Environmental DNA in lake sediment reveals biogeography of native trout diversity

by

Hedin Thomas Nelson-Chorney

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

Master of Science

in

Conservation Biology

Department of Renewable Resources
University of Alberta

Abstract

Understanding historical species distributions is vital to the conservation and restoration of native species, yet such information is often qualitative. Here, we show that the paleolimnological history of threatened freshwater fishes can be reconstructed using species diagnostic markers amplified from environmental DNA deposited in lake sediments (lake sediment DNA). This method was validated through the detection of lake sediment DNA from non-native trout (Yellowstone cutthroat trout; *Oncorhynchus clarkii bouvieri)*, which corroborated historical records of human-mediated introductions. Moreover, we discovered native trout (westslope cutthroat trout; *Oncorhynchus clarkii lewisi)* lake sediment DNA that predated human-mediated introductions of freshwater fishes in a watershed with high topographical relief. This unexpected result revealed that the population was of native origin and requires immediate conservation protection. Our findings demonstrate that lake sediment DNA can be used to determine the colonization history of freshwater fishes and the structure of ecosystems, aiding in the identification of native ranges, novel native diversity, and introductions of non-native species.

Preface

This thesis is an original composition by Hedin Thomas Nelson-Chorney. A version of this thesis has been published as: **Nelson-Chorney HT,** Davis CS, Poesch MS, Vinebrooke RD, Carli CM, and Taylor MK. 2019. Environmental DNA in lake sediment reveals biogeography of native genetic diversity. *Frontiers in Ecology and the Environment* **https://doi.org/10.1002/fee.2073**. The idea for this study was originally conceived by the late Charlie Pacas. It was expanded to its current form by myself and Dr. Mark Taylor. Dr. Mark Poesch was the primary supervisor and assisted with funding, field collection, and manuscript revisions. Dr. Mark Taylor served as co-supervisor and assisted in funding, design, field coordination, sample collection, staff support, and manuscript revisions. Dr. Corey Davis provided primary support for genetic methods and analysis as well as manuscript revisions. Dr. Rolf Vinebrooke assisted in field collection and manuscript revisions. Christopher Carli supported field collection and provided valuable input in manuscript revisions. This project was funded by Parks Canada Agency, Fisheries and Oceans Canada, the Alberta Conservation Association, and the University of Alberta.

Acknowledgements

I would never have survived the rigors of post-secondary education without the undying love and support of my parents, Anna Nelson and James Chorney. They have always provided me with the right set of tools to succeed and for this, I am forever grateful.

This thesis was funded by Parks Canada Agency, Fisheries and Oceans Canada, the Alberta Conservation Association, and the University of Alberta.

I thank Dr. Mark Poesch for taking a chance on this project and providing guidance on how to formulate a successful thesis. Thanks for always having my back and for pushing me to be a better biologist.

Thanks to Dr. Mark Taylor for sticking with me and this project and for providing countless hours of support. Your love of fisheries research has always been inspiring and it has been an absolute honor to have worked alongside you all these years.

Thanks to Dr. Corey Davis for taking on the rigorous genetic portion of this thesis. It certainly was not the easiest undertaking and this thesis would not have been possible without the countless phone calls, meetings, sample preparation and help with all things genetic.

Thanks to Dr. Rolf Vinebrooke who gave me my first chance at being a field biologist in 2010 and for the continuing support in subsequent years. You have always supported research opportunities in the Rocky Mountains and your knowledge in alpine limnology is unmatched.

Thanks to Chris Carli for all the logistical and field support. It has been a pleasure to work alongside you and develop a life-long friendship.

Thanks to Chris Groves, Julie Timmins, Dan Rafla, Bill Hunt, and the aquatics crew at Parks Canada for helping me gather field samples. Thanks to Fonya Irvine, Ryan Kovach, and Clint Muhlfeld for their supportive comments on this thesis. A special thanks to Tyana Rudolfsen for her numerous reviews and comments on the manuscript and for pushing me to always be better. Thanks to Ali Buckingham for her GIS support.

Finally, thanks to all the great friends and family who have helped shape my life. I would not be the man I am today without your friendship, love, help and support.

Table of Contents

List of Figures

List of Tables

List of Appendices

1. Introduction

Freshwater fishes are among the most endangered group of vertebrates due to the cumulative impacts of habitat modification, non-native species introductions and over-exploitation (Dudgeon *et al.* 2006). Losses to freshwater biodiversity are occurring at increasingly higher rates than those in terrestrial or marine environments (Dudgeon *et al.* 2006). In fact, the average abundance of freshwater vertebrates worldwide declined by 81% between 1970 to 2012 while those in marine and terrestrial environments fell by 36 and 38%, respectively (McLellan *et al.* 2014). Despite these alarming declines, understanding the historical distribution of imperiled species is hampered due to a lack of quantitative approaches to identify native ranges. Without this information, it is challenging to accurately identify areas of native genetic diversity for conservation.

It is very difficult to reconstruct the histories of naturally occurring (native) and introduced (non-native) fishes in freshwater environments, particularly for endangered, rare, and closely related species. Several lines of paleolimnological evidence (e.g., stratigraphic changes in the abundance of large-bodied invertebrates (Lamontagne & Schindler 1994), stable isotope signatures (Finney *et al.* 2002), or changes in algal communities (Carpenter & Leavitt 1991)) are typically used as proxies for direct enumeration of fish occupancy. However, such inferences may incorrectly estimate the presence or absence of aquatic species due to a lag effect from the beginning of an environmental alteration to the biological response (Magnuson 1990). Further, these indirect measures on lake communities lack the adequate taxonomic resolution necessary to distinguish fish species (Lamontagne & Schindler 1994).

Genetic material shed by an aquatic organism into the environment, or environmental DNA (eDNA), is an increasingly powerful tool for assessing contemporary patterns of freshwater biodiversity (Thomsen *et al.* 2012). Although freshwater eDNA studies may be constrained by dilute concentrations of DNA in the water column, examining sedimentary eDNA may offer an approach to identify the historical distribution of a species as it becomes concentrated in lake sediments through a settling process (Turner *et al.* 2014). In particular, sediments in remote high-elevation lakes provide an optimal environment for DNA preservation as degradation is expected to be relatively slow under cold and unproductive conditions (Barnes *et al.* 2014). The stratigraphic deposition of eDNA in lake sediments can provide details of historic species occupancy (Domaizon *et al.* 2017) as it can be preserved for thousands of years as sedimentary eDNA (lake sediment DNA; Olajos *et al.* 2018, Stager *et al.* 2015). Moreover, lake sediments are fully saturated and compacted which prevents the downward migration of water and aqueous DNA (Giguet-Covex *et al.* 2014). This provides undisturbed temporal succession of lake sediment DNA. To date, however, lake sediment DNA has not been used to simultaneously determine the histories of native and non-native fish populations.

Despite the immense socio-economic and ecological value that native freshwater fishes provide, many species have exhibited marked declines in geographic range and population size (Schindler *et al.* 2010). Specifically, westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) populations have experienced significant declines in their distribution as a result of introgressive hybridization with closely related species, anthropogenic alterations to habitat and increased competition with non-native species

(Shepard *et al.* 2005). Recovery efforts have focused on restoring the species to its former range; however, this historical area remains largely unknown (Shepard *et al.* 2005). The identification of pre-anthropogenic populations is critical to species recovery as these areas may contain sources of native genetic diversity for recovery and conservation programs. However, incomplete records of human-mediated introductions and topographical barriers to fish migration have made it difficult to understand the biogeographical history of westslope cutthroat trout in lakes throughout western North America.

Westslope cutthroat trout are found in lakes and streams across the continental divide of the Rocky Mountains in British Columbia, Alberta, Idaho, and Montana with isolated populations in Oregon and Washington (Behnke 1992). Despite having the largest historical range of any of the cutthroat trout subspecies (Behnke 1992), most populations of westslope cutthroat are now located in isolated headwater lakes and streams (Muhlfeld *et al.* 2014). This trend is indicative of populations across the entire range of westslope cutthroat trout, regardless if the land is public, private or protected (Mayhood and Taylor 2011). Banff National Park (BNP) in Alberta, Canada is home to the most northern native populations of westslope cutthroat trout (Mayhood 1995). Expansive human-mediated introductions of non-native rainbow (*Oncorhynchus mykiss*) and Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) have developed widespread genetic introgression with native westslope cutthroat trout (Muhlfeld *et al.* 2009). Coupled with displacement from non-native brook trout (*Salvelinus fontinalis*) (Shepard 2004), there are now only ten known populations of native westslope cutthroat trout in BNP (Mayhood and Taylor 2011). However, incomplete stocking records and

undocumented human-mediated introductions have made it difficult to determine the biogeographical history of many populations.

We explored the ancestry of freshwater fishes in mountain lakes by combining high-throughput sequencing technologies with standard paleolimnological sampling techniques. Genomic regions containing species specific diagnostic single nucleotide polymorphisms (SNPs) were amplified from lake sediment DNA and sequenced to detect the historical presence of two common cutthroat trout species. Our objectives were to test whether lake sediment DNA evidence revealed historical events of human-mediated non-native trout introductions and to reconstruct the unknown history of a westslope cutthroat trout population that was thought to have been artificially propagated.

2. Materials and Methods

Study Area

We focused on eight lakes within Banff National Park, Alberta, Canada (Table 1) that currently support self-sustaining populations of westslope cutthroat trout and an additional two lakes that are known to contain non-native fishes. Although all lakes are located in remote subalpine habitat in the Bow River watershed, they have differing physical characteristics and histories of human-mediated fish introductions (Table 1). As a control, we also sampled Oesa Lake (51° 21' N, 116° 45' W), a remote, closed-basin lake perched above an alpine bench in Yoho National Park, British Columbia, Canada. No historical record of human-mediated fish introductions or recent evidence of an extant fish population (Messner *et al.* 2013) made sediment from Oesa Lake an ideal negative control.

Paleolimnology

Two lake sediment cores were collected from the deepest portion of each lake using a mini-Glew gravity corer during the winters of 2014 and 2015. All cores from the lakes ranged in total length from 12-30 cm, and captured a horizontal sediment-water interface (Table 1). In general, water content level was typical of cores obtained from other lakes in the area where sediments are of low organic content, ranging from <10 to 20% (Phillips *et al.* 2011). The stratigraphies of all cores from these small and deep lakes appeared well stratified, changing gradually from black gyttja in the upper sediments to grey silt further down each core. Cores were extruded on site at 0.5 cm intervals. To prevent DNA contamination across samples, all extruding equipment was

thoroughly cleaned with a 60% bleach solution between sections. To further prevent cross-contamination between sections, only the central square centimeter was collected from each sectioned sediment interval for lake sediment DNA analysis. Replicate 0.1 - 0.2g sub-samples were taken from each core and stored in a -80°C freezer and one was used for lake sediment DNA analysis. The remainder of each section was lypholized and analyzed by MyCore Scientific for Pb-210 radioisotopic analysis. Dating of sections was interpreted with the constant rate of supply model (Appleby *et al.* 1979). The standard deviation of age estimate in years was determined by:

$$STD\ (age\ estimate\ in\ years) = \frac{1}{(Pb{-}210\ Decay\ Constant)} \times ln\left(\frac{Total\ Excess\ 210\ Pb}{Interval\ Excess\ 210\ Pb}\right) \times \frac{Coefficient\ of\ Variation\ (sediment\ accumyulation\ rate)}{100}$$

Age estimate precision of each section include error associated with weighing, radiochemical extraction, radionuclide counting, and all uncertainties in the quadrature (Binford 1990). Dating accuracy decreases with age of the core due to excess levels of Pb-210 in deeper intervals of sediments approaching background levels of Pb-210. To prevent misinterpreting lake sediment DNA results, sediment cores (i.e. lakes) were only included in subsequent analysis if the confidence interval at each age point did not overlap with adjacent dates and their associated error. Further, only discrete points with reported confidence intervals surrounding the age of the sediment were included in lake sediment DNA analysis. Marvel and Mystic Lakes (Figure 1) had the only sediment cores that reached these stringent criteria and were the only cores used for lake sediment DNA extraction and sequencing (Figure 3 and 4).

Lake sediment DNA Extraction and Sequencing

The PowerSoil DNA Isolation Kit (Mo Bio Laboratories, USA) was used to extract lake sediment DNA from sediment samples in a clean laboratory that had never been exposed to salmonid DNA prior to this study. DNA from each sediment core was extracted individually over a two day period and were physically separated from other cores. Two diagnostic loci for each of westslope cutthroat trout, Yellowstone cutthroat trout, and rainbow trout were used (Table 2) (Kalinowski *et al.* 2011; Campbell *et al.* 2012). These three species were chosen as they have well documented histories of human-mediated introduction across the landscape and have hybridized extensively throughout North America. An additional primer pair was used as a positive lake sediment DNA extraction metric to test for the presence of the calanoid copepod, *Hesperodiaptomus arcticus,* which is ubiquitous throughout the study area (Table 2; Messner *et al*. 2013). A negative extraction control (sterile water sample extracted at the same times as sediment samples) was performed for each lake on each day of DNA extraction and was subjected to the same amplification and sequencing protocols as the sediment samples. Loci were amplified in two multiplexed sets (of 4 and 3 loci) using the following PCR conditions: 4μL of extracted lake sediment DNA, 5μL of Qiagen PCR Multiplex MasterMix (Qiagen), 0.5μL transposon tailed (Illumina), locus specific forward primer, 0.5μL transposon tailed, locus specific reverse primer, and 0.1μL Platinum *Taq* HiFi (Invitrogen). Thermocycling conditions consisted of an initial denaturation period of 95°C for 15min followed by 12 cycles of 95°C for 30sec, 60°C for 90sec, and 72°C for 30sec, then a final extension of 60°C for 30min. All lake sediment DNA amplifications were performed in the same run which also included a negative PCR control. PCR

products from the two reactions were then pooled within individual, purified with AMPure XP beads (Agencourt) according to the manufacturer's directions, and diluted to 1:20 with ddH_2O. Purified PCR products were individually indexed using an Illumina Nextera XT dual-indexed PCR (Illumina) that included: 5μL diluted pooled amplicons, 5μL forward index primer, 5μL reverse index primer, 25μL 2x KAPA HiFi PCR reaction mix, and 10μL ddH_2O. Index PCR thermocycling conditions were the same as the initial PCR and barcoded amplicons were once again purified with AMPure XP beads to remove unincorporated primers and primer dimers. The pooled amplicon library was sequenced on an Illumina MiSeq platform using 300 cycle V2 chemistry.

Bioinformatics

Amplicon alignment and SNP genotyping was completed using Geneious R9 (Biomatters, New Zealand; https://doi.org/10.5281/zenodo.3402577). Reads were mapped to seven (6 trout and one zooplankton) reference sequences. Reference sequences were standardized to 51bp, which allowed for 25bp mapping identity on each side of the SNP site. Sequences were mapped using the standard Geneious algorithm with the following custom sensitivities: minimum mapping quality=20, minimum overlap identity=95%, maximum ambiguity=1, and maximum mismatches per read=6%. All other settings remained at the manufacturer's default conditions. To prevent the possibility of field and laboratory contamination from influencing our results, we examined read counts in field negative, extraction negative and PCR negative controls. Species reads that were detected in any of the controls were removed from further analysis (Appendix 1). The "find variations/SNP" function in Geneious R9 was

used to call SNP sites. Unexpected sequences were removed following BLAST identification to remove bacterial sequence contamination.

3. Results

Lake sediment DNA evidence of Yellowstone cutthroat trout corroborated historical reports of its introduction (ca. 1925) and subsequent persistence in Marvel Lake (Figure 2, Table 2, Mayhood and Taylor 2011). Although Marvel Lake was considered to be naturally fishless due to the surrounding geographic relief, our lake sediment DNA analysis revealed that westslope cutthroat trout had been present prior to documented human-mediated introductions. Contemporary eDNA analyses in the top layer of lake sediment show that both westslope and Yellowstone cutthroat trout, or hybrids of the two species, currently reside within Marvel Lake.

Elsewhere, our lake sediment DNA analyses revealed a surprising observation in Mystic Lake with important ramifications for conservation and management (Figure 2). Prior to this study, the absence of official reports of human-mediated introductions in conjunction with the presence of presumed topographical barriers to fish migration led managers to designate Mystic Lake as naturally fishless. Consequently, the extant population of westslope cutthroat trout in the lake was thought to be a product of an illegal introduction and therefore, was not granted conservation protection. However, lake sediment DNA analysis indicate that westslope cutthroat trout persisted in the lake since at least 1911, which predated all official human-mediated introductions in the area.

To confirm that cross-contamination of samples did not interfere with our results, we analyzed a sediment core from a known fishless lake (Oesa Lake) that was treated as a field negative control. Here, a lack of lake sediment DNA agreed with previous fisheries inventory data confirming its fishless status (Messner *et al*. 2013) and confirmed that our field sampling method did not result in across sample contamination (Appendix 1). Further, the ubiquitous presence of lake sediment DNA from the endemic alpine zooplankton species, *Hesperodiaptomus arcticus*, across all lake sediment cores confirmed that DNA had been preserved sufficiently in cold mountain lakes to yield extractable and amplifiable amounts of DNA.

4. Discussion

By using sedDNA from lake sediment cores, we show that the colonization histories of freshwaters fishes can be reconstructed to identify native populations of a threatened species. Further, we were able to document human-mediated introductions of non-native species and their subsequent establishment. These findings highlight the potential value of lake sediment DNA records as an approach that can address previously intractable questions regarding the historical distribution of aquatic species and freshwater biodiversity (Stager *et al.* 2015).

Lake sediment DNA provides a powerful approach to reconstruct the evolutionary history of freshwater organisms. As freshwater biodiversity continues to decline worldwide, it is essential that the genetic diversity of imperiled populations remain intact. The ability of lake sediment DNA to identify historical remnant populations and simultaneously find non-native species is paramount to the conservation of native freshwater fishes. This approach may provide powerful insight into the legacy of human induced changes to freshwater ecosystems including community level changes associated with historical human-mediated introductions.

Although contemporary sampling of tissue can provide information about the ancestry of freshwater fish populations that potentially interbreed, lake sediment DNA analysis of sediment cores can reveal unknown introductions that have altered native fish diversity. Hybridization and genetic introgression due to human-mediated introductions of non-native species have resulted in the extinction of native populations, lineages, and species of freshwater fishes (Rhymer & Simberloff 1996). This is particularly true in fishes such as salmonids, where extensive hybridization has already

occurred (Allendorf *et al.* 2001) and is likely to continue due to ongoing habitat loss, non-native species introductions, and climate change (Muhlfeld *et al.* 2014). In many cases, disjunct, non-hybridized populations are the last remaining sources of native genetic diversity. For example, westslope cutthroat trout, a threatened species in Canada, have decreased in geographical area by up to 95%, in large part through hybridization with rainbow trout (Mayhood & Taylor 2011). The few remaining disjunct populations are the last sources of native diversity for the species in Alberta and provides important genetic material that may be available for restoration efforts.

While genetic introgression in native trout is prominent across the landscape, it is crucial to identify and protect populations that do not show signs of non-native introgression. For example, genetic material from pure individuals may be used to save populations that are experiencing fragmentation-mediated genetic erosion (Robinson *et al.* 2017). Genetic rescue from the individual translocation of native genetic diversity has been shown to offset the deleterious effects of in-breeding depression and increase the local genetic pool, resulting in an increase in fitness (Whiteley *et al.* 2015). Further, to maintain viable populations, conservation translocation to areas free from non-native genetic diversity may provide refuge for the last remaining individuals of a native lineage. As lake sediment DNA can detect low-levels of non-native species throughout an entire population of fishes, it may be a useful method for identifying areas free of non-native genetic diversity and therefore, identify suitable locations for translocation.

Importantly, previous microsatellite analysis revealed very low levels of admixture (<4%) in contemporary samples of trout from Marvel Lake (Mayhood and Taylor 2011) yet no parental Yellowstone cutthroat trout remain. In other words, Yellowstone

cutthroat trout genes are at relatively low abundance in the system. Thus, the ability to detect the presence of non-native genetic material, even when relatively rare, reveals the sensitivity of lake sediment DNA and its potential value for describing community level biodiversity.

Although the Marvel Lake population has potentially experienced introgressive hybridization, pure parental westslope cutthroat trout still exist in the population and chemical or selective (mechanical) suppression could be explored to remove fish with non-native genetic diversity (Kovach 2018). Given the growing evidence for selection acting against non-native admixture in westslope cutthroat trout (Muhlfeld *et al.* 2009; Kovach *et al.* 2015), the removal of Yellowstone cutthroat trout genes from this population may increase the fitness and ecological function of the remaining native westslope cutthroat trout population.

Limitations in Pb-210 radioisotopic analysis confined our results to the past 100 years. Additional research is needed to understand the long-term viability of lake sediment DNA. As the sediment cores observed here showed consistent stratification, carbon dating may be an effective method to use in conjunction with lake sediment DNA to explore generational trends in freshwater fish populations. Further, it may be possible to explore the relative abundance of specific functional genes, such as those for influencing migration timing in pacific salmon (Prince *et al.* 2017), to determine historical fluctuations in migration timing and thus, long-term patterns of natural selection and local adaptation.

While our approach utilized only one PCR replicate per DNA extraction per sediment section, no false negatives were found in either core as evidenced by the constant fish identification chronology in Mystic Lake and the direct response to a human-mediated introduction in Marvel Lake. Further, our stringent contamination thresholds and absence of target loci reads in both field and laboratory controls suggest that there were no false positive results. However, future lake sediment DNA studies exploring the long-term presence of multiple taxa may wish to increase PCR replication to reduce the possibility of false negatives (Ficetola *et al.* 2015). This may be especially important if quantitative PCR (qPCR) is used to determine historical fluctuations in species abundance.

Lake sediment DNA revealed the century long presence of native westslope cutthroat trout in two lakes thought to contain only introduced fishes. Although it is possible that there were indigenous introductions of fishes into Marvel and Mystic Lakes prior to 1900, we have presented the first evidence that fish were present before any documented introductions. This discovery substantially changed the delineated range of native westslope cutthroat trout by adding two headwater lakes into their known native range in Alberta, where only a few native populations remain (Mayhood & Taylor 2011). Thus, these findings have immediate conservation implications, affording protection to populations that were previously of lower conservation value.

Our findings have broad implications for the conservation and restoration of freshwater species and ecosystems. Although aquatic conservation commonly focuses on the loss of native habitat to set recovery targets, it is rare to have evidence of a species' historical biogeography to identify restoration areas where long-term

persistence of the species is possible. Lake sediment DNA is an environmentally low-impact way to reconstruct the historical presence of aquatic species while providing a greater understanding of the legacy effects of human-mediated species translocations.

5. Conclusion

Although eDNA is becoming an increasingly popular tool for the rapid assessment of aquatic species, there is little consensus in the literature on how to design valid molecular eDNA assays or sampling strategies (Barnes *et al.* 2014; Ficetola *et al.* 2015; Goldberg *et al.* 2016; Spens *et al.* 2016). Closely related species, such as westslope cutthroat trout and Yellowstone cutthroat trout, present the specific challenge of finding diagnostic markers that are ubiquitous across the native range of each species (Wilcox *et al.* 2013). To further complicate matters, pervasive human-mediated introductions and hybridization events across North America have made it difficult to determine which populations and individuals contain native genetic material (Mayhood & Taylor 2011). Additionally, when this study was conceived, there was little to no information on how to sample eDNA from lake sediments or how to prevent contamination in the field and laboratory.

In particular, this study faced immense difficulty in determining which molecular markers should be used given that diagnostic markers from previous studies were never tested in fish from Banff National Park. Further, as the histories of salmonids in Banff National Park were not fully understood, it was difficult to discern which populations contained native genetic ancestry. In an attempt to better understand these problems, we conducted a pilot project where we used previously published diagnostic SNP markers (one for each of rainbow trout, westslope cutthroat trout, and Yellowstone cutthroat trout) to determine if it was possible to extract lake sediment DNA and if the markers chosen were reliable (Kalinowski *et al.* 2011). Elk Lake was chosen for this project as previous non-diagnostic microsatellite analysis had shown that the westlope

cutthroat trout population in the lake were “pure” (>0.99 westslope cutthroat trout genes). While we were successful at extracting and amplifying lake sediment DNA using an Ion Torrent Personal Genome Machine (PGM), we also found unintended polymorphisms in our SNP markers, which were previously thought to be diagnostic.

After discovering that the SNP markers chosen were unreliable at differentiating rainbow trout, westslope cutthroat trout, and Yellowstone cutthroat trout, we expanded our marker set to include 4 diagnostic markers for each species (Kalinowski *et al.* 2011; Campbell *et al.* 2012). These new markers were subsequently tested on wild fish from 23 populations in Banff National Park, Montana, and Alberta. Two populations of hatchery raised fish from Alberta were also tested to determine if observed polymorphisms originated from a hatchery strain. After sequencing again on the Ion Torrent PGM, we found a number of markers failed to amplify and that there were unknown diagnostic polymorphisms that were adjacent to existing SNP sites. Although a number of markers did not amplify, the abundance of unknown diagnostic SNP sites discovered allowed us to use these new sites for further analysis.

During the bioinformatics analysis of the Ion Torrent PGM data, we found that the Ion Torrent platform appeared to have a relatively high amount of sequencing error. Further, Ion Torrent’s BaseSpace application was difficult to navigate the SNP data that was inputted and did not seem like an appropriate tool for large-scale analysis. To reduce sequencing error, all future genotyping was completed on an Illumina MiSeq platform using 300 cycle V2 chemistry. Additionally, all future bioinformatics was completed using the software program, Geneious R9.

Due to the sensitivity of high-throughput sequencing, we used stringent contamination protocols that were previously described. These protocols arose after we discovered the potential for laboratory contamination during the PCR reactions. As the fin clips have a larger amount of extracted DNA than sediment samples, we chose to run both sets of samples on different themocyclers and in different rooms to prevent fin clip DNA from contaminating our lake sediment DNA samples. Further, we completed all PCR reactions for the lake sediment DNA samples where salmonid tissue had never been prior to this study.

While these protocols succeeded in reducing contamination in samples, we experienced contamination from an unlikely source. Many of our sediment samples were contaminated with *Ralstonia* spp. and *E. coli*, which originated from the Powersoil DNA extraction kit that was used. Unfortunately, sequences from these bacteria were similar enough to the salmonid reference sequences used for analysis that they consumed a large number of Illumina sequence reads. Ultimately, this led to low numbers of actual salmonid sequences relative to bacterial sequences. Further, it made the bioinformatics screening of each section difficult as all bacterial sequences had to be manually removed from further analysis in Geneious R9.

Despite these problems, we were able to overcome these obstacles and present meaningful results. I would strongly advise anyone who is conducting future eDNA studies to fully understand if the genetic markers chosen are truly diagnostic. The large biogeographical range of many aquatic species can produce unknown polymorphisms which may appear to make a genetic marker work in one area but not another, despite the same species being present in both locations. I would also suggest that an

occupancy approach be taken when analyzing eDNA samples as multiple replicates for each sample will help better understand the distribution and detection of eDNA in the environment.

6. Literature Cited

Allendorf, F.W., Leary, R.F., Spruell, P. & Wenburg, J.K. (2001). The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.*, 16, 613–622.

Appleby, P.G., Oldfield, F., Thompson, R., Huttunen, P. & Tolonen, K. (1979). 210Pb dating of annually laminated lake sediments from Finland. *Nature*, 280, 53–55.

Barnes, M., Turner, C., Jerde, C., Renshaw, M., Chadderton, W. & Lodge, D.M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environ. Sci. Technol.*, 48, 1819–1827.

Behnke RJ. 1992. Native trout of western North America. *Am Fish Soc Monogr (USA) no 6*.

Binford, M. (1990). Calculation and uncertainty analysis of 210Pb dates for PIRLA project lake sediment cores. *J. Paleolimnol.*, 3, 253–267.

Campbell, N.R., Amish, S.J., Pritchard, V.L., Mckelvey, K.S., Young, M.K., Schwartz, M.K., *et al.* (2012). Development and evaluation of 200 novel SNP assays for population genetic studies of westslope cutthroat trout and genetic identification of related taxa. *Mol. Ecol. Resour.*, 12, 942–949.

Carpenter, S.R. & Leavitt, P.R. (1991). Temporal Variation in a Paleolimnological Record Arising from a Trophic Cascade. *Ecology*, 72, 277–285.

Domaizon, I., Winegardner, A., Capo, E., Gauthier, J. & Gregory-Eaves, I. (2017). DNA-based methods in paleolimnology: new opportunities for investigating long-term dynamics of lacustrine biodiversity. *J. Paleolimnol.*, 58, 1–21.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., *et al.* (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev. Camb. Philos. Soc.*, 81, 163–82.

Ficetola, G.F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., De Barba, M., *et al.* (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Mol. Ecol. Resour.*, 15, 543–556.

Finney, B.P., Gregory-Eaves, I., Douglas, M.S. V. & Smol, J.P. (2002). Fisheries productivity in the northeastern Pacific Ocean over the past 2,200 years. *Nature*, 416, 729–733.

Giguet-Covex, C., Pansu, J., Arnaud, F., Rey, P.-J., Griggo, C., Gielly, L., *et al.* (2014). Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nat. Commun.*, 5, 3211.

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., *et al.* (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods Ecol. Evol.*, 7, 1299–1307.

Kalinowski, S.T., Novak, B.J., Drinan, D.P., deM Jennings, R. & Vu, N. V. (2011). Diagnostic single nucleotide polymorphisms for identifying westslope cutthroat trout (Oncorhynchus clarki lewisi), Yellowstone cutthroat trout (Oncorhynchus clarkii bouvieri) and rainbow trout (Oncorhynchus mykiss). *Mol. Ecol. Resour.*, 11, 389–393.

Kovach, R.P. (2018). Long-term population dynamics and conservation risk of migratory bull trout in the upper Columbia River basin. *Can. J. Fish. Aquat. Sci.*, https://doi.org/10.1139/cjfas-2017-0466.

Kovach, R.P., Muhlfeld, C.C., Boyer, M.C., Lowe, W.H., Allendorf, F.W. & Luikart, G. (2015). Dispersal and selection mediate hybridization between a native and invasive species. *Proceedings. Biol. Sci.*, 282, 20142454.

Lamontagne, S. & Schindler, D.W. (1994). Status of Fish Populations in Canadian Rocky Mountain Lakes Inferred from Subfossil Chaoborus (Diptera: Chaoboridae) Mandibles. *Can. J. Fish. Aquat. Sci.*, 51, 1376–1383.

Magnuson, J.J. (1990). Long-Term Ecological Research and the Invisible Present. *Bioscience*, 40, 495–501.

Mayhood D. 1995. The fishes of the Central Canadian Rockies Ecosystem. FWR Freshwater Research Limited Report 950408 prepared for Parks Canada, Banff National Park.

Mayhood, D.W. & Taylor, E.B. (2011). Contributions to a recovery plan (Oncorhynchus clarkii lewisi) in Alberta : Threats and limiting factors.

McLellan, R., Iyengar, L., Jeffries, B., Oerlemans, N. & Editors. (2014). *Living planet report 2014: species and spaces, people and places*. *World Wide Fund Nat.*

Messner, J., Maclellan, M. & Vinebrooke, R.D. (2013). Higher temperatures enhance the effects of invasive sportfish on mountain zooplankton communities. *Freshw. Biol.*, 58, 354–364.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., *et al.* (2009). Hybridization rapidly reduces fitness of a native trout in the wild. *Biol. Lett.*, 5, 328–31.

Muhlfeld, C.C., Kovach, R.P., Jones, L.A., Al-Chokhachy, R., Boyer, M.C., Leary, R.F., *et al.* (2014). Invasive hybridization in a threatened species is accelerated by climate change. *Nat. Clim. Chang.*, 4, 620–624.

Olajos, F., Bokma, F., Bartels, P., Myrstener, E., Rydberg, J., Öhlund, G., *et al.* (2018). Estimating species colonization dates using DNA in lake sediment. *Methods Ecol. Evol.*, 9, 535–543.

Phillips, V.J.A., St. Louis, V.L., Cooke, C.A., Vinebrooke, R.D. & Hobbs, W.O. (2011). Increased Mercury Loadings to Western Canadian Alpine Lakes over the Past 150 Years. *Environ. Sci. Technol.*, 45, 2042–2047.

Prince, D.J., O'Rourke, S.M., Thompson, T.Q., Ali, O.A., Lyman, H.S., Saglam, I.K., *et al.* (2017). The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Sci. Adv.*, 3, e1603198.

Rhymer, J.M. & Simberloff, D. (1996). Extinction by Hybridization and Introgression. *Annu. Rev. Ecol. Syst.*, 27, 83–109.

Robinson, Z.L., Coombs, J.A., Hudy, M., Nislow, K.H., Benjamin H., L. & Whiteley, A.R. (2017). Experimental Test of Genetic Rescue in Isolated Populations of Brook Trout. *Mol. Ecol.*, 26, 4418–4433.

Schindler, D.E., Hilborn, R., Chasco, B., Boatright, C.P., Quinn, T.P., Rogers, L.A., *et al.* (2010). Population diversity and the portfolio effect in an exploited species. *Nature*, 465, 609–612.

Shepard BB. 2004. Factors That May Be Influencing Nonnative Brook Trout Invasion and Their Displacement of Native Westslope Cutthroat Trout in Three Adjacent Southwestern Montana Streams. *North Am J Fish Manag* **24**: 1088–100.

Shepard, B.B., May, B.E. & Urie, W. (2005). Status and Conservation of Westslope Cutthroat Trout within the Western United States. *North Am. J. Fish. Manag.*, 25, 1426–1440.

Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T., *et al.* (2016). Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. *Methods Ecol. Evol.*, 8, 635–645.

Stager, J., Sporn, L., Johnson, M. & Regalado, S. (2015). Of Paleo-Genes and Perch: What if an" Alien" Is Actually a Native? *PLoS One*, e0119071.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., *et al.* (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.*, 21, 2565–2573.

Turner, C.R., Uy, K.L. & Everhart, R.C. (2014). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biol. Conserv.*, 183, 93–102.

Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C. & Tallmon, D.A. (2015). Genetic rescue to the rescue. *Trends Ecol. Evol.*, 30, 42–49.

Wilcox, T.M., McKelvey, K.S., Young, M.K., Jane, S.F., Lowe, W.H., Whiteley, A.R., *et al.* (2013). Robust detection of rare species using environmental DNA: the importance of primer specificity. *PLoS One*, 8, e59520.

Figures

Figure 1. Map of Western Canada, highlighting the location of Banff National Park and showing the locations of Mystic and Marvel Lakes within Banff National Park. Shaded areas are the major drainages within the study area.

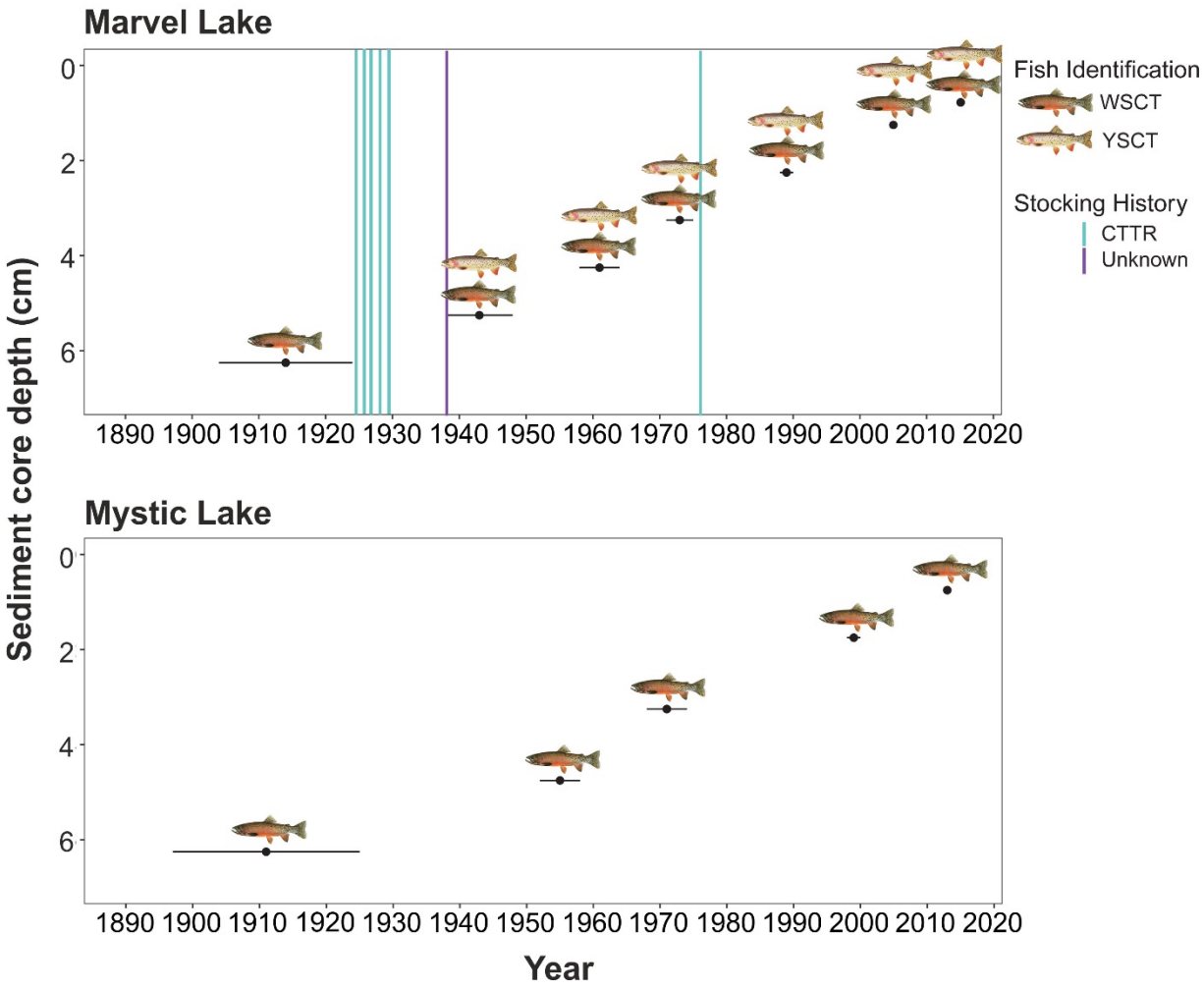


Figure 2. Historical fish presence in Marvel and Mystic Lakes. Lake sediment DNA analysis revealed a shift in species composition following the introduction of fish in Marvel Lake in the mid to late 1920s. Points are stratigraphically plotted using Pb-210 radioisotopes. Horizontal error bars represent all possible error in the dating process. Fish presence/absence for each point is illustrated with an icon of westslope cutthroat trout (WSCT) and/or Yellowstone cutthroat trout (YSCT). Vertical bars represent individual human-mediated introductions with colors respective of different fish species.

As sub-species data was not present in original reports, CTTR represents all cutthroat trout variants. Fish images © Joseph Tomelleri.

Figure 3. Log Pb^{210} profile for each core. Error bars represent 1 standard deviation.

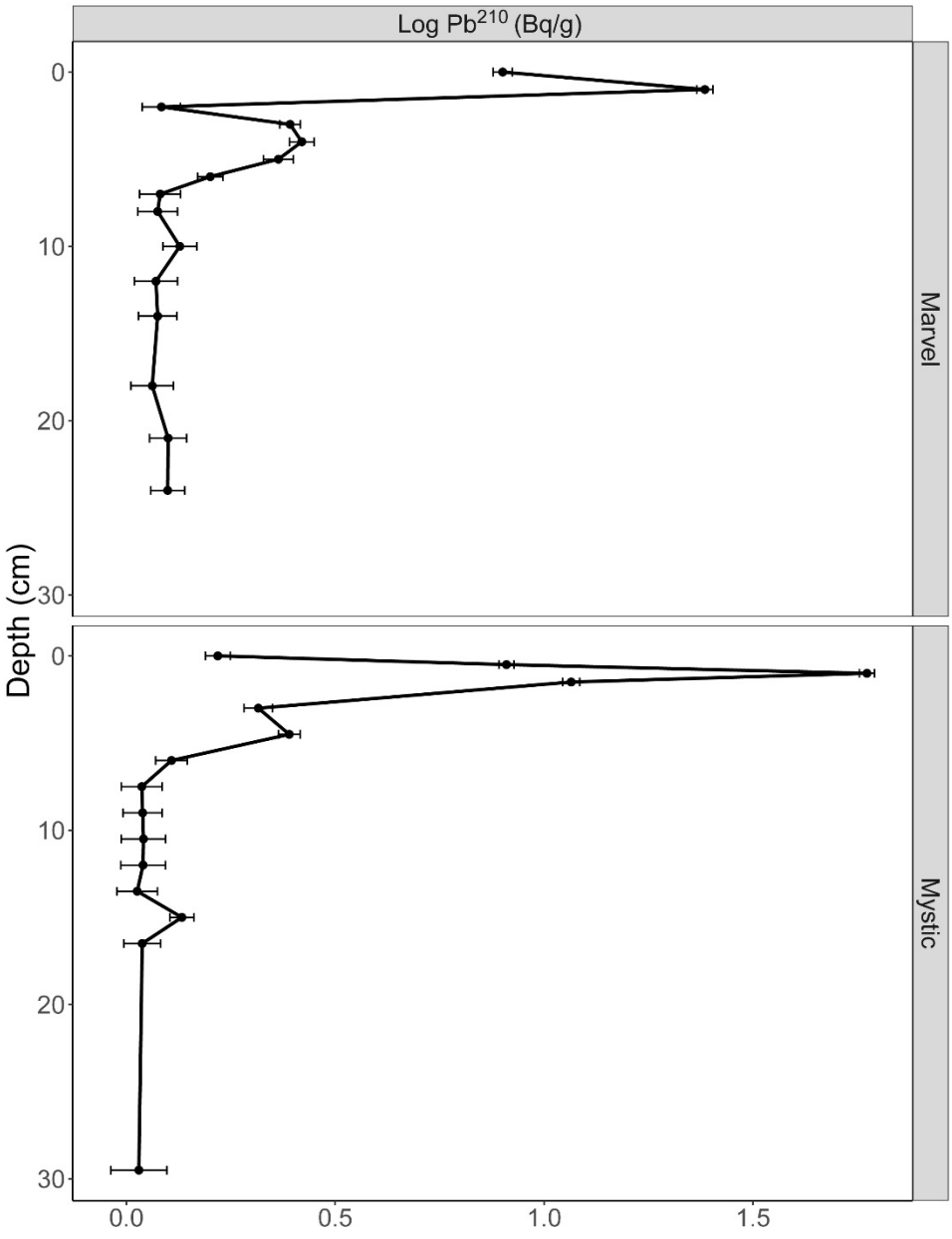
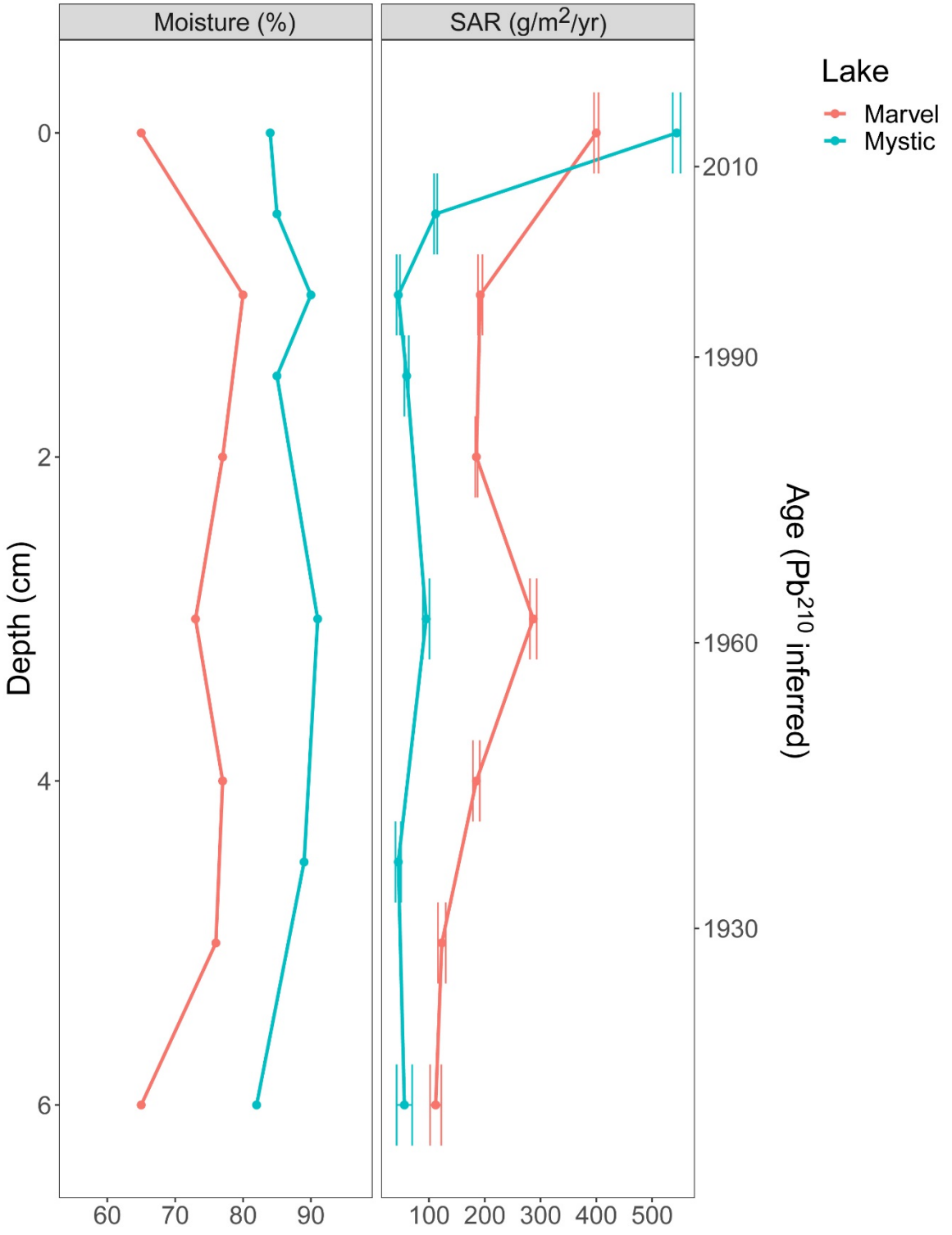


Figure 4. Percent of moisture and sediment accumulation rate for each core. Error bars represent the coefficient of variation.



Tables

Table 1. Select physical characteristics and status of fish presence from study lakes in Banff and Yoho National Parks, Canada.

Lake	Elevation (m a.s.l.)	Surface Area (ha)	Maximum Depth (m)	Fish Status	Last Stocked	Sediment Core Depth (cm)
Baker	2226	32.1	9	Present	1969	25.5
Big Fish	2227	14.6	44	Present	N/A	18.5
Deer	2186	77.4	23	Present	1966	19.5
Elk	2129	3.9	7	Present	N/A	12.0
Egypt	2042	15.7	46	Present	1965	17.5
Luellen	1981	30.2	16	Present	1953	24.0
Marvel	1798	195	67	Present	1976	24.5
Mystic	2013	8.7	17	Present	N/A	30.0
Oesa	2270	16.7	39	Fishless	N/A	15
Sawback	2044	17.8	24	Present	N/A	20

Table 2. Assay name, SNP database identification, forward and reverse primer sequences, and genotypes for rainbow trout (RBTR), westslope cutthroat trout (WSCT), and yellowstone cutthroat trout (YSCT). Hespero assay was developed from genbank popset 219665046 and is diagnostic for *Hesperodiaptomus arcticus.*

Assay Name	dbSNP	Primer sequences (5'-3')	RBTR	WSCT	YSCT
Ocl_mtC1C2-490	ss494474935	F:ACCACGCTCTTATGATTGTTCTTCTTATC R:CAAACGATCTCGATTTCTTGAGAATCA	A	A	G
Ocl_clk3W4[1]	ss244236265	F:GCAGGGCAGCACTCCA R:CTGGTGTCTCGTAACAGGTTCTG	C	A	C
Ocl_gshpx-357	ss494474974	F:GAGATCCTGAGGTCCCTGAAGTAT R:AAGTGGAAATTTGGGCTCAAAGC	C	A	A
Ocl_txnip-393	ss494475108	F:GGTCATTACTGTAAATCCGCCAGAT R:GCCATTTCAAAAGGCTGCATGT	G	G	A
Thymo-320	ss177926246	F:TCTGACATTATGATATGACACATGAA R:CATTGAAATGACGTGGAATCA	A	G	G
Ocl_WD_98683L	ss494475067	F:GGCGGAATGATACCAGAGTG R:GGAGTGTCCTCTTGCCAGGT	G	A	G
Hespero	N/A	F - ACTCCGTGACGCAATGTGAA R - TGTTTCAACCGACCCTGACC			

References

Allendorf, F.W., Leary, R.F., Spruell, P. & Wenburg, J.K. (2001). The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.*, 16, 613–622.

Appleby, P.G., Oldfield, F., Thompson, R., Huttunen, P. & Tolonen, K. (1979). 210Pb dating of annually laminated lake sediments from Finland. *Nature*, 280, 53–55.

Barnes, M., Turner, C., Jerde, C., Renshaw, M., Chadderton, W. & Lodge, D.M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environ. Sci. Technol.*, 48, 1819–1827.

Binford, M. (1990). Calculation and uncertainty analysis of 210Pb dates for PIRLA project lake sediment cores. *J. Paleolimnol.*, 3, 253–267.

Campbell, N.R., Amish, S.J., Pritchard, V.L., Mckelvey, K.S., Young, M.K., Schwartz, M.K., *et al.* (2012). Development and evaluation of 200 novel SNP assays for population genetic studies of westslope cutthroat trout and genetic identification of related taxa. *Mol. Ecol. Resour.*, 12, 942–949.

Carpenter, S.R. & Leavitt, P.R. (1991). Temporal Variation in a Paleolimnological Record Arising from a Trophic Cascade. *Ecology*, 72, 277–285.

Domaizon, I., Winegardner, A., Capo, E., Gauthier, J. & Gregory-Eaves, I. (2017). DNA-based methods in paleolimnology: new opportunities for investigating long-term dynamics of lacustrine biodiversity. *J. Paleolimnol.*, 58, 1–21.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., *et al.* (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev. Camb. Philos. Soc.*, 81, 163–82.

Ficetola, G.F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., De Barba, M., *et al.* (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Mol. Ecol. Resour.*, 15, 543–556.

Finney, B.P., Gregory-Eaves, I., Douglas, M.S. V. & Smol, J.P. (2002). Fisheries productivity in the northeastern Pacific Ocean over the past 2,200 years. *Nature*, 416, 729–733.

Giguet-Covex, C., Pansu, J., Arnaud, F., Rey, P.-J., Griggo, C., Gielly, L., *et al.* (2014). Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nat. Commun.*, 5, 3211.

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., *et al.* (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods Ecol. Evol.*, 7, 1299–1307.

Kalinowski, S.T., Novak, B.J., Drinan, D.P., deM Jennings, R. & Vu, N. V. (2011). Diagnostic single nucleotide polymorphisms for identifying westslope cutthroat trout (Oncorhynchus clarki lewisi), Yellowstone cutthroat trout (Oncorhynchus clarkii bouvieri) and rainbow trout (Oncorhynchus mykiss). *Mol. Ecol. Resour.*, 11, 389–393.

Kovach, R.P. (2018). Long-term population dynamics and conservation risk of migratory bull trout in the upper Columbia River basin. *Can. J. Fish. Aquat. Sci.*, https://doi.org/10.1139/cjfas-2017-0466.

Kovach, R.P., Muhlfeld, C.C., Boyer, M.C., Lowe, W.H., Allendorf, F.W. & Luikart, G. (2015). Dispersal and selection mediate hybridization between a native and invasive species. *Proceedings. Biol. Sci.*, 282, 20142454.

Lamontagne, S. & Schindler, D.W. (1994). Status of Fish Populations in Canadian Rocky Mountain Lakes Inferred from Subfossil Chaoborus (Diptera: Chaoboridae) Mandibles. *Can. J. Fish. Aquat. Sci.*, 51, 1376–1383.

Magnuson, J.J. (1990). Long-Term Ecological Research and the Invisible Present. *Bioscience*, 40, 495–501.

Mayhood D. 1995. The fishes of the Central Canadian Rockies Ecosystem. FWR Freshwater Research Limited Report 950408 prepared for Parks Canada, Banff National.

Mayhood, D.W. & Taylor, E.B. (2011). Contributions to a recovery plan (Oncorhynchus clarkii lewisi) in Alberta : Threats and limiting factors.

McLellan, R., Iyengar, L., Jeffries, B., Oerlemans, N. & Editors. (2014). *Living planet report 2014: species and spaces, people and places*. *World Wide Fund Nat.*

Messner, J., Maclellan, M. & Vinebrooke, R.D. (2013). Higher temperatures enhance the effects of invasive sportfish on mountain zooplankton communities. *Freshw. Biol.*, 58, 354–364.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., *et al.* (2009). Hybridization rapidly reduces fitness of a native trout in the wild. *Biol. Lett.*, 5, 328–31.

Muhlfeld, C.C., Kovach, R.P., Jones, L.A., Al-Chokhachy, R., Boyer, M.C., Leary, R.F., *et al.* (2014). Invasive hybridization in a threatened species is accelerated by climate change. *Nat. Clim. Chang.*, 4, 620–624.

Olajos, F., Bokma, F., Bartels, P., Myrstener, E., Rydberg, J., Öhlund, G., *et al.* (2018). Estimating species colonization dates using DNA in lake sediment. *Methods Ecol. Evol.*, 9, 535–543.

Phillips, V.J.A., St. Louis, V.L., Cooke, C.A., Vinebrooke, R.D. & Hobbs, W.O. (2011). Increased Mercury Loadings to Western Canadian Alpine Lakes over the Past 150 Years. *Environ. Sci. Technol.*, 45, 2042–2047.

Prince, D.J., O'Rourke, S.M., Thompson, T.Q., Ali, O.A., Lyman, H.S., Saglam, I.K., *et al.* (2017). The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Sci. Adv.*, 3, e1603198.

Rhymer, J.M. & Simberloff, D. (1996). Extinction by Hybridization and Introgression. *Annu. Rev. Ecol. Syst.*, 27, 83–109.

Robinson, Z.L., Coombs, J.A., Hudy, M., Nislow, K.H., Benjamin H., L. & Whiteley, A.R. (2017). Experimental Test of Genetic Rescue in Isolated Populations of Brook Trout. *Mol. Ecol.*, 26, 4418–4433.

Schindler, D.E., Hilborn, R., Chasco, B., Boatright, C.P., Quinn, T.P., Rogers, L.A., *et al.* (2010). Population diversity and the portfolio effect in an exploited species. *Nature*, 465, 609–612.

Shepard BB. 2004. Factors That May Be Influencing Nonnative Brook Trout Invasion and Their Displacement of Native Westslope Cutthroat Trout in Three Adjacent Southwestern Montana Streams. *North Am J Fish Manag* **24**: 1088–100.

Shepard, B.B., May, B.E. & Urie, W. (2005). Status and Conservation of Westslope Cutthroat Trout within the Western United States. *North Am. J. Fish. Manag.*, 25, 1426–1440.

Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T., *et al.* (2016). Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. *Methods Ecol. Evol.*, 8, 635–645.

Stager, J., Sporn, L., Johnson, M. & Regalado, S. (2015). Of Paleo-Genes and Perch: What if an" Alien" Is Actually a Native? *PLoS One*, e0119071.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., *et al.* (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.*, 21, 2565–2573.

Turner, C.R., Uy, K.L. & Everhart, R.C. (2014). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biol. Conserv.*, 183, 93–102.

Uutala A. 1990. Chaoborus (Diptera: Chaoboridae) mandibles-paleolimnological indicators of the historical status of fish populations in acid-sensitive lakes. *J Paleolimnol* **4**: 139–51.

Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C. & Tallmon, D.A. (2015). Genetic rescue to the rescue. *Trends Ecol. Evol.*, 30, 42–49.

Wilcox, T.M., McKelvey, K.S., Young, M.K., Jane, S.F., Lowe, W.H., Whiteley, A.R., *et al.* (2013). Robust detection of rare species using environmental DNA: the importance of primer specificity. *PLoS One*, 8, e59520.

Appendices

Appendix 1. Read counts for Marvel and Mystic Lakes including field positive controls, extraction negative controls, and fish counts. Also included are a field negative control (Oesa Lake) and PCR negative control. Read counts were only conducted on sections of sediment that had discrete, non-overlapping dating information.

Supplementary Table 1.1 Read Counts for Marvel Lake including field positive controls (*H.arcticus*), Yellowstone cutthroat trout (YSCT), westslope cutthroat trout (WSCT), and rainbow trout (RBTR).

Marvel Lake Read Counts							
Top of Section (cm)	Bottom of Section (cm)	Age (years)	Standard Deviation (Years)	WSCT Read Count	YSCT Read Count	RBTR Read Count	Field Positive Control Read Count
0	0.5	2014	0	5	3	0	61524
1	1.5	2005	0	7	6	0	50179
2	2.5	1989	1	17	5	0	66369
3	3.5	1973	2	13	4	0	52759
4	4.5	1961	3	8	9	0	64531
5	5.5	1943	5	6	3	0	58687
6	6.5	1914	10	44	0	0	70762

Supplementary Table 1.2 Read Counts for Mystic Lake including field positive controls (*H.arcticus*), Yellowstone cutthroat trout (YSCT), westslope cutthroat trout (WSCT), and rainbow trout (RBTR).

Mystic- Fish Count							
Top of Section (cm)	Bottom of Section (cm)	Age (years)	Standard Deviation (Years)	WSCT Read Count	YSCT Read Count	RBTR Read Count	Field Positive Control Read Count
0	0.5	2014	0	9	0	0	318
1.5	2.0	1999	1	76	0	0	6024
3	3.5	1971	3	15	0	0	770
4.5	5.0	1955	3	26	0	0	5857
6	6.5	1911	14	53	0	0	297

Supplementary Table 1.3 Extraction negative controls read counts for Marvel Lake and Mystic Lake for *H.arcticus*, Yellowstone cutthroat trout (YSCT), westslope cutthroat trout (WSCT), and rainbow trout (RBTR).

Extraction Control Negatives				
Lake	H.arcticus Read Count	WSCT Read Count	YSCT Read Count	RBTR Read Count
Marvel	1	0	0	0
Mystic	1	0	0	0

Supplementary Table 1.4 PCR negative controls read counts for *H.arcticus*, Yellowstone cutthroat trout (YSCT), westslope cutthroat trout (WSCT), and rainbow trout (RBTR).

PCR Negative Controls				
	H.arcticus Read Count	WSCT Read Count	YSCT Read Count	RBTR Read Count
PCR Negative Controls	6	0	0	0

Supplementary Table 1.5 Field extraction negative control read counts from Oesa Lake for *H.arcticus*, Yellowstone cutthroat trout (YSCT), westslope cutthroat trout (WSCT), and rainbow trout (RBTR).

Oesa- Field Negative Control				
Sediment Core Depth (cm)	H.arcticus Read Count	WSCT Read Count	YSCT Read Count	RBTR Read Count
0-2.5	26	0	0	0

Appendix 2. Raw data for all study lakes that were subjected to Pb 210 radioisotopic analysis.

Supplementary Table 2.1 Raw Pb-210 radioisotopic data from MyCore Scientific Ltd.

Lake	Section of Core Top (cm)	Section of Core Bottom (cm)	Moisture (%)	Pb-210 (Bq/g)	Precision (%)	Age (year)	Sediment Accumulation Rate (g/m2/yr)	Coefficient of Variation in SAR (%)	Standard Deviation (years)
Moose Lake									
	0	0.5	81%	0.439	2	2014	252	5	0
	0.5	1	74%	0.420	2	2011	238	5	0
	1	1.5	70%	0.381	2	2005	224	5	0
	1.5	2	65%	0.247	3	1998	291	7	1
	2	2.5	66%			1991	351	Interpolated	
	3	3.5	54%	0.115	5	1980	463	18	6
	3.5	4	37%			1974	495	Interpolated	
	4.5	5	57%	0.084	4	1960	431	27	14
	5	5.5	56%			1955	465	Interpolated	
	6	6.5	56%	0.067	4	1946	467	44	30
	6.5	7	58%			1941	417	Interpolated	
	7.5	8	58%	0.064	5	1930	315	50	42
	8	8.5	55%			1921	295	Interpolated	
	9	9.5	56%	0.055	5	1893	161	79	95
	9.5	10	53%						
	10.5	11	41%	0.042	6				
	11	11.5	40%						
	12	12.5	37%	0.027	7				
	12.5	13	39%						
	13.5	14	63%	0.045	7				
	14	14.5	60%						
	15	15.5	51%	0.039	6				
	15.5	16	55%						
	16.5	17	57%	0.049	5				

	17	17.5	59%						
	21.5	22	31%	0.043	7				
Egypt Lake									
	0	0.5	95%	0.848	2	2014	105	4	0
	0.5	1	90%	1.079	2	2011	73	4	0
	1	1.5	89%	1.306	2	2007	54	4	0
	1.5	2	87%	1.288	2	2000	44	4	1
	2	2.5	86%			1987	50	Interpolated	
	3	3.5	84%	0.253	7	1944	48	15	10
	3.5	4	83%			1925	50	Interpolated	
	4.5	5	86%	0.068	5				
	5	5.5	85%						
	6	6.5	84%	0.061	6				
	6.5	7	83%						
	7.5	8	81%	0.045	7				
	8	8.5	82%						
	9	9.5	86%	0.068	6				
	9.5	10	84%						
	10.5	11	88%	0.069	6				
	11	11.5	87%						
	12	12.5	86%	0.045	6				
	12.5	13	84%						
	13.5	14	87%	0.061	6				
	14	14.5	85%						
	15	15.5	86%	0.057	6				
	15.5	16	84%						
	16.5	17	83%	0.049	8				
	17	0	83%	0.053	7				
Big Fish Lake									
	0	0.5	55%	0.167	3	2014	523	14	0
	0.5	1	67%	0.345	3	2011	197	8	0

	1	1.5	69%	0.353	2	2001	139	6	1
	1.5	2	73%	0.361	2	1989	93	6	2
	2	2.5	65%			1974	120	Interpolated	
	3	3.5	43%	0.035	7				
	3.5	4	65%						
	4.5	5	67%	0.057	5				
	5	5.5	65%						
	6	6.5	47%	0.031	8				
	6.5	7	38%						
	7.5	8	63%	0.042	6				
	8	8.5	64%						
	9	9.5	66%	0.045	6				
	9.5	10	65%						
	10.5	11	55%	0.046	6				
	11	11.5	65%						
	12	12.5	61%	0.043	6				
	12.5	13	64%						
	13.5	14	64%	0.025	8				
	14	14.5	67%						
	15	15.5	69%	0.053	8				
	15.5	16	63%						
	16.5	17	57%	0.019	10				
	17	17.5	67%						
	18	18.5	55%	0.040	7				
Baker Lake									
	0	0.5	90%	1.164	2	2014	109	4	0
	0.5	1	87%	1.026	2	2004	91	4	1
	1	1.5	86%	0.881	2	2000	96	4	1
	1.5	2	86%	0.883	2	1995	82	4	1
	2	3	85%			1989	79	Interpolated	
	3	3.5	85%	0.648	3	1973	58	6	3

	3.5	4.5	83%			1959	66	Interpolated	
	4.5	5	79%	0.178	3	1912	57	17	20
	5	6	82%			1890	51	Interpolated	
	6	6.5	80%	0.106	3				
	6.5	7.5	84%						
	7.5	8	80%	NA	NA				
	8	9	80%						
	9	9.5	77%	0.101	3				
	9.5	10.5	76%						
	10.5	11	76%	0.087	4				
	11	12	77%						
	12	12.5	79%	0.101	4				
	12.5	13.5	75%						
	13.5	14	80%	0.087	4				
	14	15	82%						
	15	15.5	74%	0.080	4				
	15.5	16.5	76%						
	16.5	17	80%	0.088	4				
	17	25	77%						
	25	25.5	44%	0.027	7				
Marvel Lake									
	0.0	0.5	65%	0.901	2.3	2014	400	4	0
	0.5	1.0	97%			2011	277	Interpolated	
	1.0	1.5	80%	1.385	1.9	2005	192	4	0
	1.5	2.0	74%			1997	184	Interpolated	
	2.0	2.5	77%	0.084	4.6	1989	185	2	1
	2.5	3.0	72%			1980	204	Interpolated	
	3.0	3.5	73%	0.392	2.5	1973	287	6	2
	3.5	4.0	61%			1968	235	Interpolated	
	4.0	4.5	77%	0.420	3.0	1961	185	6	3
	4.5	5.0	68%			1953	155	Interpolated	

	5.0	5.5	76%	0.364	3.6	1943	123	7	5
	5.5	6.0	76%			1929	112	Interpolated	
	6.0	6.5	65%	0.201	3.0	1914	112	10	10
	6.5	7.0	57%			1899	129	Interpolated	
	7.0	7.5	40%	0.081	4.9	1886	550	97	124
	7.5	8.0	42%			1883	670	Interpolated	
	8.0	8.5	44%	0.075	4.7	1881	920	182	242
	8.5	9.0	37%			1880	159	Interpolated	
	10.0	10.5	52%	0.128	4.1	1870	64	20	29
	10.5	11.0	52%						
	12.0	12.5	52%	0.071	5.1				
	12.5	13.0	51%						
	14.0	14.5	20%	0.075	4.6				
	14.5	15.0	32%						
	18.0	18.5	31%	0.062	5.1				
	18.5	19.0	49%						
	21.0	21.5	62%	0.100	4.5				
	21.5	22.0	65%						
	24.0	24.5	59%	0.099	4.1				
Sawback Lake									
	0.0	0.5	85%	0.324	2.9	2014			0
	0.5	1.0	44%	0.085	5.2	2012			0
	1.0	1.5	62%	0.441	2.5	2008	572	5	0
	1.5	2.0	63%			2004	443	3	
	2.0	2.5	80%	0.721	2.3	1998	345	4	1
	2.5	3.0	73%			1987	226	Interpolated	
	3.0	3.5	53%	0.286	3.1	1976	147	4	2
	3.5	4.0	58%			1969	152	Interpolated	
	4.0	4.5	58%	0.195	3.5	1961	202	6	4
	4.5	5.0	40%			1953	193	Interpolated	
	5.0	5.5	56%	0.124	3.9	1944	190	7	6

	5.5	6.0	54%			1937	187	Interpolated	
	6.0	6.5	52%	0.107	4.1	1927	198	9	8
	6.5	7.0	38%			1916	169	Interpolated	
	8.0	8.5	38%	0.052	4.2	1905	140	10	23
	8.5	9.0	40%			1898	146	Interpolated	
	10.0	10.5	38%	0.043	5.7	1890	209	21	40
	10.5	11.0	41%			1882	198	Interpolated	
	12.0	12.5	37%	0.034	5.1	1874	191	32	79
	12.5	13.0	40%			1867	196	Interpolated	
	14.0	14.5	33%	0.043	4.8	1854	225	57	50
	14.5	15.0	41%				127	Interpolated	
	16.0	16.5	37%	0.026	6.6		65	31	
	16.5	17.0	34%						
	18.0	18.5	33%	0.020	7.0				
	18.5	19.0	32%						
	19.5	20.0	33%	0.027	5.6				
Deer Lake									
	0.0	0.5	86%	0.728	2.6	2014	258	5	0
	0.5	1.0	84%	0.769	2.1	2013	236	4	0
	1.0	1.5	81%	0.817	1.9	2011	208	4	0
	1.5	2.0	81%	0.808	1.8	2008	189	4	0
	2.0	3.5	80%			2003	199	Interpolated	
	3.5	4.0	78%	0.515	3.0	1987	159	6	2
	4.0	5.0	79%			1980	148	Interpolated	
	5.0	5.5	78%	0.398	2.2	1965	107	5	2
	5.5	6.5	78%			1956	127	Interpolated	
	6.5	7.0	77%	0.156	3.7	1934	137	12	10
	7.0	8.0	75%			1926	156	Interpolated	
	8.0	8.5	77%	0.086	4.1	1909	182	30	32
	8.5	9.5	75%			1904	169	Interpolated	
	9.5	10.0	73%	0.080	4.4	1889	115	36	46

	10.0	11.0	72%						
	11.0	11.5	66%	0.054	5.0				
	11.5	12.5	66%						
	12.5	13.0	74%	0.055	4.7				
	13.0	14.0	74%						
	14.0	14.5	64%	0.052	5.5				
	14.5	15.5	62%						
	15.5	16.0	44%	0.038	6.1				
	16.0	17.0	43%						
	17.0	17.5	43%	0.048	5.2				
	17.5	19.0	44%						
	19.0	19.5	53%	0.171	5.1				
Luellen Lake									
	0.0	0.5	77%	0.304	1.7	2014	444	3	0
	0.5	1.0	82%	0.656	1.4	2012	181	3	0
	1.0	1.5	78%	0.480	1.5	2009	227	3	0
	1.5	2.0	69%	0.297	1.7	2005	337	3	0
	2.0	3.0	72%			1998	344	Interpolated	
	3.0	3.5	66%	0.193	2.2	1990	348	4	1
	3.5	4.5	64%			1985	391	Interpolated	
	4.5	5.0	59%	0.119	2.4	1975	406	6	2
	5.0	6.0	48%			1971	507	Interpolated	
	6.0	6.5	50%	0.067	3.2	1958	597	11	6
	6.5	7.5	50%			1953	533	Interpolated	
	7.5	8.0	50%	0.064	4.4	1943	413	15	10
	8.0	9.0	57%			1934	353	Interpolated	
	9.0	9.5	57%	0.057	4.8	1921	267	18	17
	9.5	10.5	58%			1912	250	Interpolated	
	10.5	11.0	57%	0.047	4.8	1892	175	27	33
	11.0	12.0	57%						
	12.0	12.5	43%	0.034	5.6				

	12.5	13.5	54%						
	13.5	14.0	41%	0.032	6.4				
	14.0	15.0	51%						
	15.0	15.5	35%	0.032	6.0				
	15.5	16.5	32%						
	16.5	17.0	36%	0.032	6.0				
	17.0	23.5	40%						
	23.5	24.0	43%	0.030	6.7				
Elk Lake									
	0.0	1.0	48%	0.086	3.5	Not Dated			
	1.0	2.0	44%	0.079	3.8				
	2.0	3.0	37%	0.067	4.1				
	3.0	4.0	25%	0.050	4.2				
	4.0	5.0	28%	0.045	5.6				
	5.0	6.0	37%	0.070	4.1				
	6.0	7.0	41%	0.080	3.5				
	7.0	8.0	32%	0.059	5.4				
	8.0	9.0	41%	0.058	6.1				
	9.0	10.0	38%	0.042	5.7				
	10.0	11.0	41%	0.052	5.3				
	11.0	12.0	46%	0.052	5.4				
Mystic Lake									
	0.0	0.5	84%	0.219	3.0	2014	544	7	0
	0.5	1.0	85%	0.910	1.8	2013	112	3	
	1.0	1.5	90%	1.773	1.8	2006	45	3	
	1.5	2.0	85%	1.065	2.0	1999	60	4	1
	2.0	3.0	82%			1991	75	Interpolated	
	3.0	3.5	91%	0.316	3.4	1971	95	6	3
	3.5	4.5	87%			1969	77	Interpolated	
	4.5	5.0	89%	0.390	2.6	1955	45	5	3
	5.0	6.0	86%			1943	51	Interpolated	

6.0	6.5	82%	0.108	3.8	1911	56	14	14
6.5	7.5	84%						
7.5	8.0	73%	0.037	4.9				
8.0	9.0	85%						
9.0	9.5	87%	0.039	4.7				
9.5	10.5	88%						
10.5	11.0	88%	0.041	5.3				
11.0	12.0	83%						
12.0	12.5	86%	0.040	5.3				
12.5	13.5	86%						
13.5	14.0	85%	0.026	4.8				
14.0	15.0	89%						
15.0	15.5	87%	0.133	2.9				
15.5	16.5	86%						
16.5	17.0	86%	0.038	4.4				
17.0	29.5	86%						
29.5	30.0	74%	0.030	6.7				