PRIMARY RESEARCH PAPER

Use of otolith microchemistry to identify subbasin natal origin and use by invasive Lake Trout in Yellowstone Lake

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Abstract Nonindigenous lake trout (*Salvelinus namaycush*) expansion in Yellowstone Lake has led to a large decline in the native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) population. We assessed whether otolith microchemistry could be used to identify subbasin natal origins and long-term use by lake trout as a potential tool for optimizing removal efforts. ^{87}Sr:^{86}Sr and Sr:Ca ratios in otolith cores were used to assess natal origins and ^{87}Sr:^{86}Sr ratios in otolith transects were used to assess movement patterns. Water chemistry was similar throughout the lake, ranging from 0.70634 to 0.70642 and 3.94 to 4.38 mmol/mol for ^{87}Sr:^{86}Sr and Sr:Ca ratios, respectively. Lake trout otoliths also showed little variation in ^{87}Sr:^{86}Sr and Sr:Ca ratios of the natal region and ^{87}Sr:^{86}Sr across otolith transects. Thus, we found that microchemical differences among Sr isotope and Sr:Ca elemental ratios in otoliths were insufficient to detect the natal origin or extensive within-lake movement that has been established from telemetry investigations. Detailed analysis of other elements or isotopes in hard-part microchemistry, in combination with other tools for detecting movement, may improve detection of natal origin and life history movement among fishes within freshwater lentic systems.

Keywords Otolith microchemistry · Strontium isotope · Sr:Ca · Invasive species suppression · Movement

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Introduction

Lake trout [*Salvelinus namaycush* (Walbaum, 1792)] are an apex predator that were introduced in many lakes and reservoirs in the western United States with the intent of controlling prey and diversifying the recreational fishery (Martinez et al., 2009). The native range of lake trout in North America includes Alaska, Canada, the Great Lakes, and New England (Scott & Crossman, 1973). In their native range, lake trout are a dominant predator in lake food webs (Ryder et al., 1981) and support valuable sport fisheries (Healey, 1978). Lake trout are a cold-adapted (< 10 °C), deep-

water dwelling species that are largely inaccessible to avian and terrestrial piscivorous wildlife. Additionally, lake trout can be extremely long-lived (30 + years, if unexploited; Muir et al., 2012) and are capable of capturing prey at least half their body length (Ruzycki et al., 2003). Prior to spawning in autumn, adult lake trout often move extensively within lakes seeking spawning sites (Rahrer, 1968; Kapuscinski et al., 2005).

Introductions of lake trout have led to declines in native and nonnative sport fishes, leading to programs to reduce their numbers (Martinez et al., 2009; Hansen et al., 2019). Lake trout were first discovered in Yellowstone Lake in 1994 (Kaeding et al., 1996), and subsequent otolith microchemistry research confirmed they were nonnative to the lake (Munro et al., 2005). The presence of this highly predatory species in the Yellowstone Lake ecosystem has caused precipitous declines in the once abundant Yellowstone cutthroat trout [*Oncorhynchus clarkii bouvieri* (Jordan & Gilbert, 1883)] population (Ruzycki et al., 2003; Koel et al., 2005) as well as a number of cascading effects on other species in the ecosystem (Tronstad et al., 2010; Koel et al., 2019). This introduction has been particularly troubling as Yellowstone Lake is one of the last strongholds for genetically pure Yellowstone cutthroat trout (Gresswell, 2011).

An intensive, large-scale lake trout suppression effort was implemented by Yellowstone National Park (YNP) in 1995 to reduce their abundance, by gillnetting adults on spawning sites, resulting in notable declines in abundance (Koel et al., 2020a; Syslo et al., 2020). Novel techniques to increase mortality of lake trout embryos on spawning sites are also being developed (Thomas et al., 2019; Koel et al., 2020b). A total of 14 spawning sites have been located and verified by the presence of gametes (Fig. 1; Koel et al., 2020b) with additional sites identified as probable spawning areas (Williams, 2019). An understanding of the relative importance of spawning sites in producing pre-recruit lake trout could help direct gillnetting operations and embryo suppression efforts to the most productive sites and increase efficiency of the suppression program. Additionally, it is not known if lake trout tend to primarily occupy the lake subbasin of natal origin throughout their life, or if they move and tend to occupy other subbasins for long periods. In this study, we assessed the utility of using otolith microchemistry to identify such patterns. A greater

knowledge of natal origin and general subbasin movement patterns and long-term use in Yellowstone Lake would be valuable to resource managers for directing suppression efforts in the future.

Otolith microchemistry has been proven to be an important tool for identifying fish movement patterns, habitat use, and natal origins in freshwater and marine environments (Gillanders & Kingsford, 1996; Kennedy et al., 2000; Zimmerman & Reeves, 2002; Muhlfeld et al., 2012; Humston et al., 2017). Though a variety of elements can be incorporated into otoliths, Sr and Sr isotopes have been used most extensively as they have proven to be the most accurate for recording environmental history (Kennedy et al., 2000; Gibson-Reinemer et al., 2009; Pangle et al., 2010). Most work in freshwater has been done in lotic systems, which show unique chemical signatures among different tributaries and mainstem habitats correlated with varying underlying geology within river drainages (Kennedy et al., 2000; Humston & Harbor, 2006; Gibson-Reinemer et al., 2009; Muhlfeld et al., 2012). In a review of the literature, we found few studies have used otolith microchemistry to discern differences in fish spawning and rearing in different subbasins within freshwater lakes (Brazner et al., 2004; Dufour et al., 2005; Pangle et al., 2010; Prachiel et al., 2014). The lack of studies in lentic freshwater systems may be largely due to mixing of the water throughout lakes and reservoirs owing to wind and underwater currents, which can result in homogenous water chemistry throughout a water body (Dufour et al., 2005; Pangle et al., 2010). However, Yellowstone Lake has several distinct subbasins and unique geothermal features (Kaplinski, 1991; Morgan et al., 2003) that could result in the development of spatially distinct water chemistries that could be imparted into lake trout otoliths.

The primary objectives of this study were to test the potential of using otolith microchemistry to (1) identify the primary subbasin natal origins that most greatly contribute to lake trout recruitment in Yellowstone Lake and (2) identify patterns of large-scale lake trout movement and long-term occupancy of subbasins throughout their life history in the lake.
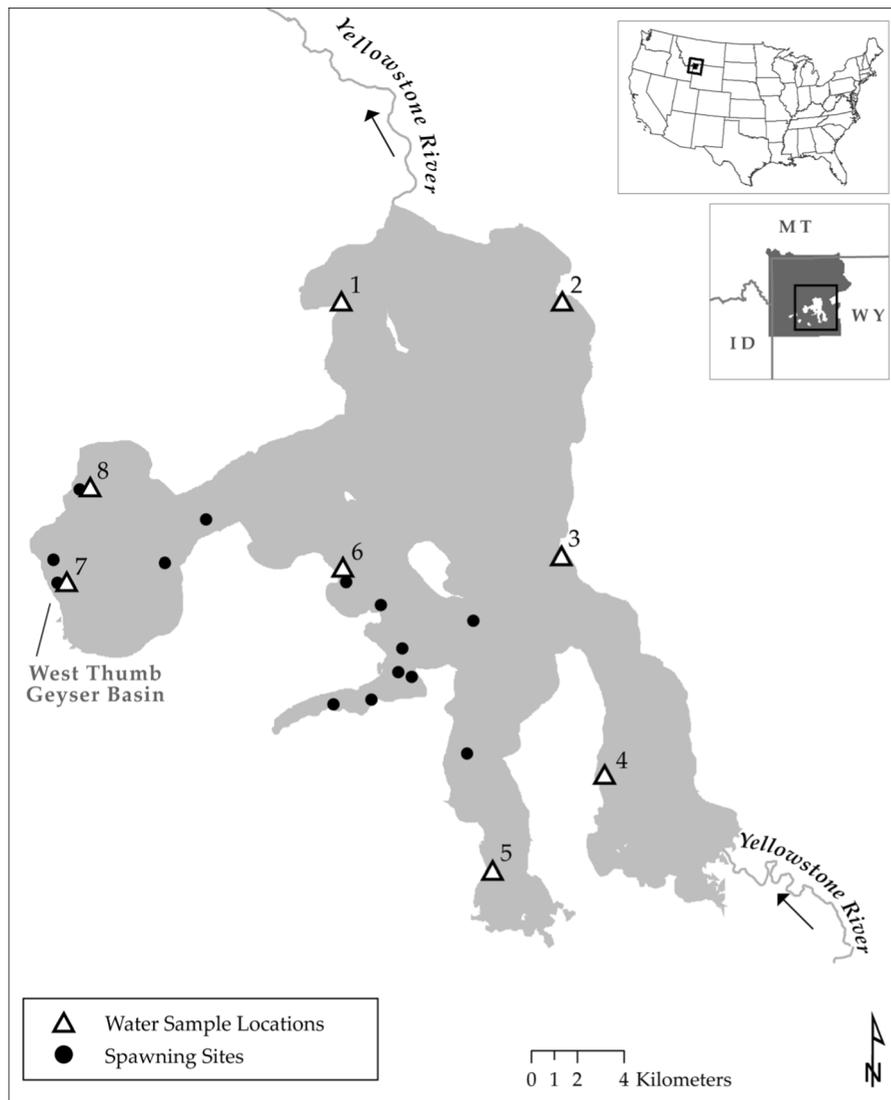
Fig. 1 Yellowstone Lake in Yellowstone National Park with verified lake trout spawning sites and locations of water samples collected for analysis of ^{87}Sr:^{86}Sr and Sr:Ca (mmol/mol) ratios

Methods

Study area

Yellowstone Lake is the largest alpine lake (above 2000 m) in North America and has a surface area of 34,000 ha, 239 km of shoreline, mean depth of 48 m, maximum depth of 137 m, and volume of 1.5×10^{10} m^3 (Kaplinski, 1991). The lake has a thermal structure that is typically unstable with a weak and variable thermocline at a depth of 12–15 m during July–September (Koel et al., 2019). The lake freezes

over by late December and can remain frozen until late May or early June.

Water collection and analysis

Lake water samples were collected from 8 locations, three near verified lake trout spawning sites (Fig. 1; Williams 2019; Koel et al., 2020b) and others within distinct subbasins. An additional sample was taken near the West Thumb Geyser Basin and was used to assess whether geothermal inputs influenced water chemistry. Water samples were collected immediately

after the lake was ice free on May 22 and 23, 2013, near the timing of lake trout fry emergence (Marsden et al., 2005; Simard et al., 2019), when natal signatures are incorporated into otoliths. Water samples were collected using a Van Dorn water sampler near the lake bottom where lake trout fry are likely to congregate (Marsden et al., 2005). All of the lake water samples were collected in water depths less than 10 m at approximately 0.5 m above the substrate. Prior to sampling, the water sampler was acid washed with 12 N hydrochloric acid diluted to 6 N with Milli-Q® water, and then triple rinsed with Milli-Q® water. To minimize risk of contamination between lake water samples, the sampler was triple rinsed using Milli-Q® water, followed by a triple rinse with water from the sample location. Water samples were filtered with a sterile 0.2-micron Whatman® syringe filter and a sterile 50 ml syringe. The water samples were stored in acid-washed polyethylene bottles and fixed with two drops of nitric acid (HNO_3).

Microchemical analysis of water samples was conducted at the Woods Hole Oceanographic Institution (WHOI) using solution-based inductively coupled plasma mass spectrometry (ICPMS). For ^{87}Sr:^{86}Sr ratios, a portion of each sample was first evaporated and re-dissolved in 50% HNO_3 then eluted through a Sr-specific cation exchange resin. The remaining sample was again evaporated and re-dissolved in 1 mL of 5% HNO_3 for analysis with a Thermo Finnigan Neptune multiple collector ICPMS. Strontium isotope ratios were calculated by correcting for interferences of ^{87}Rb on ^{87}Sr and ^{86}Kr on ^{86}Sr using methods described by Jackson & Hart (2006). All Sr isotope ratios were normalized to the NIST SRM987 standard. For elemental ratios, samples were first diluted tenfold using a 2% HNO_3 solution prior to ICPMS measurement of ^{48}Ca and ^{88}Sr. Liquid standards and instrument blanks of 2% HNO_3 were run every 4 samples. Instrument mass bias was corrected using certified values from a river water standard (SLRS-4, NRC), and additional internal laboratory river water standard was used to assess measurement precision. External precision (relative standard deviation, RSD) of Sr:Ca ratios for the laboratory standard ($n = 3$) was 1.5%.

Otolith collection and preparation

Lake trout otoliths were sampled as part of the annual gill netting assessment conducted during early August in all lake regions (Syslo et al., 2011). Captured lake trout were measured (mm, total length) and the otoliths extracted using non-metallic forceps to minimize risk of contamination. Otoliths were rinsed with purified Milli-Q® water to remove any remaining tissue and stored in clean microcentrifuge vials. Twenty of the largest lake trout (> 400 mm) were randomly selected for otolith microchemical analysis. The lake trout ranged in age from 3 to 6 years old, with $n = 3$ age 3 fish, $n = 4$ age 4 fish, $n = 11$ age 5 fish, and $n = 2$ age 6 fish. Otoliths from larger fish were preferentially selected because these fish were more likely to have occupied more habitats throughout the lake than younger fish and thus may exhibit a variety of different microchemical signatures in their otoliths reflective of within-lake movement.

Otoliths were prepared for laser ablation similar to the methods described by Muhlfeld et al., (2012). One sagittal otolith from each fish was randomly selected, cleaned with a nylon brush, triple rinsed, and then dried for 24 h in a laminar flow hood. Otoliths were then mounted on petrographic slides, sulcus side up, using cyanoacrylate glue, and sanded and polished using 600- and 1500-grit sandpaper and 0.5- and 0.1 μm lapping paper until the plane of the nucleus became visible (Muhlfeld et al., 2012; Garcez et al., 2014). After polishing, otoliths were again scrubbed with a nylon brush, triple rinsed, and soaked in Milli-Q® water overnight followed by remounting on a new slide.

Otolith microchemical analysis was performed using laser ablation ICPMS equipped with a 213-nm laser. Otolith ^{87}Sr:^{86}Sr and Sr:Ca ratios were sampled concurrently using a Thermo Finnigan Neptune™ multiple collector ICPMS and quantified following analytical methods described in Muhlfeld et al., (2012). Otolith laser ablation transects were scanned from the otolith core to the edge along the longest plane of the otolith, where circular and annular bands appeared widest, using a beam diameter of 75 μm, a repetition rate of 20 Hz, and a scan speed of 5 μm s^{-1}. Sample processing was randomized to minimize potential systematic bias from instrument drift. For quality assurance, a certified reference material (MACS-3) was run every 5 samples to assess

instrument drift and changes in mass bias ([87]Sr:[86]Sr = 0.70759; Weber et al., 2015). The mean [87]Sr:[86]Sr ratio ($\pm$ 1 SD) for MACS-3 throughout the analysis was 0.70763 ± 0.0002 ($n = 5$), which represents an accuracy within 2 SD of the certified value. External precision (RSD) for Sr:Ca ratios based on repeated measurements of a certified reference material (FEBS-1; Sturgeon et al., 2005) was < 2%. Elemental ratios were normalized to this standardized reference material as described by Jackson & Hart (2006).

Data analysis

To detect distinct natal origins and movements, the differences in otolith microchemical signatures of subbasins must, at a minimum, exceed the measurement error of the LA-ICPMS instrument. We estimated measurement error from repeated ablations of standard reference materials. These measurement error values calculated for [87]Sr:[86]Sr ($\pm$ 2 SD or 0.0004) and Sr:Ca ratios ($\pm$ 2 SD or 0.45 mmol/mol) were then used as bounds to assess whether absolute differences in water or otolith chemistry were large enough to distinguish specific subbasins. Water samples and otolith signatures with absolute differences within 1 SD of the mean measurement error were considered indistinguishable from one another.

For natal origin, otolith Sr elemental and isotope ratios were determined as the mean of the first five readings from each laser ablation transect. To assess movement among subbasins, we compared [87]Sr:[86]Sr transects across the otolith axis from the core to the edge. We focused on [87]Sr:[86]Sr transects to assess movement patterns because this ratio is minimally affected by physical and biological factors that have been shown to alter the incorporation of Sr:Ca elemental ratios throughout the life of a fish (Kennedy et al., 2000; Sturrock et al., 2015). Variation in [87]Sr:[86]Sr along each transect was estimated in relation to the measurement error similar to the water and otolith core comparisons described above (see also Kennedy et al. 2000). However, measurement error for otolith transects was calculated from all of the MACS-3 standard reference material 'reads' pooled together ($\pm$ 2 SD or 0.0016) as opposed to the integrated mean value for each transect as was the case when identifying natal origins. Using all of the standard reference material results pooled together is necessary for assessing variation within ablation transects as this represents the scale of measurement error for ICPMS of a known material. Shifts in average [87]Sr:[86]Sr along transects outside of the measurement error bounds were considered potential evidence for movement between chemically distinct lake sampling sites (Gibson-Reinemer et al., 2009).

We also calculated the partition coefficient between water and otolith Sr:Ca ($D_{Sr:Ca}$) using the methods described by Muhlfeld et al., (2012) with the following equation:

$$DSr : Ca \ = \ (Sr : Ca)otolith / (Sr : Ca)water$$

Knowledge of Sr:Ca partition coefficients is useful for predicting otolith Sr:Ca values from water samples (Muhlfeld et al., 2012) and for examining how environmental factors can influence elemental uptake when compared across systems (Izzo et al., 2018).

Results

There was little variation in [87]Sr:[86]Sr and Sr:Ca among water samples or lake trout otoliths from Yellowstone Lake (Fig. 2). Lake water [87]Sr:[86]Sr ranged from 0.70634 to 0.70642 and Sr:Ca ratios ranged from 3.94 to 4.38 mmol/mol. Otolith natal signatures ranged from 0.70615 to 0.70647 and 1.11 to 1.57 mmol/mol for [87]Sr:[86]Sr and Sr:Ca ratios, respectively. All water and otolith natal signature measurements were highly similar for [87]Sr:[86]Sr, with all values within the ICPMS measurement error range ($\pm$ 0.0008). Water and otolith core Sr:Ca values differed, with a calculated $D_{Sr:Ca}$ partition coefficient of 0.29, but all values within each category were within the range of measurement error for each respective Sr:Ca value ($\pm$ 0.45 mmol/mol), indicating little variation of Sr:Ca within water samples or within lake trout otolith cores.

Otolith [87]Sr:[86]Sr transect measurements showed a similar lack of variation throughout the life of lake trout (Fig. 2). All otolith transect values fell within range of estimated measurement error of the mean transect value ($\pm$ 0.0016). As a result, long-term use and occupancy of specific lake subbasins by lake trout could not be identified. Otolith Sr:Ca transect data were not used to infer movement for reasons previously explained; however, the data did indicate a positive trend in this elemental ratio with ontogeny (Fig. 2).
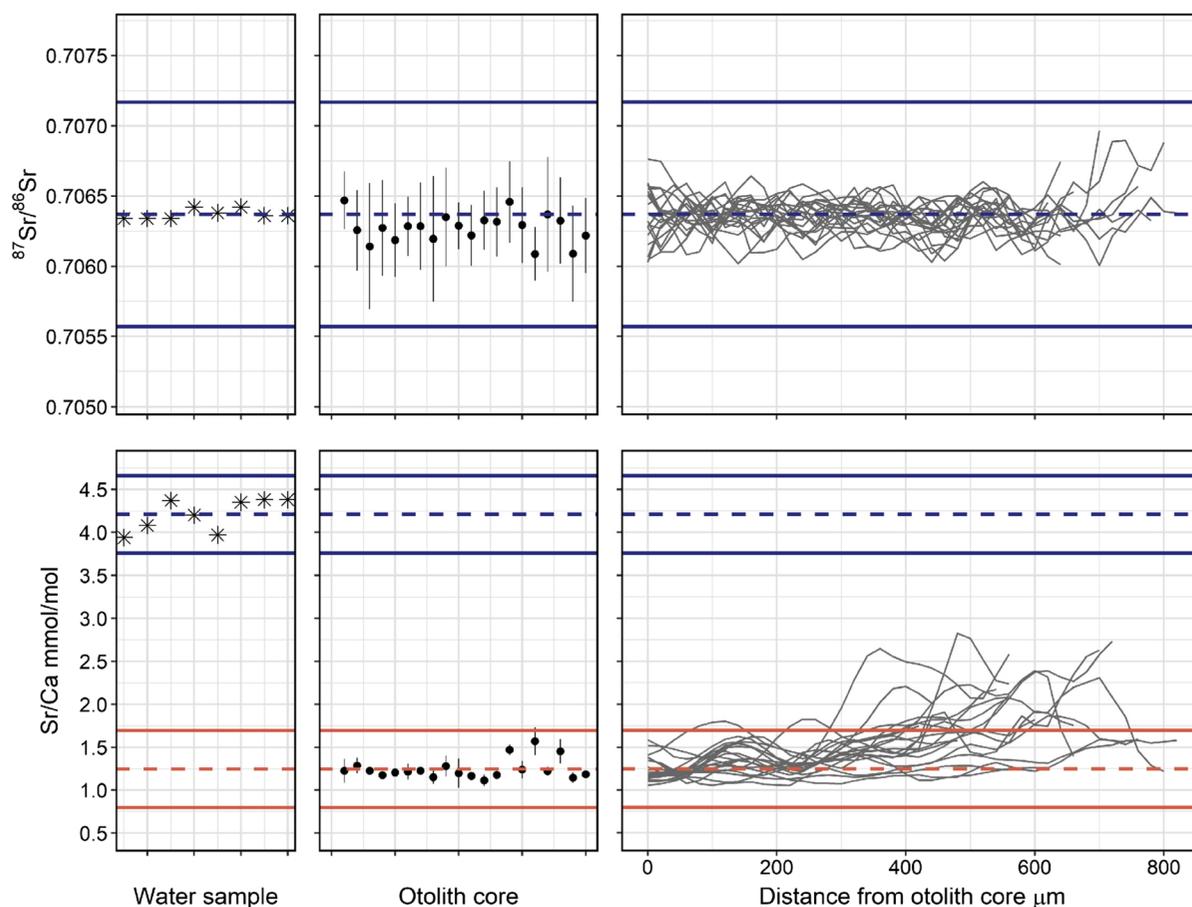
Fig. 2 Strontium isotope (^{87}Sr:^{86}Sr, upper) and Sr:Ca (lower) measured in water samples (left), otolith cores (center), and laser transects from core to otolith edge (right). The blue dashed line in the upper panel represents the mean Sr isotope of water samples and the solid blue lines reflect the scale of measurement error of the ICPMS instrument for otoliths (see text). In the lower panel, the blue lines depict the mean Sr:Ca for the water samples (dashed) and measurement error of the ICPMS (solid) for the otolith samples, and the red lines indicate the mean (dashed) and measurement error (solid) for the otolith samples. Core measures in both panels include mean (large central point) and vertical lines represent ± 1 standard deviation. Transect data are 3-point moving averages with each line representing transects from each otolith sampled

Discussion

Telemetry studies have shown that lake trout exhibit extensive seasonal movements and spawn in distinct locations within Yellowstone Lake (Williams 2019; Gutowsky et al., 2020); however, we were unable to detect specific natal origins or movement patterns within the lake using otolith microchemistry. Though our negative results were not wholly unexpected, previous studies have shown microchemistry can detect natal origin differences in freshwater and marine fish separated by as little as 5–11 km geographic distance (Kennedy et al., 2000; Chittaro & Hogan, 2013). Chittaro & Hogan (2013) suggested

that sites with long water residency times, high sediment inputs from different geologies, and those with high anthropogenic inputs facilitate development of unique isotope signatures. We hypothesized that the large, distinct subbasins in Yellowstone Lake, numerous thermal spring inputs in the lake bottom, and inlet tributaries draining different geologies (Stewart 2016), would have increased the possibility of spatially distinct chemical subregions developing in Yellowstone lake, but we did not detect such occurrences.

Although the minimum difference in water chemistry needed to impart a distinctive chemical mark has not been experimentally determined, previous

investigations in freshwater indicated that spawning and rearing source waters were distinguishable when spatial variation in water ^{87}Sr:^{86}Sr differed by $\sim$ 0.005 (Kennedy et al., 2002; Gibson-Reinemer et al., 2009). In our study, spatial variation in lake water samples was markedly lower than this threshold, differing by only 0.00008 (range 0.70634–0.70642). Our results paralleled those of the few previous freshwater lentic microchemistry investigations that also failed to identify natal origins and movement in Laurentian Great Lakes fishes that spent their entire life in open lake environments (Brazner et al., 2004; Dufour et al., 2005; Pangle et al., 2010). In these studies, the lack of spatial variation in water chemistry within lakes similarly precluded incorporation of distinctive chemical signatures despite considerable distances (tens to hundreds of kilometers) between sampling sites (Brazner et al., 2004; Dufour et al., 2005; Pangle et al., 2010). Thus far, otolith microchemistry has only successfully identified natal origin in lake fishes that have undergone spawning and rearing within lake tributaries and flooded wetlands near tributary mouths that are chemically distinctive from open lake water (Pangle et al., 2010). The lack of variation we observed in lake trout otolith microchemistry throughout their life cycle contrasts sharply with those of lake trout collected soon after their first detection in the lake, which showed a markedly different Sr:Ca signature in the early rearing portion of their otoliths indicative of spawning and rearing in a waterbody outside of Yellowstone Lake (Munro et al., 2005).

It is plausible that we were unable to detect distinct chemical signatures of natal areas due to interference from maternal contributions to otolith chemistry during embryonic development or yolk sac stages (Elsdon et al., 2008; Veinott et al., 2013). Otolith transect data did not show distinct shifts in elemental or isotopic chemistry around the core suggestive of maternal inputs. Moreover, the lack of spatial variation in water chemistry suggests that the most likely explanation is that there are no distinct natal signatures to be detected. However, we cannot exclude the possibility that maternal contributions masked their detection.

Partition coefficients between water and otoliths in our study were similar to those reported in previous investigations. We found a near 1:1 relationship between water and otolith ^{87}Sr:^{86}Sr (e.g., Kennedy et al., 2000; Muhlfeld et al., 2012; Amano et al., 2013) whereas elemental Sr:Ca in water accounted for a much lower proportion of Sr:Ca in otoliths (Gibson-Reinemer et al., 2009; Muhlfeld et al., 2012; Amano et al., 2013). Our otolith:water Sr:Ca coefficient (0.29) was nearly identical to that reported by Muhlfeld et al., (2012) (0.285) for tributaries of the North Fork Flathead River, Montana. Temporal stability of water chemistry is also an important consideration for estimating natal origin using otolith microchemistry (Pangle et al., 2010). Water Sr:Ca values in our study in 2013 (3.94 to 4.38) were similar to those from water samples collected in Yellowstone Lake in 1997–1998 (means 3.92–4.01; Munro et al., 2005), a 15-to-16-year interval, indicating high temporal stability of this chemical marker. Finally, the distinct increasing trend in otolith Sr:Ca with lake trout size independent of likely changes in water chemistry may reflect differences in elemental uptake related to onset of maturity or other environmental factors, which could be of interest for future investigations into utility of otolith chemistry for life history studies in lake trout (Sturrock et al., 2015).

In conclusion, we found that otolith microchemistry was not useful for determining natal origins and long-term subbasin use of lake trout in Yellowstone Lake. However, our results help identify the capabilities and limitations, and need for further refinements, to the rapidly emerging science of otolith microchemistry for use in the conservation and management of freshwater fishes (Prachiel et al., 2014). Detailed analysis of other elements or isotopes in hard-part microchemistry, in combination with other tools for detecting movement, may improve detection of natal origin and life history movement among fishes within freshwater lentic systems.

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