USE OF OTOLITH MICROCHEMISTRY TO IDENTIFY YELLOWSTONE CUTTHROAT TROUT AND LAKE TROUT NATAL ORIGINS AND MOVEMENT PATTERNS IN YELLOWSTONE LAKE, WYOMING

by

Kole Patrick Stewart

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 2016

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Thomas McMahon for his insight, direction, and patience throughout the course of this study. I also thank Dr. Robert Humston who provided analytical support and advice that greatly improved this research. I thank Dr. Jia Hu for review of this manuscript, as well as her suggestions, support, and encouragement. A special thanks goes to Dr. Todd Koel for working hard to obtain funding for this study as well as his continued advice throughout the duration of this research. Jurek Blusztajn from Woods Hole Oceanographic Institution provided much needed technical advice and assistance throughout the sample analysis process .This research would not have been possible without hard work of many National Park Service employees and volunteers including: Jeff Arnold, Mike Consolo, Colleen Detjens, Phil Doepke, Earl Drescher, Brian Ertel, Aza Hill, Alejandro Reyes, and Jake Williams. I thank my fellow students for their friendship, advice, and for their willingness lend a helping hand. Lastly, I would like to thank my wife Cheyenne Stewart for all of her editorial assistance, volunteer work, and positive attitude throughout the entire process; I wouldn't have been able to do this without her support.

This study would not have been possible without funding from the National Park Service and the Yellowstone Park Foundation.

TABLE OF CONTENTS

TABLE OF CONTENTS − CONTINUED

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES - CONTINUED

ABSTRACT

The Yellowstone Lake Yellowstone cutthroat trout population has declined as a result of drought, whirling disease, and the introduction of lake trout. Little is known about the recruitment patterns of cutthroat trout and lake trout in this system. Otolith microchemistry is uniquely suited for answering these questions by matching the chemical signatures found in otoliths to the same signatures found in the water fish occupy. My first objective was to identify and compare the primary spawning streams contributing to historic (1997) and recent (2013) cutthroat trout recruitment. I analyzed the chemical signatures (^{87}Sr:^{86}Sr, Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca) of 22 cutthroat trout spawning streams and the same signatures from the natal region of cutthroat trout otoliths. There was low variation among the chemical signatures of many spawning streams, thus streams were grouped into 9 clusters using a cluster analysis. Relative recruitment to each cluster was assessed using random forest models with a classification accuracy of 84.4% for known-origin cutthroat trout fry otoliths and 79.0% for simulated otolith signatures. There was a significant difference in the proportions of recruitment between historic and recent cutthroat trout spawning clusters ($X^2 = 15.40$, $p = 0.03$). The majority of historic (0.84) and recent (0.77) recruitment occurred in the same three stream clusters, with the most notable change being a decrease in recent recruitment in the stream cluster containing Pelican Creek and an increase in recruitment in tributaries in the upper Yellowstone River drainage. The second objective was to identify the spawning locations and movement patterns of lake trout within Yellowstone Lake. I analyzed the ^{87}Sr:^{86}Sr, and Sr:Ca signatures from 8 locations throughout Yellowstone Lake and the same signatures in 20 lake trout otoliths. I did not find sufficient variation within the lake water chemistry to differentiate lake regions and there was no significant differences found within in the lake trout otolith transects. This study can be used to inform future spawning stream conservation and restoration by directing managers towards spawning streams of increasing or decreasing importance. This study also highlights some of the strengths and limitations of using microchemistry studies in freshwater system

CHAPTER ONE

INTRODUCTION TO THESIS

Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri)* are a unique subspecies of cutthroat trout that are native to the Rocky Mountain regions of Montana, Wyoming, Idaho, and small portions of Utah and Nevada. Yellowstone cutthroat trout may occupy streams or lakes throughout the majority of their lives however, they spawn exclusively in streams (Behnke 2002). Yellowstone Lake and its numerous tributary streams once contained one of the largest metapopulations of genetically pure Yellowstone cutthroat trout (Varley and Gresswell 1988; Gresswell 2011). This population is primarily lacustrine-adfluvial; the majority of their life cycle is spent within the lake, with brief periods spent in streams during spawning and rearing (Varley and Gresswell 1988). Yellowstone cutthroat trout have exhibited a dramatic population decline in the Yellowstone Lake watershed over the past two decades (Gresswell 2011). This population decline has been attributed to a combination of factors including drought (Kaeding 2010), nonindigenous parasites (particularly whirling disease) (Koel et al. 2006), and the introduction and expansion of piscivorous lake trout (*Salvelinus namaycush*)(Kaeding et al. 1996).

Lake trout are a char species native to northern North America that spawn by broadcasting their eggs over cobble and boulder fields usually found in lakes at depths less than 15 meters (Scott and Crossman 1973). Most native populations in the United States are located in the northeastern states; however there are a few remnant native

populations that exist in northwestern and southwestern Montana (Snyder and Oswald 2005). In some portions of their range lake trout inhabit cold rivers and shallow lakes, however in the southern portion of their range (i.e. Montana) they are found almost exclusively in deep, cold lakes (Scott and Crossman 1973). Lake trout have been intentionally stocked throughout the United States including within Yellowstone National Park in, Shoshone Lake, and Lewis Lake. Lake trout were first discovered in Yellowstone Lake in 1994 (Kaeding et al. 1996); likely the result of an unauthorized stocking from the Lewis Lake population in 1989 (Munro et al. 2005). Due to the highly predacious nature of lake trout and the direct negative impacts they have had on the Yellowstone Lake cutthroat trout population, the National Park Service (NPS) has been removing lake trout from Yellowstone Lake in an ongoing gill and trap netting effort since 1995 (Koel et al. 2015).

Since its inception the lake trout removal effort has removed roughly 2 million lake trout from Yellowstone Lake over 20 years; over one-half of those fish (1,195,000) were removed over a 4 year period from 2012 to 2015 as a result of increased effort as well as continued population growth (Koel et al. 2015 & Personal Communication). These intensive lake trout removal efforts appear to have had a positive effect on cutthroat trout recruitment. Recent annual population assessments have shown an increase in the number of juvenile cutthroat trout within Yellowstone Lake from a low of 13 cutthroat trout per 100 m net in 2010 to 31 cutthroat per 100 m net in 2014 (Koel et al. 2015).

In addition to population assessments within the lake, the NPS has monitored spawning cutthroat trout in tributaries to Yellowstone Lake, where logistically possible. Historically, cutthroat trout were observed spawning in 68 tributaries as well as the outlet stream (Yellowstone River upstream of the Upper Yellowstone Falls) of Yellowstone Lake (Gresswell 1994). During the 1980's and 1990's numbers of spawning fish began to decrease markedly; the Clear Creek fish weir recorded just 1,438 spawning cutthroat trout in 2004, down from a peak of 70,105 spawners in 1978 (Koel et al. 2005). The decrease began prior to the introduction of lake trout, and has been attributed to a long-term drought over the past three decades (Kaeding 2010) as well as the discovery of whirling disease in 1994 (Koel et al. 2006). With the drought beginning to subside in 2010 and whirling disease not expanding, lake trout continue to assert negative impacts on the cutthroat trout population (Koel et al. 2015). While cutthroat trout recruitment has fluctuated over the past two decades with an overall negative trend, the NPS has not been able to identify changes in the contribution of specific spawning streams to the Yellowstone Lake cutthroat trout population. In the past, counts of spawning cutthroat trout in streams have been used to estimate the relative contribution of select tributaries to lake-wide recruitment (Koel et al. 2015). While this approach quantifies the number of adult spawning fish in each stream, it does not quantify how these streams contribute to the recruitment of juvenile fish to the Yellowstone Lake cutthroat trout population, or how the contributions of specific spawning streams have changed over time. Additionally, these surveys are limited by the logistics of surveying extremely remote

backcountry tributaries as well as safety concerns of surveying streams in prime grizzly bear habitat.

While lake trout removal efforts appear to be having a positive effect on the cutthroat trout population, there is a critical need to develop new and innovative approaches that improve the efficiency and reduce the cost of these efforts. Yellowstone fisheries managers are currently developing new methods to destroy lake trout embryos and kill larval fish (Koel et al. 2015). However, if these new methods are effective, they will require that the location of spawning areas be known prior to their implementation. An acoustic telemetry study is currently underway, designed to characterize lake trout movement patterns and habitat use within Yellowstone Lake (Koel et al. 2015). While traditional telemetry methods are an excellent approach for determining the movement patterns and habitat usage of large fish, this approach is confined to fish large enough for transmitter implantation, thus movement patterns in smaller fish cannot be detected.

The primary purpose of this study was to use the microchemical analysis of fish otoliths to identify the natal origins of cutthroat trout and see if they have changed over time (Chapter 2), as well as the natal origins and movement patterns of lake trout (Chapter 3) in Yellowstone Lake. Both cutthroat trout and lake trout natal origins refers to the location in which the fish were spawned. Otolith microchemistry techniques have been used to effectively identify the natal origins and movement patterns of marine (Cook 2011; Sturrock et al. 2012), anadromous (Kennedy et al. 2005; Miller et al. 2011; Barnett-Johnson et al. 2008) and freshwater fish (Humston et al. 2010; Muhlfeld et al. 2012; Amano et al. 2013). The technique uses isotopic (primarily ^{87}Sr:^{86}Sr) and elemental

ratios (primarily Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca) found in fish otoliths, which can be matched to those found in the ambient water a fish has occupied. When unique microchemical signatures exist in the water of spawning locations or across fish migration and movement pathways, the natal origins and movement patterns of a fish can be identified.

Otolith microchemistry has distinct advantages over other traditional approaches. In relation to both movement and natal origins studies, mark-recapture methods require marking large numbers of fish which can be cost prohibitive (Pangle et al. 2010), and radio telemetry can be very useful for identifying fish movement patterns; however it is limited to fish large enough for transmitter implantation (Karp 2014). In contrast, it has been observed that microchemical signatures can be reliably obtained from extremely small juvenile and larval fish otoliths (Jones and Chen 2003). Lastly, fish genetics studies may be a viable approach for identifying discrete fish stocks and natal origins of both small and large fish; however this approach is only effective for differentiating between genetically distinct groups of fish with little to no interbreeding (Allendorf and Phelps 1981; Slatkin 1987; Hartl and Clark 1989). Another distinct advantage of using otolith microchemistry is that by analyzing distinct regions of the otolith, specific microchemical signatures can be associated with specific growth stages of a fish (Begg and Waldman 1999).

Otoliths

Otolith microchemistry techniques are predicated on the unique time and environmental recording properties of otoliths. Otoliths are small calcareous structures

located in the inner ear of all teleost fish that are used for hearing and maintaining balance (Campana 1999; Popper et al. 2005). Teleost fish have three pairs of otoliths including the sagittae (the largest), lapilli, and astersci; all of which are located within three vestibules in the inner ear (Secor et al. 1991).

The utility of otoliths for learning about fish population dynamics is due to a number of unique characteristics including: 1) they grow continuously throughout the life of the fish, 2) they form daily and annual growth rings, 3) they are metabolically inert, 4) they are composed of elements incorporated from the fish's ambient environment.

Otoliths are one of the first calcified structures that appear during early development (Campana and Neilson 1985) and unlike other bones, continue to grow throughout the life of the fish (Campana and Thorrold 2001). Uniquely, otoliths continue to grow even during periods of food deprivation (Marshall and Parker 1982; Campana 1983a), stress (Campana 1983b), and exposure to low pH conditions (Geen et al. 1985). Daily growth increments (daily growth rings) form every twenty-four hour period on most teleost otoliths (Pannella 1971). Some environmental factors do affect otolith growth including: temperature, photoperiod, and feeding frequency. Nielson and Geen (1982) found that feeding frequency affected both the number and width of daily growth increments, while temperature and photoperiod only affected the width of daily growth increments. Since these three factors affect otolith growth it is easy to understand why otoliths form strong annuli in temperate regions where otolith growth is slow during winter months when the temperature, photoperiod, and feeding frequency all decrease.

Otoliths form and grow in an acellular environment while surrounded by endolymph fluid, which reduces the possibility of otoliths becoming metabolically reabsorbed or altered (Riceman 2008). This characteristic of otoliths is particularly important for fisheries research because it allows otoliths to serve as a record of time as well as an environmental tracer. This means that otoliths can be used to both age fish by counting annuli rings as well as identify the different locations occupied by a fish using unique microchemical signatures recorded in the otolith throughout the fish's life.

The microchemical composition of fish otoliths can provide a powerful tool for recording a fish's environmental history because trace elements from the ambient waters can become incorporated into the crystalline matrix. Otoliths are composed of 96-99% aragonite (a form of calcium carbonate)(Payan et al. 1999), 3-4% protein (Degans et al. 1969; Asano and Mugiya 1993; Hoff and Fuiman 1993), and less than 1% trace elements (Edmonds et al. 1992; Sie and Thresher 1992; Proctor et al. 1995; Severin et al. 1995). The protein component serves as a framework for the biomineralization process occurring during otolith formation and accretion (Borelli et al. 2001). Trace elements from the ambient environment are incorporated into the aragonite matrix as a result of substitution for calcium; elements that are of a similar chemical structure to calcium (e.g. strontium) are often substituted (Campana 1999). More than 30 elements have been detected in otoliths; most of them in trace amounts (Campana 1999).

Otolith Microchemistry Influences

The single largest factor affecting otolith microchemical composition is the chemical composition of the ambient water. The general path of elemental incorporation into otoliths from ambient water is through the blood plasma via the gills or intestines, then to the endolymph, and lastly into the crystallizing otolith (Campana 1999). Previous studies have shown a strong correlation between otolith and water Sr^{87}:Sr^{86} isotopic ratios (Kennedy et al. 2002, Muhlfeld et. al 2012), and with elemental Sr:Ca (Wells et al. 2003; Munro et al. 2005; Muhlfeld et al. 2012), Ba:Ca, and Mg:Ca ratios (Wells et al. 2003; Humston et al. 2010) in freshwater fish. A number of other elements and isotopic ratios have been used to a lesser extent with varied results. For example, Mn:Ca otolith and water ratios were not highly correlated in a study of Arctic grayling (*Thymallus arcticus*) by Amano et al. (2013); however, they were correlated in studies by Thorrold (2001), Wells et al. (2003), and Clarke et al. (2007). This discrepancy between studies may be due to the amount of variation in Mn:Ca ratios within the study areas. Study areas with higher isotopic and elemental variation provide more useful chemical signatures for discrimination between environments.

Diet, water temperature, and salinity have also been shown to effect otolith chemical composition. However, the effect of diet on otolith chemical composition is far less than the effect of absorption from ambient waters. In freshwater fish, 10% to 20% of the otolith Sr and Ca composition, respectively, are likely derived from the diet as opposed to 88% Sr and 75.5% Ca derived from ambient water (Simkiss 1974; Farrell and Campana 1996). The influence of diet on otolith microchemistry is most pronounced in

diadromous fishes with exposure to elemental concentrations (especially Sr) at both low (freshwater) and high (saltwater) concentrations. Thus for exclusively freshwater fish the influence of diet on otolith microchemistry does not play a major role because they are not exposed to such extreme fluctuations in elemental concentrations. According to Brown and Severin (2009), temperature and salinity have major influences on otolith Sr:Ca ratios in marine species, however this is not the case for freshwater and many diadromous fish. Otolith microchemistry studies of freshwater and many diadromous species indicate that temperature and salinity have a very minor effect on otolith microchemistry (Brown and Severin 2009). Since this study is only concerned with freshwater fishes, water temperature and salinity are not expected to significantly affect the otolith microchemistry of fish in the Yellowstone lake watershed.

Ontogeny and physiology may influence otolith microchemistry; however research into this topic is limited. Toole et al. (1993) noted that otolith microchemical composition could change in association with the life stages of a fish. Ontogenetic effects are most pronounced in fishes that undergo a metamorphosis, fishes such as sole and eels (Eldson et al. 2008).

Lastly, there is strong evidence for a maternal influence on the microchemical composition in the primordia within the core of fish otoliths. Volk et al. (2000) demonstrated with Pacific salmon that the Sr:Ca ratio in the otolith core of fry spawned by females that matured in seawater was roughly four times the core Sr:Ca ratio of fry spawned by females raised in freshwater. Zimmerman and Reeves (2002) also found maternal influences on the otolith primordium in their study of resident and anadromous

rainbow trout (*Oncorhynchus mykiss).* These studies indicate that in the early stages of otolith formation and growth, the isotopic and elemental composition of otoliths is influenced by the nutrients provided by the mother in the egg. In order to avoid maternal influences on otolith signatures when trying to establish natal origin, the primordia within the core of the otolith must be avoided when sampling. Sampling regions of the otolith near the core and adjacent to the primordia will provide a more accurate signature of the environment attributed to the fish's natal origin.

Otolith Microchemistry Techniques

There are a number of viable techniques for assaying otolith microchemistry including: proton induced X-ray emission, solution based inductively coupled plasma mass spectrometry, and laser ablation inductively coupled plasma mass spectrometry. Choosing the proper technique depends largely on the question being asked. Each technique has its own particular strengths and weaknesses in regards to precision, accuracy, the elements and isotopes they are capable of quantifying, the difficulty of sample preparation and analysis, and the expense of analysis. The most commonly used techniques have been reviewed in depth by Gunn et al. (1992) and Campana et al. (1997).

The portions of this study designed to investigate natal origins of cutthroat trout (Chapter 2) and spawning and movement patterns of lake trout (Chapter 3) utilized laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) due to the numerous advantages over other techniques. Laser ablation-ICPMS has the ability to detect both specific isotopic signatures and elemental concentrations at very low

detection limits (parts per billion) (Ludsin et al. 2006). Samples analyzed using LA-ICPMS are less likely to become contaminated as compared with other methods since sectioned or whole otoliths can be used (Ludsin et al. 2006). Preparing samples for LA-ICPMS analysis does require a substantial amount of time and effort, however the preparation techniques are simple and very similar to the preparation required for aging otoliths; a very commonly used process in the fisheries profession. Lastly, the time it takes to analyze otoliths using LA-ICPMS is much less than other methods, resulting in the ability to analyze more samples in a given amount of time; this is crucially important, given that laboratory fees can be in excess of $1000/day (Ludsin et al. 2006).

Sampling Design Considerations

Most studies to date have relied on thorough and extensive water sampling to ascertain what variation in microchemical signatures exist within a given study area (e.g. Clarke et al. 2007; Coghlan et al. 2007; Riceman 2008; Amano et al. 2013). Sampling all of the potential water bodies a fish may occupy within a study area is the most comprehensive approach, however when working with very large and/or complex systems, this approach may not be feasible due to time and/or funding constraints. In these cases, examining bedrock geology maps has served as an alternative method to guide sampling efforts (e.g. Kennedy et al. 2000). The microchemical signatures found in water are largely derived from the underlying bedrock geology of a stream or water body (Puckett and Bricker 1992, Liu et al. 2000), thus knowledge of the variation in bedrock geology within a given study system is an important first step for assessing the potential

variation in water microchemical signatures. Prior investigation of bedrock geology within a study area can also provide guidance when determining an adequate number of water sampling sites and locations. These prior considerations ensure that the water sampling design will accurately portray the variation in water microchemical signatures that may be found in the area of interest.

Prior to extensive sampling of fish otoliths and water throughout a study area, known origin fish and corresponding water samples should be collected in order to examine the relationship between otolith and water microchemistry. This step is important because it allows researchers to verify that the microchemical signatures found in fish otoliths are representative of the water in which they originated from. This step is also useful for determining which isotopic and element:Ca ratios will be useful for discriminating natal origins.

Objectives

The first objective of this study was to use otolith microchemistry to describe the contributions of specific spawning tributaries to the Yellowstone Lake cutthroat trout population, and determine how this has changed over time (Chapter 2). Cutthroat trout otoliths have been routinely collected during annual gillnet surveys in the lake since 2010 and sporadically prior to 2001. The availability of these samples offered the possibility to identify the primary spawning tributaries that have contributed to recent and historic cutthroat trout recruitment in the lake prior to and after the fully realized negative effects of drought, whirling disease, and lake trout on the cutthroat trout population. Otolith

microchemistry studies that have utilized both recent and historic otolith collections to examine spatial recruitment patterns are rare (e.g. Miller et al. 2011). Thus, this study provides the unique opportunity to not only examine how cutthroat trout spatial recruitment patterns may change over a broad span of time, but also how spatial recruitment patterns may shift due to ecological perturbations such as whirling disease, drought, and invasive species introduction.

The second objective of this study was to determine if using the same otolith microchemistry techniques used for cutthroat trout is also a viable method for identifying where lake trout spawn and rear within Yellowstone Lake (Chapter 3). Given that Yellowstone Lake is a relatively large lake with approximately 342 km^2 of surface area and diverse bedrock geology and thermal features, there may be variation in water microchemistry throughout the lake. If microchemical analysis of lake trout otoliths is effective, it offers the potential to identify portions of the lake that lake trout spawn and rear during the early phases of their life cycle.

Information garnered by using microchemical analysis can be used to formulate conservation, restoration and adaptive management strategies (Olley et al. 2011). These strategies could be used to protect spawning streams as well as improve the recruitment of cutthroat trout to the Yellowstone Lake population. Additionally, microchemical techniques have been very useful for identifying the movements and natal origins of invasive fishes (Crook and Gillanders 2006; Wolff et al. 2012). Any additional information pertaining to lake trout natal origins or movement patterns within

Yellowstone Lake could be valuable for developing effective management and control strategies with the ultimate goal of removing lake trout from Yellowstone Lake.

References

Allendorf, F.W., and Phelps, S.R. 1981. Use of allelic frequencies to describe population structure. Canadian Journal Fisheries and Aquatic Sciences 38:1507–1514.

Amano, Y., M. Kuwahara, T. Takahashi, K. Shirai, K. Yamane, H. Amakawa, and T. Otake. 2013. Otolith elemental and Sr isotopic composition as a natal tag for Biwa salmon *Oncorhynchus masou* subsp. in Lake Biwa, Japan. Aquatic Biology 19:85–95.

Asano, M., and Y. Mugiya 1993. Biochemical and calcium-binding properties of water-soluble proteins isolated from otoliths of the tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology 104B:201–205.

Barnett-Johnson, R., T.E. Pearson, F.C. Ramos, C.B. Grimes, and R.B. MacFarlane. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. Limnology and Oceanography 53:1633.

Begg, Gavin A., and John R. Waldman. 1999. An holistic approach to fish stock identification. Fisheries Research 43:35–44.

Behnke, R.J. 2002. Trout and salmon of North America. The Free Press New York, New York

Borelli, G., N. Mayer-Gostan, H. De Pontual, G. Boeuf, and P. Payan. 2001. Biochemical relationships between endolymph and otolith matrix in the trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Calcified Tissue International 69:356–364.

Brown, R.J., and K.P. Severin. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Canadian Journal of Fisheries and Aquatic Sciences 66:1790–1808.

Campana, S.E. 1983a. Feeding periodicity and the production of daily growth increments in otoliths of steelhead srout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Canadian Journal of Zoology 61:1591–1597.

Campana, S.E. 1983b. Factors affecting the production of daily growth increments in the otoliths of fishes. Doctoral dissertation. The University of British Columbia, Vancouver, British Columbia, Canada.

Campana, S.E., and J.D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014-1032.

Campana, S.E., S.R. Thorrold, C.M. Jones, D. Günther, M. Tubrett, H. Longerich, S. Jackson, N.M. Halden, J.M. Kalish, P. Piccoli, H. De Portual, H. Troadec, J. Panfili, D.H. Secor, K.P. Severin, S.H. Sie, R. Thresher, W.J. Teesdale, and J.L. Campbell. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences 54:2068–2079.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Campana, S.E., and S.R. Thorrold. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences 58:30–38.

Chilcote, M.W. 2003. Relationship between natural productivity and the frequency of wild fish in mixed spawning populations of wild and hatchery steelhead (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 60:1057–1067.

Clarke, A.D., K.H. Telmer, and J.M. Shrimpton. 2007. Habitat use and movement patterns for a fluvial species, the arctic grayling, in a watershed impacted by a large reservoir: evidence from otolith microchemistry. Journal of Applied Ecology 44:1156–65.

Coghlan, S.M., M.S. Lyerly, T.R. Bly, J.S. Williams, D. Bowman, and R. Hannigan. 2007. Otolith chemistry discriminates among hatchery-reared and tributary-spawned salmonines in a tailwater system. North American Journal of Fisheries Management 27:531–541.

Cook, G.S. 2011. Changes in otolith microchemistry over a protracted spawning season influence assignment of natal origin. Marine Ecology Progress Series 423:197–209.

Crook, D.A., and B.M. Gillanders. 2006. Use of otolith chemical signatures to estimate carp recruitment sources in the Mid-Murray River, Australia. River Research and Applications 22:871–879.

Degens, E.T., W.G. Deuser, and R.L. Haedrich. 1969. Molecular structure and composition of fish otoliths. Marine Biology 2:105–113.

Edmonds, J.S., R.C.J. Lenanton, N. Caputi, M. Morita, 1992. Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. Fisheries Research 13:39-51

Elsdon, T.S., B.K. Wells, S.E. Campana, B.M. Gillanders, C.M. Jones, K.E. Limburg, D.H. Secor, S.R. Thorrold, and B.D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology: An Annual Review 46:297–330.

Farrell, J., and S.E. Campana. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology 115A:103-109.

Fowler, A.J., S.E. Campana, S.R. Thorrold, and C.M. Jones. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 52:1431–1441.

Geen, G.H., J.D. Neilson, and M. Bradford. 1985. Effects of pH on the early development and growth and otolith microstructure of Chinook salmon, *Oncorhynchus tshawytscha*. Canadian Journal of Zoology 63:22–27.

Gresswell, R.E. 1994. Life-history organization of cutthroat trout in Yellowstone Lake and its management implications. Doctoral dissertation. Oregon State University, Corvallis, Oregon.

Gresswell, R.E. 2011. Biology, status, and management of the Yellowstone cutthroat trout. North American Journal of Fisheries Management 31:782–812.

Gunn, J.S., I.R. Harrowfield, C.H. Proctor, and R.E. Thresher. 1992. Electron probe microanalysis of fish otoliths—evaluation of techniques for studying age and stock discrimination. Journal of Experimental Marine Biology and Ecology 158:1–36.

Hartl, D.L., and Clark, A.G. 1989. Principles of population genetics. Sinauer, Sunderland, Massachusetts.

Hoff, G.R., and L.A. Fuiman. 1993. Morphometry and composition of red drum otoliths: changes associated with temperature, somatic growth rate and age. Comparative Biochemical Physiology 106A:209-219.

Humston, R., B.M. Priest, W.C. Hamilton, and P.E. Bugas. 2010. Dispersal between tributary and main-stem rivers by juvenile smallmouth bass evaluated using otolith microchemistry. Transactions of the American Fisheries Society 139:171–184.

Jones, C.M., Z. Chen. 2003. New techniques for sampling larval and juvenile fish otoliths

for trace-element analysis with laser-ablation sector-field inductivey-coupled-plasma mass spectrometry (SF-ICP-MS). The Big fish Bang: Proceedings of the 26th Annual Larval Fish Conference. The Norwegian Institute of Marine Research, University of Bergen. Bergen.

Karp, C. 2014. Summary of freshwater fisheries telemetry methods. Science and Technology Program, Research and Development Office. Bureau of Reclamation. Denver, CO.

Kaeding, L.R., G.D. Boltz, and D.G. Carty. 1996. Lake trout discovered in Yellowstone Lake threaten native cutthroat trout. Fisheries 21:16–20.

Kaeding, L.R., 2010. Relative contributions of climate variation, lake trout predation, and other factors to the decline of Yellowstone cutthroat trout during three recent decades. Doctoral Dissertation. Montana State University, Bozeman, Montana.

Kennedy, B.P., J.D. Blum, C.L. Folt, and K.H. Nislow. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Canadian Journal of Fisheries and Aquatic Sciences 57:2280–2292.

Kennedy, B.P., A. Klaue, J.D. Blum, C.L. Folt, and K.H. Nislow. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Canadian Journal of Fisheries and Aquatic Sciences 59:925–929.

Koel, T.M., P.E. Bigelow, P.D. Doepke, B.D. Ertel, and D.L. Mahony. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30:10–19.

Koel, T.M., D.L. Mahony, K.L. Kinnan, C. Rasmussen, C.J. Hudson, S. Murcia, and B.L. Kerans. 2006. *Myxobolus cerebralis* in native cutthroat trout of the Yellowstone Lake ecosystem. Journal of Aquatic Animal Health 18:157–175.

Koel, T.M., J.L. Arnold, P.E. Bigelow, C.R. Detjens, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2015. Native Fish Conservation Program, Yellowstone Fisheries & Aquatic Sciences: Annual Report 2012-2014, National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Liu, Z., D.E. Weller, D.L. Correll, and T.E. Jordan. 2000. Effects of land cover and geology on stream chemistry in watersheds of Chesapeake Bay. Journal of the American Water Resources Association 36:1349–1365.

Ludsin, S.A., B.J. Fryer, and J.E. Gagnon. 2006. Comparison of solution-based versus

laser ablation inductively coupled plasma mass spectrometry for analysis of larval fish otolith microelemental composition. Transactions of the American Fisheries Society 135:218–231.

Marshall, S.L., and S.S. Parker. 1982. Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences 39:542–547.

Miller, J.A., V.L. Butler, C.A. Simenstad, D.H. Backus, A.J.R. Kent, and B. Gillanders. 2011. Life history variation in Upper Columbia River chinook salmon (*Oncorhynchus tshawytscha*): a comparison using modern and ~500-year-old archaeological otoliths. Canadian Journal of Fisheries and Aquatic Sciences 68:603–617.

Muhlfeld, C.C., S.R. Thorrold, T.E. McMahon, and B. Marotz. 2012. Estimating westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) movements in a river network using strontium isoscapes. Canadian Journal of Fisheries and Aquatic Sciences 69:906–915.

Munro, A.R, T.E. McMahon, and J.R. Ruzycki. 2005. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake. Canadian Journal of Fisheries and Aquatic Sciences 62:79–87.

Neilson, J.D., and G.H. Geen. 1982. Otoliths of Chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Canadian Journal of Fisheries and Aquatic Sciences 39:1340–1347.

Olley, R., R.G. Young, G.P. Closs, E.A. Kristensen, T.O. Bickel, N.A. Deans, L.N. Davey, and S.M. Eggins. 2011. Recruitment sources of brown trout identified by otolith trace element signatures. New Zealand Journal of Marine and Freshwater Research 45:395–411.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173:1124–1127.

Payan, P., A. Edeyer, H. De Pontual, G. Borelli, G. Boeuf, and N. Mayer-Gostan. 1999. Chemical composition of saccular endolymph and otolith in fish inner ear: lack of spatial uniformity. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 277:123–131.

Popper A.N., J.U. Ramcharitar, S.E. Campana. 2005. Why otoliths? Insights from inner ear physiology and fisheries biology. Marine and Freshwater Research 56:497–504.

Proctor, C.H., R.E. Thresher, J.S. Gunn, D.J. Mills, I.R. Harrowfield, S.H. Sie, 1995. Stock structure of the southern bluefin tuna *Thunnus maccoyi*: an investigation based on probe microanalysis of otolith composition. Marine Biology 122:511–526.

Puckett, L.J., and O.P. Bricker. 1992. Factors controlling the major ion chemistry of streams in the Blue Ridge and Valley and Ridge physiographic provinces of Virginia and Maryland. Hydrological Processes 6:79–98.

Riceman, M.S. 2008. The use of otolith microchemistry to investigate natal origins and movement of lacustrine wild rainbow trout (*Oncorhynchus mykiss*) and common smelt (*Retropinna retropinna*). Master's thesis. The University of Waikato, Hamilton, New Zealand.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada, Bulletin 184. 966 pp.

Secor, D.B., J.M. Dean, and E.B. Laban. 1991. Manual for otolith removal and preparation for microstructural examination. Belle W. Baruch Institute, University of South Carolina Press. Columbia, South Carolina.

Severin, K.P., J. Carroll, B.L. Norcross. 1995. Electron microprobe analysis of juvenile walleye pollock, *Theragra chalcogramma*, otoliths from Alaska: a pilot stock separation study. Environmental Biology of Fishes 43:269–283.

Sie, S.H., and R.E. Thresher. 1992. Micro-PIXE analysis of fish otoliths: methodology and evaluation of first results for stock discrimination. International Journal of PIXE 2:357–379.

Simkiss, K. 1974. Calcium metabolism of fish in relation to ageing. Unwin Brothers Publishing. London, England.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Snyder, B. and D. Oswald. 2005. Montana's fish species of special concern: Lake trout. Status of native lake trout in Montana. Montana Fish Wildlife and Parks, Helena, Montana.

Sturrock, A.M., C.N. Trueman, A.M. Darnaude, and E. Hunter. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? Journal of Fish Biology 81:766–795.

Thorrold, S.R. 2001. Natal homing in a marine fish metapopulation. Science 291:297–299.

Toole, C.L., D.F. Markle, and P.M. Harris. 1993. Relationships between otolith microstructure, microchemistry, and early life history events in Dover sole, *Microstomus pacificus*. U.S. Fishery Bulletin 91:732–753.

Varley, J.D., and R.E. Gresswell. 1988. Ecology, status, and management of the Yellowstone cutthroat trout. American Fisheries Society Symposium 4:13–24.

Volk, E.C., A. Blakley, S.L. Schroder, and S.M. Kuehner. 2000. Otolith chemistry reflects migratory characteristics of Pacific salmonids: using otolith core chemistry to distinguish maternal associations with sea and freshwaters. Fisheries Research 46:251–266.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan, and C.M. Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132:409–424.

Westerling, A.L., M.G. Turner, E.A.H. Smithwick, W.H. Romme, and M.G. Ryan. 2011. Continued warming could transform Greater Yellowstone fire regimes by mid-21st century. Proceedings of the National Academy of Sciences 108:13165–13170.

Wolff, B.A., B.M. Johnson, A.R. Breton, P.J. Martinez, D.L. Winkelman, and B. Gillanders. 2012. Origins of invasive piscivores determined from the strontium isotope ratio ($^{87}Sr/^{86}Sr$) of otoliths. Canadian Journal of Fisheries and Aquatic Sciences 69:724–739.

Zimmerman, C.E., and G.H. Reeves. 2002. Identification of steelhead and resident rainbow trout progeny in the Deschutes River, Oregon, revealed with otolith microchemistry. Transactions of the American Fisheries Society 131:986–993.

CHAPTER TWO

USE OF OTOLITH MICROCHEMISTRY TO IDENTIFY CURRENT AND HISTORIC NATAL ORIGINS OF YELLOWSTONE CUTTHROAT TROUT IN YELLOWSTONE LAKE, WYOMING

Abstract

Yellowstone Lake is the core of the remaining habitat for genetically pure Yellowstone cutthroat trout. This population has declined substantially over the past 20 years as a result of drought, whirling disease, and especially due to the introduction of piscivorous lake trout. The objective of this study was to test the hypothesis that cutthroat trout recruitment patterns have measurably shifted in response to these environmental stressors. Natal origins of cutthroat trout were compared under historic (1997) and recent (2013) conditions using otolith microchemistry. There was unexpectedly low variation in water chemistry among the 22 sampled spawning tributaries and nearest neighbor cluster analysis identified 9 separate distinct spawning stream groups. Relative recruitment to each cluster was assessed using random forest models with a classification accuracy of 84.4% for known-origin cutthroat trout fry otoliths and 79.0% for simulated otolith signatures. There was a significant difference in the proportion of recruitment between historic and recent cutthroat trout spawning clusters (X^2 = 15.40, p = 0.03). The majority of historic (0.84) and recent (0.77) recruitment occurred in the same three stream clusters, with the most notable change a decrease in recent recruitment in the stream cluster containing Pelican Creek (high whirling disease prevalence) and an increase in

recruitment in tributaries in the upper Yellowstone River drainage. Having identified key streams clusters that are important to cutthroat trout recruitment in the Yellowstone Lake watershed both historically and recently, as well as their response to environmental stressors this study can be used to formulate future restoration and conservation efforts, focused on improving cutthroat trout recruitment in spawning streams that once contributed much greater numbers of fish.

Introduction

Yellowstone cutthroat trout (*Onchorhynchus clarkii bouvieri)* are native to the Yellowstone River drainage in Montana and Wyoming, and the upper Snake River drainage in Idaho, Nevada, Utah, and Wyoming (Behnke 2002). Yellowstone cutthroat trout populations have declined dramatically throughout their native range over the past two decades (Gresswell 2011). Yellowstone cutthroat trout indigenous to Yellowstone Lake and its numerous tributaries have also been affected. The decline in this population has been attributed to prolonged drought from the late 1990's–mid 2000's (Kaeding et al. 2010), whirling disease found in some spawning tributaries (Koel et al. 2006), and, in particular, the introduction of piscivorous lake trout (*Salvelinus namaycush*)(Koel et al. 2005). Decline in this population has been especially troubling because this watershed once supported the largest population of Yellowstone cutthroat trout (Gresswell 2011), and is currently one of the few genetically pure populations that remain (Koel et al. 2008).

Yellowstone cutthroat trout spawn exclusively in streams and, like other Salmonids, usually show high spawning fidelity to natal streams (Ball 1955). The Yellowstone Lake cutthroat trout population primarily exhibits a lacustrine-adfluvial life history with spawning occurring in tributaries during brief periods, and juvenile fish generally migrating to the lake shortly after emergence (Varley and Gresswell 1988; Kaeding and Boltz 2001; Koel et al. 2004; Ertel 2011). Spawning has been documented from April to early August (Ball 1955; Cope 1956, 1957; Jones et al. 1986) in 68 of the 124 tributaries to the lake (Jones et al. 1986). According to Ertel (2011), once fish return to the lake after spawning, they move throughout the lake without exhibiting any patterns of site fidelity within the lake.

Yellowstone cutthroat trout are considered a keystone species in the greater Yellowstone Ecosystem, with 4 mammal species, and 16 bird species documented consuming Yellowstone cutthroat trout (Bergum et al. in review). The decline in cutthroat trout numbers has resulted in cascading effects throughout the ecosystem. Cutthroat trout once composed a large portion of the spring diet of many Yellowstone grizzly bears (*Ursus arctos*), however in recent years bears few bears have been observed feeding on spawning fish (Bergum et al. in review). Similarly, once-abundant osprey nesting near the lake (*Pandion haliaetus*) are now rare (Bergum et al. in review).

To better understand the threats to cutthroat trout and mitigate for them, the National Park Service (NPS) has been monitoring the effects of the drought and whirling disease prevalence among spawning streams, and have enacted a large-scale lake trout removal program (Koel et al. 2015). In recent years the drought has subsided (Koel et al.

2015), whirling disease seems to have stabilized, and is present in only a few spawning tributaries (Koel et al 2006; Koel et al. 2015), and annual lake trout removal efforts have removed record numbers of fish (Koel et al. 2015). Cutthroat trout recruitment seems to be improving in recent years (Koel et al. 2015), however little is known about which streams are contributing to cutthroat trout recruitment or how recruitment patterns may have changed over time.

Previously, counts of adult spawning fish in streams have been used as a metric to determine which spawning streams are most important to recruitment, and how recruitment may fluctuate over time (Koel et al. 2004). However, it has not been verified that these counts correspond to actual recruitment. Furthermore, these counts were largely confined to small tributaries on the western side of Yellowstone Lake where fish could be easily observed and counted, while many important spawning streams in remote locations were not monitored due to inaccessibility (Reinhart and Mattson 1990).

The goal of this study was to use otolith microchemistry to identify historic and current cutthroat trout natal origins (streams in which fish were spawned) as well as the contributions of specific spawning streams to the population of cutthroat trout in Yellowstone Lake. The varying chemical composition of spawning tributaries, reflective of differences in underlying geology (Kennedy et al. 2000; Wells et al. 2003), impart unique chemical signatures to otoliths during early rearing, allowing for retrospective identification of natal origins by matching water and otolith composition (Campana 1999). In this study, a combination of Sr isotopes (^{87}Sr:^{86}Sr) and elemental ratios (Sr:Ca, Ba:Ca, Mg:Ca, Mn:Ca) in otoliths and water were used to identify the natal origins, and

subsequently the contributions of specific spawning streams to the population of Yellowstone cutthroat trout in Yellowstone Lake. The spawning stream contributions were compared for two time periods: 1) historically, prior to the negative effects of drought, whirling disease, and the full manifestation of lake trout population expansion and high predation pressure on cutthroat trout (1997), and 2) recently in 2013 reflective of very low cutthroat trout abundance following an increase in lake trout abundance (Koel et al. 2012).

We hypothesize that spawning streams located in regions of the lake with the high lake trout densities (streams flowing in to West Thumb and western side of the main basin), streams with the highest whirling disease prevalence, and the streams that are most susceptible to drought, may have reduced recruitment of juvenile cutthroat trout. The majority of lake trout netted from Yellowstone Lake have been netted in the West Thumb and western side of the main basin of the lake (Koel et al. 2015), thus it appears that lake trout densities may be higher in some portions of the lake than others. As a result of the uneven distribution of lake trout throughout the lake, the effects of their predation on cutthroat trout may be more pronounced in relation to spawning streams flowing into portions of the lake with higher lake trout densities. Additionally, the effects of drought and whirling disease is not uniform among spawning tributaries (Koel et al. 2006; Koel et al. 2015), therefore, we suspect that recruitment patterns among tributaries may have shifted considerably during the past~20 years as a result of large and cumulative stressors on this population.

Comparison of historic and current recruitment patterns offers a unique opportunity to evaluate fish population response to environmental stressors. Understanding how recruitment patterns have changed over time valuable for future management and restoration efforts targeted toward improving recruitment

Methods

Study Area

The study area encompasses the Yellowstone Lake drainage upstream of Upper Yellowstone Falls (Figure 2.1). Yellowstone Lake is the largest high elevation lake (2376 m) in North America (352 km^2; Kaplinski 1991). The drainage has an area of 2616 km^2 (Benson 1961) with 124 tributaries, 68 of which have documented spawning by cutthroat trout (Jones et al. 1986). Underlying bedrock in the drainage is primarily Tertiary andesite and Quaternary rhyolite, and Tertiary sedimentary deposits are present in several tributary drainages in the southern portion of the basin (USGS 1972). At lower elevations, post-glacial deltaic deposits are common, likely as a result of historically higher lake levels (Richmond 1976).

Two species of fish are native in the Yellowstone Lake drainage, the longnose dace (*Rhinichthys cataractae)* and Yellowstone cutthroat trout. Four nonnative species are present, including: redside shiner (*Richardsonius balteatus)*, lake chub (*Couesius plumbeus)*, longnose sucker (*Catostomus catostomus)* (Varley and Schullery 1998), and lake trout (Kaeding et al. 1996).

Water Chemistry

Water chemistry used to identify natal origins was determined for 22 different spawning streams. Streams were selected based on historic spawner distribution and underlying geology. Historic spawner distribution was based on previous spawner counts and observations of spawning concentrations (Reinhart 1990; Haroldson et al. 2005; Ertel 2011); spawning streams that routinely had greater than 100 spawners were included in this analysis. Maps of age and relative composition of underlying geology, shown to be important predictors of isotopic and elemental composition of surface waters (Garcez et al. 2014), were also used to ensure adequate spatial representation of tributaries likely differing in chemical composition.

Water samples were collected during summer base flow conditions in 2013 and 2014 (August and September), which coincided with the post-emergence period of juvenile rearing prior to outmigration to the lake. Due to the large size of three of the streams, two water samples were collected at different locations. To test for annual variation in water chemistry, a subset of 5 tributary streams were resampled in 2014. At each stream, 50 ml water samples were collected in the thalweg by inserting a sterile 50 ml syringe vertically into the upstream water column. Samples were filtered with a sterile 0.2 micron Whatman filter and then stored in acid washed polyethylene bottles containing two drops of nitric acid.

Water samples were processed at Woods Hole Oceanographic Institution (WHOI) using solution-based inductively coupled plasma mass spectrometry (ICPMS) for the following isotopic and elemental ratios: ^{87}Sr:^{86}Sr, Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca. For

^{87}Sr:^{86}Sr ratios, a portion of each sample was first evaporated and redissolved in 50% HNO_3 then eluted through a Sr-specific cation exchange resin. The remaining sample was again evaporated and redissolved 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 and Hart (2006). Lastly, all Sr isotope ratios were normalized to the NIST SRM987 standard. For elemental ratios, samples were first diluted 10-fold using a 2% HNO_3 solution prior to ICPMS measurement of ^{48}Ca, ^{88}Sr, ^{138}Ba, ^{25}Mg and ^{55}Mn. Liquid standards and instrument blanks of 2% HNO3 were run every six samples. Instrument mass bias was corrected using certified values from river water standard (SLRS-4, NRC), and an internal laboratory river water standard was used to assess measurement precision. External precision (relative standard deviation) of Sr:Ca ratios for the laboratory standard (n = 20) was 1.3%.

Otolith Sampling

Otoliths from known-origin fish were used to correlate natal stream water chemistry to chemical composition of cutthroat trout otoliths following the methods of Muhlfeld et al. (2012) and Olley et al. (2011). Cutthroat trout fry (~1-2 months post emergence, n = 5 - 12) were collected by dipnet from a subset of 7 natal streams (Beaverdam Creek, Columbine Creek, Cub Creek, Grouse Creek, Little Thumb Creek, Sewer Creek, and Unnamed Creek (SONYEW 1138; System of numbering Yellowstone waters) during base flow conditions in August and September 2013 coincident with water sampling. Sample sites were selected based on the underlying geology to represent the

range of variation in chemical signatures among streams. Otoliths were removed under a dissecting microscope using nonmetallic forceps, triple rinsed to remove any tissue, and stored in microcentrifuge vials prior to preparation.

Natal contributions during the two study periods were assessed using otoliths from trout captured during annual gillnet sampling conducted in 1997 and 2013. During both samplings gillnets were distributed throughout Yellowstone Lake to ensure sampling effort was equal throughout the lake, however only 11 sites were used in 1997, while 24 sites were used in 2013 (personal communication with National Park Service Fisheries Biologist Jeff Arnold). Gillnet sampling in 2013 consisted of 2 multi-panel gillnets of two sizes large (57 to 95 mm) and small (19 to 51 mm) mesh set in parallel at each site perpendicular to the shore in three depth strata: shallow near shore (above the thermocline), mid-depth (across the thermocline), and deep (below the thermocline) (personal communication). The thermocline was identified prior to sampling using a Hydrolab multi-probe sonde (Koel et al 2015). Gillnet sampling in the historic (1997) sampling consisted of 5 shallow near shore small mesh multi-panel gillnets in each location. Many of the sites sampled in 2013 (n = 24) were in the same locations as those sampled in 1997 (n=11) (personal communication).

Sagittal otoliths were removed from all trout collected in 2013 using nonmetallic forceps, triple rinsed and scrubbed with Milli-Q water. The instruments used to remove otoliths collected in 1997 are unknown. To minimize the possibility of otolith contamination the 1997 and 2013 otolith collections were both soaked overnight in Milli-Q water then scrubbed and triple rinsed with Milli-Q water. Otoliths were then air dried

and stored in acid washed microcentrifuge vials prior to further preparation. Because there was a limited selection of otoliths collected in 1997 (*n*=107) all of these otoliths were used in microchemical analysis. For the 2013 sampling, a subset of otoliths was selected for microchemical analysis using a stratified random design. One hundred otoliths were randomly selected for each of three length classes (<200, 200-400, and >400 mm) to ensure sampling fish across all age and size classes. For both sampling years, one otolith of each pair was randomly selected for further analysis. Some otoliths were damaged during processing and were removed from analysis; final sample sizes were 89 for the 1997 sample and 236 for the 2013 sample.

Otoliths were prepared following the methods of Muhlfeld et al. (2012). Each otolith was rinsed with Milli-Q water and scrubbed with a nylon brush to remove any foreign material or remaining tissue, dried under a laminar flow hood for 24 hours, mounted on petrographic slides (sulcus side up) using cyanoacrylate glue, and then sanded to approximately 40-50 microns above the plane of the nucleus using 600- and 1500-grit sandpaper and polished using 0.5- and 0.1-µm diamond lapping film. After a final scrubbing and soaking with Milli-Q water, otoliths were remounted on a new petrographic slide. Finally, otoliths were aged to verify that the variation in age classes among both historical and recently collected fish were similar. Age estimates were independently verified by at least two experienced readers. Historic and recent trout ages ranged from age 1 to age 9. The majority of both groups were age 3 to 6, which comprised 74.2% of the historic trout group and 66.5% of the recent trout group. Age 2 fish made up 10.1% and 13.1% of the historic and recent trout respectively, whereas

collectively ages 1, 7, 8, and 9 comprised 15.7% and 20.3% of the historic and recent trout groups respectively.

Otolith Microchemistry

Otolith microchemistry was performed using laser ablation ICPMS equipped with a 213-mm laser. Otolith $^{87}Sr:^{86}Sr$, Sr:Ca, and Ba:Ca ratios were obtained using a Thermo Finnigan Neptune multiple collector ICPMS, whereas elemental Mg:Ca and Mn:Ca ratios were obtained in a separate assay using a Thermo Finnigan Element2 single collector ICPMS, both of which were coupled to the same laser ablation system (Walther et al. 2008). Otoliths were ablated along a 450-micron curved transect for both assays using a beam diameter of 75 μm, a repetition rate 20 Hz, and a scan speed 5 $\mu m \cdot s^{-1}$. Transects were positioned adjacent to the core to minimize interference from a maternal signal and within the narrow region corresponding to the early growth period after emergence but prior to outmigration to the lake. Sample processing was randomized to minimize potential systematic bias from instrument drift. For quality assurance, a certified reference material (MACS-3) was run every 10 samples (Muhlfeld et al. 2012) to assess instrument drift and changes in mass bias. The mean $^{87}Sr:^{86}Sr$ ratio (±1 SD) for the certified reference material run throughout the analysis was 0.707601 ± 0.00006, which is within 1 standard deviation of the accepted value of MACS-3 (0.70759). External precision (relative SDs) for Sr:Ca and Ba:Ca ratios based on repeated measurements of a certified reference material (Sturgeon et al. 2005) was 4.0% and 0.1% (n=34) for Sr:Ca and Ba:Ca respectively. External precision for Mg:Ca and Mn:Ca ratios was 1.5% and 4.1% respectively. All results were normalized using a standardized reference material

described by Jackson and Hart (2006). Lastly, similar to Blair and Hicks (2012) all element:Ca readings that that fell below the detection limits of the LA-ICPMS and outliers greater than 5 standard deviations from the mean were excluded prior to further analysis.

Statistical Analyses

Statistical analyses were conducted using R version 3.1.1 (R Core Team 2014). Annual variation in water chemistry of five streams was evaluated by comparing the percentage difference in isotopic and elemental ratios within streams to the percentage differences among all streams. Relations between water and otolith microchemistry for each isotope ($^{87}Sr:^{86}Sr$) and elemental ratio (Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca) of known-origin cutthroat trout fry collected from the 7 natal streams were first assessed using simple linear regression. Initial examination of diagnostic quantile-quantile plots, box plots, and scatter plots found no severe violation of the parametric assumptions of normality, homoscedasticity, linearity, and equal variances. There was a near 1:1 relationship between otolith and water Sr isotopes ($r^2 = 0.99$; $p < 0.001$)(Fig 2.2); otolith and water Sr:Ca ratios were also strongly related ($r^2 = 0.74$; $p = 0.029$) (Figure 2.3). In contrast, there were no clear linear relationships between otolith and water Ba:Ca ($r^2 = 0.21$; $p = 0.36$), Mg:Ca ($r^2 = -0.21$; $p = 0.76$), and Mn:Ca $r^2 = -0.22$; $p = 0.78$) ratios. Given the regression results, only $^{87}Sr:^{86}Sr$ and Sr:Ca ratios were retained in further analyses. One-way analysis of variance (ANOVAs) tests were used to determine if there were differences in otolith $^{87}Sr:^{86}Sr$ and Sr:Ca chemical signatures among fish from known-origin streams.

Studies that estimate the natal origin of fish using otolith microchemistry typically collect known-origin fish from a variety of likely recruitment sources to develop a model for classifying the putative natal origins of unknown-origin adults (e.g., Munro et al. 2005; Muhlfeld et al. 2012; Sousa et al. 2016). In the case of Yellowstone Lake, where many potential recruitment tributaries exist, the collection of known-origin fish over many potential sources was impractical. Instead, we created a model to predict likely otolith Sr isotopic and elemental signatures based on water chemistry for the 22 study streams. A 3-step process was used to create a range of expected otolith $^{87}Sr:^{86}Sr$ and Sr:Ca ratios. First, because Sr:Ca ratios in otoliths are lower than those found in water, Sr:Ca ratios were predicted by applying a partition coefficient based on the relationship between water and otolith Sr:Ca derived from known-origin cutthroat trout fry. Partition coefficients reflect the degree that elemental uptake in otoliths reflects that found in source waters; the relationship observed for Yellowstone cutthroat trout in our study based on the slope of the linear regression for Sr:Ca ($r = 0.21$) was similar to that found for cutthroat trout in other studies (Wells et al. 2003; Muhlfeld et al. 2012). No partition coefficient was necessary to predict fish Sr isotopic ratios for each stream based on the slope of the linear regression (0.98) which was close to 1, indicating there is little partitioning of Sr isotopic ratios during incorporation into the otolith from ambient water. Second, an estimate of variation around a predicted mean value of otolith Sr was generated by averaging the standard deviation of known-origin otolith $^{87}Sr:^{86}Sr$ and Sr:Ca calculated for each stream ($n = 6$; Beaverdam Creek was excluded because only one otolith remained after preparation). Due to the higher variation in incorporation of

elemental Sr into otoliths from stream water, two standard deviations were used when generating expected values for Sr:Ca. Finally, using the R 'rnorm' function, 50 simulated otolith isotopic and elemental values were generated for each stream using the stream $^{87}Sr:^{86}Sr$ and adjusted Sr:Ca stream values as the means (Step 1) and the standard deviations calculated from the known origin fish as the standard deviations (Step 2). Comparisons of the expected and observed otolith signatures from known-origin cutthroat trout fry were used to assess the accuracy of the simulated otolith chemistry values (see below).

Ideally, stream chemical signatures are distinct enough to allow the classification of natal origins to individual streams (e.g., Muhlfeld et al. 2012; Sousa et al. 2016). However, examination of bivariate plots of stream water $^{87}Sr:^{86}Sr$ and Sr:Ca revealed considerable overlap in chemical signatures among some spawning tributaries. Therefore, prior to classification of natal origins, study streams were first grouped according to their isotopic and elemental signatures using a nearest-neighbor cluster analysis (Maechler et al. 2015). In the analysis, multiple samples of water chemistry from large streams were pooled by averaging, and stream $^{87}Sr:^{86}Sr$ and Sr:Ca ratios were standardized to a mean of zero and a standard deviation of 1.0 to accommodate for the different scales of for isotopic and elemental values. Variables in the cluster analysis were also weighted to reflect the higher precision of isotopic Sr as a predictor of natal origins than elemental Sr (Kennedy et al. 2000). Standardized stream Sr:Ca ratios were multiplied by 0.50 to reduce the variance and reflect additional weight given to Sr isotopes. A weighting factor of 0.50 was chosen as a simple means of reducing the Sr:Ca variance by one half, thus

allowing Sr isotopic ratios to drive the clustering process. Weighting of variables is not common in microchemistry studies, however given that Sr isotopic ratios are intrinsically more precise and less variable we used weighting of the variables as a means of informing the clustering process as recommended by Kaufman and Rousseeuw (2005).

Following clustering, a random forest model was constructed for the classification of natal origin of unknown-origin cutthroat trout otolith signatures. A recent comparison of natal stream origin classification methods found random forest modeling had the highest classification accuracy and least violation of statistical assumptions among discrimination methods used for otolith microchemistry studies (Mercier et al. 2011). Random forest analysis is based on many classification trees, these classification trees use rules to recursively split the data into binary groups (Breiman 2001). Each random forest classification tree was constructed using a random subset of 75% of the simulated otolith signatures data set using bootstrap resampling with replacement. The remaining 25% of the simulated otolith signatures data were then used to measure the prediction ability of that tree. For each given tree, a random predictor variable was searched at each node to find the one that maximized the within group homogeneity until further splitting resulted in no gain in within-group homogeneity (Mercier et al. 2011). The random forest model was created using the simulated otolith chemistry data for each stream; unknown origin fish were then classified to natal origin by stream cluster using the constructed random forest model. The final classifications of unknown-origin cutthroat were based on 500 bootstrapped random forests each with 2000 trees. Each tree in a random forest makes a prediction of natal origin. Once all 2000 trees are complete, a single prediction

of fish origin is obtained for each random forest based on the fish origin most often predicted by each tree in a random forest. By using an ensemble of random forests (*n* = 500) the classification accuracy was improved by once again selecting the fish origin that was most often selected by each of the random forest models. The number of clusters used to classify fish was determined iteratively by examining the classification accuracy of the random forest model when classifying the 25% simulated fish signatures withheld from the analysis. Further validation of random forest modeling was obtained by comparing the percent correct classification of microchemical signatures from known-origin trout fry to model output. Lastly, the differences in the assigned natal origins of historic and recently collected cutthroat trout samples were quantified using a Pearson's chi-squared test with Monte-Carlo simulation of p-values. Due to insufficient sample sizes (<5) of some of the historic and recent otoliths assigned to each cluster, 2000 random p-value replicates were simulated to create a reference distribution for comparison to satisfy the assumptions of the analysis (Hope 1968).

Results

Water Chemistry

There were many similarities but also some distinctive differences in ^{87}Sr:^{86}Sr and Sr:Ca ratios among streams within the Yellowstone Lake watershed (Table 2.1). Sr isotope ratios ranged from 0.705824 to 0.709242, and Sr:Ca ratios ranged from 0.92 to 6.22 mmol/mol (Table 2.1). In many instances streams in close proximity exhibited similar microchemical signatures, while other nearby streams exhibited very different signatures (Figure 2.4), this is likely attributed to the underlying geology (Figure 2.5).

For example, Chipmunk Creek and Grouse Creek, two streams that are within 5 km of one another exhibited similar Sr:Ca ratios (3.40 and 3.54 mmol/mol respectively), however their $^{87}Sr:^{86}Sr$ ratios were very different (0.706757 and 0.709242 respectively). In some instances, streams that are relatively distant from one another exhibited similar microchemical signatures; for instance Trail Creek and Little Arnica Creek had very similar $^{87}Sr:^{86}Sr$ ratios (0.707903 and 0.707732 respectively) despite their spatial separation of nearly 47 km (Figure 2.1).

Water chemistry variation among streams was much greater than the variation exhibited between replicate samples, indicated by minimal annual variability in both microchemical ratios within five streams (Figure 2.6 and Figure 2.7). The average differences between replicate samples was 0.04‰ (0.01‰–0.12‰) and 6.77% (2.03%–11.90%) for $^{87}Sr:^{86}Sr$ and Sr:Ca ratios respectively. Differences among streams averaged over both 2013 and 2014 were 0.60‰ (0.16‰–1.23‰) and 23.81% (5.11%–42.89%) for $^{87}Sr:^{86}Sr$ and Sr:Ca ratios respectively. The average difference among stream $^{87}Sr:^{86}Sr$ ratios were roughly 15 times greater than the differences between replicate stream samples. The differences in Sr:Ca ratios among streams was roughly 3.5 times greater than the average differences between replicate stream samples.

Streams were grouped into 9 clusters (Table 2.1; Figure 2.8) (n=22), based on similarities in $^{87}Sr:^{86}Sr$ and Sr:Ca ratios using nearest-neighbor cluster analysis. The number of streams in each cluster ranged from 1 to 7 streams with three separate clusters containing only 1 stream, and one cluster containing 7 streams. There were no clear spatial patterns among most stream clusters; streams in the largest cluster (cluster 2) were

distributed throughout the West Thumb, main basin, and the South and Southeast Arms of Yellowstone Lake. Only two clusters contained multiple streams that were all located in relatively close proximity to one another, cluster 6 (Cub and Pelican Creeks) and cluster 9 (Mountain and Thorofare Creeks).

Classification of Natal Origins

Simulated otoliths, known-origin otoliths, and both historic and recent unknown-origin fish were assigned to the 9 clusters with varying classification accuracies. Mean classification accuracy of simulated otolith signatures for the 9-cluster random forest model across all 500 random forests was 79.0% (range 72.3% to 84.5%). Classification accuracy of simulated otolith signatures (Table 2.2) was not the same across all clusters. The cluster-specific classification accuracy ranged from 31.6% to 100.0%. Cluster 1 and cluster 4 had the lowest classification accuracy of simulated otolith signatures with 49.3% and 31.6% correct classification, respectively. Cluster 7 and cluster 8 had the highest classification accuracy of simulated otolith signatures with 100% classified correctly to both clusters. Known-origin trout fry were classified with 84.4% (range 77.8% to 100.0%) classification accuracy (Table 2.3). These fish originated from streams in 4 of the 9 clusters.

The proportion of both historic and recent fish otolith signatures assigned to each cluster (Table 2.4) ranged from 0.0 to 0.33. The largest proportions of both historic and recent otoliths were assigned to clusters 2, 5, and 6 (0.27, 0.33, and 0.24 respectively for historic and 0.33, 0.28 and 0.16 respectively for recent) (Table 2.4; Figure 2.9, and 2.10). These three clusters contained some of the largest streams sampled in this study,

including the Upper Yellowstone River (cluster 2), the Lower Yellowstone River (cluster 5), and Pelican Creek (cluster 6) as examples. None of the sampled fish were assigned to cluster 1 (Arnica Creek and Bridge Creek) and of all otoliths analyzed, only 1 recent fish was classified to cluster 3 (Big Thumb Creek) (Table 2.4, Figure 2.10).

There was a significant difference in the estimated proportions of historic and current recruitment from the different spawning stream clusters (($X^2 = 15.40$; $p = 0.03$) The proportion of historic and recently collected cutthroat trout assigned to each cluster appeared similar within 5 of the 9 clusters, with differences of 0.0 to 0.05. However, clusters 6 (Cub and Pelican Creeks) and 7 (Flat Mountain, Little Arnica, and Trail Creeks) showed a 0.08, and 0.06 decrease in the proportion of cutthroat trout originating from these respective clusters over time. Conversely, cluster 2 (Beaverdam Creek, Cabin Creek, Chipmunk Creek, Little Thumb Creek, Solution Creek, Unnamed Creek (SONYEW 1138), and the Upper Yellowstone River) and cluster 9 (Mountain and Thorofare Creeks) showed notable proportional increases of 0.06 and 0.09 respectively.

Discussion

In order to examine how spatial recruitment patterns may have changed as a result of lake trout predation, whirling disease, and drought it is imperative to understand which spawning streams have historically and are currently contributing to cutthroat trout recruitment. Previous to this study, there was little information regarding the historic or recent contributions of specific spawning streams to cutthroat trout recruitment in Yellowstone Lake. In the past the National Park Service (NPS) has relied on counts of

actual spawning fish in tributaries as a metric for spawning stream contributions. However, these counts were confined to mostly small, easily accessible tributaries, and there was no way of knowing if the number of spawning fish is actually representative of recruitment associated with that spawning stream. The otolith microchemistry techniques used in this study addressed the lack of previous knowledge, and provides resource managers with a more detailed understanding of which spawning streams are contributing most to cutthroat trout recruitment.

The general lack of differences in stream microchemical signatures among Yellowstone Lake spawning tributaries was unexpected given the complex geology of the region. Proximal streams within many of the clusters exhibited similarities in their microchemical signatures; however there were also similarities among streams relatively distant from one another. Because the underlying bedrock geology in the northern and western portion of the drainage is primarily rhyolite, and the underlying geology in the eastern and southern portion of the drainage is primarily mixed clastic/volcanic rock, streams within these respective regions often had similarities in their microchemical signatures (Figure 2.5). Accordingly, some areas with unique bedrock types within the drainage also exhibited unique microchemical signatures. For instance, Grouse Creek was the only stream that had an abundance of conglomerate bedrock within its watershed and consequently its Sr isotopic signature was much higher than other streams. There were some anomalies in water chemistry throughout the drainage. For example, Little Thumb Creek and Solution Creek two streams flowing into the West Thumb of Yellowstone Lake exhibited similar microchemical signatures to Cabin Creek, Beaverdam Creek,

Upper Yellowstone River, and other streams originating from areas with a different dominant bedrock type. These similarities may be due to the presence of alluvial deposits at lower elevations throughout these drainages; these alluvial deposits could alter stream chemistry enough that streams draining very different geologies are exhibiting similar microchemical signatures.

In comparison to other freshwater otolith microchemistry studies, the range of variation in $^{87}Sr:^{86}Sr$ ratios across all spawning streams sampled was much lower in the Yellowstone Lake watershed. For example, Muhlfeld et al. (2012) found that $^{87}Sr:^{86}Sr$ ratios ranged from 0.71131 to 0.74679 in the Flathead River drainage, MT, a range of ~0.0355; in contrast the $^{87}Sr:^{86}Sr$ ratios found in this study area were from 0.705824 to 0.709242, a range of ~0.0034, a range of variation an order of magnitude lower. The stream Sr:Ca ratios in this study exhibited a wider range of variation (0.92 to 6.22 mmol/mol) than isotopic ratios and was similar in magnitude to other studies (e.g., 0.23 to 3.10 mmol/mol; Muhlfeld et al. 2012), however most of the streams (59.1%) were within a smaller range of variation (3.00 to 5.00 mmol/mol). Additionally, Sr:Ca ratios found in otoliths have been shown to be heteroscedastic (Wells et al. 2003; Strohm 2015), with fish from streams with high Sr:Ca ratios, like those found in the Yellowstone Lake drainage, tending to have a wider range of variability in their otolith Sr:Ca signatures than fish from streams with lower Sr:Ca ratios.

The low variation in stream and otolith chemistry in the Yellowstone Lake basin necessitated the clustering of streams. Clustering can improve classification accuracy for estimating natal origin (e.g. Wells et a1. 2003; Carlton 2011). Carlton (2011) found that

classification accuracy improved by approximately 20% when streams with similar microchemical signatures were grouped together based on similarities in microchemistry. Nine stream clusters were used in this analysis; these clusters of streams provided optimal classification accuracy while subsequently providing enough separation of streams to provide the NPS with valuable information for future management.

The random forest analysis using 9 stream clusters indicated that clusters 2, 5, and 6 included the most productive tributaries both prior to, and after the effects of drought, whirling disease and the expansion of the lake trout population. These results are not surprising given that these three clusters contain some of the largest streams within the watershed. Cluster 2 contains the Upper Yellowstone River, cluster 5 contains the Lower Yellowstone River and cluster 6 contains Pelican Creek; all streams that previous studies reported as having large numbers of spawning fish, and multiple spawning sites (Reinhart 1990; Kelly 1993; and Ertel 2011).

There were some important differences in the proportion of historic and recent fish originating from specific clusters, these temporal differences may be due to natural variation in recruitment patterns or caused by the negative effects of lake trout, whirling disease, and drought. The proportion of fish originating from cluster 6 (Cub Creek and Pelican Creek) decreased by 0.08, this decrease could be largely attributed to whirling disease. Koel et al. (2006) found that whirling disease was present in Pelican Creek with infection risk for juvenile cutthroat trout being extremely high; this may have resulted in the reduction in the number of fish originating from Pelican Creek due to juvenile mortality. The proportion of fish originating from cluster 2 (Beaverdam Creek, Cabin

Creek, Chipmunk Creek, Little Thumb Creek, Solution Creek, Unnamed Creek (SONYEW 1138), and Upper Yellowstone River) increased by 0.06, and the proportion of fish originating from cluster 9 (Mountain Creek and Thorofare Creek) increased by 0.09. It is more difficult, however, to speculate why there was an increase in fish with natal origins from streams in clusters 2 and 9. One possible explanation is that juvenile fish from some of these streams may stay in their natal streams for a slightly longer period of time after emergence, given that some of these steams are among those that are furthest from Yellowstone Lake (Mountain and Thorofare Creeks in cluster 9, and Upper Yellowstone River in cluster 2). If juvenile cutthroat trout are remaining in their natal streams for a longer period of time, increased growth may impart a greater ability to escape lake trout predation. Alternatively, in reference to cluster 4 some fish could be misclassified between cluster 9 and cluster 4 causing conflicting results. Examination of the random forest confusion matrix (Table 2.2) indicates that misclassified fish originating from cluster 4 are most likely to be assigned to cluster 9. However, Clear Creek (cluster 4) had the highest Sr:Ca ratio of any stream sampled; very few historic or recent otoliths signatures exhibited similarly extreme Sr:Ca ratios. While the random forest misclassifications indicate that more unknown-origin fish may have originated from Clear Creek, examining the cluster assignment graphs (Figure 2.9; Figure 2.10) indicates there may be conflicting results. The cluster assignment figures show a clear separation between cluster 4 (the highest Sr:Ca values) and cluster 9 and it is evident that very few fish contain high enough Sr:Ca ratios to have originated from Clear Creek. Low numbers of fish originating from Clear Creek is surprising given that greater than 70,000

spawning fish were once documented spawning in Clear Creek (Jones et al. 1979). It is therefore possible that Clear Creek may not be as important to the overall recruitment of cutthroat trout to the Yellowstone Lake population as previously thought.

Previous studies have emphasized the importance of sampling water and known-origin reference fish from all areas of potential recruitment when using otolith chemistry to assess natal origins (Campana et al. 2005). Given that Yellowstone cutthroat trout have been observed spawning in 68 tributaries to Yellowstone Lake (Gresswell 1994) it was not feasible to thoroughly sample water from all of those streams due to time and monetary constraints. While not including all potential sources of cutthroat trout recruitment may lead to some fish being misclassified, including additional streams would have undoubtedly increased the number streams in each cluster and reduced model classification accuracy.

Because of time and monetary constraints, known-origin reference fish were not collected from all streams sampled. Therefore, otolith signatures of cutthroat trout in spawning streams was simulated for each stream based on the water chemistry. Simulating data can create a potential for error, and in this case it would pertain more to the generation of Sr:Ca ratios than ^{87}Sr:^{86}Sr ratios. Because stream ^{87}Sr:^{86}Sr ratios require no adjustment with a partition coefficient and physical and environmental factors play a very small role in their incorporation into the otolith, there is less potential for error. Creating the random forest model based solely on simulated data and validating it with known origin fish, which classified with 84.4% accuracy provided support for the validity of the simulated data. To our knowledge, this is the first otolith microchemistry study that

used simulated otolith data to develop a model to classify fish to stream clusters. While it would be better to have known-origin fish to train the model, collecting and analyzing data from known-origin fish can be cost and logistically prohibitive. Here, we provide an alternative method, however we still relied heavily on knowledge gained from the collection of known-origin fish. Ideally, known-origin fish otoliths would have been collected from each cluster to insure that known-origin fish are assigned to each cluster with a high degree of accuracy, further validating the simulated data.

Results of this study can be used to direct monitoring and protection efforts to streams that are major contributors to the Yellowstone Lake cutthroat trout population. In order to maintain the biological integrity of a fish population and its resiliency to stressors and environmental stochasticity, it is very important that multiple spawning stocks be preserved (Schindler et al. 2010). Thus, maintaining the integrity of multiple sources of cutthroat trout recruitment in Yellowstone Lake will make the population more resilient to the negative effects of whirling disease, lake trout, and future climatic changes. This study has provided information on both historic and recent cutthroat trout recruitment patterns among spawning streams within the Yellowstone Lake drainage, this information can be used as a baseline for further research and monitoring. While it appears that the major spawning streams in the past are still contributing large numbers of recruits, the declines in recruitment associated with some tributaries may be a cause for concern as well as a justification for future conservation efforts. In conclusion, this study demonstrated both the strengths as well as the limitations of otolith microchemistry for determining natal origins of freshwater fish. Both recently collected and historically

collected fish were classified to streams of their natal origin. However, this study was limited by the variation in the microchemical signatures among spawning streams. Thus, in most cases otolith signatures could be classified to a group of streams with similar microchemical signatures, but not to a single stream. This study can be used as a reference to future researchers seeking to conduct otolith microchemistry studies in similar freshwater systems. The methods used in this study for grouping streams, and simulating data may be useful to other researcher with limited budgets and other logistical constraints.

Tables

TABLE 2.1. Stream $^{87}Sr:^{86}Sr$, Sr:Ca, Adjusted Sr:Ca ratios, and cluster assignment. Adjusted Sr:Ca ratios were adjusted using the partition coefficient (0.21).

Stream	$^{87}Sr:^{86}Sr$	Sr:Ca mmol/mol	Adjusted Sr:Ca mmol/mol	Cluster Assignment
Arnica Creek	0.706945	1.68	0.35	1
Bridge Creek	0.706505	2.02	0.42	1
Beaverdam Creek	0.706823	4.12	0.87	2
Cabin Creek	0.706604	3.43	0.72	2
Cabin Creek 2	0.706616	3.50	0.74	2
Chipmunk Creek	0.706757	3.40	0.71	2
Little Thumb Creek	0.706845	4.33	0.91	2
Little Thumb Creek 2	0.706758	3.85	0.81	2
Solution Creek	0.706908	3.11	0.65	2
Unnamed Creek (SONYEW 1138)	0.706657	3.54	0.74	2
Upper Yellowstone River	0.706927	3.50	0.74	2
Big Thumb Creek	0.706111	2.98	0.63	3
Clear Creek	0.705938	5.93	1.25	4
Clear Creek 2	0.705920	6.22	1.31	4
Columbine Creek	0.706476	4.04	0.85	5
Columbine Creek 2	0.706508	4.59	0.96	5
Lower Yellowstone River	0.706428	4.05	0.85	5
Sewer Ck. Creek	0.706588	4.27	0.90	5
Cub Creek	0.706217	5.39	1.13	6
Cub Creek 2	0.706225	5.62	1.18	6
Pelican Creek	0.706210	5.05	1.06	6
Flat Mountain Creek	0.707556	1.85	0.39	7
Little Arnica Creek	0.707732	0.92	0.19	7
Trail Creek	0.707903	2.76	0.58	7
Grouse Creek	0.709242	3.54	0.74	8
Mountain Creek	0.705824	4.53	0.95	9
Thorofare Creek	0.705862	4.47	0.94	9

TABLE 2.2. Confusion matrix for the 9-cluster random forest classification model. The rows represent the stream clusters in which the simulated otolith signatures were generated, and the columns represent the cluster the simulated otolith signatures were classified to. The number of simulated otolith signatures correctly classified is shown across the diagonal, the number of simulated otolith signatures outside of the diagonal represent the number of simulated otolith signatures misclassified that were misclassified. The last column is the percent correct classification (PCC) for each cluster.

Cluster	1	2	3	4	5	6	7	8	9	PCC
1	37	20	0	0	18	0	0	0	0	49.3
2	15	258	0	0	26	1	0	0	0	86.0
3	0	0	25	0	1	6	0	0	6	65.8
4	0	0	0	12	0	1	0	0	25	31.6
5	10	18	0	0	110	12	0	0	0	73.3
6	0	0	4	0	8	63	0	0	0	84.0
7	0	0	0	0	0	0	117	0	0	100.0
8	0	0	0	0	0	0	0	38	0	100.0
9	0	0	3	17	0	0	0	0	93	82.3

TABLE 2.3. Classification accuracy of known origin juvenile fish for the 9-cluster random forest model. The last column represents the percent correct classification (PCC) in each stream.

Stream	*n*	Misclassified	Classified Correctly	PCC
Beaverdam Creek	1	0	1	100.0
Columbine Creek	10	2	8	80.0
Cub Creek	4	0	4	100.0
Grouse Creek	6	1	5	83.3
Little Thumb Creek	9	2	7	77.8
Sewer Creek	9	1	8	88.9
Unnamed Creek (SONYEW 1138)	6	1	5	83.3

TABLE 2.4. Proportion of historic (n = 89), and recent (n = 236) otolith signatures assigned to each cluster. Values contained within the parenthesis is the number of fish assigned to each cluster.

Cluster	Historic Otoliths (1997)	Recent Otoliths (2013)
1	0.00 (0)	0.00 (0)
2	0.27 (24)	0.33 (78)
3	0.00 (0)	<0.01 (1)
4	0.05 (4)	0.06 (14)
5	0.33 (29)	0.28 (66)
6	0.24 (21)	0.16 (38)
7	0.09 (8)	0.03 (7)
8	0.01 (1)	0.02 (5)
9	0.02 (2)	0.11 (27)

Figures

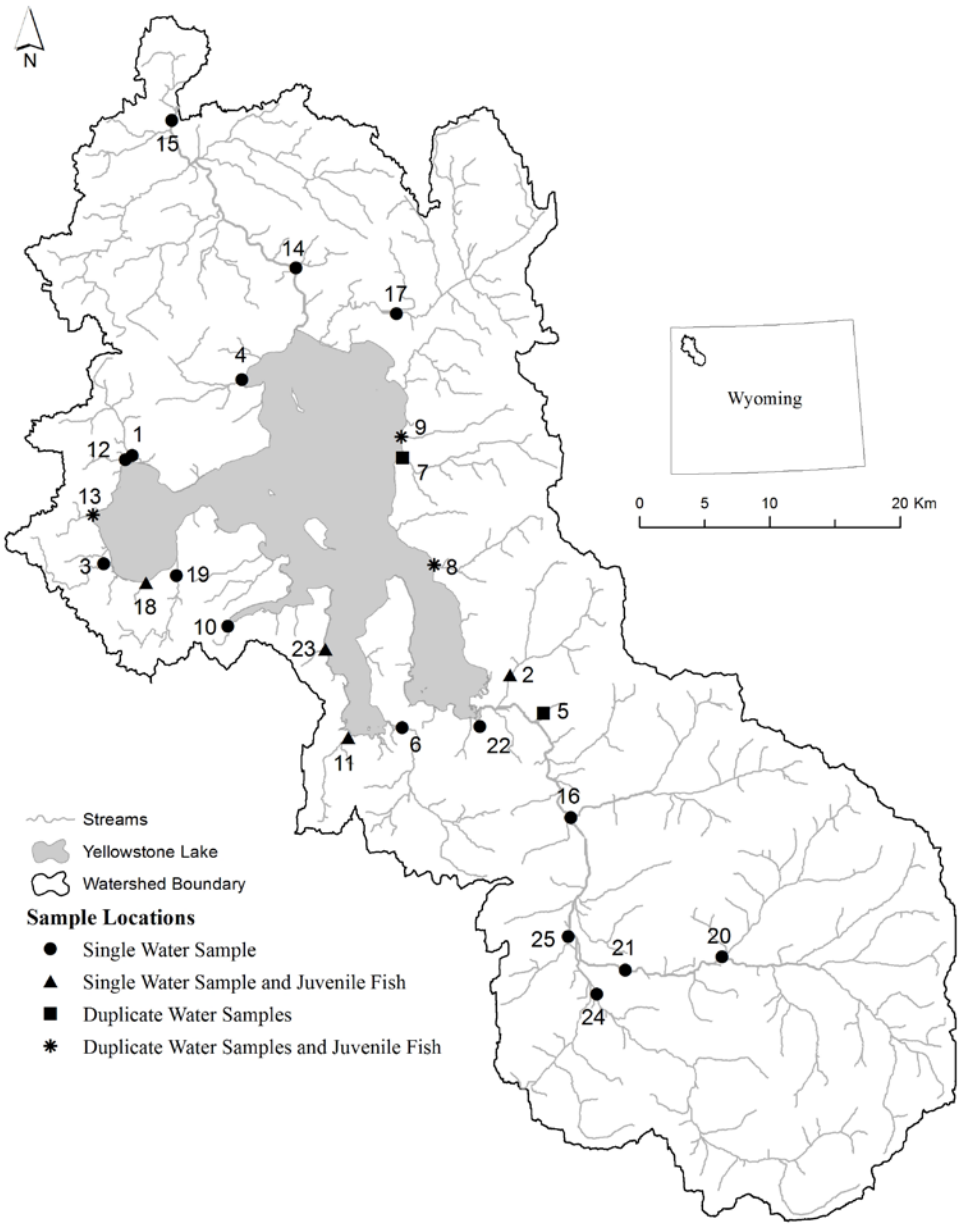

FIGURE 2.1. Map of study area in Yellowstone National Park, WY. Symbols are the stream sample locations numbered: 1. Arnica Creek, 2. Beaverdam Creek, 3. Big Thumb Creek, 4. Bridge Creek, 5. Cabin Creek, 6. Chipmunk Creek, 7. Clear Creek. 8. Columbine Creek, 9. Cub Creek, 10. Flat Mountain Creek, 11. Grouse Creek, 12. Little Arnica Creek, 13. Little Thumb Creek, 14. Lower Yellowstone River 14, 15. Mountain Creek, 16. Pelican Creek, 17. Sewer Creek, 18. Solution Creek, 19. Thorofare Creek, 20, 21. Trail Creek, 22. Unnamed Tributary, 23. Upper Yellowstone River, 24, 25.

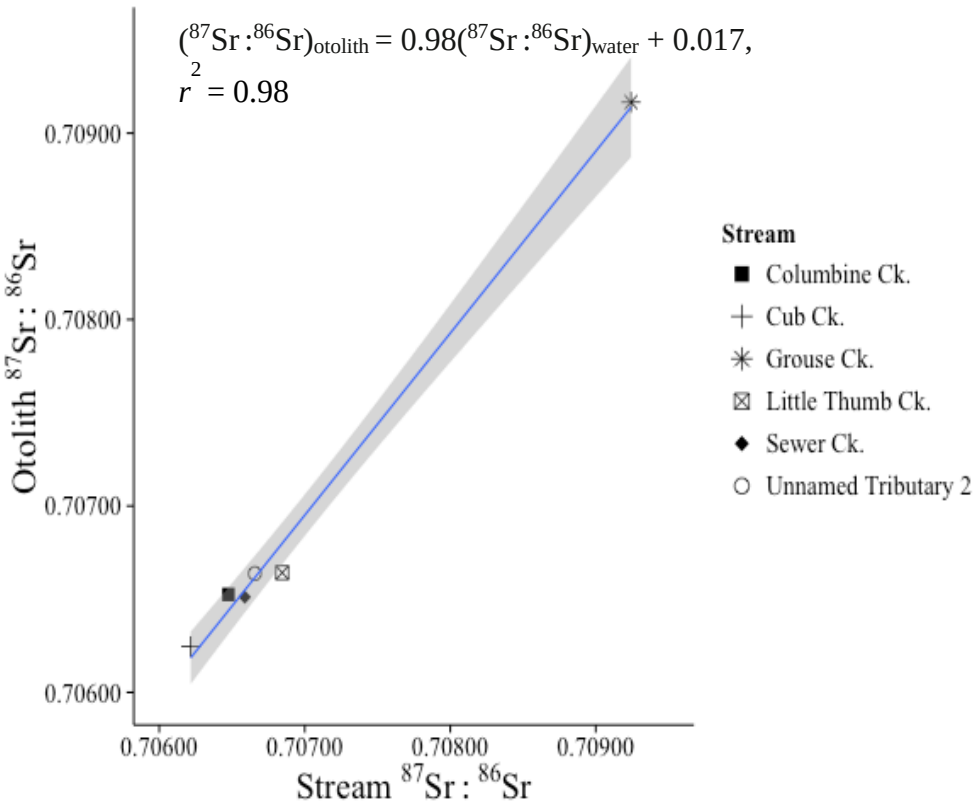

FIGURE 2.2. Mean (n=6) otolith $^{87}Sr:^{86}Sr$ ratios for known-origin cutthroat trout fry from six streams. Solid blue line represents least squares regression line; shaded region represents 95% confidence intervals for the regression line.

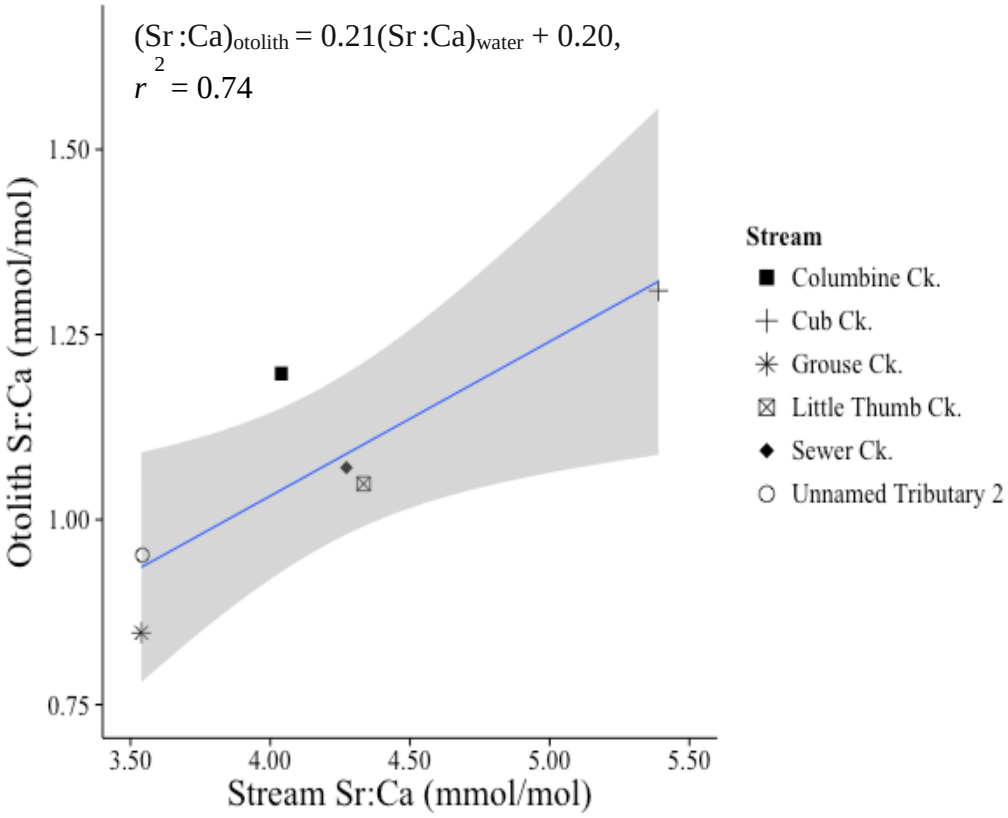

FIGURE 2.3. Mean (n=6) otolith Sr:Ca (mmol/mol) ratios in juvenile cutthroat trout collected from six streams. Solid blue line represents least squares regression line, shaded region represents 95% confidence intervals for the regression line.

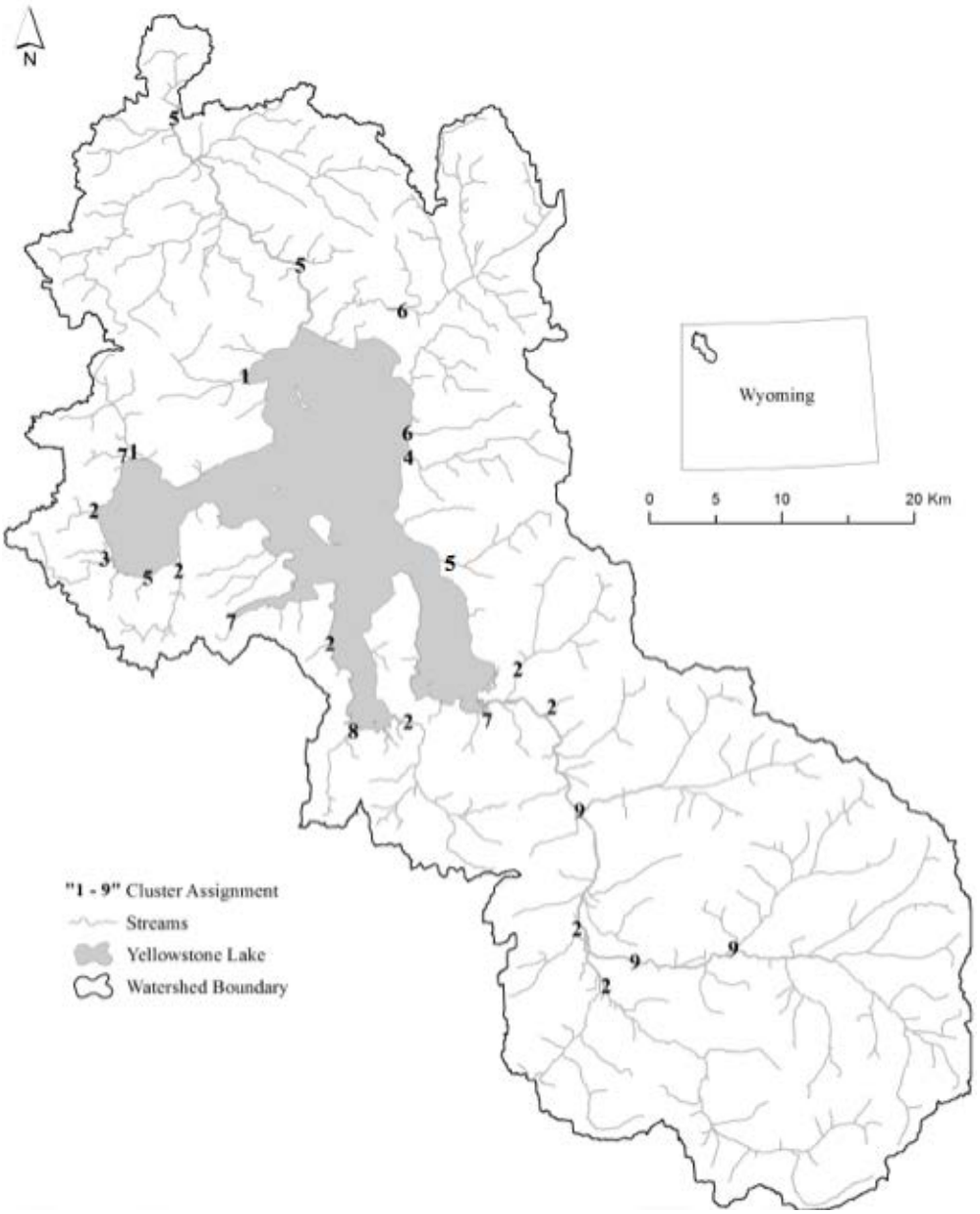

FIGURE 2.4. Cluster assignment of streams (n =22) including sample locations in streams sampled in more than a single location (n = 24) (the upper and lower Yellowstone River)

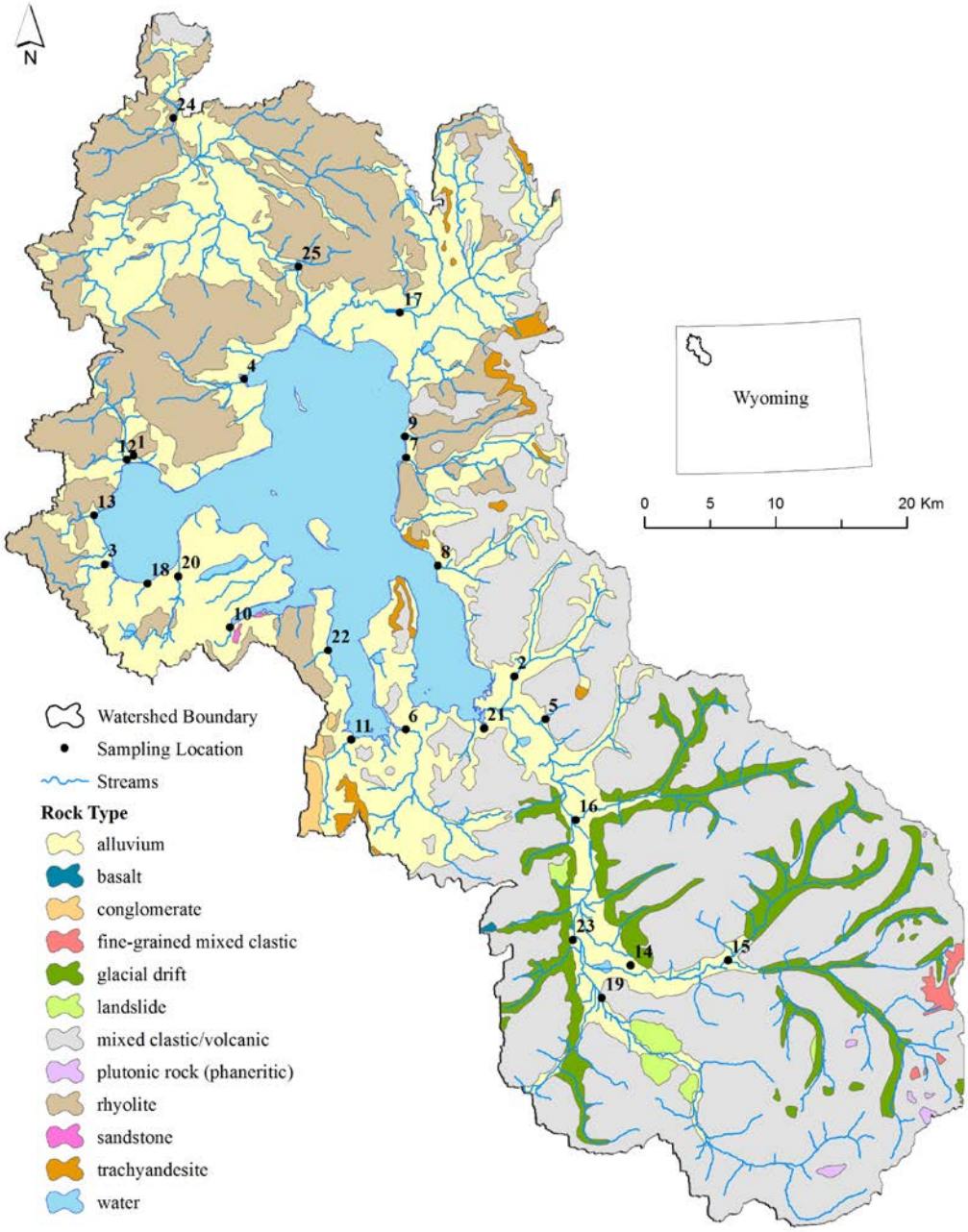

FIGURE 2.5. Underlying geology of the study area in Yellowstone National Park, WY. The stream sample locations are numbered: 1. Arnica Creek, 2. Beaverdam Creek, 3. Big Thumb Creek, 4. Bridge Creek, 5. Cabin Creek, 6. Chipmunk Creek, 7. Clear Creek. 8. Columbine Creek, 9. Cub Creek, 10. Flat Mountain Creek, 11. Grouse Creek, 12. Little Arnica Creek, 13. Little Thumb Creek, 14. Lower Yellowstone River 24, 25. Mountain Creek, 16. Pelican Creek, 17. Sewer Creek, 18. Solution Creek, 19. Thorofare Creek, 14, 15. Trail Creek, 21. Unnamed Tributary, 22. Upper Yellowstone River, 19, 23.

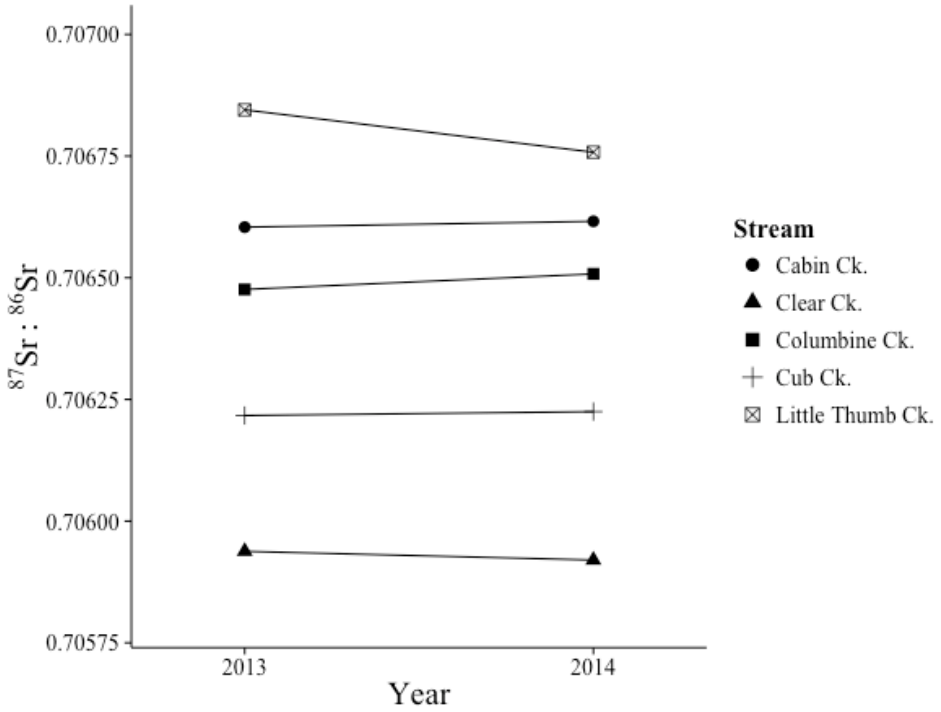

FIGURE 2.6. Annual variability in stream $^{87}Sr:^{86}Sr$ ratios.

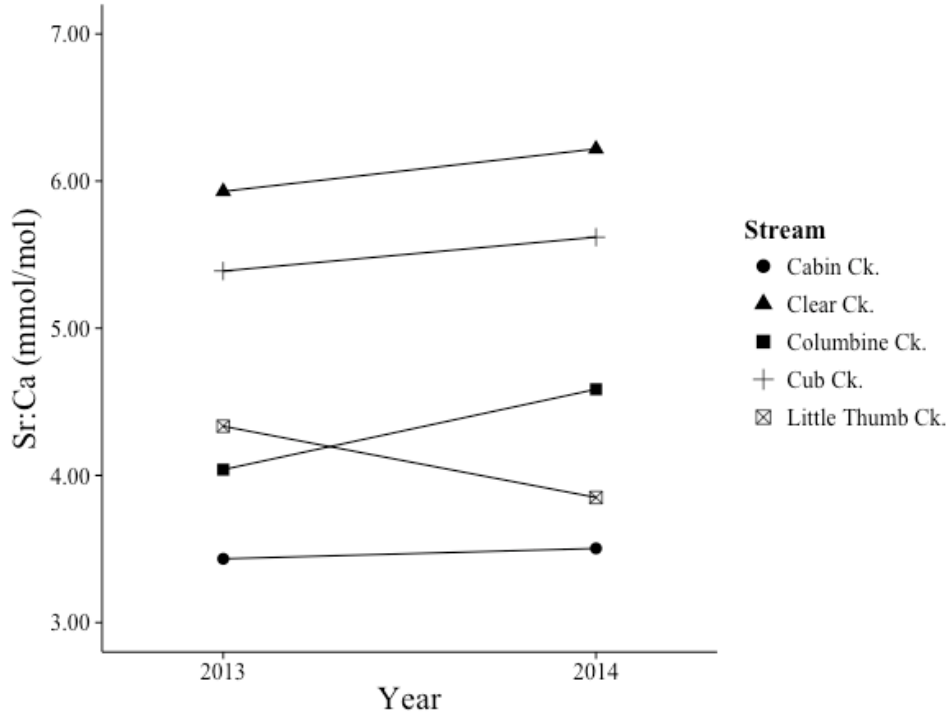

FIGURE 2.7. Annual variability in stream Sr:Ca ratios.

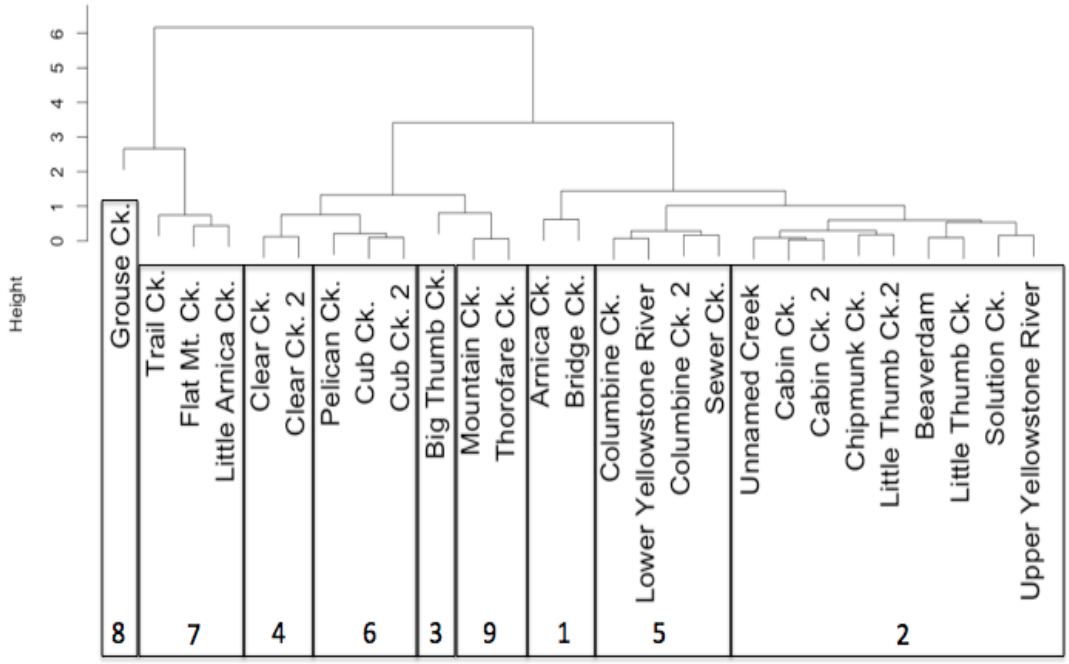

FIGURE 2.8. Nearest neighbor cluster analysis dendrogram with 9 clusters. The y-axis 'height' is a measure of the closeness of clusters based on where they split. Streams with the number 2 are replicate samples. Black boxes contain all streams in a single cluster; numbers in the lower portion of the boxes correspond to cluster in which each stream was assigned.

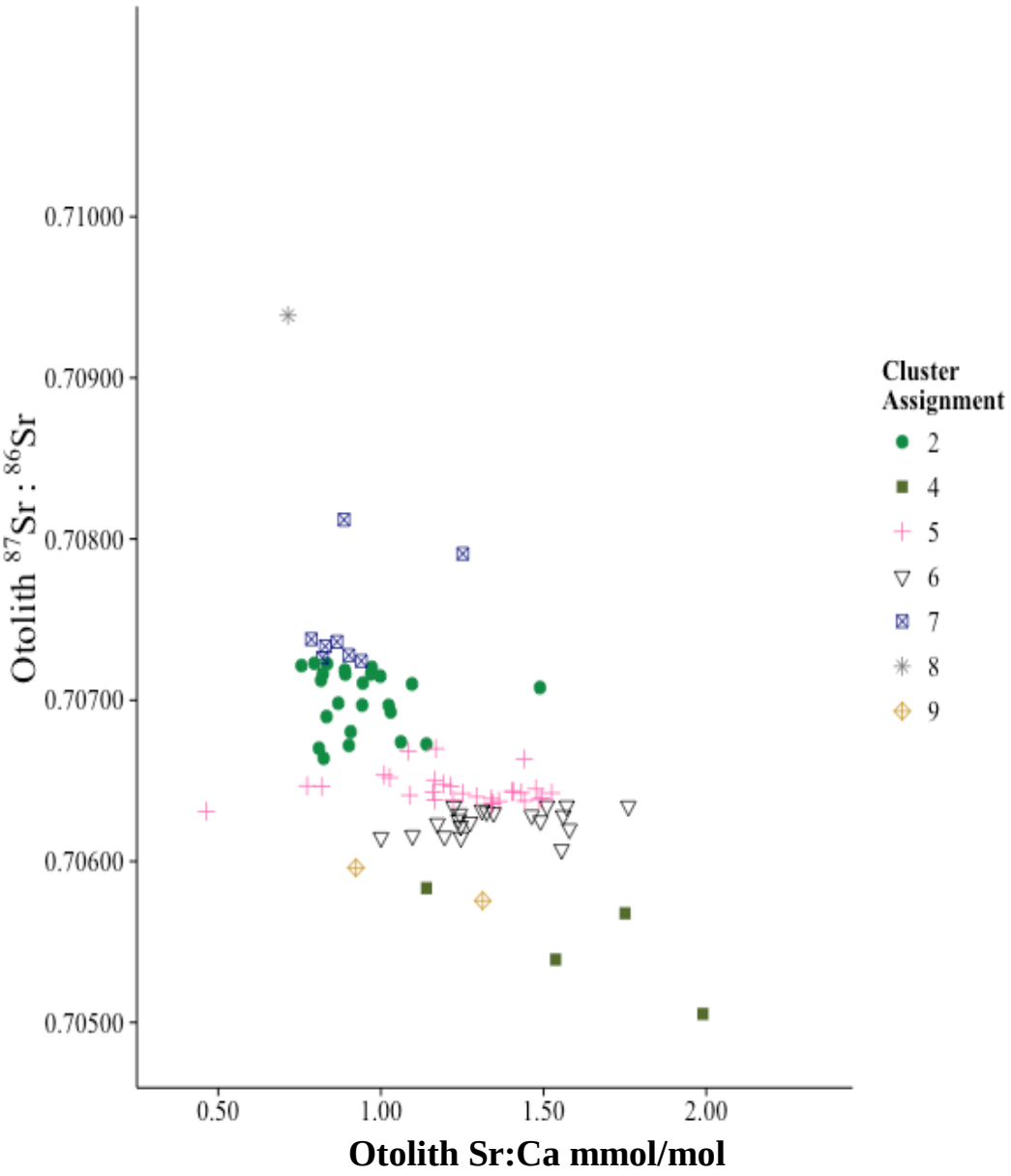

FIGURE 2.9. Cluster assignment of the historic cutthroat trout otolith signatures.

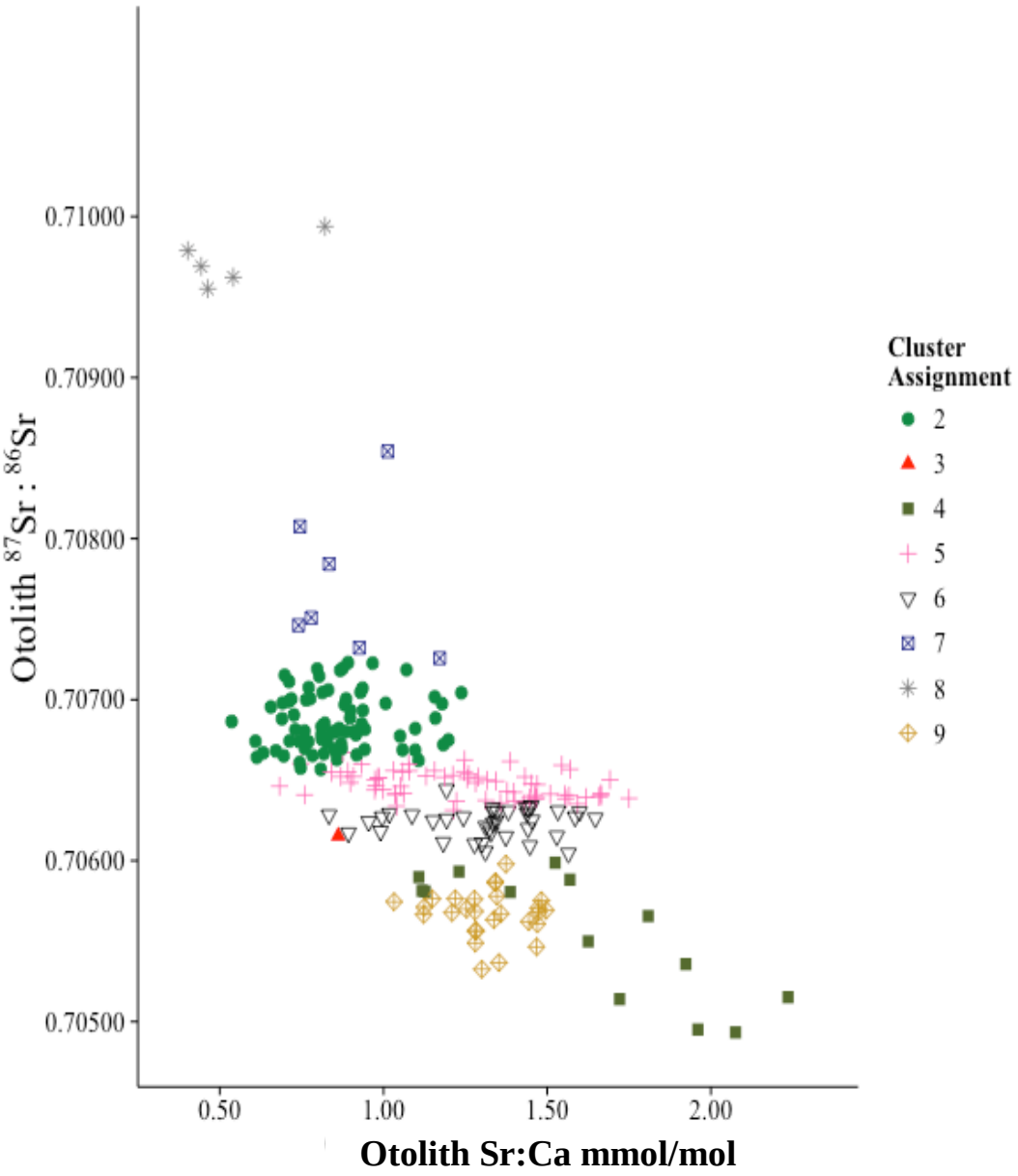

FIGURE 2.10. Cluster assignment of recent cutthroat trout otolith signatures.

References

Ball, O.P. 1955. Some aspects of homing in cutthroat trout. Proceedings of the Utah Academy of Science, Arts, and Letters 32:75-80.

Behnke, R.J. 2002. Trout and salmon of North America. The Free Press. New York.

Benson, N.G. 1961. Limnology of Yellowstone Lake in relation to the cutthroat trout. U.S. Fish and Wildlife Service. Research Report 56:1–38.

Bergum, D. J., K. A. Gunther, and L. M. Baril. In review. Impacts of Yellowstone cutthroat trout decline on mammals and birds in Yellowstone National Park.

Blair, J.M., and B.J. Hicks. 2012. Otolith microchemistry of koi carp in Waikato region, New Zealand: a tool for identifying recruitment locations? Inland Waters 2:109–118.

Breiman, L. 2001. Random forests. Machine Learning 45:5–32.

Breiman, L., A. Cutler, A. Liaw, and M. Wiener. 2014. Breiman and Cutler's random forests for classification and regression. R package version 4.6–10.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Campana, S.E. 2005. Otolith science entering the 21st century. Marine and freshwater Research 56:485–489.

Carlson, S.A. 2011. Identifying tributaries to Jackson Lake important for Snake River cutthroat trout recruitment. University of Wyoming National Park Service Research Center Annual Report 34: 5.

Ertel, B.D. 2011. Distribution, movements, and life-history characteristics of Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* in the Upper Yellowstone River drainage. Master's thesis. Montana State University, Bozeman, Montana.

Fowler, A.J., S.E. Campana, S.R. Thorrold, and C.M. Jones. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 52:1431–1441.

Garcez, R.C.S., R. Humston, D. Harbor, and C.E.C. Freitas. 2014. Otolith geochemistry in young-of-the-year peacock bass *Cichla temensis* for investigating natal

dispersal in the Rio Negro (Amazon - Brazil) River system. Ecology of Freshwater Fish 24:242-251.

Gresswell, R.E. 1994. Life-history organization of cutthroat trout in Yellowstone Lake and its management implications. Doctoral dissertation. Oregon State University, Corvalis, Oregon.

Gresswell, R.E. 2011. Biology, status, and management of the Yellowstone cutthroat trout. North American Journal of Fisheries Management 31:782–812.

Gunn, J.S., I.R. Harrowfield, C.H. Proctor, and R.E. Thresher. 1992. Electron probe microanalysis of fish otoliths—evaluation of techniques for studying age and stock discrimination. Journal of Experimental Marine Biology and Ecology 158:1–36.

Haroldson, M.A., K.A. Gunther, D.P. Reinhart, S.R. Podruzny, C.Cegelski, L.Waits, T. Wyman, and J. Smith. 2005. Changing numbers of spawning cutthroat trout in tributary streams of Yellowstone Lake and estimates of grizzly bears visiting streams from DNA. Ursus 16:167–180.

Hope, A.C.A. 1968. A simplified Monte-Carlo significance test procedure. Journal of the Royal Statistical Society 30:582–598.

Jackson, M., and S. Hart. 2006. Strontium isotopes in melt inclusions from Samoan basalts: implications for heterogeneity in the Samoan Plume. Earth and Planetary Science Letters 245:260–277.

Jones, R. D., R. E. Gresswell, D. E. Jennings, S. M. Rubrecht, and J. D. Varley. 1979. Fishery and aquatic management program in Yellowstone National Park. U.S. Fish and Wildlife Service, technical report 1978, Yellowstone National Park, Wyoming.

Jones, R.D., D.G. Carty, R.E. Gresswell, C.J. Hudson, L.D. Lentsch, and D.L. Mahony. 1986. Fishery and aquatic management program in Yellowstone National Park. U. S. Fish and Wildlife Service, Technical Report for 1985, Yellowstone National Park, Wyoming.

Kaeding, L.R., G.D. Boltz, and D.G. Carty. 1996. Lake trout discovered in Yellowstone Lake threaten native cutthroat trout. Fisheries 21:16-20.

Kaeding, L.R., and G.D. Boltz. 2001. Spatial and temporal relations between fluvial and allacustrine Yellowstone cutthroat trout, *Oncorhynchus clarki bouvieri*, spawning in the Yellowstone River, outlet stream of Yellowstone Lake. Environmental Biology of Fishes 61:395–406.

Kaeding, L.R., 2010. Relative contributions of climate variation, lake trout predation, and other factors to the decline of Yellowstone cutthroat trout during three recent decades. Doctoral Dissertation. Montana State University, Bozeman, Montana.

Kaplinski, M.A. 1991. Geomorphology and geology of Yellowstone Lake, Yellowstone National Park, Wyoming. Master's thesis. Northern Arizona University, Flagstaff, Arizona.

Kaufman, L., and P.J. Rousseeuw. 2005. Finding groups in data: An introduction to cluster analysis. Wiley and Sons Inc. Hoboken, New Jersey pp. 11.

Kelly, B.M. 1993. Ecology of Yellowstone cutthroat trout and an evaluation of potential effects of angler wading in the Yellowstone River. Master's thesis. Montana State University, Bozeman, Montana.

Kennedy, B.P., J.D. Blum, C.L. Folt, and K.H. Nislow. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Canadian Journal of Fisheries and Aquatic Sciences 57:2280–2292.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2004. Yellowstone Fisheries Aquatic Sciences. US National Park Service, Yellowstone Center for Resources Yellowstone National Park, Wyoming.

Koel, T.M., P.E. Bigelow, P.D. Doepke, B.D. Ertel, and D.L. Mahony. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30:10–19.

Koel, T.M., D.L. Mahony, K.L. Kinnan, C. Rasmussen, C.J. Hudson, S. Murcia, and B.L. Kerans. 2006. *Myxobolus Cerebralis* in native cutthroat trout of the Yellowstone Lake ecosystem. Journal of Aquatic Animal Health 18:157–175.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2008. Yellowstone Fisheries & Aquatic Sciences: annual report, 2007. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, YCR-2008-02.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2012. Yellowstone Fisheries & Aquatic Sciences: Annual report, 2009–2010. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, YCR-2011-11.

Koel, T.M., J.L. Arnold, P.E. Bigelow, C.R. Detjens, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2015. Native Fish Conservation Program, Yellowstone Fisheries & Aquatic Sciences: Annual Report 2012–2014. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, K. Hornik, M. Studer, and P. Roudier. 2015. cluster: cluster analysis extended. R package version 1.15–3.

Mercier, L., A.M. Darnaude, O. Bruguier, R.P. Vasconcelos, H.N. Cabral, M.J. Costa, M. Lara, D.L. Jones, D. Mouillot. 2011. Selecting statistical models and variable combinations for optimal classification using otolith microchemistry. Ecological Applications 21:1352–1364.

Miller, J.A., V.L. Butler, C.A. Simenstad, D.H. Backus, A.J.R. Kent, B. Gillanders. 2011. Life history variation in upper Columbia River Chinook salmon (*Oncorhynchus tshawytscha*): a comparison using modern and ~500-year-old archaeological otoliths. Canadian Journal of Fisheries and Aquatic Sciences 68:603-617.

Muhlfeld, C.C., S.R. Thorrold, T.E. McMahon, and B. Marotz. 2012. Estimating westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) movements in a river network using strontium isoscapes. Canadian Journal of Fisheries and Aquatic Sciences 69:906–915.

Munro, A.R, T.E. McMahon, and J.R. Ruzycki. 2005. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake. Canadian Journal of Fisheries and Aquatic Sciences 62:79–87.

Olley, R., R.G. Young, G.P. Closs, E.A Kristensen, T.O. Bickel, N.A.Deans, L.N. Davey, and S.M. Eggins. 2011. Recruitment sources of brown trout identified by otolith trace element signatures. Marine and Freshwater Research 45:395–411.

Pangle, K.L., S.A. Ludsin, and B.J. Fryer. 2010. Otolith microchemistry as a stock identification tool for freshwater fishes: testing its limits in Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 67:1475–1489.

Reinhart, D.P. 1990. Grizzly bear habitat use on cutthroat trout spawning streams in tributaries of Yellowstone Lake. Master's thesis. Montana State University, Bozeman, Montana.

Reinhart, D.P. and D.J. Mattson. 1990. Bear use of cutthroat trout spawning streams in Yellowstone National Park. In Bears: Their Biology and Management, Vol. 8, A selection of papers from the Eighth International Conference on Bear Research and Management, Victoria, British Columbia, Canada, February 1989. International Association for Bear Research and Management, New York, New York. 343–350.

Richmond, G.M. 1976. Surficial geological history of the Canyon Village quadrangle. Yellowstone National Park, Wyoming, for use with map 1-642. U.S. Geological Survey Bulletin 1427.

Schindler, D.E., R. Hilborn, B. Chasco, C.P. Boatright, T.P. Quinn, L.A. Rogers, and M.S. Webster. 2010. Population diversity and the portfolio effect in an exploited species. Nature 465:609–612.

Schullery, P. and Varley, J.D., 1995. Cutthroat trout and the Yellowstone Lake ecosystem. The Yellowstone Lake crisis: confronting a lake trout invasion. A Report to the Director of the National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, USA, pp.12-21.

Sousa, R.G.C., R. Humston, and C.E.C. Freitas. 2016. Movement patterns of adult peacock bass *Cichla temensis* between tributaries of the middle Negro River basin (Amazonas–Brazil): an otolith geochemical analysis. Fisheries Management and Ecology 23:76-87.

Strohm, D. 2015. Matching watershed and otolith chemistry to establish natal origin of an endangered desert lake sucker. Master's Thesis. Utah State University. Logan, Utah. United States Geological Survey. 1972. Geologic map of Yellowstone National Park. U.S. Geological Survey, Miscellaneous Geological Investigations Report 711.

Varley, J.D., and R.E. Gresswell. 1988. Ecology, status, and management of the Yellowstone cutthroat trout. American Fisheries Society Symposium 4:13–24.

Varley, J. D., and P.D. Schullery. 1998. Yellowstone fishes: ecology, history, and angling in the park. Stackpole Books, Mechanicsburg, Pennsylvania.

Walther, B.D., S.R. Thorrold, and J.E. Olney. 2008. Geochemical signatures in otoliths record natal origins of American shad. Transactions of the American Fisheries Society 137:57–69.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan, and C.M. Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132:409–424.

CHAPTER THREE

AN INVESTIGATION OF LAKE TROUT SPAWNING LOCATIONS AND MOVEMENT WITHIN YELLOWSTONE LAKE VIA OTOLITH MICROCHEMISTRY

Abstract

The introduction of lake trout to Yellowstone Lake has had detrimental effects on the ecosystem, including a large decline in the once-abundant Yellowstone cutthroat trout population. This study used otolith microchemistry to identify spawning locations and movement patterns of lake trout within Yellowstone Lake as a potential tool for optimizing lake trout removal efforts. The microchemical signatures of 20 adult lake trout otoliths and water chemistry from 8 locations throughout Yellowstone Lake were analyzed using inductively coupled plasma mass spectrometry. Strontium isotopic (^{87}Sr:^{86}Sr) and Sr:Ca ratios were used to assess lake trout natal origins and ^{87}Sr:^{86}Sr ratios in otolith transects were used to assess lake trout movement patterns. The water chemistry throughout Yellowstone Lake was highly similar, 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. In turn, lake trout otoliths showed very little variation in both ^{87}Sr:^{86}Sr and Sr:Ca ratios of the natal region and ^{87}Sr:^{86}Sr signatures across otolith transects representing the entire life of lake trout inhabiting the lake (3-6 years old). All the natal signatures as well as the transect data were within the measurement error (± 0.00031 and ± 0.46 for ^{87}Sr:^{86}Sr and Sr:Ca ratios respectively) of the equipment used to obtain the microchemical signatures,

further indicating that variation in otolith microchemical signatures was very small throughout the life of the lake trout. Yellowstone Lake water chemistry was very uniform throughout this large lake system, and the corresponding low variation in lake trout otolith microchemistry suggested that lake trout spawn and rear their entire lives in Yellowstone Lake and do not venture into tributaries during their life span. Study results illustrate the strengths and limitations of using otolith microchemistry analysis in freshwater lentic systems for assessing natal origins and movement.

Introduction

Lake trout (*Salvalinus namaycush*) are an apex predator (Post et al. 2000) native to northern North America. In some portions of their range lake trout inhabit cold rivers and shallow lakes, however in the southern portion of their range they are found almost exclusively in deep, cold lakes (Scott and Crossman 1973). This species has been intentionally introduced in many lakes and reservoirs in the western United States with the intent of providing additional angling opportunities (Martinez et al. 2009). The introduction of lake trout to new lakes can cause native and nonnative fishes to decline (Martinez et al. 2009). Nonnative lake trout were first discovered in Yellowstone Lake in 1994 (Kaeding et al. 1996), and subsequent otolith microchemistry research indicated they were likely introduced in the late 1980's (Munro et al. 2005). The presence of this highly predacious species in the Yellowstone Lake ecosystem has devastated the once abundant Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) population (Ruzycki et al. 2003; Koel et al. 2005). This introduction has been particularly troubling

due to Yellowstone Lake being one of the last strong holds for genetically pure Yellowstone cutthroat trout (Varley and Gresswell 1988), and the importance of this cutthroat trout population within the ecosystem (Koel et al. 2005).

The National Park Service (NPS) has been conducting an intensive large-scale lake trout removal effort since 1995 using gillnetting and trap netting (Koel et al. 2015). In recent years, the program has been identifying new and novel methods for locating lake trout spawning locations in order to target removals of spawning fish as well as reductions of eggs and fry (Koel et al. 2015). Yellowstone Lake is very large high elevation (2356 m.) lake with a surface area of 341 km^2 and maximum depth of 107 m (Kaplinski 1991) making it difficult to discern lake trout movement and spawning patterns. A greater knowledge of lake trout spawning locations and movement patterns within Yellowstone Lake would therefore be valuable to resource managers for directing future removal efforts.

The use of otolith microchemistry techniques has proven to be a powerful method for identifying fish movement patterns, habitat usage, and natal origins (locations in which the fishes were spawned) (e.g. Muhlfeld et al. 2012; Miller et al. 201; Garcez et al. 2014). Otolith microchemistry relies on the unique time and environmental recording properties of fish otoliths (Campana 1999). Otoliths are paired metabolically inert bone structures found in a fish's inner ear, these structures form and grow continually throughout the fish's life (Campana 1999). Being metabolically inert indicates that after material is accreted onto the otolith-growing surface it is never reabsorbed, regardless of the physical and/or environmental stresses. During accumulative otolith growth, fish

incorporate the microchemical signatures of their ambient environment into their otolith, creating a record of their past environmental history (Campana 1999).

In the past two decades, the use of otolith microchemistry techniques has been used extensively to reconstruct environmental histories of anadromous, catadromous, and marine fishes (e.g., Gillanders and Kingsford 1996; Ingram and Weber 1999; Zimmerman and Reeves 2002; Ohji et al. 2006). Many recent studies have focused on fishes that occupy freshwater lotic systems during some stage of their lifecycle (e.g., Clarke et al. 2007; Muhlfeld et. al. 2012; Amano et al. 2013). To date, few studies have explored the effectiveness of using otolith microchemistry to identify movement patterns and natal origins of fish that reside wholly in freshwater lentic systems such as lake trout in Yellowstone Lake. Munro et al. (2005) sought to identify the time and source of the introduced lake trout in Yellowstone Lake. This study was able to identify the likely time and source (Lewis Lake, Yellowstone National Park) from which lake trout originated, however this study did not assess the efficacy of using microchemistry to identify specific spawning areas of lake trout or movement patterns with the lake. In our review of the literature, only one study was found that demonstrated that there was potential for using otolith microchemistry to delineate stock differences in fish spawning and rearing in different parts of a large lake (Dufour et al. 2005).

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 could result in homogenous microchemical signatures throughout a water body. Yellowstone Lake is a unique lake in terms of overall topography, geology and

geothermal influences. Yellowstone Lake has a north south oriented main basin and six distinct sub-basins (Kaplinski 1991). Given the large size of Yellowstone Lake, if there is sufficient geologic variation within the lake's sub-basins, then water samples collected from these sub-basins may exhibit unique microchemical signatures. It has been previously established that Yellowstone Lake has a unique Sr:Ca signature as compared to Lewis Lake, Heart Lake, and Shoshone Lake (Munro et al 2005), all of which are in close proximity to Yellowstone Lake; this may indicate that the underlying geology of Yellowstone Lake sub-basins could in effect cause a diversity of microchemical signatures throughout the lake. Additionally the geothermal processes that occur within and adjacent to Yellowstone Lake may influence the chemical composition of Yellowstone Lake and result in regions with unique microchemical signatures. Lastly, utilizing a suite of elemental and isotopic ratios (^{87}Sr:^{86}Sr, and Sr:Ca) that have proven useful in many recent studies may provide additional discriminatory power necessary to differentiate sub-basins of Yellowstone Lake. As a very large lake with unique thermal influences, and diverse underlying geology Yellowstone Lake provides an excellent template to determine if microchemistry techniques can be applied to lakes.

The primary objectives of this study were to use otolith microchemistry to: 1) identify the primary spawning locations contributing to lake trout recruitment in Yellowstone Lake, and 2) identify patterns in lake trout movement within the subbasins of Yellowstone Lake. Information gained from this study would be informative to local managers for directing lake trout removal efforts to regions of the lake where lake trout are spawned and areas they are concentrated.

Methods

Water Collection and Analysis

Lake water samples were collected from 8 locations within Yellowstone Lake: northwest main basin, northeast main basin, southwest main basin, southeast main basin, Southeast Arm, South Arm, West Thumb, and near West Thumb Geyser Basin (Figure 3.1). The sample collected near West Thumb Geyser Basin was used to assess whether geothermal inputs influence the localized water microchemistry.

Water samples from Yellowstone Lake were collected immediately after the lake was ice free on May 22 and 23, 2013. This timing was selected because it is prior to thermal stratification, and to reduce the effects of lake mixing due to wind. This timing also allowed for water collection as close as possible to the timing of lake trout fry emergence, as noted by Marsden et al. (2005). 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 locations with water depths less than 10 meters, and whenever possible near areas lake trout are known to spawn within Yellowstone Lake (information provided by NPS fisheries personnel). Each sample was collected approximately 0.5 meters above the substrate to avoid contaminating the water samples with lake substrate. Prior to sampling, the Van Dorn water sampler was acid washed with 12N hydrochloric acid diluted to 6N with Milli-Q water, and triple rinsed with Milli-Q water prior to collecting any water samples. The Van Dorn sampler was not acid washed between lake samples, however it was triple rinsed using Milli-Q water, followed by a triple rinse with the sample location water prior

to collection to minimize risk of sample cross contamination. Each water sample was filtered using 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).

Water samples were processed at 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 redissolved in 50% HNO_3 then eluted through a Sr-specific cation exchange resin. The remaining sample was again evaporated and redissolved 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 and Hart (2006). Lastly, all Sr isotope ratios were normalized to the NIST SRM987 standard. For elemental ratios, samples were first diluted 10-fold using a 2% HNO_3 solution prior to ICPMS measurement of ^{48}Ca, and ^{88}Sr. Liquid standards and instrument blanks of 2% HNO3 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) of Sr:Ca ratios for the laboratory standard (n = 3) was 1.5%.

Otolith Collection and Preparation

Since 2010 the NPS has conducted an annual gill netting assessment during the first 10 days of August, to monitor cutthroat trout and lake trout populations (Syslo et al.

2011). This assessment consists of 2 multi-panel gillnets of two sizes large (57 to 95 mm) and small (19 to 51 mm) mesh set in parallel at each site perpendicular to the shore in three depth strata: shallow near shore (above the thermocline), mid-depth (across the thermocline), and deep (below the thermocline) (personal communication with NPS fisheries biologist Jeff Arnold). The thermocline was identified prior to sampling using a Hydrolab multi-probe sonde (Koel et al 2015). All lake trout netted during this assessment were collected and returned to the Yellowstone fisheries lab for further processing, including otolith extraction.

Twenty of the largest lake trout (> 400 mm) collected in 2013 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. Only otoliths from large fish were selected because these fish are more likely to have occupied more habitats throughout the lake than younger fish, and thus may exhibit different microchemical signatures in their otoliths. The otoliths were removed from the fish with non-metallic forceps, minimizing the risk of metal contamination. Otoliths were rinsed with Milli-Q water to remove any remaining tissue, and then stored in clean microcentrifuge vials prior to further preparation.

One sagittal otolith from each fish was randomly selected for laser ablation. Otoliths were prepared similar to the methods described by Muhlfeld et al. (2012). Each otolith was scrubbed with a nylon brush and triple rinsed to remove any foreign material or remaining tissue, then dried under a laminar flow hood for twenty-four hours. The otoliths were then mounted on petrographic slides, sulcus side up, using cyanoacrylate

glue. Once the glue hardened the otoliths were sanded to approximately 40-50 microns above the plane of the nucleus similar to Garcez et al. (2014). The otoliths were sanded first using 600- and 1500-grit sandpaper then polished using 0.5- and 0.1-μm diamond lapping film. Once the sanding and polishing process was complete, the otoliths were once again scrubbed with a nylon brush and triple rinsed, soaked in Milli-Q water overnight and remounted on a new slide.

Otolith microchemical analysis was performed using laser ablation ICPMS equipped with a 213-mm laser. Otolith $^{87}Sr:^{86}Sr$, and Sr:Ca ratios were obtained using a Thermo Finnigan Neptune multiple collector ICPMS. Otolith laser ablation transects were from the otolith core to the edge using a beam diameter of 75 μm, a repetition rate 20 Hz, and a scan speed 5 $\mu m \cdot s^{-1}$. 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. The mean $^{87}Sr:^{86}Sr$ ratio (± 1 SD) for the certified reference material run throughout the analysis was 0.70765 ± 0.00022, which is within 1 standard deviation of the accepted value of MACS-3 (0.70759). External precision (relative SDs) for Sr:Ca ratios based on repeated measurements of a certified reference material (Sturgeon et al. 2005) was between 1.0 and 2.0%. All results were normalized using a standardized reference material described by Jackson and Hart (2006).

Data Analysis

In order to determine distinct natal origins, the variation in the microchemical signatures of the water and otoliths must be greater than the measurement error

associated with the LA-ICPMS method. Measurement error (2 standard deviations) was calculated using the LA-ICPMS results from MACS-3 standard reference material run prior to starting and after every five otolith samples throughout the analysis. Measurement error was calculated for both $^{87}Sr:^{86}Sr$ and Sr:Ca ratios. To quantify the microchemical signatures found in natal region of the otolith, the mean of the first five readings from each laser ablation transect were selected to represent the natal region of the otolith, the area of the otolith associated with the immediate post-hatching life stages of the fish. The measurement error values calculated for both $^{87}Sr:^{86}Sr$ and Sr:Ca ratios were then used to examine differences in water and the calculated mean otolith values. Water samples and otolith signatures with overlapping measurement error were considered indistinguishable from one another

Movement among lake subbasins was assessed by comparing Sr isotope transects measured across the otolith axis from the core to the edge. Strontium isotopic ratios were used to assess movement patterns because this ratio is minimally affected by physical and biological factors that have been shown to alter the incorporation of other elemental ratios throughout the life a fish (Kennedy et al. 2000). Variation in Sr isotope ratio along each transect was estimated in relation to the measurement error (±2 standard deviations) similar to the water and otolith core comparisons described above. In the case of otolith transects, measurement error was calculated from all of MACS-3 standard reference material results pooled together as opposed to an overall mean value for each standard run as was the case when identifying natal origins. Using all of the standard reference material results pooled together is important when identifying movement patterns

because each point on a transect is evaluated separately when seeking to identify fish movement patterns. The measurement error was then used to evaluate whether any of the lake trout transect values exhibited signatures that were not likely to be attributed to instrument error alone but rather movement between chemically distinct lake sampling sites.

Results

There was little variation in Sr isotopic and elemental ratios among water samples or lake trout otoliths from Yellowstone Lake. Lake water Sr isotope ratios ranged from 0.70634 to 0.70642 and Sr:Ca ratios ranged from 3.94 to 4.38 mmol/mol(Table 3.1). 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 (Table 3.2). All water and otolith natal signature measurements were within the measurement error range for Sr isotopes (0.00031) and Sr:Ca ratios (± 0.46). The variation among water samples and natal otolith signatures were within the measurement error of the LA-ICPMS method, and thus regions of Yellowstone Lake water chemistry were highly similar as were the natal signatures of lake trout otoliths (Figure 3.2 and 3.3).

Otolith transect measurements showed a similar lack of variation across all lake trout otoliths. The measurement error (±2 standard deviations) for the otolith transect data as calculated from the pooled MACS-3 standard results was ±0.00054. All otolith transect values were within the measurement error of the machine indicating that

variation in otolith signatures and lake chemistry is not large enough to identify lake trout movement patterns throughout Yellowstone Lake (Figure 3.4).

Discussion

This study was initiated in an attempt to identify the natal origins and movement patterns of lake trout in Yellowstone Lake in order to provide the NPS with valuable information for future lake trout removal efforts. It appears that this may be one of the first studies that sought to use otolith microchemistry to identify the natal origins and movement patterns of fish that reside wholly within a lake throughout their entire lives. Studies by Dufour et al. (2005) and Pangle et al. (2010) also attempted to identify the natal origins of two fish species inhabiting the Great Lakes that spawned within the lakes as well as inundated river mouths and lakeside marshes. These studies were successful in identifying fish natal origins when fish originated from sites located in inundated river mouths and lakeside marshes; however, as in our study, they were unable to identify fish originating from separate natal origins within the lake. While the results of Dufour et al. (2005) and Pangle et al. (2010) seem to suggest that studies seeking to identify natal origins and movement among lake regions may be difficult and preclude the initiation of similar studies, both of these studies occurred in the Great Lakes, which may not be representative of the microchemical characteristics of all lakes throughout North America.

Prior qualitative inspection of Yellowstone Lake suggested the Yellowstone Lake may be an ideal lake study system for using otolith microchemistry techniques to inform

lake trout management. Chittaro and Hogan (2012) demonstrated that the natal origins of marine reef fish could be identified from locations separated by as little as 11 km. Chittaro and Hogan (2012) also suggest that study areas with long water residency times, high terrigenous sediment inputs, and those with high anthropogenic pollutant inputs are easier to distinguish based on their water chemistry. These factors suggested that Yellowstone Lake may be an ideal study system due to its large embayments, thermal influences (a potential surrogate to anthropogenic pollutants), and multiple stream inputs. A previous otolith microchemistry study by Munro et al. (2005) indicated that water Sr:Ca ratios throughout Yellowstone Lake may be homogenous and stable over time, and thus transplanted lake trout could be identified by marked changes in the Sr:Ca ratios found in lake trout otoliths. However, there are two important differences between the study by Munro et al. (2005) and this study. First, we utilized more precise techniques for microchemical analysis, LA-ICPMS rather than ToF-SIMS (Time of Flight-Secondary Ion Mass Spectrometry), thus allowing for the analysis of water chemistry at the parts per billion level rather than the parts per million level (Jones and Chen 2003). Secondly, we used Sr isotopic signatures in water and otoliths which are less affected by environmental and physiological variables (Kennedy et al. 2000) as opposed to using Sr:Ca ratios alone to identify natal origins and movement. However, our more precise measurement techniques also showed a lack of variation in otolith microchemistry in lake trout throughout their life history, providing further confirmation that lake trout spawn and rear entirely with Yellowstone Lake.

There was a lack of variation in both Yellowstone Lake water chemistry, and otolith microchemical signatures, indicating that identification of natal origins and movement patterns of lake trout would not be possible. Amano et al. (2013) found that water from different natal streams were distinguishable when water Sr isotopic and elemental ratios differed by approximately 0.0012 (unit less ratio) and 1.0 mmol/mol respectively. Studies that have identified fish movement patterns have noted discernible changes in Sr isotopic signatures of fish otoliths coincident with their movement to a chemically distinct waterbody (Kennedy et al. 2002; Gibson-Reinemer et al. 2009; Muhlfeld et al. 2012). For example, Gibson-Reinemer et al. (2009) noted a marked change in otolith Sr isotopic signatures from 0.7112 to 0.7170, a 0.0058 difference, coincident with fish being moved from one hatchery to another. In contrast to these studies, water Sr isotopic and Sr:Ca ratios throughout Yellowstone Lake only ranged from 0.7063 to 0.7064, and 3.94 to 4.38 mmol/mol respectively, a 0.0001 and 0.44 mmol/mol difference. This study showed similar results as Munro et al. (2005), water chemistry was homogenous throughout Yellowstone Lake, and consequently otoliths from lake trout that had spent their entire lives within the lake showed homogenous microchemical signatures despite extensive movement of lake trout throughout Yellowstone Lake documented by Sandstrom et al. (2014).

Though the primary objectives of this study were not achieved there is still valuable information that can be gained. Partition coefficients that facilitate the comparison of lake trout otolith and water Sr:Ca signatures can be calculated that may be useful for other researchers. Because the water was chemically homogenous throughout

the lake, the lake trout can be assumed as all having the same natal origin (Yellowstone Lake), and thus a partition coefficient ($D_{Sr:Ca}$) can be calculated using the methods described by Muhlfeld et al. (2012) with the following equation:

$$D_{Sr:Ca} = (Sr:Ca)_{otolith} / (Sr:Ca)_{water}$$

Previously the only Sr:Ca partition coefficient available for lake trout was calculated by Munro et al. (2005) as 0.24, however this partition coefficient was specific to $^{88}Sr:^{43}Ca$, while many other studies use the $^{88}Sr:^{48}Ca$ ratio (e.g. Muhlfeld et al. 2012), thus a $^{88}Sr:^{48}Ca$ specific partition coefficient for this ratio was calculated as 0.29 using data from this study.

While some may suggest that it is unnecessary to conduct further research using similar techniques in other large lakes, it may be that the specific suite of isotopic and elemental ratios being used is inadequate. In the future researchers may discover a new suite of microchemical ratios or otolith characteristics that are more conducive to the study of fish natal origins and movement patterns throughout large lakes. It may also be possible that further refinement of otolith microchemistry techniques may provide the resolution necessary to discriminate among different regions within a lake.

Tables

Table 3.1 Lake water $^{87}Sr:^{86}Sr$ and Sr:Ca (mmol/mol) signatures.

Sample	$^{87}Sr:^{86}Sr$	Sr:Ca
1	0.70634	3.94
2	0.70634	4.08
3	0.70634	4.37
4	0.70642	4.20
5	0.70638	3.97
6	0.70642	4.35
7	0.70636	4.38
8	0.70636	4.38

Table 3.2. Otolith natal $^{87}Sr:^{86}Sr$ and Sr:Ca (mmol/mol) signatures.

Otolith	$^{87}Sr:^{86}Sr$	Sr:Ca
1	0.70647	1.22
2	0.70636	1.28
3	0.70634	1.22
4	0.70647	1.18
5	0.70629	1.21
6	0.70631	1.21
7	0.70642	1.22
8	0.70647	1.15
9	0.70643	1.28
10	0.70629	1.20
11	0.70624	1.16
12	0.70633	1.11
13	0.70632	1.18
14	0.70646	1.47
15	0.70637	1.24
16	0.70615	1.57
17	0.70637	1.22
18	0.70644	1.45
19	0.70623	1.14
20	0.70628	1.18

Figures

Figure 3.1. Yellowstone Lake water-sampling locations.

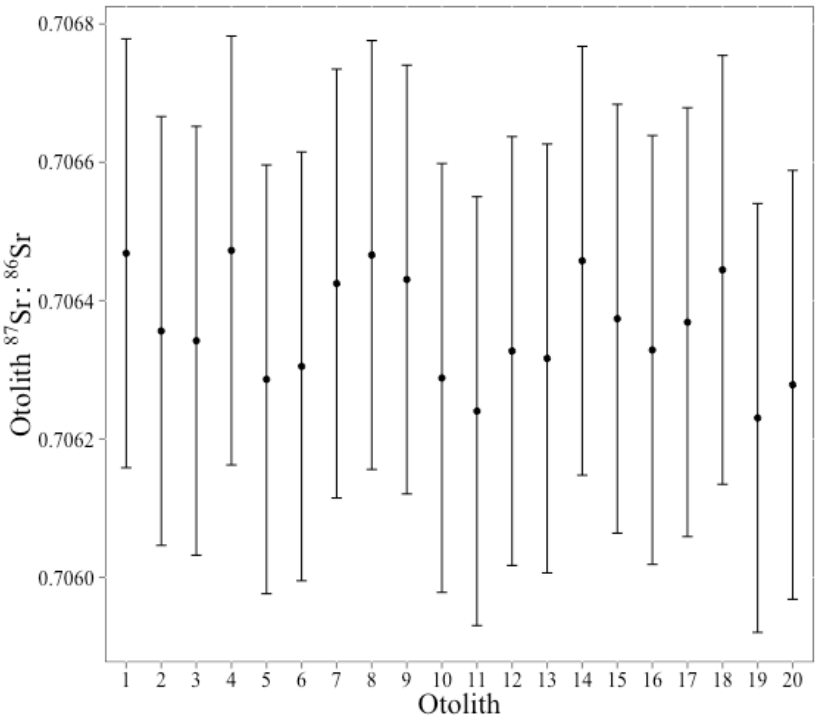

Figure 3.2. Otolith $^{87}Sr:^{86}Sr$ signatures from the natal region of each of the 20 study otoliths. Error bars represent measurement error (2 standard deviations = ±0.00031). The mean water Sr isotopic signature from all 8 lake water samples was 0.70637; each of the otolith signatures is within the measurement error for the mean water $^{87}Sr:^{86}Sr$ value (0.70637).

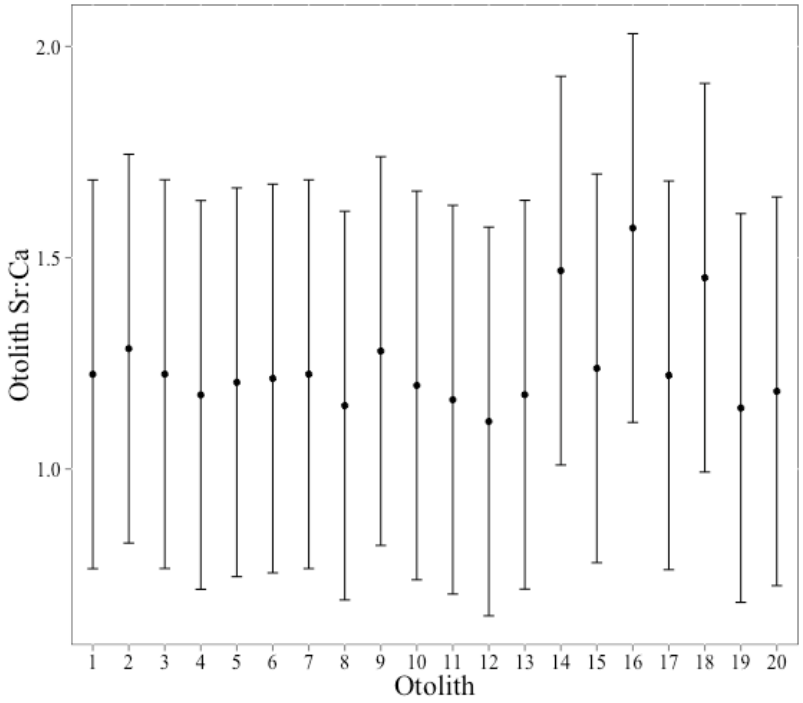

Figure 3.3. Otolith Sr:Ca signatures from the natal region of each of the 20 study otoliths. The error bars represent measurement error (2 standard deviations = ±0.46). The mean water Sr:Ca signature was 1.22 after adjustment with the partition coefficient (0.29; see Discussion); while some otolith Sr:Ca ratios may appear distinct each of the otolith signatures is within the measurement error for the mean water Sr:Ca value (4.20 mmol/mol).

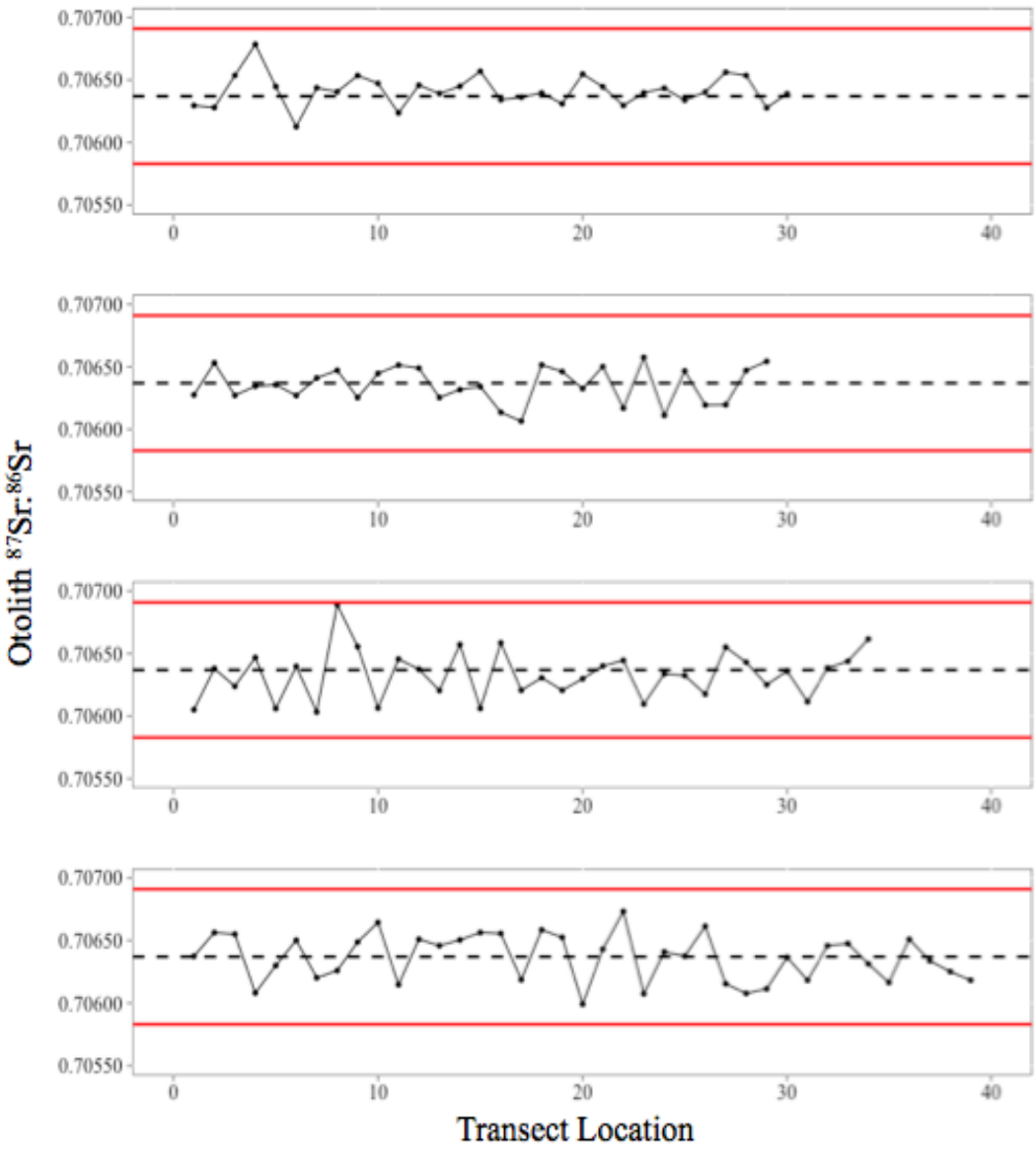

Figure 3.4. Otolith $^{87}Sr:^{86}Sr$ signatures from the otolith core to the outer edge of the otolith. The x axis (Transect Location) represents the location on the otolith starting at the core at 0, and ending at the edge. The dashed black line represents the mean lake water $^{87}Sr:^{86}Sr$ signature (0.70637), and the red lines represent the measurement error (2 standard deviations = ±0.00054). These four otolith transects were randomly chosen to illustrate the lack of variation found in $^{87}Sr:^{86}Sr$ signatures from the otolith core to edge, these otolith are representative of the variation found among other otolith transects.

References

Amano, Y., M. Kuwahara, T. Takahashi, K. Shirai, K. Yamane, H. Amakawa, and T. Otake. 2013. Otolith elemental and Sr isotopic composition as a natal tag for Biwa salmon *Oncorhynchus masou* Subsp. in Lake Biwa, Japan. Aquatic Biology 19:85–95.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Chittaro, P.M., and J.D. Hogan. Patterns of connectivity among populations of a coral reef fish. Coral Reefs 32:341–354.

Clarke, A.D., K.H. Telmer, and J.M. Shrimpton. 2007. Habitat use and movement patterns for a fluvial species, the Arctic grayling, in a watershed impacted by a large reservoir: Evidence from otolith microchemistry. Journal of Applied Ecology 44:1156–1165.

Dufour, E., W.P. Patterson, T.O. Höök, and E.S. Rutherford. 2005. Early life history of Lake Michigan alewives (*Alosa pseudoharengus*) inferred from intra-otolith stable isotope ratios. Canadian Journal of Fisheries and Aquatic Sciences 62:2362–2370.

Garcez, R.C.S., R. Humston, D. Harbor, and C.E.C. Freitas. 2014. Otolith geochemistry in young-of-the-year peacock bass *Cichla temensis* for investigating natal dispersal in the Rio Negro (Amazon - Brazil) River system. Ecology of Freshwater Fish 24:242–251.

Gibson-Reinemer, D.K., B.M. Johnson, P.J. Martinez, D.L. Winkelman, A.E. Koenig, and J.D. Woodhead. 2009. Elemental signatures in otoliths of hatchery reared rainbow trout (*Oncorhynchus mykiss*): distinctiveness and utility for detecting origins and movement. Canadian Journal of Fisheries and Aquatic Science 66:513–524.

Gillanders, B.M., and M.J. Kingsford. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Marine Ecology Progress Series 141:13–20.

Ingram, B.L., and P.K. Weber. 1999. Salmon origin in California's Sacramento–San Joaquin River system as determined by otolith strontium isotopic composition. Geology 27:851–854

Jackson, M., and S. Hart. 2006. Strontium isotopes in melt inclusions from Samoan basalts: implications for heterogeneity in the Samoan Plume. Earth and Planetary Science Letters 245:260–277.

Jones, C.M., Z. Chen. 2003. New techniques for sampling larval and juvenile fish otoliths for trace-element analysis with laser-ablation sector-field inductivey-coupled-plasma mass spectrometry (SF-ICP-MS). The Big fish Bang: Proceedings of the 26th Annual Larval Fish Conference. The Norwegian Institute of Marine Research, University of Bergen. Bergen.

Kaeding, L.R., G.D. Boltz, and D.G. Carty. 1996. Lake trout discovered in Yellowstone Lake threaten native cutthroat trout. Fisheries 21:16–20.

Kaplinski, M. A. 1991. Geomorphology and geology of Yellowstone Lake, Yellowstone National Park, Wyoming. Master's Thesis. Northern Arizona University. Flagstaff, Arizona.

Kennedy, B.P., J.D. Blum, C.L. Folt, and K.H. Nislow. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Canadian Journal of Fisheries and Aquatic Sciences 57:2280–2292.

Kennedy, B.P., C.L. Folt, and K.H. Nislow. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Canadian Journal of Fisheries and Aquatic Sciences 59:925–929

Koel, T.M., P.E. Bigelow, P.D. Doepke, B.D. Ertel, and D.L. Mahony. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30:10–19.

Koel, T.M., J.L. Arnold, P.E. Bigelow, C.R. Detjens, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2015. Native Fish Conservation Program, Yellowstone Fisheries & Aquatic Sciences 2012-2014, Yellowstone National Park. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Marsden, E.J., B.J. Ellrott, R.M. Claramunt, J.L. Jonas, and J.D. Fitzsimons. 2005. A comparison of lake trout spawning, fry emergence, and habitat use in lakes Michigan, Huron, and Champlain. Journal of Great Lakes Research 31:492–508.

Martinez, P.J., P.E. Bigelow, M.A. Deleray, W.A. Fredenberg, B.S. Hansen, N.J. Horner, S.K. Lehr, R.W. Schneidervin, S.A. Tolentino, and A.E. Viola. 2009. Western lake trout woes. Fisheries 34:424–442.

Muhlfeld, C.C., S.R. Thorrold, T.E. McMahon, and B. Marotz. 2012. Estimating westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) movements in a river network using strontium isoscapes. Canadian Journal of Fisheries and Aquatic Sciences 69:906–915.

Munro, A.R., T.E. McMahon, and J.R. Ruzycki. 2005. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake. Canadian Journal of Fisheries and Aquatic Sciences 62:79–87.

Ohji, M., Harino, H., and Arai, T. 2006. Differences in organotin accumulation among ecological migratory types of the Japanese eel *Anguilla japonica*. Estuary Coastal and Shelf Science 69:270–290.

Pangle, K.L., S.A. Ludsin, and B.J. Fryer. 2010. Otolith microchemistry as a stock identification tool for freshwater fishes: testing its limits in Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 67:1475–1489.

Post, D.M., M.L. Pace, and N.G. Hairston. 2000. Ecosystem size determines food-chain length in lakes. Nature (London) 405:1047–1049.

Ruzycki, J.R., D.A. Beauchamp, and D.L. Yule. 2003. Effects of introduced lake trout on native cutthroat trout in Yellowstone Lake. Ecological Applications 13: 23–37.

Sandstrom, P.T., R.E. Gresswell, B. Farokhkish, and M.J. Parsley. 2014. Identifying movement patterns and spawning patterns of lake trout (*Salvalinus namaycush*) in Yellowstone Lake. Investigators Annual Report for 2013. Montana Institute on Ecosystems, Bozeman, Montana

Scott, W.B. and E.J Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada, Bulletin 184:966.

Sturgeon, R.E., S.N. Willie, L. Yang, R. Greenberg, R.O. Spatz, Z. Chen, C. Scriver, V. Clancy, J.W. Lam, and S. Thorrold. 2005. Certification of a fish otolith reference material in support of quality assurance for trace element analysis. Journal of Analytical Atomic Spectrometry 20:1067–1071.

Syslo, J. M., C.S. Guy, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and T.M. Koel. 201. Response of non-native lake trout (*Salvalinus namaycush*) to 15 years of harvest in Yellowstone Lake, Yellowstone National Park. Canadian Journal of Fisheries and Aquatic Sciences 68: 2132–2145.

Varley, J. D., and R. E. Gresswell. 1988. Ecology, status, and management of the Yellowstone cutthroat trout. In American Fisheries Society Symposium 4:13–24.

Zimmerman, C. E., and Reeves, G. H. 2002. Identification of steelhead and resident rainbow trout progeny in the Deschutes River, Oregon, revealed with otolith microchemistry. Transactions of the American Fisheries Society 131:986–993.

CHAPTER FOUR

CONCLUSION

The purpose of this study was to identify the primary spawning streams contributing to the Yellowstone Lake population of Yellowstone cutthroat trout both historically and in recent years (Chapter 2) as well as to identify the natal origins and movement patterns of lake trout within Yellowstone Lake (Chapter 3). This study used otolith microchemistry techniques in an effort to accomplish these objectives by matching otolith and water microchemical signatures.

For the portion of the study dedicated to identifying both historical (1997 collection) and recent (2013 collection) cutthroat trout natal origins, water microchemistry throughout the drainage did not exhibit enough variation to clearly separate all spawning streams based on their microchemical signatures, thus streams were grouped or "clustered" together based on their similarities in their microchemical signatures. There were 9 clusters of streams with the largest cluster containing 7 streams and the smallest cluster containing a single stream.

Simulated otolith data was used to create a Random Forest classification model based on the observed variation in microchemical signatures from known-origin fish collected in 7 spawning streams. This approach saved both time and funds, by eliminating the time and effort necessary to sample fish in one of the most remote locations in the contiguous United States, and the analytical time necessary to analyze the otolith microchemical signatures from every spawning stream included in this analysis.

The classification of known-origin fish using the constructed model provided confidence that the random forest model was classifying fish to the correct cluster of streams based on their microchemical signatures The classification accuracy of the random forest model was 79.0% and 84.4% for simulated fish otolith data and known-origin fish otolith data respectively.

All historic and recent otolith were assigned to a stream cluster and differences among the historic and recent otolith collections were found. The majority of unknown-origin cutthroat trout from both the historical and recent collections were assigned to clusters 2 (Beaverdam Creek, Cabin Creek, Chipmunk Creek, Little Thumb Creek, Solution Creek, Unnamed Creek (SONYEW 1138), and Upper Yellowstone River), 5 (Columbine Creek, Lower Yellowstone River, and Sewer Creek), and 6 (Cub Creek, and Pelican Creek) (Table 2.4; Figures 2.9 and 2.10). The results of the fish classifications were not surprising given that each of these clusters contained a relatively large spawning stream. For example, cluster 2 contained the Upper Yellowstone River, cluster 5 contained the Lower Yellowstone River, and Cluster 6 contained Pelican Creek; the second largest tributary to Yellowstone Lake (Varley and Gresswell 1988; Gresswell et al. 1994). Between the historic and recent otolith collections there was a 0.06 increase in fish originating from cluster 2, a 0.08 decrease in fish originating from cluster 6, and a 0.09 increase in fish originating from cluster 9 (Table 2.4).

Changes in spawning stream contributions over time may be a consequence of drought, lake trout and whirling disease, or potentially the result of natural variation and normal shifts in the ecosystem over time. The decrease in fish originating from cluster 6

in particular seems to be likely as a result of whirling disease, given that whirling disease is most prevalent in Pelican Creek a major cutthroat trout spawning stream (Koel et al. 2006; Koel et al. 2015) The increase in the proportion of cutthroat trout originating from clusters 2 and 9 could be due to the reduced effects of lake trout predation on cutthroat trout originating from these streams, some of these streams are located much further from the lake than the majority of other spawning streams (e.g. Mountain Creek, Thorofare Creek, and the Upper Yellowstone River), thereby removing these fish from the effects of lake trout predation during their earliest and most vulnerable life stages. Alternatively, the proportional increase may be an artifact of decreasing numbers in other clusters. Study results point to the complexities of estimating recruitment in a large system and suggest further research is warranted.

This study has clearly identified groups of spawning streams that are major contributors to the cutthroat trout population in Yellowstone Lake, this information will be useful for guiding future management, restoration actions, and inform research. Management and restoration actions can be prioritized to streams that are currently or have historically contributed large numbers of recruits to the Yellowstone Lake cutthroat trout population. Future research that has the potential to expand upon the results of this study would be useful for understanding the contributions of individual streams that were grouped together within a cluster. This study has provided the baseline information necessary for such a study by clearly defining which groups of streams are contributing most to cutthroat trout recruitment, thus future research can focus on clarifying individual stream contributions.

Results of Yellowstone Lake water samples and lake trout otolith data indicated that elemental and isotopic Sr is essentially uniform throughout Yellowstone Lake. These results are similar to previous studies in the Great Lakes that also found a lack of distinctive chemical signatures within large lakes (e.g. Dufour et al. 2005; Pangle et al. 2010). However, given the unique topography of Yellowstone Lake and the thermal influences to specific regions of the lake, this ecosystem provided a unique opportunity to try otolith microchemistry once again in a much different system than previous Great Lakes studies. While unsuccessful this study can be used as a reference by other researchers seeking to conduct similar studies.

This otolith microchemistry study of cutthroat trout and lake trout natal origins and movement patterns within the Yellowstone Lake ecosystem has revealed both the strengths as well as the limitations of otolith microchemistry. It is my hope that the methods used in this study will be useful to future researchers as well as to the conservation of Yellowstone cutthroat trout in Yellowstone Lake. It is important that new and innovative approaches be employed in order to conserve native fish populations. While these approaches may be limited and not achieve the desired resolution or results it is still important that studies of this nature be initiated to further the state of the science as well as improve the efficiency of future conservation efforts.

References

Dufour, E., W.P. Patterson, T.O. Höök, and E.S. Rutherford. 2005. Early life history of Lake Michigan alewives (*Alosa pseudoharengus*) inferred from intra-otolith stable isotope ratios. Canadian Journal of Fisheries and Aquatic Sciences 62:2362–2370.

Gresswell, R.E. 1994. Life-history organization of cutthroat trout in Yellowstone Lake and its management implications. Doctoral Dissertation. Oregon State University. Corvallis, Oregon.

Koel, T.M., D.L. Mahony, K.L. Kinnan, C. Rasmussen, C.J. Hudson, S. Murcia, and B.L. Kerans. 2006. *Myxobolus Cerebralis* in native cutthroat trout of the Yellowstone Lake ecosystem. Journal of Aquatic Animal Health 18:157–175.

Koel, T.M., J.L. Arnold, P.E. Bigelow, C.R. Detjens, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2015. Native Fish Conservation Program, Yellowstone Fisheries & Aquatic Sciences 2012-2014, Yellowstone National Park. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Pangle, K.L., S.A. Ludsin, and B.J. Fryer. 2010. Otolith microchemistry as a stock identification tool for freshwater fishes: testing its limits in Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 67:1475–1489.

Varley, J.D., and R.E. Gresswell. 1988. Ecology, status, and management of the Yellowstone cutthroat trout. American Fisheries Society Symposium 4:13–24.

REFERENCES

Allendorf, F.W., and Phelps, S.R. 1981. Use of allelic frequencies to describe population structure. Canadian Journal Fisheries and Aquatic Sciences 38:1507–1514.

Amano, Y., M. Kuwahara, T. Takahashi, K. Shirai, K. Yamane, H. Amakawa, and T. Otake. 2013. Otolith elemental and Sr isotopic composition as a natal tag for Biwa salmon *Oncorhynchus masou* subsp. in Lake Biwa, Japan. Aquatic Biology 19:85–95.

Asano, M., and Y. Mugiya 1993. Biochemical and calcium-binding properties of water-soluble proteins isolated from otoliths of the tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology 104B:201–205.

Ball, O.P. 1955. Some aspects of homing in cutthroat trout. Proceedings of the Utah Academy of Science, Arts, and Letters 32:75-80.

Barnett-Johnson, R., T.E. Pearson, F.C. Ramos, C.B. Grimes, and R.B. MacFarlane. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. Limnology and Oceanography 53:1633.

Begg, Gavin A., and John R. Waldman. 1999. An holistic approach to fish stock identification. Fisheries Research 43:35–44.

Behnke, R.J. 2002. Trout and salmon of North America. The Free Press New York, New York .

Benson, N.G. 1961. Limnology of Yellowstone Lake in relation to the cutthroat trout. U.S. Fish and Wildlife Service. Research Report 56:1–38.

Bergum, D. J., K. A. Gunther, and L. M. Baril. In review. Impacts of Yellowstone cutthroat trout decline on mammals and birds in Yellowstone National Park.

Blair, J.M., and B.J. Hicks. 2012. Otolith microchemistry of koi carp in Waikato region, New Zealand: a tool for identifying recruitment locations? Inland Waters 2:109–118.

Borelli, G., N. Mayer-Gostan, H. De Pontual, G. Boeuf, and P. Payan. 2001. Biochemical relationships between endolymph and otolith matrix in the trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Calcified Tissue International 69:356–364.

Breiman, L. 2001. Random forests. Machine Learning 45:5–32.

Breiman, L., A. Cutler, A. Liaw, and M. Wiener. 2014. Breiman and Cutler's random forests for classification and regression. R package version 4.6–10.

Brown, R.J., and K.P. Severin. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Canadian Journal of Fisheries and Aquatic Sciences 66:1790–1808.

Campana, S.E. 1983a. Feeding periodicity and the production of daily growth increments in otoliths of steelhead srout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). Canadian Journal of Zoology 61:1591–1597.

Campana, S.E. 1983b. Factors affecting the production of daily growth increments in the otoliths of fishes. Doctoral dissertation. The University of British Columbia, Vancouver, British Columbia, Canada.

Campana, S.E., and J.D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014-1032.

Campana, S.E., S.R. Thorrold, C.M. Jones, D. Günther, M. Tubrett, H. Longerich, S. Jackson, N.M. Halden, J.M. Kalish, P. Piccoli, H. De Portual, H. Troadec, J. Panfili, D.H. Secor, K.P. Severin, S.H. Sie, R. Thresher, W.J. Teesdale, and J.L. Campbell. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences 54:2068–2079.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Campana, S.E., and S.R. Thorrold. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences 58:30–38.

Campana, S.E. 2005. Otolith science entering the 21st century. Marine and freshwater Research 56:485–489.

Carlson, S.A. 2011. Identifying tributaries to Jackson Lake important for Snake River cutthroat trout recruitment. University of Wyoming National Park Service Research Center Annual Report 34: 5.

Chilcote, M.W. 2003. Relationship between natural productivity and the frequency of wild fish in mixed spawning populations of wild and hatchery steelhead (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 60:1057–1067.

Chittaro, P.M., and J.D. Hogan. Patterns of connectivity among populations of a coral reef fish. Coral Reefs 32:341–354.

Clarke, A.D., K.H. Telmer, and J.M. Shrimpton. 2007. Habitat use and movement patterns for a fluvial species, the arctic grayling, in a watershed impacted by a large reservoir: evidence from otolith microchemistry. Journal of Applied Ecology 44:1156–65.

Coghlan, S.M., M.S. Lyerly, T.R. Bly, J.S. Williams, D. Bowman, and R. Hannigan. 2007. Otolith chemistry discriminates among hatchery-reared and tributary-spawned salmonines in a tailwater system. North American Journal of Fisheries Management 27:531–541.

Cook, G.S. 2011. Changes in otolith microchemistry over a protracted spawning season influence assignment of natal origin. Marine Ecology Progress Series 423:197–209.

Crook, D.A., and B.M. Gillanders. 2006. Use of otolith chemical signatures to estimate carp recruitment sources in the Mid-Murray River, Australia. River Research and Applications 22:871–879.

Degens, E.T., W.G. Deuser, and R.L. Haedrich. 1969. Molecular structure and composition of fish otoliths. Marine Biology 2:105–113.

Dufour, E., W.P. Patterson, T.O. Höök, and E.S. Rutherford. 2005. Early life history of Lake Michigan alewives (*Alosa pseudoharengus*) inferred from intra-otolith stable isotope ratios. Canadian Journal of Fisheries and Aquatic Sciences 62:2362–2370.

Edmonds, J.S., R.C.J. Lenanton, N. Caputi, M. Morita, 1992. Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. Fisheries Research 13:39-51

Elsdon, T.S., B.K. Wells, S.E. Campana, B.M. Gillanders, C.M. Jones, K.E. Limburg, D.H. Secor, S.R. Thorrold, and B.D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology: An Annual Review 46:297–330.

Ertel, B.D. 2011. Distribution, movements, and life-history characteristics of Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* in the Upper Yellowstone River drainage. Master's thesis. Montana State University, Bozeman, Montana.

Farrell, J., and S.E. Campana. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology 115A:103-109.

Fowler, A.J., S.E. Campana, S.R. Thorrold, and C.M. Jones. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 52:1431–1441.

Garcez, R.C.S., R. Humston, D. Harbor, and C.E.C. Freitas. 2014. Otolith geochemistry in young-of-the-year peacock bass *Cichla temensis* for investigating natal dispersal in the Rio Negro (Amazon - Brazil) River system. Ecology of Freshwater Fish 24:242-251.

Geen, G.H., J.D. Neilson, and M. Bradford. 1985. Effects of pH on the early development and growth and otolith microstructure of Chinook salmon, *Oncorhynchus tshawytscha*. Canadian Journal of Zoology 63:22–27.

Gibson-Reinemer, D.K., B.M. Johnson, P.J. Martinez, D.L. Winkelman, A.E. Koenig, and J.D. Woodhead. 2009. Elemental signatures in otoliths of hatchery reared rainbow trout (*Oncorhynchus mykiss*): distinctiveness and utility for detecting origins and movement. Canadian Journal of Fisheries and Aquatic Science 66:513–524.

Gillanders, B.M., and M.J. Kingsford. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Marine Ecology Progress Series 141:13–20.

Gresswell, R.E. 1994. Life-history organization of cutthroat trout in Yellowstone Lake and its management implications. Doctoral dissertation. Oregon State University, Corvallis, Oregon.

Gresswell, R.E. 2011. Biology, status, and management of the Yellowstone cutthroat trout. North American Journal of Fisheries Management 31:782–812.

Gunn, J.S., I.R. Harrowfield, C.H. Proctor, and R.E. Thresher. 1992. Electron probe microanalysis of fish otoliths—evaluation of techniques for studying age and stock discrimination. Journal of Experimental Marine Biology and Ecology 158:1–36.

Haroldson, M.A., K.A. Gunther, D.P. Reinhart, S.R. Podruzny, C.Cegelski, L.Waits, T. Wyman, and J. Smith. 2005. Changing numbers of spawning cutthroat trout in tributary streams of Yellowstone Lake and estimates of grizzly bears visiting streams from DNA. Ursus 16:167–180.

Hartl, D.L., and Clark, A.G. 1989. Principles of population genetics. Sinauer, Sunderland, Massachusetts.

Hoff, G.R., and L.A. Fuiman. 1993. Morphometry and composition of red drum otoliths: changes associated with temperature, somatic growth rate and age. Comparative Biochemical Physiology 106A:209-219.

Hope, A.C.A. 1968. A simplified Monte-Carlo significance test procedure. Journal of the Royal Statistical Society 30:582–598.

Humston, R., B.M. Priest, W.C. Hamilton, and P.E. Bugas. 2010. Dispersal between tributary and main-stem rivers by juvenile smallmouth bass evaluated using otolith microchemistry. Transactions of the American Fisheries Society 139:171–184.

Ingram, B.L., and P.K. Weber. 1999. Salmon origin in California's Sacramento–San Joaquin River system as determined by otolith strontium isotopic composition. Geology 27:851–854

Jackson, M., and S. Hart. 2006. Strontium isotopes in melt inclusions from Samoan basalts: implications for heterogeneity in the Samoan Plume. Earth and Planetary Science Letters 245:260–277.

Jones, R. D., R. E. Gresswell, D. E. Jennings, S. M. Rubrecht, and J. D. Varley. 1979. Fishery and aquatic management program in Yellowstone National Park. U.S. Fish and Wildlife Service, technical report 1978, Yellowstone National Park, Wyoming.

Jones, R.D., D.G. Carty, R.E. Gresswell, C.J. Hudson, L.D. Lentsch, and D.L. Mahony. 1986. Fishery and aquatic management program in Yellowstone National Park. U. S. Fish and Wildlife Service, Technical Report for 1985, Yellowstone National Park, Wyoming.

Jones, C.M., Z. Chen. 2003. New techniques for sampling larval and juvenile fish otoliths for trace-element analysis with laser-ablation sector-field inductivey-coupled-plasma mass spectrometry (SF-ICP-MS). The Big fish Bang: Proceedings of the 26th Annual Larval Fish Conference. The Norwegian Institute of Marine Research, University of Bergen. Bergen.

Karp, C. 2014. Summary of freshwater fisheries telemetry methods. Science and Technology Program, Research and Development Office. Bureau of Reclamation. Denver, CO.

Kaeding, L.R., G.D. Boltz, and D.G. Carty. 1996. Lake trout discovered in Yellowstone Lake threaten native cutthroat trout. Fisheries 21:16–20.

Kaeding, L.R., and G.D. Boltz. 2001. Spatial and temporal relations between fluvial and allacustrine Yellowstone cutthroat trout, *Oncorhynchus clarki bouvieri*, spawning in the Yellowstone River, outlet stream of Yellowstone Lake. Environmental Biology of Fishes 61:395–406.

Kaeding, L.R., 2010. Relative contributions of climate variation, lake trout predation, and other factors to the decline of Yellowstone cutthroat trout during three recent decades. Doctoral Dissertation. Montana State University, Bozeman, Montana.

Kaplinski, M.A. 1991. Geomorphology and geology of Yellowstone Lake, Yellowstone National Park, Wyoming. Master's thesis. Northern Arizona University, Flagstaff, Arizona.

Kaufman, L., and P.J. Rousseeuw. 2005. Finding groups in data: An introduction to cluster analysis. Wiley and Sons Inc. Hoboken, New Jersey pp. 11.

Kelly, B.M. 1993. Ecology of Yellowstone cutthroat trout and an evaluation of potential effects of angler wading in the Yellowstone River. Master's thesis. Montana State University, Bozeman, Montana.

Kennedy, B.P., J.D. Blum, C.L. Folt, and K.H. Nislow. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Canadian Journal of Fisheries and Aquatic Sciences 57:2280–2292.

Kennedy, B.P., A. Klaue, J.D. Blum, C.L. Folt, and K.H. Nislow. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Canadian Journal of Fisheries and Aquatic Sciences 59:925–929.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2004. Yellowstone Fisheries Aquatic Sciences. US National Park Service, Yellowstone Center for Resources Yellowstone National Park, Wyoming.

Koel, T.M., P.E. Bigelow, P.D. Doepke, B.D. Ertel, and D.L. Mahony. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30:10–19.

Koel, T.M., D.L. Mahony, K.L. Kinnan, C. Rasmussen, C.J. Hudson, S. Murcia, and B.L. Kerans. 2006. *Myxobolus cerebralis* in native cutthroat trout of the Yellowstone Lake ecosystem. Journal of Aquatic Animal Health 18:157–175.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2008. Yellowstone Fisheries & Aquatic Sciences: annual report, 2007. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, YCR-2008-02.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2012. Yellowstone Fisheries & Aquatic Sciences: Annual report, 2009–2010. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, YCR-2011-11.

Koel, T.M., J.L. Arnold, P.E. Bigelow, C.R. Detjens, P.D. Doepke, B.D. Ertel, and M.E. Ruhl. 2015. Native Fish Conservation Program, Yellowstone Fisheries & Aquatic Sciences: Annual Report 2012-2014, National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Liu, Z., D.E. Weller, D.L. Correll, and T.E. Jordan. 2000. Effects of land cover and geology on stream chemistry in watersheds of Chesapeake Bay. Journal of the American Water Resources Association 36:1349–1365.

Ludsin, S.A., B.J. Fryer, and J.E. Gagnon. 2006. Comparison of solution-based versus laser ablation inductively coupled plasma mass spectrometry for analysis of larval fish otolith microelemental composition. Transactions of the American Fisheries Society 135:218–231.

Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, K. Hornik, M. Studer, and P. Roudier. 2015. cluster: cluster analysis extended. R package version 1.15–3.

Marsden, E.J., B.J. Ellrott, R.M. Claramunt, J.L. Jonas, and J.D. Fitzsimons. 2005. A comparison of lake trout spawning, fry emergence, and habitat use in lakes Michigan, Huron, and Champlain. Journal of Great Lakes Research 31:492–508.

Marshall, S.L., and S.S. Parker. 1982. Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences 39:542–547.

Martinez, P.J., P.E. Bigelow, M.A. Deleray, W.A. Fredenberg, B.S. Hansen, N.J. Horner, S.K. Lehr, R.W. Schneidervin, S.A. Tolentino, and A.E. Viola. 2009. Western lake trout woes. Fisheries 34:424–442.

Mercier, L., A.M. Darnaude, O. Bruguier, R.P. Vasconcelos, H.N. Cabral, M.J. Costa, M. Lara, D.L. Jones, D. Mouillot. 2011. Selecting statistical models and variable combinations for optimal classification using otolith microchemistry. Ecological Applications 21:1352–1364.

Miller, J.A., V.L. Butler, C.A. Simenstad, D.H. Backus, A.J.R. Kent, and B. Gillanders. 2011. Life history variation in Upper Columbia River chinook salmon (*Oncorhynchus tshawytscha*): a comparison using modern and ~500-year-old archaeological otoliths. Canadian Journal of Fisheries and Aquatic Sciences 68:603–617.

Muhlfeld, C.C., S.R. Thorrold, T.E. McMahon, and B. Marotz. 2012. Estimating westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) movements in a river network using strontium isoscapes. Canadian Journal of Fisheries and Aquatic Sciences 69:906–915.

Munro, A.R, T.E. McMahon, and J.R. Ruzycki. 2005. Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake. Canadian Journal of Fisheries and Aquatic Sciences 62:79–87.

Neilson, J.D., and G.H. Geen. 1982. Otoliths of Chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Canadian Journal of Fisheries and Aquatic Sciences 39:1340–1347.

Ohji, M., Harino, H., and Arai, T. 2006. Differences in organotin accumulation among ecological migratory types of the Japanese eel *Anguilla japonica*. Estuary Coastal and Shelf Science 69:270–290.

Olley, R., R.G. Young, G.P. Closs, E.A. Kristensen, T.O. Bickel, N.A. Deans, L.N. Davey, and S.M. Eggins. 2011. Recruitment sources of brown trout identified by otolith trace element signatures. New Zealand Journal of Marine and Freshwater Research 45:395–411.

Pangle, K.L., S.A. Ludsin, and B.J. Fryer. 2010. Otolith microchemistry as a stock identification tool for freshwater fishes: testing its limits in Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 67:1475–1489.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173:1124–1127.

Payan, P., A. Edeyer, H. De Pontual, G. Borelli, G. Boeuf, and N. Mayer-Gostan. 1999. Chemical composition of saccular endolymph and otolith in fish inner ear: lack of spatial uniformity. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 277:123–131.

Popper A.N., J.U. Ramcharitar, S.E. Campana. 2005. Why otoliths? Insights from inner ear physiology and fisheries biology. Marine and Freshwater Research 56:497–504.

Post, D.M., M.L. Pace, and N.G. Hairston. 2000. Ecosystem size determines food-chain length in lakes. Nature (London) 405:1047–1049.

Proctor, C.H., R.E. Thresher, J.S. Gunn, D.J. Mills, I.R. Harrowfield, S.H. Sie, 1995. Stock structure of the southern bluefin tuna *Thunnus maccoyi*: an investigation based on probe microanalysis of otolith composition. Marine Biology 122:511–526.

Puckett, L.J., and O.P. Bricker. 1992. Factors controlling the major ion chemistry of streams in the Blue Ridge and Valley and Ridge physiographic provinces of Virginia and Maryland. Hydrological Processes 6:79–98.

Reinhart, D.P. 1990. Grizzly bear habitat use on cutthroat trout spawning streams in tributaries of Yellowstone Lake. Master's thesis. Montana State University, Bozeman, Montana.

Reinhart, D.P. and D.J. Mattson. 1990. Bear use of cutthroat trout spawning streams in Yellowstone National Park. In Bears: Their Biology and Management, Vol. 8, A selection of papers from the Eighth International Conference on Bear Research and Management, Victoria, British Columbia, Canada, February 1989. International Association for Bear Research and Management, New York, New York. 343–350.

Riceman, M.S. 2008. The use of otolith microchemistry to investigate natal origins and movement of lacustrine wild rainbow trout (*Oncorhynchus mykiss*) and common smelt (*Retropinna retropinna*). Master's thesis. The University of Waikato, Hamilton, New Zealand.

Richmond, G.M. 1976. Surficial geological history of the Canyon Village quadrangle. Yellowstone National Park, Wyoming, for use with map 1-642. U.S. Geological Survey Bulletin 1427.

Ruzycki, J.R., D.A. Beauchamp, and D.L. Yule. 2003. Effects of introduced lake trout on native cutthroat trout in Yellowstone Lake. Ecological Applications 13: 23–37.

Sandstrom, P.T., R.E. Gresswell, B. Farokhkish, and M.J. Parsley. 2014. Identifying movement patterns and spawning patterns of lake trout (*Salvalinus namaycush*) in Yellowstone Lake. Investigators Annual Report for 2013. Montana Institute on Ecosystems, Bozeman, Montana

Schindler, D.E., R. Hilborn, B. Chasco, C.P. Boatright, T.P. Quinn, L.A. Rogers, and M.S. Webster. 2010. Population diversity and the portfolio effect in an exploited species. Nature 465:609–612.

Schullery, P. and Varley, J.D., 1995. Cutthroat trout and the Yellowstone Lake ecosystem. The Yellowstone Lake crisis: confronting a lake trout invasion. A Report to the Director of the National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming, USA, pp.12-21.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada, Bulletin 184:966.

Secor, D.B., J.M. Dean, and E.B. Laban. 1991. Manual for otolith removal and preparation for microstructural examination. Belle W. Baruch Institute, University of South Carolina Press. Columbia, South Carolina.

Severin, K.P., J. Carroll, B.L. Norcross. 1995. Electron microprobe analysis of juvenile walleye pollock, *Theragra chalcogramma*, otoliths from Alaska: a pilot stock separation study. Environmental Biology of Fishes 43:269–283.

Sie, S.H., and R.E. Thresher. 1992. Micro-PIXE analysis of fish otoliths: methodology and evaluation of first results for stock discrimination. International Journal of PIXE 2:357–379.

Simkiss, K. 1974. Calcium metabolism of fish in relation to ageing. Unwin Brothers Publishing. London, England.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Snyder, B. and D. Oswald. 2005. Montana's fish species of special concern: Lake trout. Status of native lake trout in Montana. Montana Fish Wildlife and Parks, Helena, Montana.

Sousa, R.G.C., R. Humston, and C.E.C. Freitas. 2016. Movement patterns of adult peacock bass *Cichla temensis* between tributaries of the middle Negro River basin (Amazonas–Brazil): an otolith geochemical analysis. Fisheries Management and Ecology 23:76-87.

Strohm, D. 2015. Matching watershed and otolith chemistry to establish natal origin of an endangered desert lake sucker. Master's Thesis. Utah State University. Logan, Utah. United States Geological Survey. 1972. Geologic map of Yellowstone National Park. U.S. Geological Survey, Miscellaneous Geological Investigations Report 711.

Sturgeon, R.E., S.N. Willie, L. Yang, R. Greenberg, R.O. Spatz, Z. Chen, C. Scriver, V. Clancy, J.W. Lam, and S. Thorrold. 2005. Certification of a fish otolith reference material in support of quality assurance for trace element analysis. Journal of Analytical Atomic Spectrometry 20:1067–1071.

Sturrock, A.M., C.N. Trueman, A.M. Darnaude, and E. Hunter. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? Journal of Fish Biology 81:766–795.

Syslo, J. M., C.S. Guy, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and T.M. Koel. 201. Response of non-native lake trout (*Salvalinus namaycush*) to 15 years of harvest in Yellowstone Lake, Yellowstone National Park. Canadian Journal of Fisheries and Aquatic Sciences 68: 2132–2145.

Thorrold, S.R. 2001. Natal homing in a marine fish metapopulation. Science 291:297–299.

Toole, C.L., D.F. Markle, and P.M. Harris. 1993. Relationships between otolith microstructure, microchemistry, and early life history events in Dover sole, *Microstomus pacificus*. U.S. Fishery Bulletin 91:732–753.

Varley, J.D., and R.E. Gresswell. 1988. Ecology, status, and management of the Yellowstone cutthroat trout. American Fisheries Society Symposium 4:13–24.

Varley, J. D., and P.D. Schullery. 1998. Yellowstone fishes: ecology, history, and angling in the park. Stackpole Books, Mechanicsburg, Pennsylvania.

Volk, E.C., A. Blakley, S.L. Schroder, and S.M. Kuehner. 2000. Otolith chemistry reflects migratory characteristics of Pacific salmonids: using otolith core chemistry to distinguish maternal associations with sea and freshwaters. Fisheries Research 46:251–266.

Walther, B.D., S.R. Thorrold, and J.E. Olney. 2008. Geochemical signatures in otoliths record natal origins of American shad. Transactions of the American Fisheries Society 137:57–69.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan, and C.M. Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132:409–424.

Westerling, A.L., M.G. Turner, E.A.H. Smithwick, W.H. Romme, and M.G. Ryan. 2011. Continued warming could transform Greater Yellowstone fire regimes by mid-21st century. Proceedings of the National Academy of Sciences 108:13165–13170.

Wolff, B.A., B.M. Johnson, A.R. Breton, P.J. Martinez, D.L. Winkelman, and B. Gillanders. 2012. Origins of invasive piscivores determined from the strontium isotope ratio ($^{87}Sr/^{86}Sr$) of otoliths. Canadian Journal of Fisheries and Aquatic Sciences 69:724–739.

Zimmerman, C.E., and G.H. Reeves. 2002. Identification of steelhead and resident rainbow trout progeny in the Deschutes River, Oregon, revealed with otolith microchemistry. Transactions of the American Fisheries Society 131:986–993.