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An evaluation of fin ray microchemistry to describe movement of White Sturgeon in the Kootenai River basin: insights and limitations

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Introduction: White Sturgeon *Acipenser transmontanus* in the Kootenai River basin is listed as endangered in the United States and Canada. Declines have been mainly attributed to poor recruitment exacerbated by the environmental effects of Libby Dam in Montana. Reduced primary production downstream of Libby Dam has been identified as one factor limiting growth of White Sturgeon, thereby limiting natural reproduction through delayed sexual maturity. However, estimating changes in growth over time without knowledge of fish location (i.e., Kootenai River vs. Kootenay Lake) is difficult. The objective of this project was to use microchemistry analysis to describe the movement of White Sturgeon within the Kootenai River basin.

Methods: Water samples ($n = 27$) and White Sturgeon fin rays ($n = 162$) collected in the Kootenai River basin were measured for strontium isotope (^{87}Sr:^{86}Sr) ratios using laser ablation multicollector inductively coupled plasma mass spectrometry. All water samples and a subset of fin rays ($n = 8$) were also analyzed for trace elements (e.g., Sr, Ba, Mg). Fin ray annuli were measured and tested using knowledge of known age; known locations from physical capture events were assigned to age at capture.

Results: Strontium isotope analysis was unable to detect differences in the Kootenai River and Kootenay Lake using water or fin ray samples. The Kootenai River and Kootenay Lake were distinguishable using trace element data from water samples, but not fin rays. The discrepancy with trace elements appears to be associated with the physiology of fin ray growth.

Discussion: Although this study did not meet its original objective of describing the movement of White Sturgeon in the Kootenai River basin from fin ray microchemistry, our results provide insight into the potential influence of physiology on microchemistry analysis. In particular, fin ray microchemistry of slow-growing fishes may be possible in freshwater systems with further investigation into the physiological processes associated with growth and the incorporation of elements into calcified structures.
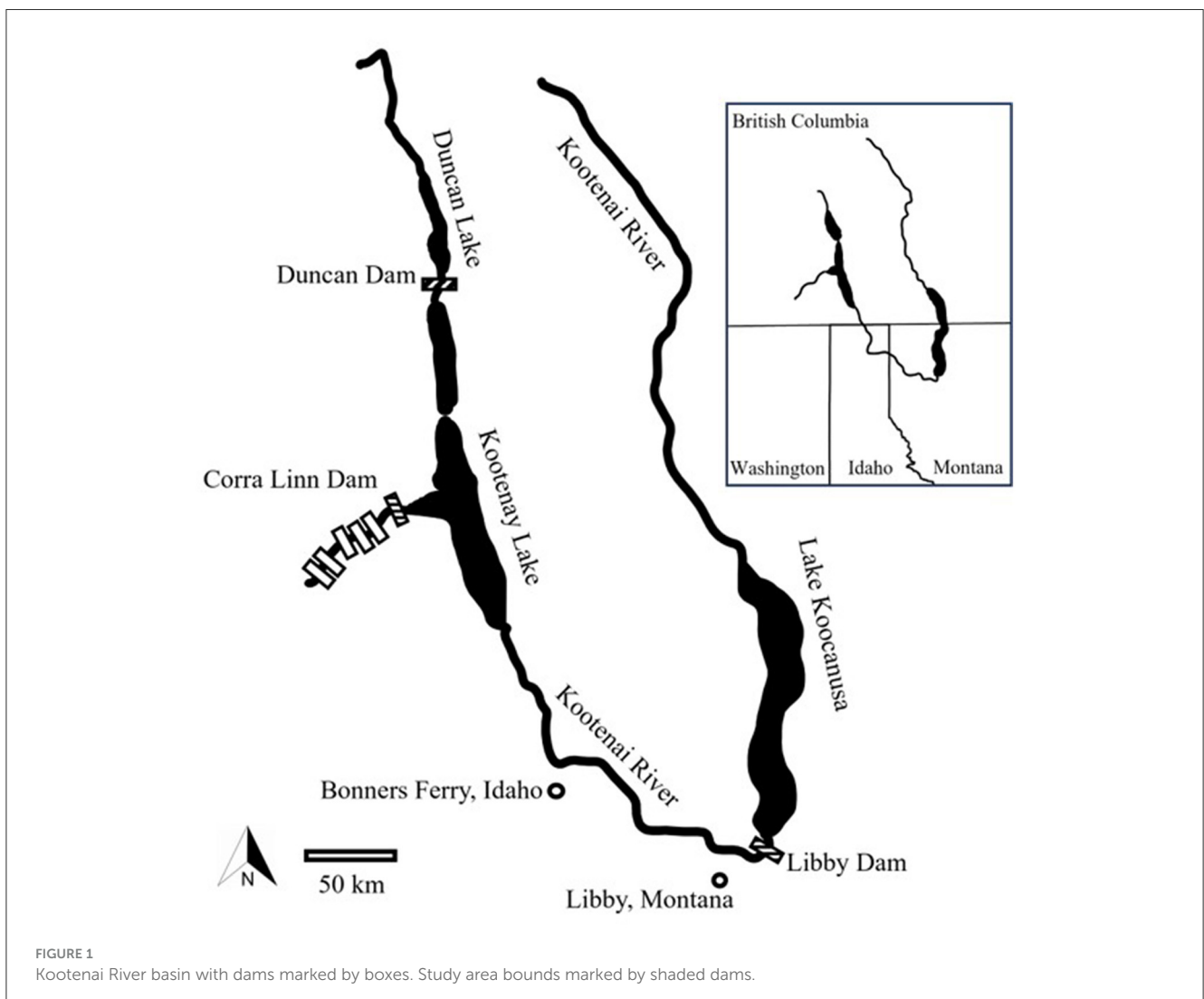
1 Introduction

Sturgeons (Acipenseridae) across the world are imperiled due to a variety of anthropogenic disturbances such as modification to flow, nutrient, and substrate dynamics (Dynesius and Nilsson, 1994; Birstein et al., 1997; Pikitch et al., 2005; Haxton et al., 2016). Sturgeons are particularly sensitive to changes in habitat because of their specific flow and habitat requirements (Birstein et al., 1997; Cech and Doroshov, 2004). The requirements for successful spawning span from environmental cues for migration (e.g., peak flows, temperature) to requirements for developing juveniles post-hatch (e.g., suitable prey, interstitial spaces; Haxton et al., 2016). In addition to vulnerabilities associated with habitat modification, sturgeons are globally exploited for caviar and meat. Sturgeons are late maturing and spawn intermittently, putting them at high risk for recruitment overfishing (Boreman, 1997). The combination of environmental changes and increased anthropogenic pressures have led to the listing of multiple species of sturgeon in the United States, including the Kootenai River population of White Sturgeon *Acipenser transmontanus*, as endangered under the Endangered Species Act (Haxton et al., 2016).

White Sturgeon is native to the Pacific Coast of North America from northern Mexico to the Aleutian Islands in Alaska (Hildebrand et al., 2016). White Sturgeon populations are declining across the species' distribution due to their sensitivity to habitat degradation, overharvesting, and climate change (Paragamian et al., 2002; van Poorten and McAdam, 2010; Hildebrand et al., 2016). White Sturgeon also display multiple life history types, all of which include a freshwater component (Hildebrand et al., 2016). White Sturgeon are long-lived fish (>80 years) that typically mature between 10 and 20 years of age, with males maturing at younger ages than females (Hildebrand et al., 2016). Individuals spawn periodically (every 2–10 years) during high flows associated with spring runoff (Parsley et al., 1993; Paragamian et al., 2002; Paragamian, 2012). White Sturgeon eggs require large substrate for incubation and post-hatch larvae require free-flowing water to drift into protective habitat (Parsley et al., 1993; Paragamian et al., 2002; Hildebrand et al., 2016; Counihan and Chapman, 2018). Because of the spawning ecology and early life history requirements of White Sturgeon, populations in human-altered rivers experience challenges to reproduction and recruitment (Hildebrand et al., 2016). Recruitment failure is considered the cause of decline
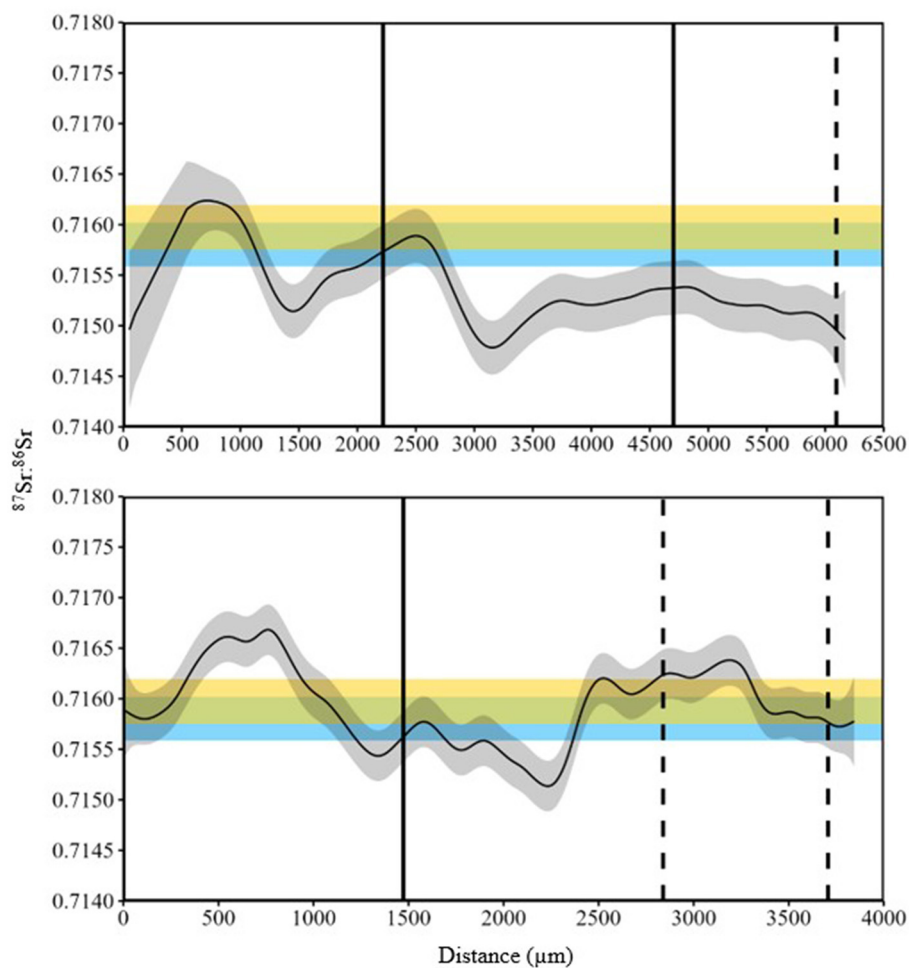

FIGURE 1
Kootenai River basin with dams marked by boxes. Study area bounds marked by shaded dams.

FIGURE 2
^{87}Sr:^{86}Sr (solid line) data from line scans of two White Sturgeon (top panel age 16 yrs, 69.5 cm fork length; bottom panel age 20 yrs, 98.9 cm fork length) sampled in 2015 in the Kootenai River basin. Gray shading is the interquartile range of ^{87}Sr:^{86}Sr values. Distance was measured as micrometers from the lumen to the edge of the fin ray. Blue shading is the distribution of ^{87}Sr:^{86}Sr values for Kootenay Lake and yellow shading is the distribution of ^{87}Sr:^{86}Sr values for Kootenai River. Solid vertical lines represent capture in Kootenai River, dashed vertical lines represent capture in Kootenay Lake.

for several White Sturgeon populations (Anders et al., 2002; Hildebrand et al., 2016). A White Sturgeon population of particular concern is the Kootenai River population of British Columbia, Idaho, and Montana (Paragamian, 2012).

White Sturgeon in the Kootenai River basin was listed as endangered under the Endangered Species Act in 1994 by the United States and under the Species at Risk Act by Canada in 2006 (Paragamian, 2012). Declines have been attributed to poor recruitment exacerbated by the environmental effects of Libby Dam in Montana (Hildebrand et al., 2016). Libby Dam was constructed in 1972 on the Kootenai River to provide flood control, hydroelectric power, and recreational opportunity. Changes to temperature and discharge regimes downstream of Libby Dam have affected environmental cues for White Sturgeon spawning, and dampened spring flows have led to the sedimentation of downstream spawning habitat (Paragamian et al., 2002; Paragamian and Wakkinen, 2002). Evidence of White Sturgeon recruitment failure was documented within the first decade after construction of Libby Dam (Partridge, 1983).

One of the most concerning trends that may be related to the absence of natural reproduction is the especially slow growth of White Sturgeon in the Kootenai River. White Sturgeon in the Kootenai River are estimated to exceed 30 years of age without reaching sexual maturity, which is thought to have negatively influenced natural recruitment (Anders et al., 2002; Paragamian and Beamesderfer, 2003; Paragamian et al., 2005). Slow growth of White Sturgeon in the Kootenai River is likely a combination of environmental effects, such as cold water temperatures and low nutrient concentrations (Beamesderfer, 1993; Paragamian and Beamesderfer, 2003; Watkins et al., 2017). Hatchery supplementation of White Sturgeon may also play an important role in reduced growth rates of White Sturgeon (i.e., density dependence; Justice et al., 2009). The effects of hatchery supplementation on growth have been observed in other White Sturgeon populations. For example, growth of White Sturgeon in the upper Columbia River basin declined as density increased from hatchery supplementation (Crossman et al., 2023). A better understanding of which factors regulate growth of White Sturgeon

TABLE 1 Mean microchemistry values from water samples for all sites sampled in Kootenai River and Kootenay Lake in 2015 and 2023.

Sample size (*n*)	Element	Mean	SD
Kootenai River			
15	^{87}Sr:^{86}Sr	0.71580	0.00047
9	Sr:Ca	0.00318	0.00004
9	Ba:Ca	0.00114	0.00008
9	Mg:Ca	0.26187	0.01960
Kootenay Lake			
12	^{87}Sr:^{86}Sr	0.71597	0.00048
12	Sr:Ca	0.00408	0.00029
12	Ba:Ca	0.00100	0.00001
12	Mg:Ca	0.22011	0.00157

Standard deviation (SD) is listed for each elemental ratio.

in the Kootenai River would help guide conservation efforts in the system.

The causes for slow growth of various fishes in the Kootenai River have been studied extensively (Snyder and Minshall, 1996; Watkins et al., 2017; Hardy et al., 2022). Libby Dam traps sediment and nutrients in Koocanusa Reservoir which has led to a decrease in primary production in downstream habitats. Nutrient addition began in 2005 to address the loss in primary production and is currently the largest riverine nutrient addition project in the world (Hardy et al., 2020, 2022). Nutrient addition has successfully increased primary production in the Kootenai River near the treatment site and has had a positive effect on growth of Largescale Suckers *Catostomus macrocheilus* (Watkins et al., 2017; Hardy et al., 2022). Conversely, the density of Mountain Whitefish *Prosopium williamsoni* increased with nutrient addition which resulted in decreased growth. Inferences regarding the slow growth of White Sturgeon in the Kootenai River basin have been limited by a lack of knowledge about White Sturgeon movements between the Kootenai River and Kootenay Lake. White Sturgeon hatch and rear in the Kootenai River but may use both lake and river habitats during adulthood (Paragamian and Wakkinen, 2002; Paragamian et al., 2002; Justice et al., 2009). Managers hypothesize that growth is faster in Kootenay Lake than in the Kootenai River. Because White Sturgeon are long-lived, tracking an entire lifetime of movement is difficult (Nelson et al., 2013). White Sturgeon outlive the batteries of telemetry tags and adequate technology to monitor passive integrated transponder (PIT) tags using instream antennae in large rivers such as the Kootenai River has been absent until recently. Challenges in documenting the movement of White Sturgeon have left managers uncertain about how much time White Sturgeon spend in the Kootenai River or Kootenay Lake as they grow. Microchemistry analysis is an alternative method that has been used to evaluate movement of fishes in various habitats (Pracheil et al., 2014; Tzadik et al., 2017). As such, microchemistry analysis was identified as a potential solution to the challenges

of characterizing White Sturgeon movement in the Kootenai River basin.

Microchemistry analysis is the measurement of elements that are incorporated into calcified fin rays over a fish's life (Pracheil et al., 2014). Elements and their isotopes are released into water and subsequently integrated into fin rays via respiration and diet. Some elements and isotopes are incorporated into calcified structures at values that reflect what is available in the environment (Wolff et al., 2013; Willmes et al., 2016; Sellheim et al., 2017; Hegg et al., 2019). As fish grow and move, isotopic ratios change to reflect the new environment, thereby tracking migrations throughout a fish's life. Additionally, age and growth measurements from calcified structures can be paired with movements described by microchemistry analysis to describe the life history of the fish (e.g., Hale and Swearer, 2008; Allen et al., 2009). Various elemental and isotopic ratios have been used in microchemistry analysis, but in freshwater systems, isotopes of strontium (^{87}Sr:^{86}Sr) are often most useful in distinguishing among stream systems with diverse geologies (e.g., Brown and Severin, 2009; Hegg et al., 2013; Brennan et al., 2016). Strontium is isostructural to calcium (Ca) and replaces Ca in calcified structures more predictably than other elements (Pracheil et al., 2014). The most reliable isotopic ratio for analysis is ^{87}Sr:^{86}Sr because Sr is stable in calcified structures and at specific locations over time (Kennedy et al., 2000; Pracheil et al., 2014). Although ^{87}Sr:^{86}Sr analysis is most common in freshwater, trace element (TE) analysis is another method that can be paired with ^{87}Sr:^{86}Sr analysis to improve the ability to assign and discriminate among locations (Hegg et al., 2019).

Measurement of the TE value in a calcified structure is typically reported as the ratio of the element of interest to total Ca (Campana, 1999). Analysis of TE is most often used to detect anadromy, but some examples of successful freshwater TE studies exist (e.g., Howland et al., 2001; Clarke et al., 2015). Many elements have been used to describe movement, but results can be unpredictable as certain elements (e.g., Sr) can demonstrate phenomena such as life-long accumulation (Mugiya and Satoh, 1997; Howland et al., 2001). Some studies indicate that physiological processes may be dominant over environmental influences for particular elements (Sturrock et al., 2014). However, experimental studies of calcified structures in fishes indicate that Ca, Sr, and barium (Ba) are most useful in TE analysis (Hale and Swearer, 2008; Jarić et al., 2011; Veinott et al., 2014; Luque et al., 2017; Vignon et al., 2023). In this study, analyses of TE focused on Sr, Ba, and magnesium (Mg) because the incorporation of these elements into calcified structures of fishes has been well studied, they are commonly found throughout aquatic environments, and have been shown to reflect environmental concentrations (Doubleday et al., 2013; Pracheil et al., 2014). Additionally, Sr and Ba are considered metabolically inert and easily measured in calcified structures.

In both ^{87}Sr:^{86}Sr and TE analysis, otoliths are the preferred and most studied calcified structure in fishes (Veinott and Evans, 1999; Hobbs et al., 2019). However, otoliths are relatively small in acipenserids and composed primarily of vaterite (Brennan and Cailliet, 1989; Baremore and Rosati, 2014; Pracheil et al., 2017). The structure of vaterite is poorly understood and often produces misleading results in microchemistry analyses (Pracheil
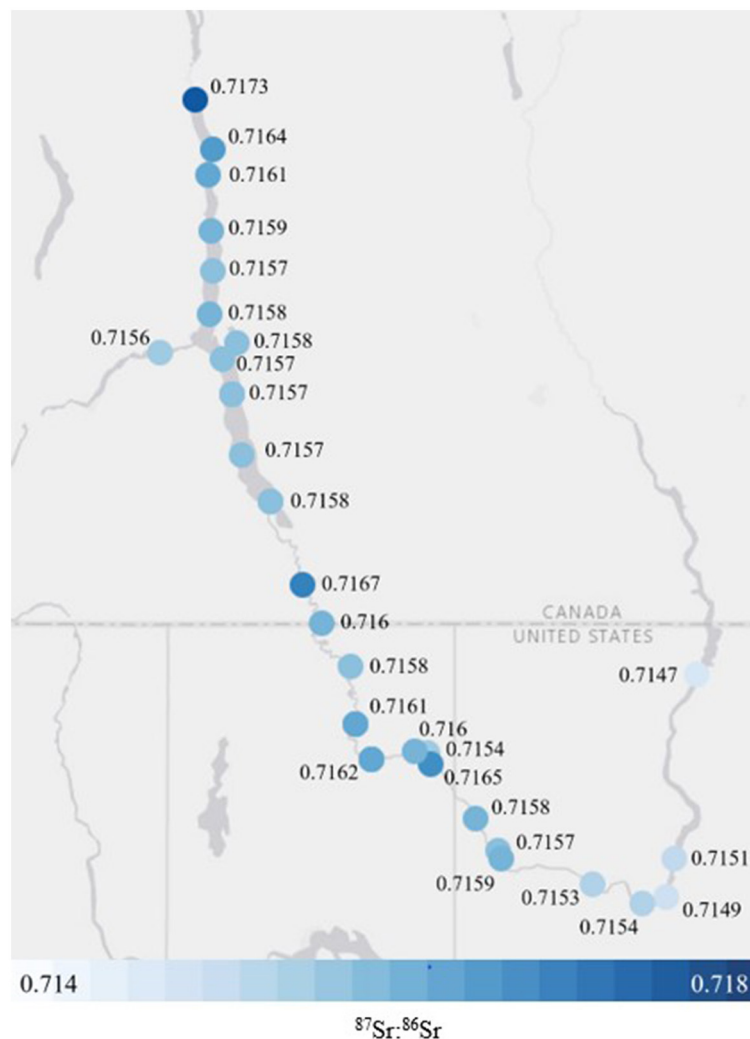
FIGURE 3
^{87}Sr:^{86}Sr values for water samples collected in the Kootenai River basin in 2015 ($n = 6$) and 2023 ($n = 21$).

et al., 2019a). Collection of otoliths is lethal, and many sturgeon populations have imperiled status. For these reasons, fin rays are often used for microchemistry studies of sturgeons (Sellheim et al., 2022). Calcification of fin rays in White Sturgeon begins 11–36 days after hatch, providing a nearly complete life history of ^{87}Sr:^{86}Sr and TE values (Sweeney et al., 2020). Fin rays are composed of hydroxyapatite, a calcium phosphate mineral, as opposed to the calcium carbonate matrices found in otoliths (Pracheil et al., 2014; Whitledge, 2017). When analyzed using laser ablation (LA) multicollector (MC) inductively coupled plasma mass spectrometry (ICPMS), fin rays demonstrate a CaPO polyatomic interference because of their hydroxyapatite composition (Woodhead et al., 2005; Vroon et al., 2008; Willmes et al., 2016). By increasing the plasma sampling depth in LA-MC-ICPMS, oxide levels can be reduced to eliminate interference. This technique makes fin ray microchemistry a reliable alternative to otolith microchemistry. Given the imperiled status of White Sturgeon and a lack of information about their life histories, the objective of this study was to use fin ray microchemistry analysis

to describe the movement of White Sturgeon throughout the Kootenai River basin.

2 Materials and methods

2.1 Study area

The headwaters of the Kootenai River originate in eastern British Columbia (Figure 1). The river flows south into Montana and turns northwest near Libby, Montana. From there, the Kootenai River continues northwest through Idaho and into British Columbia's Kootenay Lake. From the west arm of Kootenay Lake, the river continues west to its confluence with the Columbia River at Castlegar, British Columbia. Natural barriers at Bonnington Falls, British Columbia, and Kootenai Falls, Montana, isolated fishes over 10,000 years ago (Northcote, 1973); Kootenay Lake was also formed during this period (Hildebrand et al., 1999). The Kootenai River basin has been subjected to substantial anthropogenic development
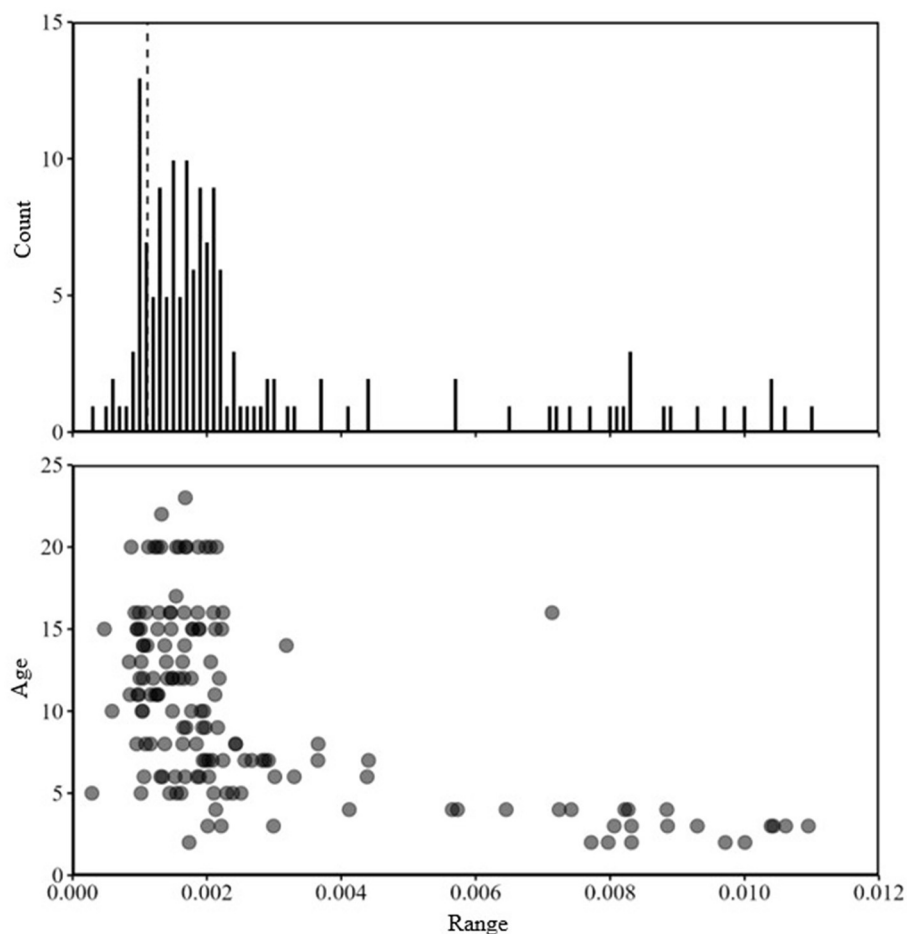
FIGURE 4
Frequency plot of the range (maximum minus minimum) of ^{87}Sr:^{86}Sr values from each line scan of White Sturgeon fin rays ($n = 162$) collected in 2015 from the Kootenai River (top panel). The vertical dashed line is the greatest estimated difference between Kootenai River and Kootenay Lake from water samples. Range of ^{87}Sr:^{86}Sr values from each line scan plotted by fish age (in yrs) at capture (bottom panel).

(e.g., mining, timber harvest, levees) including eight dams. Corra Linn, Duncan, and Libby dams define the distribution of White Sturgeon in the Kootenai River. Corra Linn Dam is located at the outlet of Kootenay Lake. Upstream, Duncan Dam prevents fish movement to the Duncan River basin at the north end of Kootenay Lake. Libby Dam is 50 km upriver from a natural barrier, Kootenai Falls, and is the most upstream dam in the Kootenai River. Construction of Libby Dam created Lake Koocanusa.

Geologic heterogeneity drives the ability of microchemistry to discriminate between aquatic habitats (Kennedy et al., 2000; Hegg et al., 2013). Four main geologic features influence drainage into the study area (Horton, 2017): the Purcell Mountains to the north, Salish Mountains to the east, and Cabinet Mountains to the south. Geologic history of these mountain ranges began during Mesoproterozoic, about 1,000–1,600 Ma. To the west are the Selkirk Mountains which began forming during the Cretaceous Period between 145 and 66 Ma. Kootenay Lake is highly influenced by inflow from the Kootenai River, but Kootenay Lake also has many other tributaries that drain diverse geology (Cui et al., 2017). The western shore of Kootenay Lake is composed mainly of Paleozoic (541–252 Ma) and Mesozoic (252–66 Ma) geologic

assemblages. The eastern and southern portions of the lake are primarily Neoproterozoic (900–550 Ma) and Mesozoic geologic units. In general, the geology surrounding Kootenay Lake and its tributaries is composed of younger formations that would be expected to have higher ^{87}Sr:^{86}Sr values than the Mesoproterozoic formations dominating the Kootenai River drainage upstream of Kootenay Lake (Kennedy et al., 2000; Dunnigan et al., 2023).

2.2 Water sample collection and processing

Water samples were collected from the Kootenai River ($n = 6$) and Kootenay Lake ($n = 6$) in 2011 by Hobbs (2013) and indicated that discrimination between the two systems was possible using ^{87}Sr:^{86}Sr analysis. However, ^{87}Sr:^{86}Sr values from water samples collected from the Kootenai River ($n = 6$) in September 2015 were not consistent with Hobbs (2013). As such, additional water samples were collected from the Kootenai River ($n = 9$) and Kootenay Lake ($n = 12$) in 2023. In February 2023, samples were collected from the surface of the Kootenai River during a period of
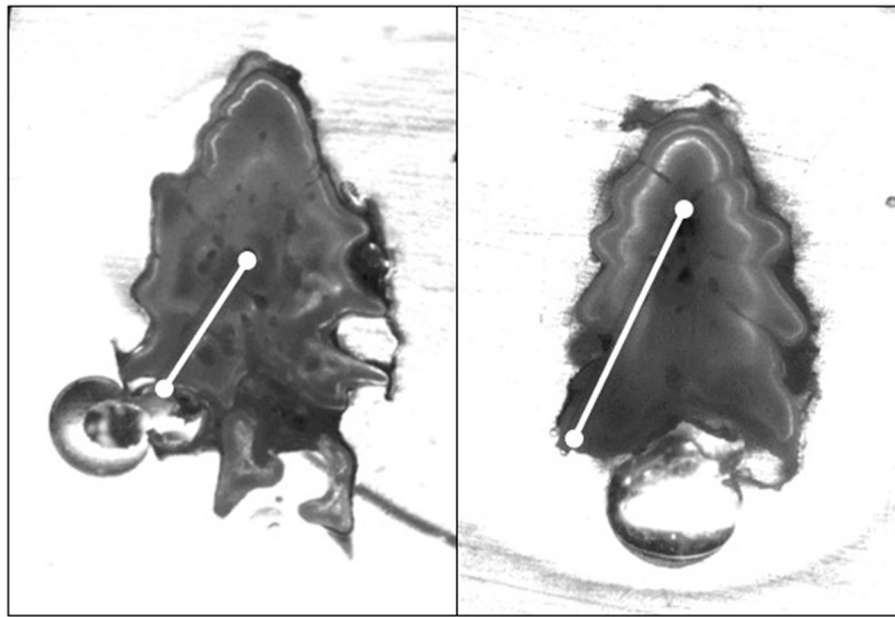
FIGURE 5
Images of White Sturgeon fin rays sampled in 2015 from the Kootenai River basin. Both are age-2 fish that displayed high rubidium values and abnormally high ^{87}Sr:^{86}Sr values (see Figure 6). Line scan path is highlighted by the white line.

low flow to ensure samples were minimally affected by runoff from tributaries. In March 2023, samples were collected from 30 m depth in Kootenay Lake during a period of mixing to best approximate an average annual ^{87}Sr:^{86}Sr value. Prior to sample collection, all 50 mL vials, lids, and 10 mL syringes were acid washed, rinsed with ultrapure water, air dried, and stored in Whirl Pak bags (Nasco, Fort Atkinson, Wisconsin; Shiller, 2003). Samples were collected using 0.2 µm pore filters (GE, Pittsburgh, Pennsylvania). Water samples were transported to the Interdisciplinary Center for Plasma Mass Spectrometry at the University of California-Davis (UC Davis) where they were analyzed to obtain ^{87}Sr:^{86}Sr values using a Nu Plasma HR (Nu032) MCICP mass spectrometer with a desolvating nebulizer inlet system (Nu Instruments DNS-100; North Wales, United Kingdom). The MCICP instrument was calibrated throughout sampling as described by Sellheim et al. (2017).

2.3 Fin ray collection

White Sturgeon were sampled in the Kootenai River from March 13 to October 26, 2015, by Idaho Department of Fish and Game (IDFG) and British Columbia Ministry of Water, Land, and Resource Stewardship. Fish were sampled using gill nets and setlines as part of annual monitoring (Hardy et al., 2020). Weighted multifilament gill nets had stretch mesh sizes of 2.5, 5.1, and 7.6 cm. Setlines were 23 m long with six baited hooks of various sizes (12/0, 14/0, 16/0). Fork length (FL; cm) and weight (kg) were measured from all captured White Sturgeon. All White Sturgeon were scanned for PIT tags and data were entered into an interagency database as each fish was processed. If a PIT tag identified the fish as known-age and hatchery-origin,

a fin ray sample was collected. A section from the most anterior pectoral fin ray was collected using side-cutting pliers. The section was cut just distal to the origin of the fin ray and then 25 mm distally from the first cut (Koch et al., 2008; Nguyen et al., 2016). Fin ray samples were stored in coin envelopes and allowed to air dry.

2.4 Fin ray ^{87}Sr:^{86}Sr analysis

Fin rays from White Sturgeon were mounted in epoxy and sectioned using a Buehler low-speed saw (Buehler, Lake Bluff, Illinois) following standard techniques (Koch and Quist, 2007). Sections of fin rays (0.5 mm) were sanded until annuli were clearly visible. Cross sections were mounted onto petrographic slides using Crystalbond 509-3 (Aremco, Valley Vintage, New York) and cleaned with ultrapure water. Slides contained between six and ten cross sections of White Sturgeon fin rays. All samples were taken to UC-Davis for strontium isotope analysis using LA-MC-ICPMS with a New Wave Research UP213 (Thermo Scientific, Fremont, California) laser ablation system coupled with a Nu Plasma HR (Nu Instruments Nu032; North Wales, United Kingdom) multiple-collection, high-resolution, double-focusing plasma mass spectrometer system. Line scans of fin rays from White Sturgeon proceeded from the lumen to posterior lobe edge at a beam width of 80 µm, scanning speed of 10 µm/s, laser pulse frequency of 20 Hz, and 90–100% laser power. In addition to measuring ^{87}Sr:^{86}Sr, isotopic masses were measured for ^{87}Sr:^{86}Sr, ^{88}Sr, and ^{85}Rb:^{88}Sr. At the beginning and end of each slide containing fin ray samples, instrumental accuracy was evaluated by measuring a White Seabass *Atractoscion nobilis* otolith, Green Sturgeon *Acipenser medirostris*
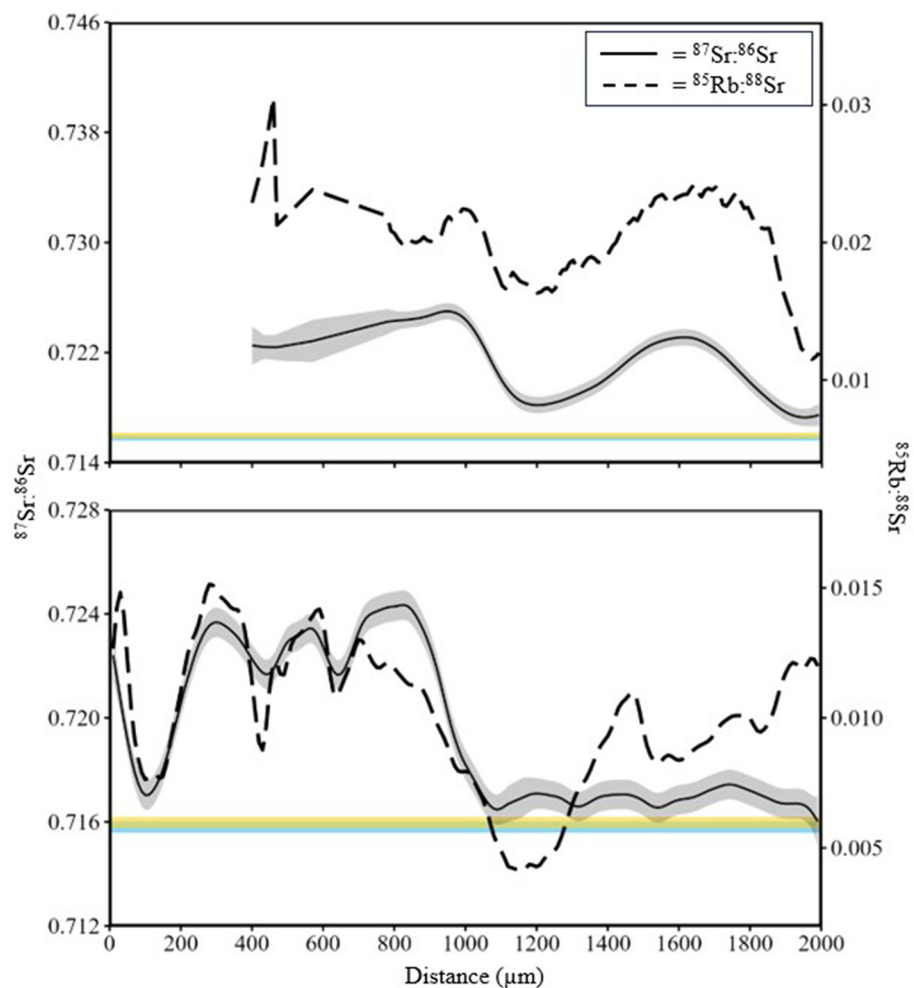
FIGURE 6
^{87}Sr:^{86}Sr and ^{85}Rb:^{88}Sr data from line scans of two White Sturgeon (top panel age 2 yr, 31.5 cm fork length; bottom panel age 2 yr, 31.6 cm fork length) sampled in 2015 in the Kootenai River basin (see Figure 5). Gray shading is the interquartile range of ^{87}Sr:^{86}Sr values. Distance was measured as micrometers from the lumen to the edge of the fin ray. Blue shading is the distribution of ^{87}Sr:^{86}Sr values for Kootenay Lake and yellow shading is the distribution of ^{87}Sr:^{86}Sr values for Kootenai River.

fin ray, and White Sturgeon fin ray with accepted standard Sr ratio values. Data collection and reduction followed methods detailed by Willmes et al. (2018) using the IsoFishR app in Program R (R Core Team, 2024). Reduced data were then smoothed using a thin-plate regression spline ($k = 100$) and generalized cross validation using the mgcv package in R (Wood, 2017). After processing fin rays for microchemistry, fin ray cross sections were viewed with a dissecting microscope using transmitted light. Using knowledge of known ages, a single reader measured the distance between annuli on the ablation transect with Image-Pro Plus software (Media Cybernetics, Inc., Bethesda, Maryland).

2.5 Fin ray trace element analysis

Preliminary results from ^{87}Sr:^{86}Sr analysis of water and fin ray samples suggested low discriminatory power between the Kootenai River and Kootenay Lake. Therefore, we conducted TE

analysis in an attempt to differentiate between habitats. Water samples collected for ^{87}Sr:^{86}Sr analysis in 2023 ($n = 21$) were analyzed for TE concentrations at UC Davis Interdisciplinary Center for Plasma Mass Spectrometry using an Agilent 8900 QQQ-ICPMS (Agilent Technologies Inc., Palo Alto, California) via a peristaltic pump and a 0.4 mL/min MicroMist nebulizer. Prior to analysis, the QQQ-ICPMS instrument was tuned and calibrated following methods detailed in Sweeney et al. (2020).

White Sturgeon fin rays from the Kootenai River basin were analyzed for trace element concentrations at UC Davis. To efficiently use allotted time and resources, we chose a small subsample ($n = 8$) of fin rays for TE analysis. The subsample reflected the distribution of ages (2–23 years) of the larger sample. In addition, subsampled fish had recapture histories from both the Kootenai River and Kootenay Lake. Fin rays were ablated using a Photon Machines 193 nm ArF Excimer laser (Photon Machines Inc., Redmond, Washington) with a HelEx dual-volume LA cell (Teledyne CETAC Technologies, Omaha, Nebraska) coupled
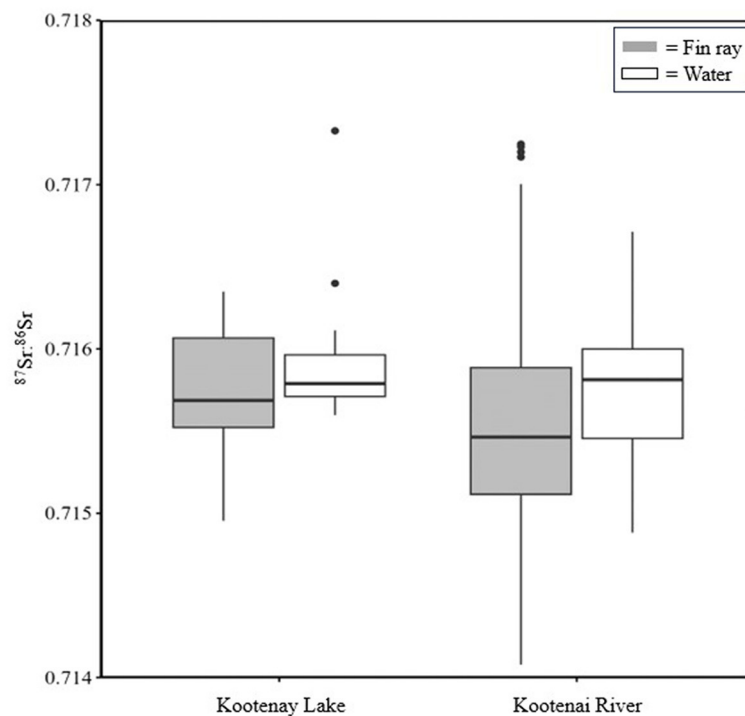
FIGURE 7
Comparison of ^{87}Sr:^{86}Sr data from the Kootenai River and Kootenay Lake. Fin ray data were collected from White Sturgeon captured in Kootenai River in 2015. Microchemistry data were assigned to either Kootenay Lake or the Kootenai River based on capture location and measured between the nearest 10 μm on either side of annuli for each recapture event. Water samples were collected in the Kootenai River basin in 2015 ($n = 6$) and 2023 ($n = 21$).

to an Agilent 7700x Quadrupole ICPMS (Agilent, Santa Clara, California). Line scans of fin rays from White Sturgeon proceeded from the lumen to the lateral edge at a beam width of 40 μm, scanning speed of 5 μm/s, and laser pulse frequency of 10 Hz. Prior to data collection, a transect of a larger spot size (150 μm) was ablated at a higher speed (100 μm/s) as a cleaning run. Data collection and reduction followed methods detailed by Sweeney et al. (2020). Isotopic masses were measured for ^{88}Sr, ^{137}Ba, ^{44}Ca, magnesium (^{24}Mg), manganese (^{55}Mn), iron (^{57}Fe), zinc (^{66}Zn), and lead (^{208}Pb). A single reader measured the distance between annuli on the ablation transect using known ages from the inter-agency database and Image-Pro Plus software.

2.6 Data summary and analysis

Mean ^{87}Sr:^{86}Sr, Sr:Ca, Ba:Ca, and Mg:Ca values were obtained from ICPMS analysis for each water sample collected in 2015 and 2023. Values of ^{87}Sr:^{86}Sr, Sr:Ca, Ba:Ca, and Mg:Ca were collected from fin rays at annuli with recapture data (see Figure 2 for example). We estimated the range of ^{87}Sr:^{86}Sr profile for each fish as the maximum value observed minus the minimum value observed to compare the range in ^{87}Sr:^{86}Sr data from fish to that observed in water chemistry. Microchemistry data between the nearest 10 μm on either side of annuli were obtained for each recapture and assigned to either Kootenay Lake or the Kootenai River based on capture location. Only recaptures from age-5 and

older ($n = 34$) White Sturgeon were used for ^{87}Sr:^{86}Sr analysis in an effort to minimize error from elevated rubidium (Rb) measurements observed in young fish. Because of the small sample size (14 observations from eight White Sturgeon) and no apparent influence of Rb in the TE profiles, all observations for TEs were included. Water and fin ray data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests (Allen et al., 2009; Avigliano et al., 2019). Data did not meet parametric assumptions and were compared using the Wilcoxon rank sum test (Blair and Higgins, 1980). A type-I error rate of α = 0.05 was used for all statistical tests.

3 Results

Mean ^{87}Sr:^{86}Sr values from water samples were similar [Wilcoxon test statistic (W) = 99, P = 0.68; Table 1] between the Kootenai River and Kootenay Lake. Additionally, no spatial pattern existed in values observed throughout the basin that would indicate a gradient of values associated with environment (Figure 3). In total, 162 White Sturgeon fin rays were ablated using LA-MC-ICPMS to measure ^{87}Sr:^{86}Sr. White Sturgeon varied in length from 23 to 112 cm FL and in age from 2 to 23 years. The range of ^{87}Sr:^{86}Sr values detected by water samples was small compared to the range of values observed in White Sturgeon fin rays (Figure 4). The maximum difference between the highest ^{87}Sr:^{86}Sr and lowest ^{87}Sr:^{86}Sr values in water samples was 0.0024. In contrast, differences between the maximum and
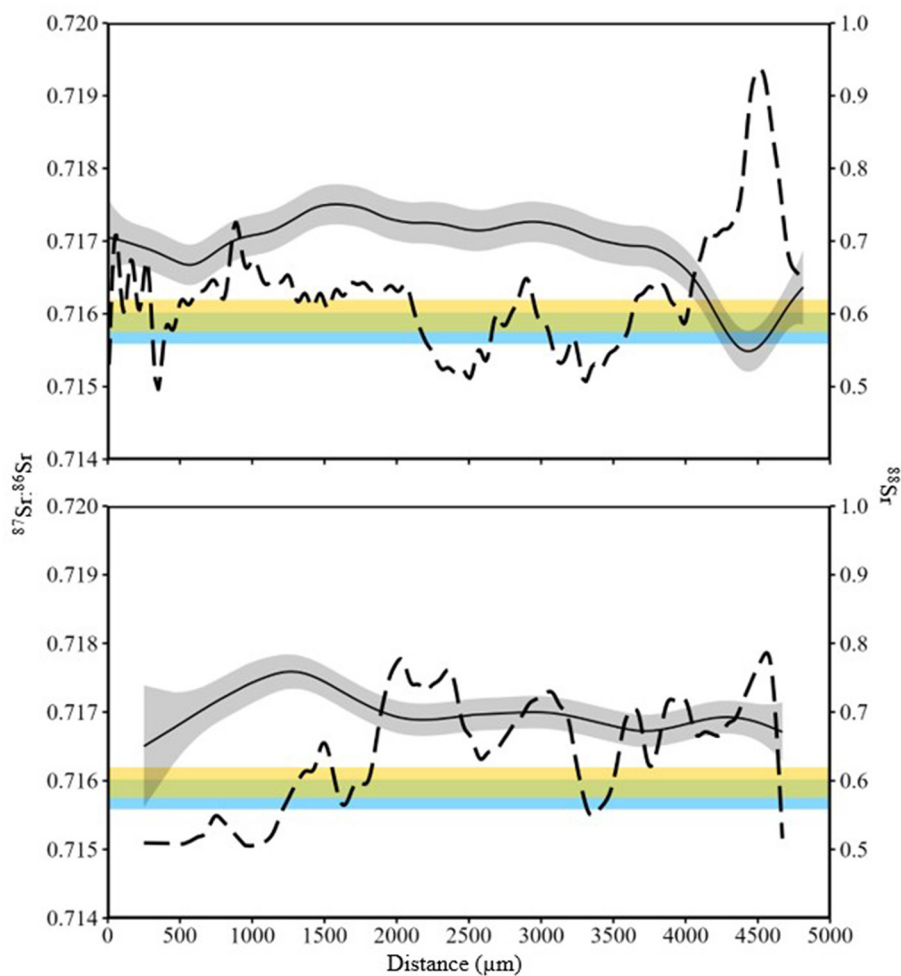
FIGURE 8
^{87}Sr:^{86}Sr (solid line) and ^{88}Sr (dashed line) from line scans of two White Sturgeon (top panel age 7 yr, 49.3 cm fork length; bottom panel age 8 yr, 73.3 cm fork length) sampled in 2015 in the Kootenai River basin. Gray shading is the interquartile range of ^{87}Sr:^{86}Sr values. Distance was measured as micrometers from the lumen to the edge of the fin ray. Blue shading is the distribution of ^{87}Sr:^{86}Sr values for Kootenay Lake and yellow shading is the distribution of ^{87}Sr:^{86}Sr values for Kootenai River.

minimum values of ^{87}Sr:^{86}Sr on individual White Sturgeon fin rays varied from 0.00029 to 0.011. Age-5 and younger White Sturgeon typically had a higher range of ^{87}Sr:^{86}Sr values than older White Sturgeon (Figure 4). White Sturgeon with a highly vascularized lumen were often observed with elevated Rb and ^{87}Sr:^{86}Sr values near the lumen (Figures 5, 6). This pattern was particularly common in fin rays from age-5 and younger White Sturgeon.

Summary statistics for ^{87}Sr:^{86}Sr were calculated at locations on the fin ray with a known location (i.e., based on recapture; see Figure 2 for an example). ^{87}Sr:^{86}Sr values from fin rays when fish were in Kootenay Lake were significantly different from values measured when fish were in the Kootenai River ($W = 1{,}310$, $P = 0.02$). Despite significant test results, shifts in the distribution of these data could not reliably describe differences in location (Figure 7). Collectively, results from the analysis of ^{87}Sr:^{86}Sr from water samples and White Sturgeon fin rays suggested that ^{87}Sr:^{86}Sr was not useful for describing whether White Sturgeon inhabited the Kootenai River or Kootenay Lake. Although ^{87}Sr:^{86}Sr exhibited low

variability across fin rays, high variability in total strontium (^{88}Sr) from fin rays indicated that TE may provide insight not available with ^{87}Sr:^{86}Sr (Figure 8).

Mean values of all TE values measured from water samples differed significantly between the Kootenai River and Kootenay Lake (Table 1). Mean Sr:Ca in water samples was lower in the Kootenai River than in Kootenay Lake, whereas Ba:Ca and Mg:Ca were highest in the Kootenai River. Patterns in TEs from eight White Sturgeon (29–106 cm FL; 2–23) fin rays were inconsistent with observations from water samples. For instance, Sr:Ca and Ba:Ca had a clear inverse relationship in water samples, but the same pattern was absent from all fin rays (Figure 9). All peaks in line scans that might be interpreted as movement were almost exclusively associated with annuli. Mg:Ca appeared to increase over time and, similar to Ba:Ca, failed to exhibit an inverse relationship with Sr:Ca (Figure 10). Sr:Ca and Ba:Ca from locations on fin rays where White Sturgeon were recaptured in Kootenay Lake were not significantly different than values measured when fish were in the Kootenai River ($W = 13$–26, $P = 0.16$–0.90). In contrast, Mg:Ca
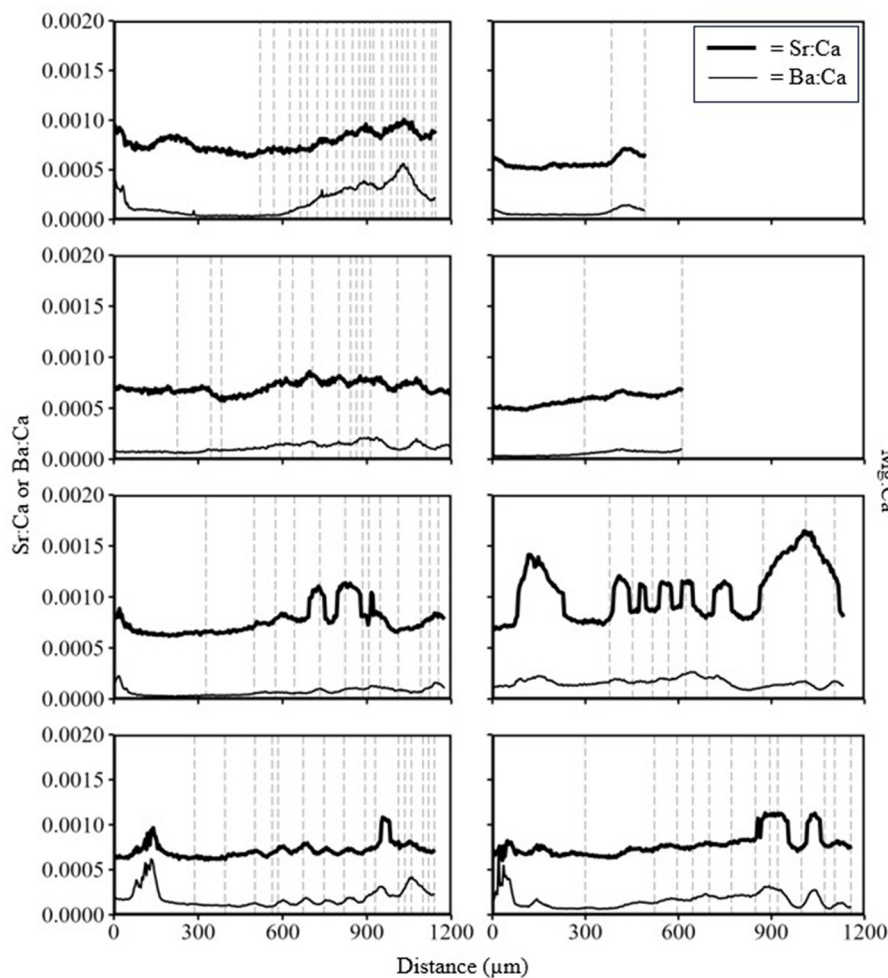
FIGURE 9
Trace element line scan of eight White Sturgeon fin rays collected from the Kootenai River basin in 2015. Vertical dashed lines are annuli. Distance was measured as micrometers of the line scan from the lumen to the edge of the fin ray.

was significantly different (i.e., higher in Kootenay Lake) between known recapture locations ($W = 45$, $P \leq 0.05$).

4 Discussions

Microchemistry has become an important tool in fish science. Describing movement of fishes with ^{87}Sr:^{86}Sr is accomplished by comparing the isotopic ratio found in calcified structures to that of water in the study area. Analyses of ^{87}Sr:^{86}Sr have been used to describe locations of fishes at many spatial scales, from small streams (Kennedy et al., 2000) to the ocean coastline (Fink-Jensen et al., 2022). In this study, ^{87}Sr:^{86}Sr analyses were unable to detect movement of White Sturgeon given the homogeneity of water chemistry in the Kootenai River basin. Using ^{87}Sr:^{86}Sr is best conducted in systems with high geologic heterogeneity since the age of geologic formations in the watershed is reflected in the water chemistry (Kennedy et al., 2000). Although the geology of the Kootenai River basin is heterogenous, the geography of the basin leads to mixing of water in the mainstem waterbodies. Both

the Kootenai River and Kootenay Lake have major tributaries that drain from the Selkirk and Purcell mountain ranges. Upstream of Libby Dam, Koocanusa Reservoir has major tributaries that drain the Purcell and Salish mountain ranges. Mixed water from Koocanusa Reservoir forms the Kootenai River downstream of Libby Dam further homogenizing ^{87}Sr:^{86}Sr values. Multivariate analysis that pair TE analysis with ^{87}Sr:^{86}Sr analysis have been used in other systems to discriminate between water bodies (Hale and Swearer, 2008; Allen et al., 2018; Pracheil et al., 2019b). Because ^{87}Sr:^{86}Sr values alone were unable to discriminate between the Kootenai River and Kootenay Lake, TE analysis was completed for a subsample of White Sturgeon in this research.

Analysis of TE describes the location of fishes by comparing the amount a specific element in a water body to that found in calcified structures. Different elements may be measured in microchemistry analysis, each with varying levels of influence from either diet or environment (Doubleday et al., 2013; Tzadik et al., 2017). Concentrations of TE may vary in response to geology, pollution, and other environmental inputs (Campana, 1999). Elements subject to physiological influence (e.g., Zn, Mg) are
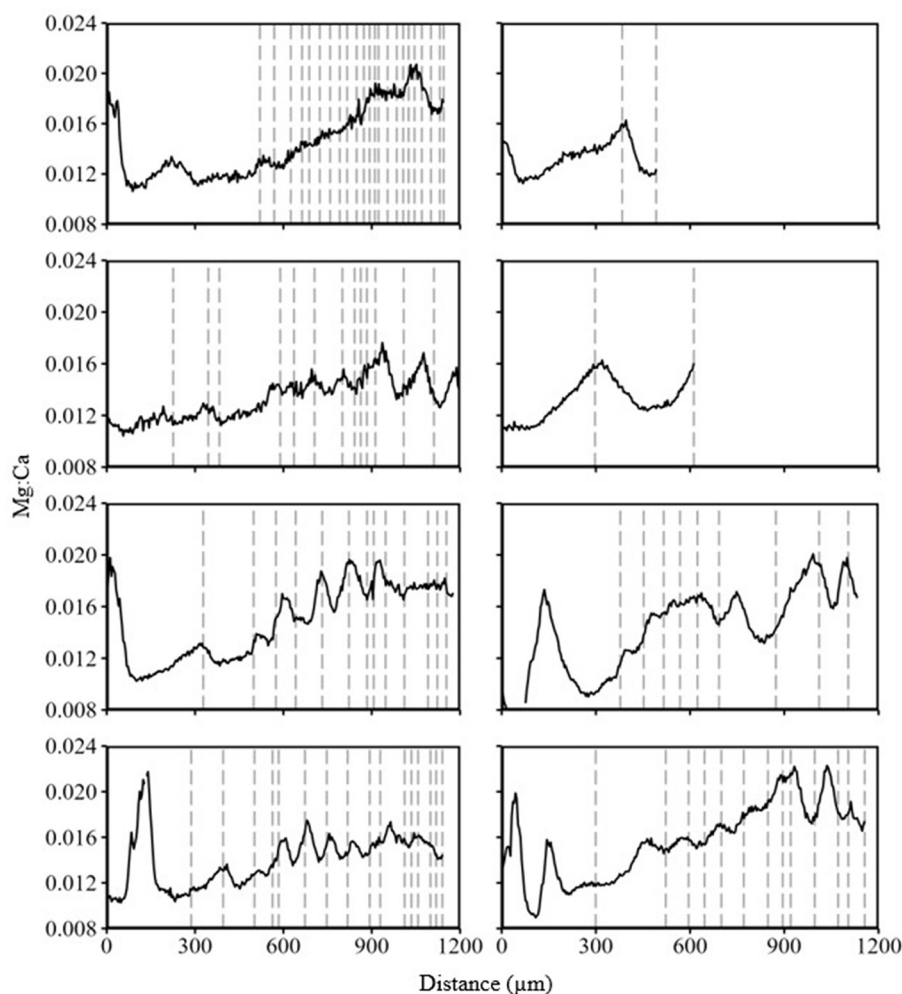
FIGURE 10
Trace element line scan of eight White Sturgeon fin rays collected from the Kootenai River basin in 2015. Black lines represent Mg:Ca values. Vertical dashed lines are annuli. Distance was measured as micrometers of the line scan from the lumen to the edge of the fin ray.

best paired with elements that have well-understood incorporation processes (e.g., Ba, Sr) for discrimination between water bodies (Clarke et al., 2007; Loewen et al., 2016; Thomas and Swearer, 2019). Systems with distinct chemical transitions (e.g., freshwater to salt water; Limburg, 1995; Allen et al., 2009; Sellheim et al., 2022) are well-studied, but fewer analyses of TE have been conducted in freshwater systems (Clarke et al., 2015; Avigliano et al., 2019). Studies using sturgeon fin rays found that TEs were able to detect natal origin of river sturgeons between the Mississippi and Missouri river basins (Phelps et al., 2012) and describe movement of Shovelnose Sturgeon *Scaphirhynchus platorynchus* in the Mississippi and Wisconsin rivers (Pracheil et al., 2019b). In the Kootenai River basin, TE analysis was able to distinguish between water samples from the Kootenai River and Kootenay Lake. Unfortunately, TEs in White Sturgeon fin rays provided no discriminatory power, thereby suggesting that biological processes may have influenced TEs in fin rays or that the small annuli size, coupled with the relatively large ablation spot size was unable to resolve movements.

The processes regulating elemental incorporation into otoliths are generally known, but processes of incorporation in fin rays are less understood (Reis-Santos et al., 2023). Research into the processes of elemental incorporation in otoliths is extensive and has increased confidence in the interpretation of microchemistry results (Farrell and Campana, 1996; Elsdon and Gillanders, 2005; Brown and Severin, 2009; Clarke et al., 2015). Some processes involved in the growth of fin rays are documented (e.g., annuli formation, vascularization), but the cellular and molecular processes are not (Tzadik et al., 2017). Studies into fin ray physiology have largely been experimental studies on environmental effects or comparison to otoliths (e.g., Clarke et al., 2007; Sellheim et al., 2017; Sweeney et al., 2020). Rude et al. (2014) found that dietary influences from marine-derived hatchery feed changed the microchemistry in fin rays of juvenile Muskellunge *Esox masquinongy*. The authors also reported that once Muskellunge consumed a wild diet, there was no influence on fin ray microchemistry because wild diets had a similar chemical composition to the environment. A review by Loewen et al. (2016)

found that incorporation of elements into fin rays was highly influenced by growth, reproduction, and environmental conditions even for elements that are considered reliable geologic markers. Methodologically, the chemical structure of fin rays can also create challenges during ablation. In this study, both the hydroxyapatite structure and morphological characteristics of White Sturgeon fin rays influenced methods of ablation.

The morphology of sturgeon fin rays presents several challenges with regard to microchemistry analysis. Fin rays are flexible structures, and their morphology can be altered by growth or damage (Whitledge, 2017). A variety of techniques have been used to overcome morphological issues when conducting microchemistry analysis on fin rays. For example, spot analysis is sometimes used when morphology prevents effective line scans (Pracheil et al., 2014; Sellheim et al., 2017). White Sturgeon from the Kootenai River grow particularly slow, and most fin ray samples taken from the Kootenai River basin exhibited crowding of annuli. The hydroxyapatite structure of fin rays delivers a low signal when ablated, requiring a large spot size (40–80 μm) to ablate enough material to register a signal with both LA-MC-ICPMS and quadrupole ICPMS. The large spot size negates the benefits of using spot analysis (i.e., targeted ablation) because multiple annuli were often ablated simultaneously. Therefore, portions of our microchemistry results were a mixture of multiple years' worth of annuli data, further complicating inferences.

The original goal of this research was to provide insight on the population ecology of White Sturgeon that could be used to inform future conservation efforts in the Kootenai River system. Specifically, we sought to characterize movement of White Sturgeon with the aim of describing factors influencing growth across different habitats. Unfortunately, we were unable to answer precipitating research objectives but our process provided an important cautionary account and path for future research in fin ray microchemistry analysis and physiology. All study systems and fishes present unique conditions that influence methods used in microchemistry analysis. Water chemistry can change daily (e.g., tidal systems; Sellheim et al., 2022), seasonally (e.g., spring runoff; Hüssy et al., 2016), or over several years (e.g., pollution; Daros et al., 2021) making water chemistry one of the most important factors for recognizing the scope of inference in microchemistry study design. Considering data from fishes, understanding how elements are incorporated into the calcified structure of choice and how elemental incorporation may change over time is important. Lastly, additional attention given to determining the appropriate method for collection of microchemistry data may improve use of these techniques as microchemistry analysis can be conducted using different equipment and ablation methods (Pracheil et al., 2014). In many scientific fields, the majority of "null results" go unpublished or even unreported (e.g., Franco et al., 2014). Non-reporting of null results can lead to wasted effort in future research. Observations from this study support previous research findings from otolith microchemistry that elements commonly measured in otolith TE analysis can be more influenced by physiology than environment (Sturrock et al., 2014). Research of physiological processes in fin ray formation is necessary to improve interpretations of microchemistry analysis in fin rays.

Data availability statement

The datasets presented in this article are the property of the Idaho Department of Fish and Game but can be made available upon reasonable request to the corresponding author.

Ethics statement

The animal study was approved by all fish handling associated with the creation of this data set was performed in accordance with Idaho Department of Fish and Game and American Fisheries Society guidelines. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CG: Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Visualization. MQ: Writing – original draft, Writing – review & editing, Conceptualization, Formal analysis, Funding acquisition, Investigation. RH: Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Investigation. MW: Writing – original draft, Writing – review & editing, Methodology, Software. LL: Writing – original draft, Writing – review & editing, Methodology. SW: Writing – original draft, Writing – review & editing. TS: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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