification using laser ablation split stream (LASS)

The vertebrae and otoliths were analyzed using the laser ablation split stream (LASS) technique developed by Hegg et al. (2020) at the Washington State University Radiogenic Isotope and Geochronology Laboratory (RIGL). This approach allows for simultaneous collection of Sr isotope and trace element data during a single laser analysis. Laser transects were positioned perpendicular to the growth bands in the vertebrae and otoliths and averaged between 1 and 7 mm long (Figure 3).

The standard NIST-610 glass was analyzed with the samples and was used to normalize the measured trace element concentrations, and a modern marine shell ($^{87}Sr/^{86}Sr$ of 0.70918) was analyzed with the samples to normalize the measured $^{87}Sr/^{86}Sr$ values, as described by Hegg et al. (2020). Data reduction used a custom-built data reduction scheme operating in the Iolite

software package (Hegg et al., 2020; Paton et al., 2011). All isotope and trace element data are reported in Appendix S3. Limits of detection for each trace element analysis are reported in Appendix S4 following Longerich et al. (1996).

RESULTS

Application of HT-qPCR to archaeological DNA yielded positive species identification

All archaeological and modern samples were successfully extracted, amplified on the HT-qPCR platform and identified to species. The nine archaeological samples had DNA concentrations between 0.07–0.30 ng/μL and were determined to include three Pink, three Chum, and three Chinook, all species which spawn within the Skagit River. In all nine cases, there was 100% amplification across replicates for the identified species. For five of the nine samples, the identified species was the only assay with amplification meeting the threshold for inference of positive detection from environmental samples in Elmore et al. (2024; amplification in >1 replicate). Four samples also had very low-levels of amplification in one to two of the four technical replicates for another species. However, the Ct values for these additional amplifications were 4–16 PCR cycles delayed, indicating that target sample DNA was >200 × more concentrated than low-level contaminants.

The initial DNA concentrations of the nine modern samples ranged between 2.99–17.33 ng/μL and were diluted prior to HT-qPCR. All modern samples from known species taxonomically assign to their true species identification (100% amplification rates and Ct values >9 earlier than the next nearest taxon). No species were detected in the no-template and extraction-negative controls, and both positive controls showed amplification for all 10 taxa.

Microchemistry

Modern otolith and vertebra pairs

Six modern otolith-vertebra pairs were analyzed from two Chinook, two Sockeye, and two Steelhead fish (Figure 4). Both Chinook otolith transects initially exhibit high $^{87}Sr/^{86}Sr$ values in the core of around ~0.708, which drop to ~0.705. At approximately 500 μm from the core, the values rebound to ~0.709 and plateau for the remainder of the transect. The same trend is visible in the Sr/Ca ratios across these transects. The Sr/Ca value is low near the core (~0.2) and then rises to ~0.4 at a distance of 500 μm and plateaus until the end of life. The Ba/Ca ratio varies inversely, being highest within 500 μm from the core (0.03–0.04), and then drops to ~0.01 for the remainder of life.

The $^{87}Sr/^{86}Sr$ ratio is generally flat across the corresponding Chinook vertebrae, consistently at the value of ~0.709. The same is true for the Sr/Ca ratios, which consistently range between 0.3 and 0.4. The Ba contents are more variable and there are discernable intervals of high Ba/Ca closer to the centrum core (~0.15) which fall to nearly 0 at a distance of 1,500 μm. Both vertebrae show an increase in Ba/Ca up to ~0.05 at about 2,500 μm, but then the value decreases again until the end of life.

Transects of the Sockeye otoliths have the lowest $^{87}Sr/^{86}Sr$ values closest to the core, at ~0.705. In both fish, the $^{87}Sr/^{86}Sr$ value increases to ~0.709 at 1000 μm, and plateaus until the end of life. Sockeye 1 (SOCK 1) shows a sharp decrease in $^{87}Sr/^{86}Sr$ for a short interval just before the end of life to a value of ~0.695, which is not seen in Sockeye 3. For both fish, the trends and inflections in the $^{87}Sr/^{86}Sr$ values are mirrored by the Sr/Ca ratios. Both otoliths have

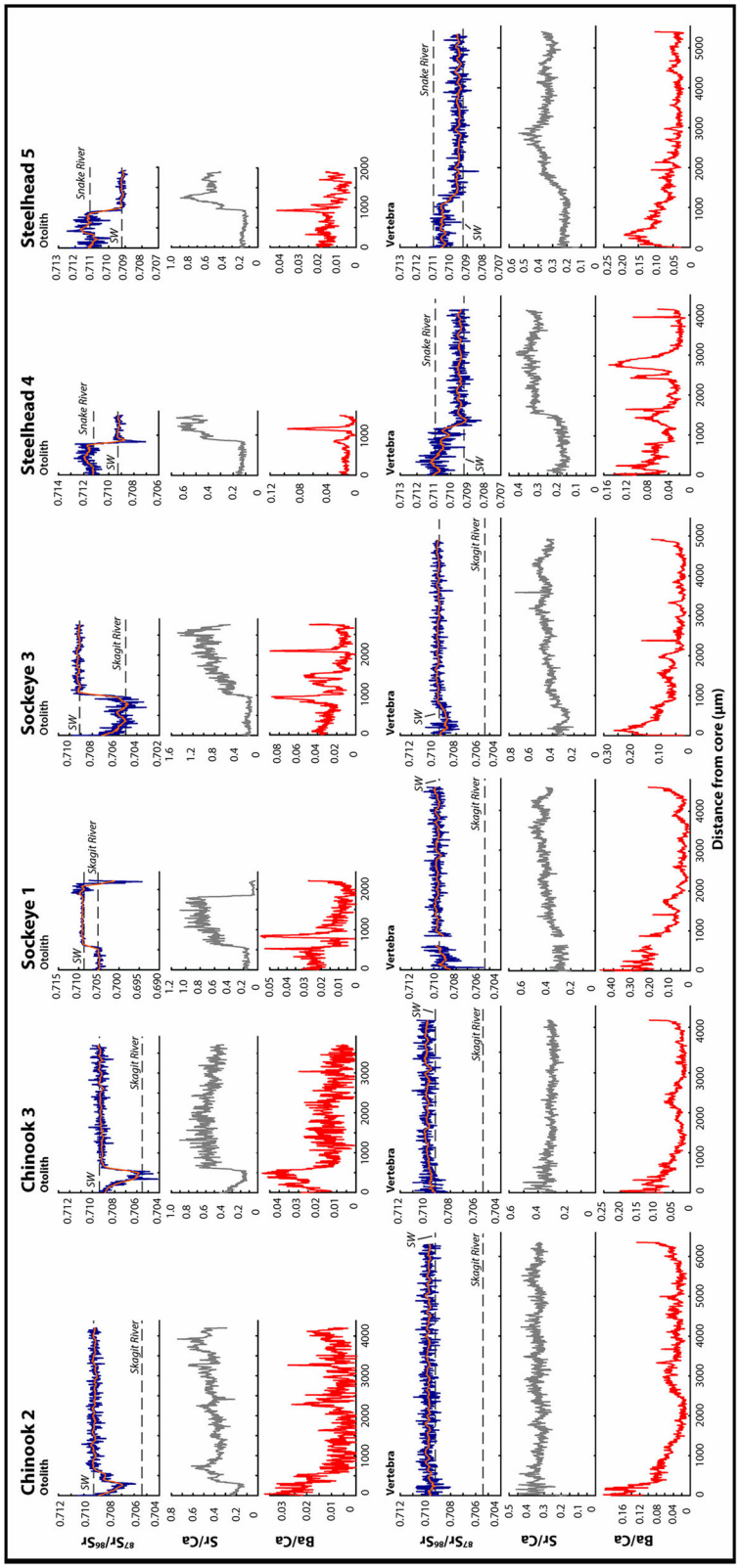

FIGURE 4 Sr isotope ($^{87}Sr/^{86}Sr$) and trace element results from pairs of otoliths and vertebrae from contemporary Chinook, Sockeye, and Steelhead. All plots progress from core (young) to rim (old) from left-to-right. The x-axis represents distance (µm) along the transects. The red line on the Sr isotope plots represents the average of the five $^{87}Sr/^{86}Sr$ ratios before and after each time slice.

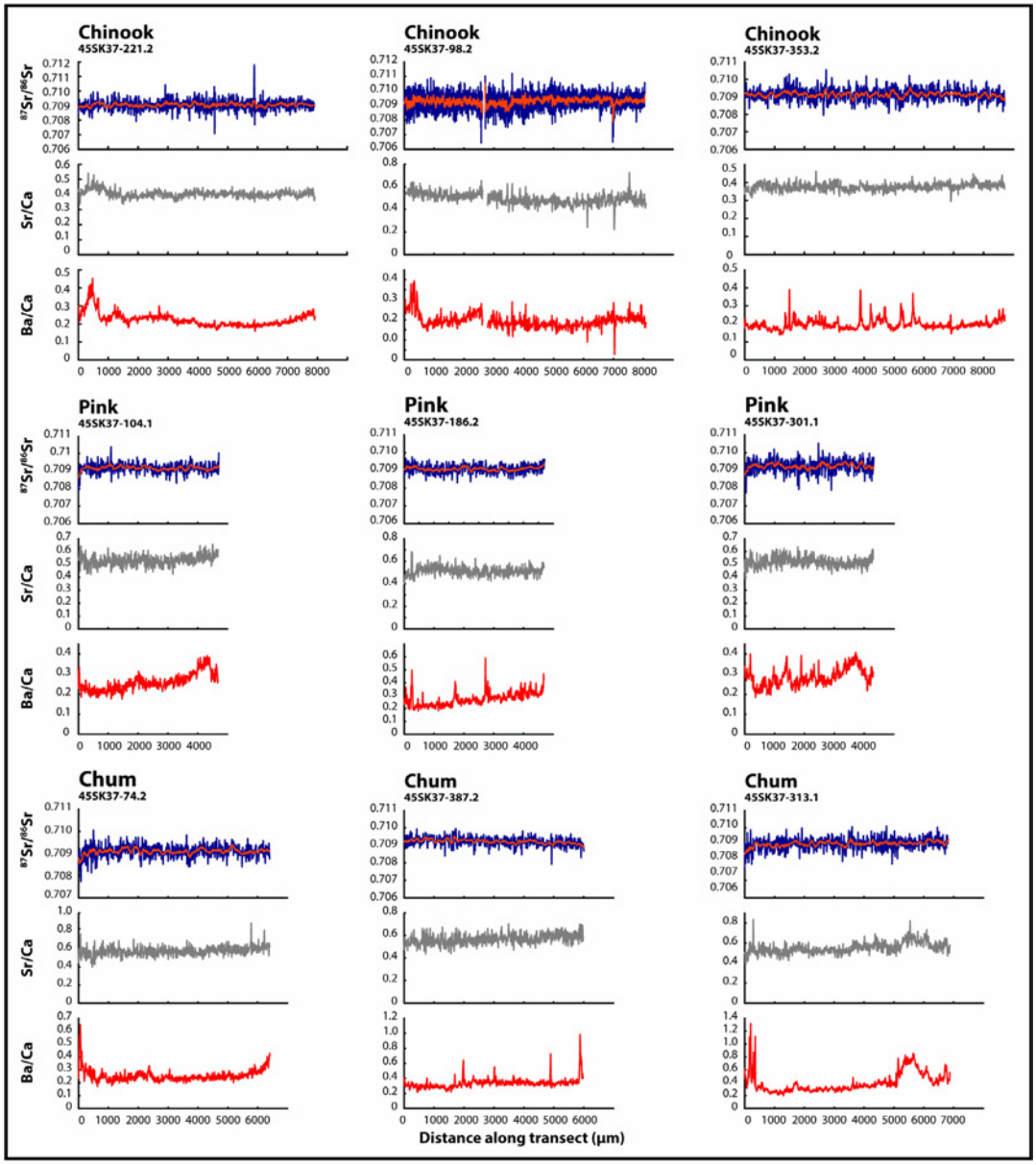

FIGURE 5 Sr isotope ($^{87}Sr/^{86}Sr$) and trace element results from archaeological vertebrae of three *Salmonidae* species. All plots progress from core (young) to rim (old) from left to right. The x-axis represents distance (µm) along the transects. The red line on the Sr isotope plots represents the average of the five $^{87}Sr/^{86}Sr$ ratios before and after each time slice.

the lowest Sr/Ca values near the core (~0.2), which rise to values between 0.8 to 1.2. In Sockeye 1, just as with the $^{87}Sr/^{86}Sr$ values, the Sr/Ca drops near the end of life. The Ba concentrations generally vary inversely to Sr, where both otoliths have the highest Ba/Ca ratios near the core. In Sockeye 1, there is an uptick in Ba/Ca near the end of life, matching the behavior seen in the Sr/Ca and $^{87}Sr/^{86}Sr$ data.

Transects across the Sockeye vertebrae have relatively consistent $^{87}Sr/^{86}Sr$ values of ~0.709, except for an interval within 1,000 µm of the centrum core. In this segment, the $^{87}Sr/^{86}Sr$ value is slightly lower at ~0.708. The Sr/Ca ratios along these transects show similar behavior, where

the lowest values (~0.2) are within 1,000 μm of the centrum core, and then gradually rise to 0.4 to 0.5 for the rest of life. The Ba/Ca ratio varies inversely, where values are the highest near the core (~0.2) and then fall for the remainder of life.

The Steelhead otoliths have the highest $^{87}Sr/^{86}Sr$ values near the cores of ~0.711. In both otoliths, the value remains elevated in the first 1,000 μm of the transects, and then drops to ~0.709 until the end of life. There are inflections at similar points in the corresponding Sr/Ca and Ba/Ca ratios. Early in life, the Sr/Ca ratio is <0.2, and increases to 0.6 until the end of life. The Ba/Ca ratio behaves inversely, where the core regions have higher values of ~0.02, which drop to <0.01 later in life.

Steelhead vertebrae have elevated $^{87}Sr/^{86}Sr$ values in the regions closest to the centrum core at 0.711, just as observed in the otoliths. The $^{87}Sr/^{86}Sr$ value drops to 0.709 later in life after a distance of ~1,000 μm from the core of each vertebra. The core region has the lowest Sr/Ca ratios of ~0.2, which increase to 0.4 after about ~1,000 μm. The Ba/Ca ratio varies inversely, where it is higher in the centrum core at a value of ~0.10, and falls below 0.01 after ~1,000 μm.

Archaeological vertebrae

For this exploratory study, nine salmonid archaeological vertebrae were analyzed (Figure 5). Strontium isotope, Sr/Ca, and Ba/Ca ratios were determined across vertebral transects, which include 3 different salmonid species (Table 1).

Three Chum vertebrae have generally uniform $^{87}Sr/^{86}Sr$ values of 0.709, although sample 45SK37–74 does show a small downward excursion near the centrum core to ~0.708. The Sr/Ca ratios are also consistent across these transects, ranging between 0.4 and 0.6. The Ba content is more variable where samples 45SK37–313 and 45SK37–74 have elevated Ba/Ca ratios (0.3–1.0) within ~500 μm of the centrum core, which drop later in life, and then rise again after about 5,000 μm. The Ba/Ca ratio is generally flat throughout life in 45SK37–387.

The three vertebrae from Pink Salmon also have relatively uniform $^{87}Sr/^{86}Sr$ values of 0.709 and Sr/Ca ratios between 0.4 and 0.6. As with the Chum vertebrae, the Ba/Ca ratios of these transects show more variance. Samples 45SK37–186 and 45SK37–301 both show a small, 100–200 μm long interval of elevated Ba/Ca, up to ~0.3, near the centrum core. The Ba/Ca ratio drops after this but then rises again later in life. Sample 45SK37–104 does not have an interval of high Ba/Ca near the core, but the ratio does rise later in life as in the other Pink vertebrae.

Transects across three Chinook vertebrae have consistent $^{87}Sr/^{86}Sr$ values of 0.709 and Sr/Ca ratios of 0.3–0.5 like the Pink and Chum vertebrae. Like the other archaeological vertebrae, these transects also have variable Ba/Ca ratios. Two Chinook vertebrae have elevated Ba/Ca ratios near the centrum core, up to 0.4, which drop later in life. The other Chinook vertebra 45SK37–353.2 has a generally consistent Ba/Ca ratio of 0.2, but the transect is punctuated by sharp brief spikes with values as high as 0.4.

DISCUSSION

To effectively assess shifting biodiversity baselines, we need tools to characterize the diversity that existed in the past as well as the present. These tools further require innovative scholarship foregrounding Indigenous practices and understandings of ecosystems through time (Berkes et al., 2000). Below, we consider the utility of this multidisciplinary method in assessing *Salmonidae* species diversity and life history variants across time before considering how this approach might be used in species' conservation and management practices informed by Indigenous practices.

HT-qPCR offers a resource-efficient method for identifying species from archaeological vertebrae

Many Indigenous communities are interested in using ancient samples to reconstruct the species composition of past fisheries (e.g., Morin, Royle, et al., 2021; Rodrigues et al., 2018). High throughput-qPCR is a cost- and time-effective method that can be used widely to estimate the structure and dynamism of *Salmonidae* species' populations across time. The HT-qPCR biochip is particularly conducive to archaeological DNA applications for many reasons. First, the assays are species-specific, therefore greatly reducing the probability of exogenous or non-target DNA amplification introduced from modern sources. Second, the assays are also highly sensitive and can detect DNA down to 2–10 copies per reaction (Elmore et al., 2024). Third, the target amplicon sizes are small (<200 bp), making it ideal for highly degraded DNA. This tool is also very resource-conscientious, utilizing a small amount of extracted archaeological DNA per reaction. This is particularly useful where template DNA is highly limited, often with no opportunities for additional extractions as often is the case with archaeological DNA. While not all species assays exist for rapid HT-qPCR detection, pipelines for rapid assay validation have substantially reduced the investment required to add taxa (Kronenberger et al., 2022) and most HT-qPCR platforms can flexibly incorporate >48 assays in a single chip (e.g., OpenArray, Fluidigm, SmartChip).

A potential limitation of the HT-qPCR platform in some archaeological DNA systems is the reliance on assays based on contemporary genetic resources. First, unlike salmonids, some taxonomic groups lack good molecular databases, although current biodiversity sequencing efforts are beginning to fill this gap. Further, assays may perform poorly when contemporary genetic patterns are not a good reflection of ancient patterns (Wilcox et al., 2024). These assays cannot detect species and lineages which are no longer extant and may lose efficacy for lineages with rapid sequence change. A possible alternative could be metabarcoding – sequencing of amplicons from conserved primer sets which are less reliant on species-specific optimization (Taberlet et al., 2012). For instance, metabarcoding has successfully been used to characterize biological communities from soil cores taken from two-million year old ecosystems in Greenland (Kjær et al., 2022). This approach, however, can be limiting as species-specific genetic reference sequences are often taxonomically biased and limited in species with high speciation rates (Kronenberger et al., 2022; Wilcox et al., 2024). Additionally, this approach tends to be more resource-intensive, requiring more genetic sequencing and bioinformatics tools and expertise to identify communities of species (summarized in Deiner et al., 2018).

Collagen peptide mass fingerprinting, or zooarchaeology by mass spectrometry (ZooMS), is another method for taxonomic determinations of unidentifiable archaeological bone using collagen peptide biomarkers (Buckley, 2018; Richter et al., 2022). This minimally destructive, cost-effective, and quick analytical technique has successfully been used to distinguish between five species of anadromous salmon vertebrae (Efford et al., 2023; Richter et al., 2020). However, no comprehensive database exists for collagen peptitide biomarkers, therefore provisional markers must be independently verified, and no real reporting standards exist (Richter et al., 2022). Furthermore, we are unaware of any diagnostic peptide markers for Steelhead, Cutthroat, or charr species common to the Northwest. High throughput sequencing paired with exponentially growing publicly available molecular databases offer the ability to easily utilize previously developed assays or design new ones for rapid specific-species HT-qPCR assays. Isolating DNA from samples and using PCR to amplify taxonomically diagnostic sequences is not new and numerous approaches to taxonomic identification from eDNA and aDNA have been developed and significantly validated (Deiner et al., 2018; Wilcox et al., 2020; Wilcox et al., 2024). Isolating DNA also provides the opportunity for using metabarcoding to try to identify species not specifically targeted in a HT-qPCR assay (Deiner et al., 2018) and for population genetics analyses (Essel et al., 2023).

Contemporary otoliths-vertebrae pairs reflect shared marine signatures

The fundamental assumption of fish migration studies based on microchemical data is that the measured compositions of hard parts (e.g., otoliths, bones, spines, scales) reflect the composition of the environment at the time of growth (Campana, 1999). To assess the utility of vertebrae microchemistry for understanding fish migration we evaluate the otolith data which are proven to provide this information. The otolith microchemistry of all modern samples depicts anadromous life histories. The low $^{87}Sr/^{86}Sr$ values near the otolith cores of the contemporary Sockeye and Chinook Salmon otoliths (~0.705) match the reported Sr isotope composition of Skagit River water by Bacon et al. (2004), representing the juvenile freshwater stage of life. The cores of the Steelhead otoliths have higher $^{87}Sr/^{86}Sr$ values of ~0.711, which match the composition of waters in the Salmon River, in the Snake River basin reported by Hegg et al. (2013) representing juvenile freshwater residence. The shift of $^{87}Sr/^{86}Sr$ values to 0.709 observed in all otoliths represents migration into the open ocean. This is also visible in the Sr/Ca and Ba/Ca ratios that rise and fall, respectively, during this transition.

Although the otoliths exhibit anadromous trends in $^{87}Sr/^{86}Sr$, Sr/Ca, and Ba/Ca, this is less apparent in the corresponding vertebrae. The transects across the Chinook vertebrae reveal largely uniform marine $^{87}Sr/^{86}Sr$ values of ~0.709, without evidence of the early freshwater stage. The youngest regions of the Sockeye vertebrae do exhibit slightly lower values (~0.708), which could be evidence of freshwater residency early in life, although these $^{87}Sr/^{86}Sr$ values are significantly higher than the Skagit River Sr isotope composition (~0.705). In contrast, the freshwater stage of life is unequivocal in the Sr isotope data from the Steelhead Trout vertebrae, where the youngest regions exhibit $^{87}Sr/^{86}Sr$ values of ~0.711, consistent with both the corresponding otolith data and the composition of water in the Snake River basin where these fish reared (Hegg et al., 2013). The shift of the Sr isotope value to 0.709 observed in both Steelhead Trout vertebrae clearly represents migration to the open ocean.

Each of these species presents a different case where vertebrae microchemistry is variably able to accurately record anadromous behavior. This observation aligns with previous findings by Koch et al. (1992) and Zazzo et al. (2006), who concluded that vital processes such as ossification affect how bone material can be utilized to reconstruct life histories. Zazzo et al. (2006) documented that salmon vertebrae do not fully ossify in the first year of life. If a fish migrates to the open ocean before vertebrae ossification can "freeze-in" the geochemical signature of the juvenile environment, the primary freshwater signal may be overprinted by the composition of the marine environment. We therefore expected the juvenile freshwater signal to be more clearly present in vertebrae from salmonid species that spend longer periods in freshwater before migration to the open ocean, such as Steelhead Trout.

Of the three contemporary species sampled, Chinook Salmon has a life history (i.e., ocean type) that spends the least time in freshwater (weeks) before migration to the open ocean (Healey, 1991; Huber et al., 2011; Shared Strategy for Puget Sound, 2007; Tomelleri & Behnke, 2002; Wydoski & Whitney, 2003). As expected, the Chinook vertebrae Sr isotope data fail to record the short freshwater period in their microchemistry. Sockeye Salmon spend a relatively longer period in freshwater (one year) (Huber et al., 2011; Quinn, 2018; Tomelleri & Behnke, 2002; Wood, 1995; Wydoski & Whitney, 2003), and the Sr isotope data from these vertebrae show evidence of freshwater residence. However, the disagreement between the Sr compositions of the cores of the Sockeye otoliths and vertebrae indicates the freshwater signal is partially preserved in vertebrae, perhaps diluted by marine Sr as ossification was only partially complete upon ocean migration (Zazzo et al., 2006). Steelhead, which can exhibit a number of migratory expressions including anadromy, amphidromy, and potadromy, can remain in freshwater longest after coming out of the gravel (1 to 3 years). This should allow the most time for ossification and preservation of the freshwater signal in vertebrae (Zazzo

et al., 2006). Our dataset clearly reflects this as the Steelhead Trout vertebrae provide the clearest evidence of the freshwater life stage.

It is difficult, however, to interpret the timing of this transition more precisely based on the structures visible in these vertebrae. Although all sectioned vertebrae in this study contain fine-scale banding as seen in Hofkamp and Butler (2017), the presence of distinct annular features, such as lighter and darker bands commonly seen in otoliths, is less obvious in cross-section (Figure 3). Regardless, our dataset provides a more generalized view into life history. Each vertebrae transect starts in the centrum center and progresses to the outermost edge of the bone crossing all visible growth bands and encompassing the entire lifespan of the fish. The otoliths from each specimen were analyzed in exactly the same way, from core to outermost rim, so we are confident these transects also cover the full lifespan. The trends of the $^{87}Sr/^{86}Sr$, Sr/Ca, and Ba/Ca ratios across the Sockeye and Steelhead vertebrae mirror those in the corresponding otoliths. The youngest, centermost regions all have freshwater compositions, which shift to marine values later in life, consistent with anadromous behavior. Future work should be focused on establishing more quantitative age links (e.g., year of life) between vertebrae microchemistry and growth structures.

The application of the LASS method allows for the simultaneous assessment of isotopic and trace element data. Variation of the Sr isotope values, Ba/Ca ratios, and Sr/Ca ratios across the Steelhead vertebrae clearly record the anadromous life histories of these fish. In the Sockeye vertebrae, we observe that the centermost (juvenile) growth bands exhibit elevated Ba/Ca and subdued Sr/Ca ratios, aligning with the composition of Ba-rich and Sr-poor freshwater (Brown & Severin, 2009; Smith & Kwak, 2014; Tabouret et al., 2010). This lends support to our interpretation that the lower $^{87}Sr/^{86}Sr$ values in these centermost growth bands genuinely reflect the freshwater phase of life in these fish. Further, as life progresses, the Sr/Ca and Ba/Ca ratios are inversely correlated, consistent with migration to the open ocean (e.g., Smith & Kwak, 2014). This demonstrates the utility of LASS as several proxies can be concurrently evaluated, greatly helping interpret the more complicated microchemistry record in vertebrae.

Although the contemporary Chinook vertebrae exhibit consistent Sr/Ca and $^{87}Sr/^{86}Sr$ ratios, there is greater variability in their Ba/Ca ratios, which follow the expected pattern for anadromous fish: elevated during the early stages in freshwater and subsequently decreasing after migration to the open ocean. In the case of ocean-type Chinook Salmon, which usually spend more time in the estuary before heading to sea, high Ba/Ca ratios may reflect time in the estuary. The complex dynamics of fish movement across the salinity gradient during the process of acclimating to saline water, combined with an increase in barium at very low salinities due to particle desorption (Walther, 2019), could result in high Ba in low salinity along with a more oceanic $^{87}Sr/^{86}Sr$ value due to forays across the salt wedge. Additionally, both Chinook vertebrae display a notable peak in Ba/Ca midway through their lifespan, not mirrored in the $^{87}Sr/^{86}Sr$ or Sr/Ca data. Do these increases in Ba/Ca signify anything about the life histories of these fish and why is this not reflected in the $^{87}Sr/^{86}Sr$ and Sr/Ca ratios? Given that both fish share the same Ba/Ca trends, and that they were caught together and likely share the same life histories, it is plausible these patterns indeed reveal aspects of their migrations. If true, the high Ba/Ca interval during the juvenile stage indeed represents residence in freshwater. The discrepancy between the Ba/Ca and $^{87}Sr/^{86}Sr$ or Sr/Ca ratios may be attributed to fundamental chemical differences between Sr and Ba, such as Sr having a smaller ionic radius influencing how these elements are mobilized during bone resorption or before ossification. Pre-migration Ba concentrations in bone or collagen may persist through the ossification process, whereas Sr does not. The increase in Ba/Ca midway through life occurs beyond the ossification period, indicating the absence of a corresponding shift in the $^{87}Sr/^{86}Sr$ or Sr/Ca ratios cannot be attributed to vital processes. This observation might reflect the geochemical composition of the open ocean. Localized zones in the open ocean experience deep upwelling that brings Ba-rich water to the surface (Hsieh & Henderson, 2017). It is plausible that these peaks in Ba/Ca reflect the passage of these fish through upwelling zones during the open ocean phase of their lives (Mohan et al., 2018).

Archaeological vertebrae

The effects of diagenesis postdeposition must be considered whenever interpreting chemical or isotopic data from archaeological remains. Buried archaeological bone material is especially vulnerable to intrusion by sediment pore water, which may facilitate leaching (Kendall et al., 2018), shifting the original Sr-isotope composition of a material toward the $^{87}Sr/^{86}Sr$ of the matrix at the burial site (Alexander Bentley, 2006). The Sr-isotope composition of the bedrock and derivative sediments in the Cascade Range is well-characterized by decades of geochemical studies that document essentially all geologic material has an $^{87}Sr/^{86}Sr$ of ~0.705, reflecting the relatively young Cenozoic geology (Church & Tilton, 1973). Traverses across all nine vertebrae in this study have uniform $^{87}Sr/^{86}Sr$ of ~0.709, markedly higher than the composition of the surrounding geology and adjacent Skagit river water (Bacon et al., 2004). Therefore, even without direct samples of the matrix sediments from the Tronsdal site, we are confident the isotopic and trace element compositions of these vertebrae are not altered by the composition of the surrounding environment but are primary from before deposition reflecting the marine residence of these fish (Miller et al., 2011).

The $^{87}Sr/^{86}Sr$ values and Sr concentrations across all archaeological vertebrae are relatively consistent, dominated by the marine signal, and do not preserve evidence of the freshwater stage of life. This is consistent with the results of the archaeological DNA sequencing that identifies all vertebrae are from obligately anadromous species (Quinn, 2018; Tomelleri & Behnke, 2002; Wood, 1995; Wydoski & Whitney, 2003). The absence of freshwater intervals in these vertebrae is also consistent with the genomic data, as these species have known life histories that only reside in freshwater for short periods and likely migrated to the open ocean before complete ossification of their vertebrae. Therefore, the absence of their initial freshwater residency is not surprising. Although many life histories may not be visible with the granularity of this data (i.e., odd or even-year Pink, fry or delta-rearing ocean-type Chinook), other life histories such as winter- or summer-run Steelhead or stream-type Chinook should be identifiable with an expanded sample size because of their extended freshwater rearing period prior to migration.

Two of the archaeological Chinook vertebrae, like the modern Chinook vertebrae, also have more variable Ba concentrations that follow the expected patterns for anadromous behavior—high early in life, and then dropping during migration to the ocean. There are perhaps other examples, as seen in the modern Chinook vertebrae, of decoupling between Sr and Ba due to vital processes during ossification. However, transects across many of the archaeological vertebrae have variable Ba content later in life when bone growth was more consistent, and the issue of ossification is less important. Therefore, we are more confident that these variations in Ba content are recording information about the marine environment. Dissolved (bioavailable) Ba content increases with depth in the ocean, so surface waters in zones of ocean upwelling are recognized to be locally Ba rich. Interaction between fish and ocean upwelling zones has been studied using the Ba content of shark vertebrae (Mohan et al., 2018). Therefore, the variable Ba content of these vertebrae is consistent with the ocean period, likely reflecting passage through local zones where Ba contents may have been elevated (Baumann et al., 2015; Lin et al., 2013; Woodson et al., 2013).

Can vertebrae be used to understand anadromy?

The results of our study demonstrate that archaeological vertebrae are uniquely suited for pairing the perspectives of microchemistry and genetics on salmonid populations in deep time. Our microchemical data from modern Sockeye and Steelhead vertebrae and otoliths establish that vertebrae can preserve a record of migration between freshwater and open ocean. Although

future work is needed to more precisely ascertain the timing (e.g., year of life) of these movements from vertebrae analyses, our approach using the LASS method gives a general account of anadromous behavior. Our dataset also identifies the important caveat that the resolution of this technique is strongly correlated with the duration of premigration freshwater residence. This means the methodology may be more effective for tracking ocean-going behavior in species that migrate out of freshwater later in life (Steelhead and Sockeye) rather than in species which spend short periods in freshwater after hatching (ocean-type Chinook). Still, this method, with larger sample sizes, could be used to estimate the proportion of ocean-type and stream-type life histories, and potentially the duration of stream residence, based on the length of elevated Ba in the vertebrae. The proportion of life histories present in salmon populations is an important measure of life history diversity and has been an important conservation question in historical and contemporary populations (Bottom et al., 2008; Burke, 2004; Chittaro et al., 2019; Hegg et al., 2018).

The added advantage of vertebrae over otoliths in archaeological studies, other than being more often recovered during excavations, is that vertebral bones carry genetic material, whereas otoliths do not (Campana, 1999). The results from nine archaeological vertebrae demonstrate that aDNA can be successfully extracted and sequenced for species identification. With this information and microchemical data, comparisons can be made between modern and archaeological vertebrae of fish of the same species in the same river system; differences between the length and microchemistry of the freshwater interval in modern and archaeological vertebrae may signal changes in the behavior of fish populations through time. Comparisons at this level were not possible in our study because all archaeological samples were from species with short freshwater residence where ossification was outpaced by migration to the marine environment, as seen in the modern and archaeological Chinook vertebrae that lack clear evidence of the freshwater period. Therefore, future work should focus on an expanded set of archaeological vertebrae to include samples from species that spend longer periods in freshwater (i.e., Steelhead), which may allow for more meaningful comparisons with contemporary fish behavior. Another potential application of this approach is monitoring marine migration of salmonid species which are not obligately anadromous in the archaeological record (e.g., *O. mykiss*: Bull Trout, Coastal Cutthroat Trout). The aDNA methods can identify archaeological vertebrae belonging to these species, and growth intervals in these bones with marine $^{87}Sr/^{86}Sr$, Sr/Ca, and Ba/Ca in transects across these vertebrae would further confirm they belong to an ocean-going variant. It would be impossible to gain this level of insight from otoliths as they lack genetic material for extraction and sequencing.

The LASS approach used here provides an additional dimension with trace elements for understanding salmonid life histories. The Ba/Ca and Sr/Ca ratios of the Sockeye and Steelhead vertebrae record anadromy, complementing the Sr isotope data. The Chinook vertebrae indicate the decoupled behavior of Ba and Sr during ossification, where Sr may be preferentially redistributed due to its different ionic properties. If true, it may be possible to use Ba/Ca ratios to track anadromy when ossification complicates other proxies. Ultimately, there are several variables that affect the ability of salmonid vertebrae to record anadromy, but the approach outlined in this paper presents a powerful coarse-grained approach for studying fish migration in the absence of otoliths.

CONCLUSION

Salmonid fisheries successfully supported Northwest Indigenous economies and food systems for over 9,000 years before settler colonial contact, commercial development, and contemporary restoration radically redefined the structure of local populations (Butler & O'Connor, 2004; McKechnie & Moss, 2016; Morin et al., 2023). Consideration of these long-term population

dynamics can inform species management plans and help to avoid "shifting baseline syndrome" (Pauly, 1995; Thurow et al., 2020). By combining the application of genetics (HT-qPCR), geochemistry (LASS), and zooarchaeology, our multidisciplinary method offers a novel approach to *Salmonidae* taxonomic determinations and migratory life history variants. Our work demonstrates that applying HT-qPCR technology to archaeological salmonid vertebrae can provide accurate, efficient, and cost-effective means for species identification, which is necessary for characterizing past species' distributions and population dynamics. From the nine archaeological vertebrae, we identified three salmonid species and determined them to have short freshwater residence periods as a result of the dominantly marine Sr values. Compared with contemporary otoliths and vertebrae, we demonstrate these skeletal tissues could invariably provide clear records of marine migrations, but attenuation of Sr in freshwater was complicated and seemed directly related to species-specific life history variation. The combination of these two methods in the Northwest and in other regions thus could clarify species- and life history-specific behaviors and dynamics across the Holocene (Thompson et al., 2019). Documenting these population parameters across hundreds to thousands of years is critical in establishing population baselines and dynamics so scientists, natural resource managers, and policymakers may make informed decisions regarding contemporary populations (Hofman et al., 2015; Oosting et al., 2019).

Perspectives from Indigenous voices about the stewardship practices that have sustained these fisheries for millennia can inform future management goals (Reeder-Myers et al., 2022). We see several avenues for future work where our approach could be expanded to this end. First, we recognize that assessing population species composition and life history diversity requires additional samples across multiple archaeological sites and time periods in both the Skagit River and more broadly across the Northwest Coast and Columbia-Fraser Plateau to generate statistically meaningful inferences. Second, additional contemporary paired samples of otoliths and vertebrae could be assessed across additional species with diverse life history types. We estimate there may be at minimum 15 life history types across Sockeye, Coho, Chinook, Steelhead, Coastal Cutthroat, Dolly Varden, and Bull Trout that may be identifiable given the granularity of this approach. Third, estimating age at emigration, immigration, and harvest could be determined in future studies through comparison of microchemical data with vertebrae annuli (Hofkamp & Butler, 2017). These data could be compared with known life histories to consider the ways past Indigenous peoples employed stewardship practices to sustainably manage salmonid populations according to culturally defined criteria (Morin, Royle, et al., 2021; Rodrigues et al., 2018; Royle et al., 2018; Sanchez, 2020). Fourth, incorporating paleoenvironmental data could lend insight to the ways past communities made decisions within challenging climatic conditions and could again inform contemporary strategies as global temperatures continue to increase. Finally, integrating population genetic approaches are being more frequently employed with ancient samples to estimate changes in genetic diversity of historic populations compared to contemporary stocks (Johnson et al., 2018; Thompson et al., 2019).

Conservation has emphasized the significance of the loss of interspecific genetic diversity for species' persistence but often has lacked methodologies to evaluate how this diversity has changed compared to generations prior. For highly impacted species such as salmonids, where genetic diversity and life history strategies are closely tied, loss of diversity diminishes their ability to adapt to environmental change. As climate change and contemporary anthropogenic activities continue to reshape ecosystems, the breadth of salmonid populations and migratory patterns in deep time are considerations for fisheries managers.

Knowledge of such dynamic past populations can help to inform strategies to mitigate contemporary anthropogenic impacts to fisheries and landscapes. As Oosting et al. (2019), Atlas et al. (2021), and Hatch et al. (2023) note, collaborative frameworks that integrate multiple lines of evidence can help to generate innovative solutions to anthropogenic-induced environmental and climatic change. Data generated from our outlined approach may inform efforts to incorporate Indigenous knowledges and practices and historical reference points in management plans.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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