Environmental DNA in lake sediment reveals biogeography of native genetic diversity

Hedin T Nelson-Chorney[1,2*], Corey S Davis[3], Mark S Poesch[1], Rolf D Vinebrooke[3], Christopher M Carli[4], and Mark K Taylor[1,2]

Understanding the historical distributions of species is vital to the conservation and restoration of native species, yet such information is often qualitative. 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 sedDNA). This method was validated through the detection of lake sedDNA from non-native trout (Yellowstone cutthroat trout, *Oncorhynchus clarkii bouvieri*), which corroborated historical records of human-mediated introductions. We also discovered native trout (westslope cutthroat trout, *Oncorhynchus clarkii lewisi*) lake sedDNA that predated human-mediated introductions of freshwater fishes in a watershed with high topographical relief. This unexpected result revealed that the westslope population was of native origin and requires immediate conservation protection. Our findings demonstrate that lake sedDNA 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.

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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). Biodiversity losses are occurring at an increasingly higher rate in freshwater ecosystems than in terrestrial or marine environments (Dudgeon *et al.* 2006). The average abundance of freshwater vertebrates worldwide declined by 81% between 1970 to 2012, while vertebrate abundance in marine and terrestrial environments fell by 36% and 38%, respectively (McLellan *et al.* 2014). Despite these conspicuous declines, understanding the historical distribution of imperiled species is hampered by 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.

Reconstructing the histories of naturally occurring (native) and introduced (non-native) fishes in freshwater environments is difficult, particularly for endangered, rare, and closely related species. Several lines of paleolimnological evidence (eg "stratigraphic" changes [in this context, changes preserved in successive layers of sedimentary deposits] in the abundance of large-bodied invertebrates [Lamontagne and Schindler 1994]), stable isotope signatures (Finney *et al.* 2002), or changes in algal communities (Carpenter and Leavitt 1991) are typically used as proxies for direct enumeration of fish occupancy. However, such inferences may result in incorrect estimates of the presence or absence of aquatic species due to a lag effect from the beginning of an environmental alteration to the subsequent biological response (Magnuson 1990). Furthermore, these indirect measures on lake communities lack the adequate taxonomic resolution necessary to distinguish fish species (Lamontagne and Schindler 1994).

Genetic material shed by an aquatic organism into the environment, known as 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 because degradation is expected to be relatively slow under cold and unproductive conditions (Figure 1; Barnes *et al.* 2014). The stratigraphic deposition of eDNA in lake sediments can provide details of historical species occupancy (Domaizon *et al.* 2017) given that it can be preserved for thousands of years as sedimentary eDNA (lake sedDNA; Stager *et al.* 2015; Olajos *et al.* 2018). Moreover, lake sediments are fully saturated and compacted, which prevents the downward migration of water and aqueous (water saturated) DNA (Giguet-Covex *et al.* 2014). This provides undisturbed temporal succession of lake sedDNA. To date, however, lake sedDNA has not been used to simultaneously determine the histories of both native and non-native fish populations.

Despite the immense socioeconomic 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

[1]*Department of Renewable Resources, University of Alberta, Edmonton, Canada *(nelsonch@ualberta.ca);* [2]*Parks Canada Agency, Banff National Park, Banff, Canada;* [3]*Department of Biological Sciences, University of Alberta, Edmonton, Canada;* [4]*Fisheries and Oceans Canada, Whitehorse, Canada*

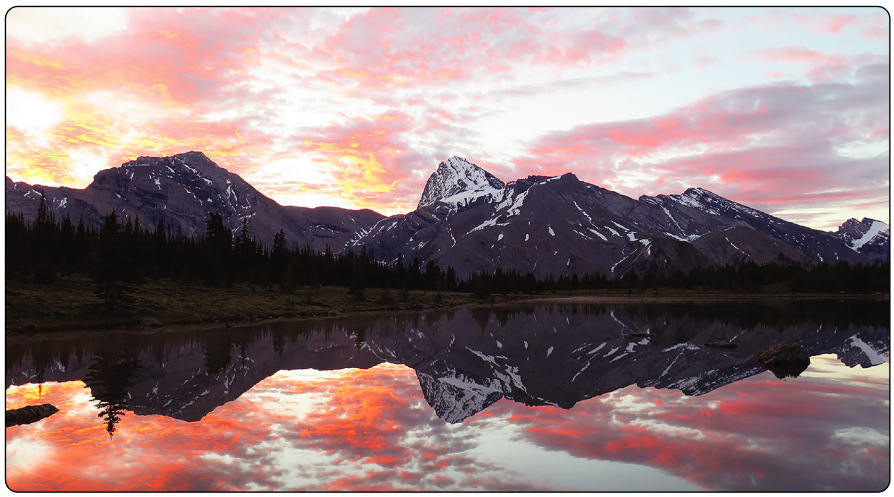

Figure 1. A typical remote, high-elevation lake in Banff National Park, Alberta, Canada, characterized by relatively little shoreline vegetation, cold water, and low levels of human use.

experienced substantial 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, but this historical area remains largely unknown (Shepard *et al.* 2005). As such, identification of pre-anthropogenic populations is critical to species recovery because these areas may contain sources of native genetic diversity that can inform 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.

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; alleles at specific loci that are fixed for a given species) were amplified from lake sedDNA and sequenced to detect the historical presence of two common cutthroat trout species. Our objectives were to test whether lake sedDNA evidence revealed historical events of human-mediated trout introductions and to reconstruct the unknown history of a westslope cutthroat trout population that was thought to have been artificially propagated.

Methods

Study area

We focused on eight lakes in Banff National Park (Alberta, Canada; WebTable 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 (WebTable 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). The absence of a 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

During the winters of 2014 and 2015, we collected two lake sediment cores from the deepest portion of each lake using a mini-Glew gravity corer (Glew 1991). All cores from the lakes ranged in total length from 12 cm to 30 cm, and captured a horizontal sediment–water interface (WebTable 1). In general, the water content level was typical of cores obtained from other lakes in the area where sediments contain low levels of organic matter, ranging from <10% to 20% of total core weight after loss on ignition (Phillips *et al.* 2011). The stratigraphies of all cores from these small and deep lakes appeared well layered, changing gradually from black gyttja (organic content) in the upper sediments to gray silt farther down each core. Cores were extruded on site at 0.5-cm intervals. To prevent DNA contamination across samples, we thoroughly cleaned all extruding equipment with a 60% bleach solution between sections. To further prevent cross-contamination between sections, we collected only the central square centimeter from each sectioned sediment interval for lake sedDNA analysis. Replicate 0.1–0.2 g subsamples were taken from each core and stored at –80°C in a freezer, with one sample used for lake sedDNA analysis. The remainder of each section was lyophilized (freeze-dried) and submitted to personnel at MyCore Scientific (Ottawa, Canada) for ^{210}Pb radioisotopic analysis. Dating of sections was interpreted with the constant rate of supply model. Age estimate precision of each section included error associated with weighing, radiochemical extraction, radionuclide counting, and all uncertainties in the differential equations used for dating. To prevent misinterpretation of lake sedDNA results, we included sediment cores (ie lakes) in subsequent analysis only if the confidence interval at each age point did not overlap with adjacent dates and their associated error. Furthermore, only discrete points with reported confidence intervals surrounding the age of the sediment were included in lake sedDNA analysis. The only sediment cores that met these stringent criteria were collected from Marvel Lake and Mystic Lake (Figure 2) and, therefore, only the cores from these two lakes were used for lake sedDNA extraction and sequencing (WebFigures 1 and 2).

Lake sedDNA extraction and sequencing

A PowerSoil DNA Isolation Kit (MO BIO Laboratories; Carlsbad, CA) was used to extract lake sedDNA from sediment samples in a "clean" laboratory (that is, one that had never been exposed to salmonid DNA prior to this study). DNA from each sediment core was extracted individually over a 2-day period and was stored in isolation from the DNA of the other cores. Two diagnostic loci with SNPs unique to westslope cutthroat trout, Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), and rainbow trout (*Oncorhynchus mykiss*) were used (WebTable 2; Kalinowski *et al.* 2011; Campbell *et al.* 2012). These three species were chosen because 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 sedDNA extraction control to test for the presence of the calanoid copepod *Hesperodiaptomus arcticus*, which is ubiquitous in high-elevation lakes throughout the study area (WebTable 2; Messner *et al.* 2013). A negative extraction control (sterile water sample extracted at the same time 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 four and three loci) using the following polymerase chain reaction (PCR) conditions: 4 µL of extracted lake sedDNA; 5 µL of PCR Multiplex MasterMix (QIAGEN; Hilden, Germany); 0.5 µL transposon tailed, locus-specific forward primer (Illumina; San Diego, CA); 0.5 µL transposon tailed, locus-specific reverse primer (Illumina; San Diego, CA); and 0.1 µL Invitrogen Platinum *Taq* HiFi (Thermo Fisher Scientific; Waltham, MA). Thermocycling conditions consisted of an initial denaturation period of 95°C for 15 min followed by 12 cycles of 95°C for 30 s, 60°C for 90 s, and 72°C for 30 s, then a final extension of 60°C for 30 min. All lake sedDNA amplifications were performed in the same run, which also included a negative PCR control. PCR products from the two reactions were then pooled and purified with Agencourt AMPure XP beads (Beckman Coulter; Brea, CA) according to the manufacturer's directions, and diluted to 1:20 with double-distilled water (ddH_2O). Purified PCR products were individually indexed using an Illumina Nextera XT dual-indexed PCR that included 5 µL diluted pooled amplicons, 5 µL forward index primer, 5 µL reverse index primer, 25 µL 2× KAPA HiFi PCR reaction mix (Kapa Biosystems; Wilmington, MA), and 10 µL ddH_2O. Index PCR thermocycling conditions were the same as the conditions in 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.

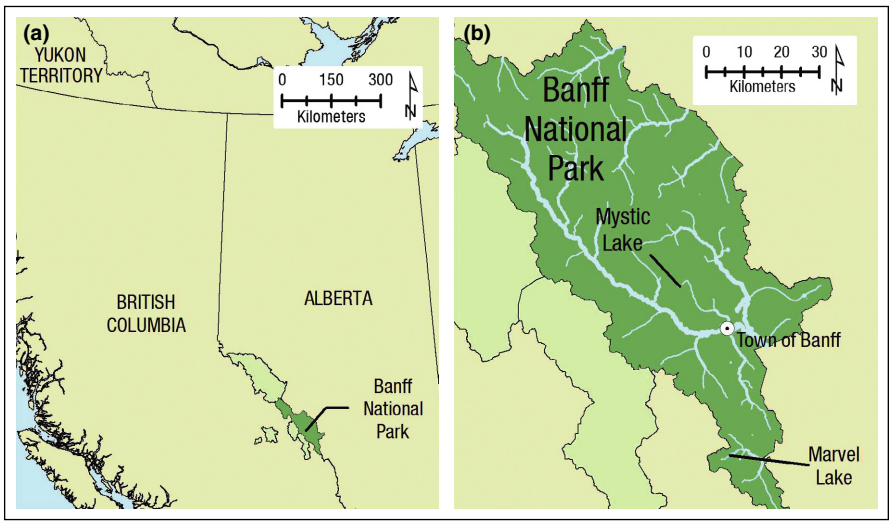

Figure 2. (a) Map of western Canada showing the location of Banff National Park. (b) The locations of Mystic Lake and Marvel Lake within Banff National Park. Shaded areas are the major drainages within the study area.

Bioinformatics

Amplicon alignment and SNP genotyping were performed with Geneious R9 (Biomatters; Auckland, New Zealand). Reads were mapped to seven (six trout and one zooplankton) reference sequences. Reference sequences were standardized to 51 base pairs (bp), which allowed for 25 bp 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 (WebTable 3). The "find variations/SNP" function in Geneious R9 was used to call SNP sites. Unexpected sequences were removed following basic local alignment search tool (BLAST) identification to remove bacterial sequence contamination.

Results

Lake sedDNA evidence of Yellowstone cutthroat trout corroborated historical reports of its introduction (circa 1925) and subsequent persistence in Marvel Lake (Figure 3; WebTable 2; Mayhood and Taylor 2011). Although Marvel Lake was assumed to be naturally fishless due to the surrounding geographic relief, our lake sedDNA analysis suggested that westslope cutthroat trout might have been present prior to documented

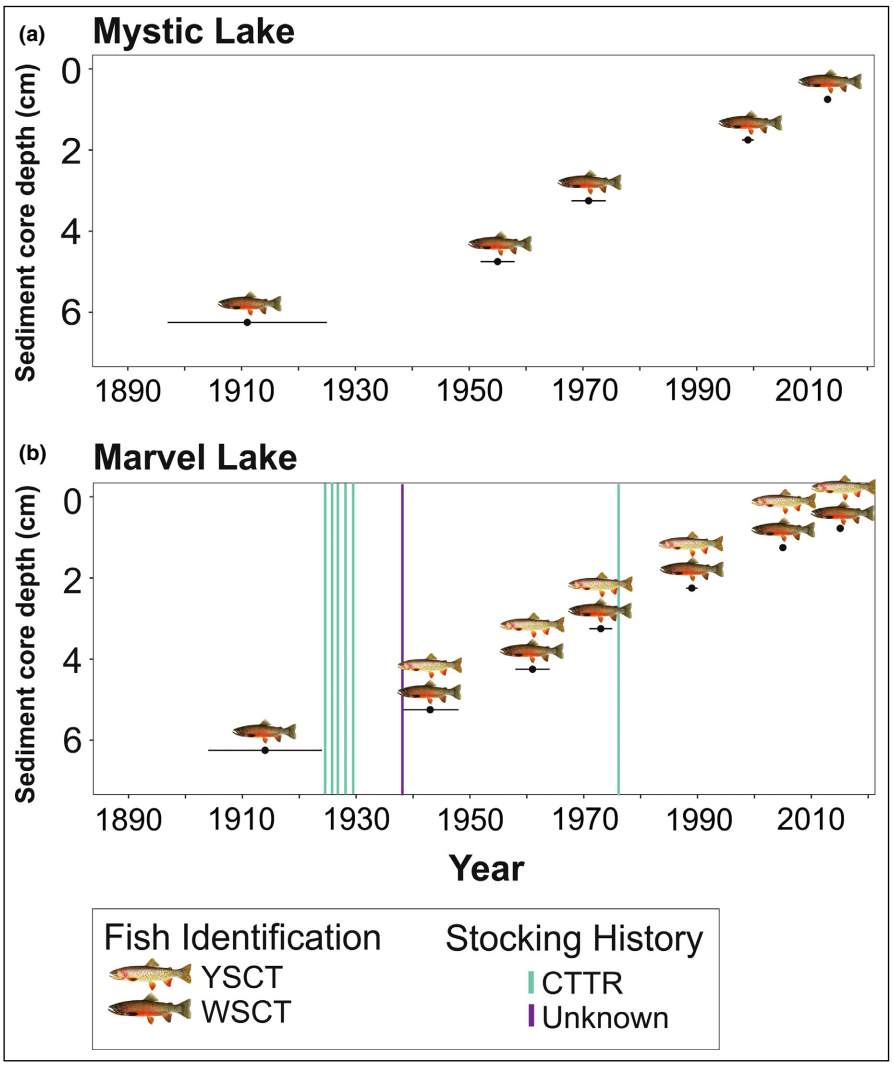

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Figure 3. Historical fish presence in (a) Mystic Lake and (b) Marvel Lake. Lake sedDNA analysis revealed a shift in species composition following the introduction of fish in Marvel Lake in the mid- to late-1920s. Points are stratigraphically plotted using ^{210}Pb 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 (*Oncorhynchus clarkii lewisi*; WSCT) and/or Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*; YSCT). Vertical bars represent individual human-mediated introductions, with colors denoting different fish species. As subspecies data were not present in the original reports, CTTR represents all cutthroat trout variants.

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, in Mystic Lake, our lake sedDNA analyses revealed an unexpected finding with important ramifications for conservation and management (Figure 3). 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, analysis of lake sedDNA collected from Mystic Lake indicated that westslope cutthroat trout have inhabited 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. For this lake, a lack of lake sedDNA 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 contamination across samples (WebTable 3). Moreover, the ubiquitous presence of lake sedDNA from the endemic alpine zooplankton species *H arcticus* across all lake sediment cores confirmed that DNA had been preserved sufficiently in cold mountain lakes to yield extractable and amplifiable amounts.

Discussion

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

Analysis of lake sedDNA provides a powerful tool for reconstructing the evolutionary history of freshwater organisms. As freshwater biodiversity continues to decline worldwide, it is essential that the genetic diversity of imperiled populations be maintained. The use of lake sedDNA analysis to identify historical remnant populations and simultaneously locate the presence of non-native species is paramount to the conservation of native freshwater fishes. This approach may provide important insights into the legacy of human-induced changes to freshwater ecosystems, including community-level changes associated with historical human-mediated species introductions.

Although contemporary sampling of tissue can provide information about the ancestry of freshwater fish populations that potentially interbreed, lake sedDNA analysis of sediment cores can reveal unknown introductions that have altered native fish diversity. Hybridization (interbreeding between

two species) and genetic introgression (gene flow from one species to another after successive rounds of hybridization with the parental species) due to human-mediated introductions of non-native species have resulted in the extinction of native populations, lineages, and species of freshwater fishes (Rhymer and Simberloff 1996). This is particularly true in fishes like 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, the historical range of westslope cutthroat trout, a threatened species in Canada, has decreased in area by up to 95%, in large part through hybridization with rainbow trout (Mayhood and Taylor 2011). The few remaining disjunct populations are the last sources of native diversity for the species in the province of Alberta and provide important genetic material that may be required 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 through the translocation of individuals harboring native genetic diversity may offset the deleterious effects of inbreeding depression and enhance the local genetic pool, resulting in an increase in fitness (Whiteley *et al.* 2015). Furthermore, to maintain viable populations, translocation to areas free from non-native genetic diversity may provide refuge for the last remaining individuals of a native lineage. As lake sedDNA 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 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. As such, the ability to detect the presence of non-native genetic material, even when relatively rare, demonstrates the sensitivity of lake sedDNA 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.

Potential sources of uncertainty in the ^{210}Pb radioisotopic dating of deeper core intervals restricted our results to the past 100 years. Although the true age in our oldest sediment sections may have been underestimated (Binford 1990), our results revealed a distinct change in fish species prior to documented introductions. Because the stratigraphies of the sediment cores were intact, carbon dating would have enabled us to extend the time frame of our investigation from decades to centuries and explore generational trends in freshwater fish populations. In addition, it may be possible to also explore the relative abundance of specific functional genes, such as those influencing migration timing in Pacific salmon (Prince *et al.* 2017), to determine historical fluctuations in migration timing and therefore 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. Furthermore, 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 sedDNA researchers 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 sedDNA revealed the century-long presence of native westslope cutthroat trout in two lakes thought to contain only introduced fishes. Although there possibly were indigenous introductions of fishes into Marvel Lake and Mystic Lake prior to 1900, we have presented the first evidence that fish may have been present before any documented introductions. This discovery substantially alters 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 and Taylor 2011). Our findings therefore have immediate conservation implications, affording protection to westslope cutthroat populations that were previously considered to be of lower conservation value.

Our results 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 that can be used to identify restoration areas where long-term persistence of the species is possible. Lake sedDNA is an environmentally low-impact method for reconstructing the historical presence of aquatic species while improving our understanding of the legacy effects of human-mediated species translocations.

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Supporting Information

Additional, web-only material may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/fee.2073/suppinfo

