NRC Research Press

RAPID COMMUNICATION

Using forensic geochemistry via fish otoliths to investigate an illegal fish introduction

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Abstract: Illegal fish introductions create some of the most challenging problems for resource managers because of their potential to harm existing recreational fisheries and their impact on species of conservation concern. Determining the origin of a suspected illegal fish introduction can aid managers in preventing the colonization and subsequent ecosystem impacts of introduced species. In this study, we used forensic geochemistry via fish otoliths to investigate an illegal walleye (*Sander vitreus*) introduction in Swan Lake, Montana, which provides critical habitat for threatened bull trout (*Salvelinus confluentus*) and native westslope cutthroat trout (*Oncorhynchus clarkii*). Core to edge geochemical profiles of $^{87}Sr/^{86}Sr$ and Sr/Ca ratios in the walleye otoliths revealed that these fish had been introduced to Swan Lake within the past growing season, and their geochemical signature matched that of walleye sampled from Lake Helena, Montana, located 309 road kilometres away. This research highlights application of a tool fisheries managers can use to identify the natal waterbody source of illegally introduced fish.

Résumé : Les introductions illégales de poissons créent certains des problèmes les plus difficiles à régler pour les gestionnaires de ressources en raison des dommages qu'elles peuvent causer aux pêches sportives et de leur impact sur des espèces dont la conservation est préoccupante. La détermination de l'origine d'une introduction illégale soupçonnée de poissons peut aider les gestionnaires à prévenir la colonisation d'espèces introduites et ses impacts subséquents sur l'écosystème. Dans cette étude, nous avons appliqué la géochimie médicolégale aux otolites de poissons pour étudier une introduction illégale de dorés jaunes (*Sander vitreus*) dans le lac Swan (Montana), qui fournit un habitat critique pour l'omble à tête plate (*Salvelinus confluentus*) et la truite fardée versant de l'Ouest indigène (*Oncorhynchus clarkii*), des espèces menacées. Les profils géochimiques du centre vers la bordure des rapports $^{87}Sr/^{86}Sr$ et Sr/Ca dans des otolites de dorés révèlent que ces poissons ont été introduits dans le lac Swan durant la dernière saison de croissance et leur signature géochimique concorde avec celle de dorés jaunes prélevés du lac Helena (Montana), situé à 309 km par la route. L'étude met en relief l'application d'un outil dont les gestionnaires des pêches peuvent se servir pour déterminer le plan d'eau natal de poissons introduits illégalement. [Traduit par la Rédaction]

Introduction

Biotic invasions have greatly altered both aquatic and terrestrial systems worldwide (Mack et al. 2000; Simberloff et al. 2013). In fresh waters, the introduction and spread of non-native fishes has decreased biodiversity and imperiled freshwater species (Jenkins 2003), consequently impacting local economies and cultural resources. The introduction of non-native fishes has a long history in North America. Federal and state agencies, including the US Fish Commission (established 1871), were founded with the mission to increase fishery resources through hatchery propagation and stocking (Nielsen 1999; Rahel 2004). Despite evidence that introducing non-native fishes can have detrimental ecosystem effects, only recently have agencies worked to assess stocking practices and manage invasive species (Johnson et al. 2009). Contemporary methods by which unauthorized fish introductions occur include illegal plants by anglers, bait bucket releases, escapes from aquaculture or aquaria, and ballast water exchange (Benson 1999; Rahel 2004). While some recent fish introductions have been supported by national or local governments (e.g., Azevedo-Santos et al. 2016), illegal planting by anglers is now the most common means by which new species are introduced to waters where they were not historically present. Illegal fish introductions may be perpetuated by the fishery profession's historical practice of stocking, leading to confusion among the public about native fish stocking efforts (Johnson et al. 2009). Such trends are even more troubling given the rise of invasive species denialism (Russell and Blackburn 2017).

Illegal fish introductions can impact water quality, aquatic habitat, and diminish angling opportunity, creating problems for fisheries managers that may necessitate chemical rehabilitation, suppression programs, or additional fish stocking to supplement the impacted fishery (Johnson et al. 2009). However, determining when and where introduced fish will become established is difficult (Garcia-Berthou 2007), and interactions between fish invaders and anthropogenic disturbance make it difficult to predict the effects invasion may have on ecosystems, recreational fisheries, and native species (Rahel and Olden 2008; Leprieur et al. 2009). Once established, the reduction or elimination of unauthorized fish species is often challenging and resource-intensive. With the exception of small isolated waters or in instances where illegal introductions are quickly detected, eradication of species is expensive and challenging (Marr et al. 2010). Therefore, management actions during the initial introduction and dispersal stages provide the greatest chance of preventing the establishment and negative impacts of introduced species (Vander Zanden and Olden 2008). To deter illegal introductions, fisheries managers can use innovative tools and technologies to identify reliably sources of illegal introductions, thus aiding law enforcement in convicting

Received 2 March 2018. Accepted 8 August 2018.

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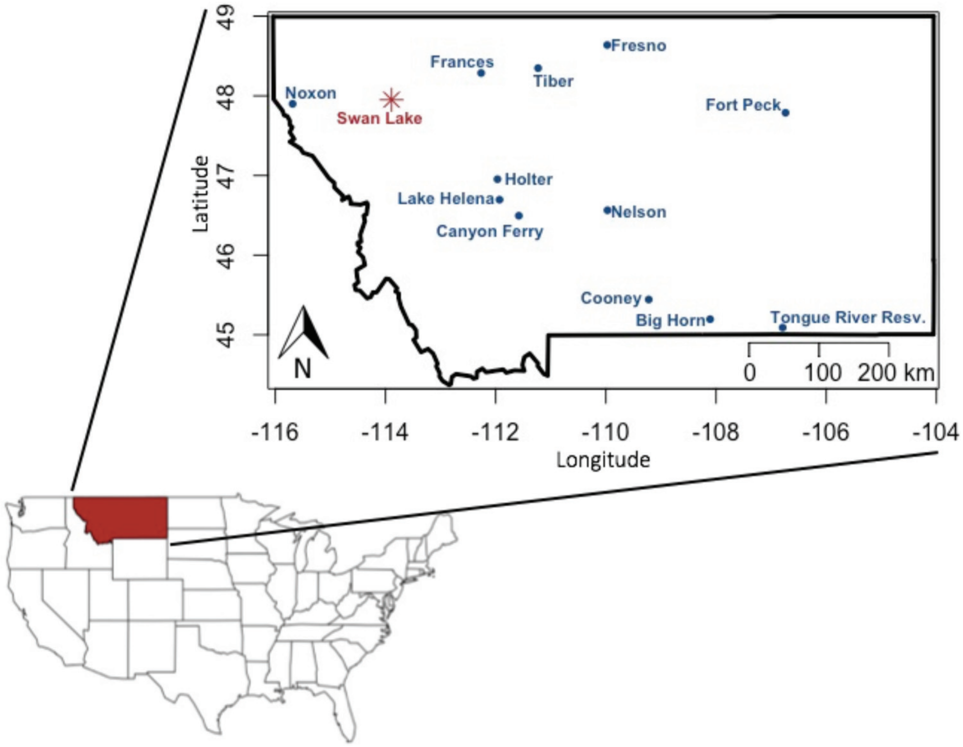
Published at www.nrcresearchpress.com/cjfas on 26 August 2018.

Fig. 1. Map of Montana showing waterbodies where walleye were sampled to build the geochemical database (in blue). Swan Lake is highlighted in red (Becker et al. 2017).



perpetrators and allowing managers to focus prevention efforts on the waterbodies most at risk.

One method for illegal fish source identification uses the chemical chronology of a fish's life history deposited in the inert concentric layers of $CaCO_3$ in otoliths (Kennedy et al. 2000; Barnett-Johnson et al. 2008). Naturally occurring strontium (Sr) substitutes for calcium (Ca) in otoliths, and its relative concentration provides a temporal and spatial record of changes in Sr concentration encountered over a fish's lifetime (Campana and Thorrold 2001; Wells et al. 2003). Sr can be found in differing concentrations among waterbodies due to landscape scale variation in geology, land use, and weathering patterns. The influence of hydroclimatic variation on spatial and temporal stability of water chemistry is moderated by drainage size, with relatively large drainages exhibiting stable $^{87}Sr/^{86}Sr$ isotope values (Wolff et al. 2012). Dissolved Sr concentrations in water are correlated with otolith Sr/Ca (Wells et al. 2003; Bourret et al. 2014) and $^{87}Sr/^{86}Sr$ isotope (Kennedy et al. 1997; Muhlfeld et al. 2005; Brennan et al. 2015) values. The elemental tracer Sr/Ca fluctuates annually and seasonally in the environment, and its uptake in fish otoliths is fractionated by temperature, salinity, and physiology. The $^{87}Sr/^{86}Sr$ geochemical signature is temporally stable and has a direct correlation with ambient water concentrations (Muhlfeld et al. 2012; Brennan et al. 2015). Both Sr/Ca and $^{87}Sr/^{86}Sr$ geochemical signatures have proven useful as forensic tools to identify the source and timing of illegal and invasive fish introductions (Munro et al. 2005; Wolff et al. 2012).

The overall goal of this study was to use forensic geochemistry techniques via fish otoliths to determine the natal origin of illegally introduced walleye (*Sander vitreus*) in a Montana lake. Our major objectives were to (*i*) compare the otoliths of walleye caught in Swan Lake with otoliths of lake trout (*Salvelinus namaycush*) of Swan Lake origin to confirm they were illegally introduced and (*ii*) to build a geochemical database, from the most likely source populations of walleye, that allowed us to assign the two walleye to their natal water body.

Methods

Study site

Our study area encompassed lakes and reservoirs throughout Montana, USA. A specific focus on Swan Lake, a deep (maximum depth 70.4 m), oligotrophic, 1323 ha natural lake in the Flathead River drainage of northwest Montana, was the result of illegal fish introductions (Fig. 1). Once a pristine environment for native fish species, Swan Lake contains critical habitat for species of conservation concern such as bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Oncorhynchus clarkii*), but has been the unfortunate recipient of illegal fish introductions, including northern pike (*Esox lucius*; discovered 1979), lake trout (discovered 1998), and walleye (discovered 2015), all of which pose substantial predation threats to native fish species. Swan Lake is isolated above Big Fork Dam, and there are no connected sources of walleye, making it impossible for walleye to colonize Swan Lake naturally.

Fish collection

Two walleye and three lake trout were caught in Swan Lake in October 2015 with large-mesh gill nets (11.5–12.5 cm) set overnight in shallow lake trout spawning habitats. In 2017, Montana Fish, Wildlife & Parks (MFWP) personnel used experimental mesh gill nets to collect three walleye for otolith geochemical analysis from each of the 13 lakes and reservoirs in Montana (Fig. 1) with the highest number of angler days. Angler days is an estimation of the number of hours spent by anglers fishing for walleye. The 13 lakes were also nearest in road kilometres to Swan Lake (Table 1). These waterbodies were sampled to build a geochemical database used to assess the potential sources of the illegally introduced walleye. Rufus Woods Lake, Washington, is another popular nearby walleye fishery, and three walleye were collected for analysis via hook and line in 2017. These waterbodies were chosen because they represent the fisheries with the highest walleye fishing pressure in the region.

Table 1. The top 13 most angled walleye fisheries in Montana based on mail-in angler creel surveys.

Water body	Angler days	Distance to Swan Lake (road km)
Canyon Ferry	3983	299
Fort Peck Reservoir	3962	742
Holter	1510	253
Tiber	1078	360
Fresno	906	444
Nelson Reservoir	881	631
Cooney Reservoir	776	616
Frances	657	432
Hauser	589	298
Tongue River Reservoir	415	929
Bighorn Reservoir	340	851
Lake Helena	228	309
Noxon Rapids Reservoir	103	261

Note: Also included is the distance to Swan Lake of each fishery via road kilometres.

Otolith geochemistry

Sagittal otoliths were extracted from collected fish with nonmetallic forceps and stored in dry paper scale envelopes. Otoliths were cleaned with Milli-Q water for 5 min and dried overnight and embedded in heat-activated Crystal Bond epoxy sulcus side up on precleaned 25 mm × 75 mm clear glass microscopy slides. Otoliths were polished with 600, 800, and 1200 wet grit silicon carbide adhesive discs in a dorsal plane until the primordium and annual growth rings were visible with reflected light and a compound light microscope.

Otolith sections were assayed for $^{87}Sr/^{86}Sr$ and Sr/Ca with a laser ablation inductively coupled plasma mass spectrometry (ICP-MS). Analysis included a Thermo Finnigan Neptune multiple collector (MC-ICP-MS) coupled to a New Wave 193 nm laser ablation system at the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Laboratory, following the methods reported by Bourret et al. (2014). $^{87}Sr/^{86}Sr$ and Sr/Ca ratios were quantified with a single ablated transect from otolith core to edge at a perpendicular angle to growth rings. Laser ablation transects were implemented using a 50 μm diameter spot size at 100% output energy, with a pulse rate of 10 Hz, and a scan rate of 5 $\mu m \cdot s^{-1}$. Analytical accuracy of LA data was evaluated by measuring the $^{87}Sr/^{86}Sr$ ratio of the US Geological Survey (USGS) MACS3 carbonate standard during each LA run. The $^{87}Sr/^{86}Sr$ LA runs of the carbonate standard mean were 0.70762 (N = 6, 2 SD ± 0.000135), which is within the accepted error value of 0.70754 (Weber et al. 2017). Sr/Ca was measured in otolith samples by converting $^{88}Sr/^{48}Ca$ to Sr/Ca $mmol \cdot mol^{-1}$, and analytical accuracy (0.3%) was assessed with repeated analysis of MACS3 solid carbonate standard (USGS).

Determining Swan Lake walleye origin

Core and edge values were gathered from each individual otolith transect by calculating the mean (M) and standard deviation (SD) of $^{87}Sr/^{86}Sr$ and Sr/Ca from 200 μm long sections at each location. We first compared the core to edge transects of the two Swan Lake walleye with the lake trout. We assumed the lake trout were born in Swan Lake and inhabited the lake for their entire life history and therefore would have a geochemical transect that we could use as a control to compare with the walleye. We hypothesized that the two walleye would have a similar core to edge geochemical transect as the lake trout if they were also naturalized in Swan Lake. We directly compared the Swan Lake walleye $^{87}Sr/^{86}Sr$ and Sr/Ca core values with the edge values. We also calculated the percent change of walleye core values and edge values. Next, we compared the $^{87}Sr/^{86}Sr$ and Sr/Ca core values of the three lake trout with the core values of the two Swan Lake walleye. To determine when the walleye were introduced to Swan Lake, we compared the location of the geochemical signatures' abrupt change on the otolith axis with the last annuli that corresponds to the fishes' last winter.

To assign the two Swan Lake walleye to their natal origin, we used linear discriminant function analysis (DFA; e.g., Wells et al. 2003; Bourret et al. 2014). $^{87}Sr/^{86}Sr$ and Sr/Ca values from the three walleye otoliths from each suspected source (Fig. 1) were pooled to form the groups in the DFA model. The groups were used as the training set in the DFA model with equal prior probability, and jackknifed leave-one-out predictions were used to assess the accuracy of the DFA model predictions. Statistical analyses were conducted in R version 3.2.3 (R development Core Team 2015).

Results

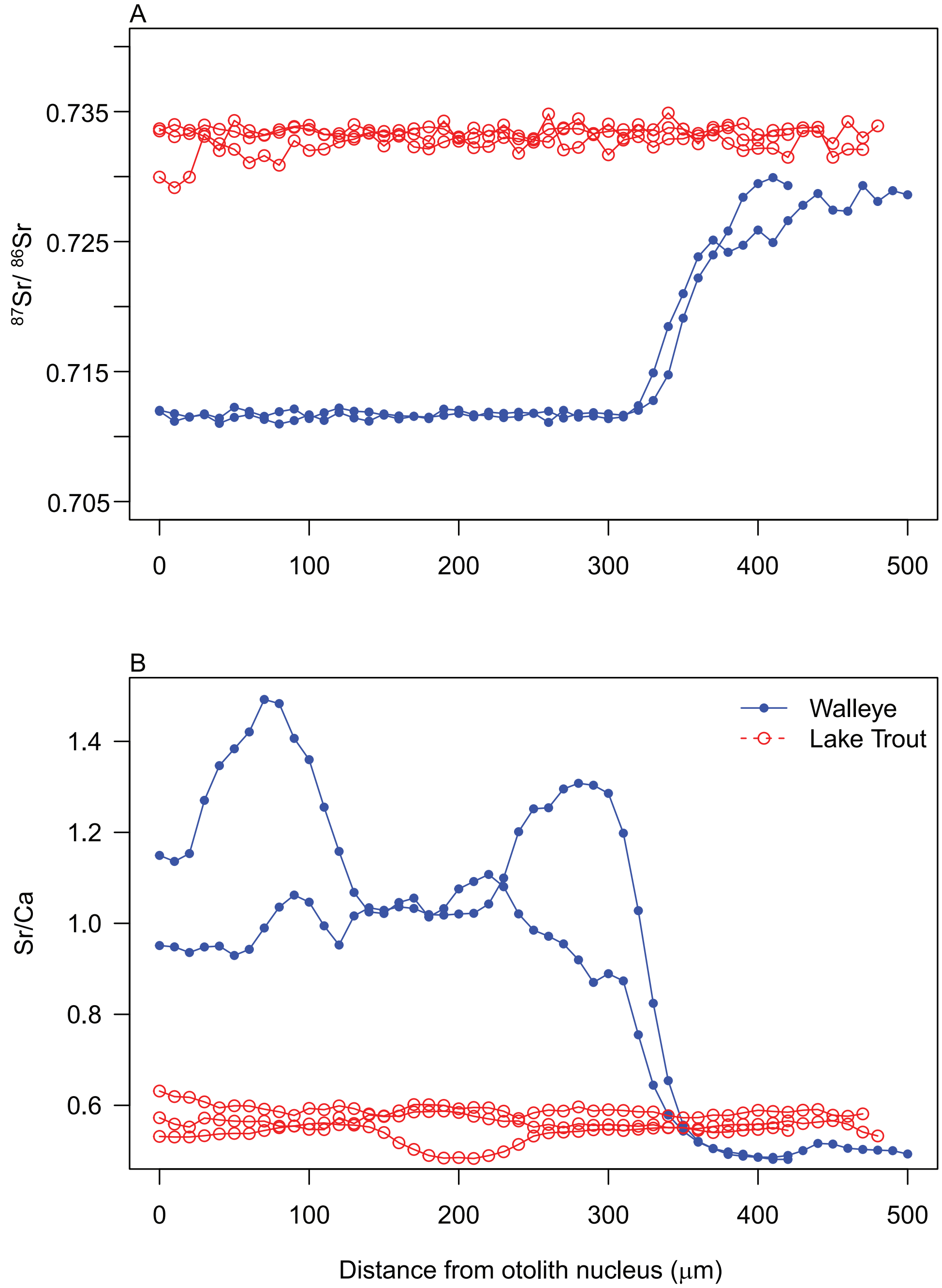
The otolith core to edge transects from the two walleye captured in Swan Lake (total lengths = 43.2 and 44.5 cm) clearly indicated they were not born in Swan Lake, given the abrupt changes in both $^{87}Sr/^{86}Sr$ and Sr/Ca starting at 330 μm from the otolith core (Fig. 2). We found a 2.2% mean change in $^{87}Sr/^{86}Sr$ and 49.44% change in Sr/Ca between core to edge geochemical signatures (Fig. 2). Lake trout from Swan Lake showed very little change in the core to edge geochemical transects, validating their use as a Swan Lake control group. The abrupt change in geochemical signatures of both walleye occurred near the edge of the otolith and further from the core then the last annulus, which indicated the walleye were introduced to Swan Lake after the annulus was formed (winter 2015) and before the fish were captured (October 2015).

The following descriptive statistics compare the mean Swan Lake walleye geochemical core values with edge values, as well as the Swan Lake walleye core values with the Swan Lake lake trout core values. The Swan Lake walleye $^{87}Sr/^{86}Sr$ core values (n = 2, M = 0.712, SD = 0.0002) were lower compared with the $^{87}Sr/^{86}Sr$ edge values (n = 2, M = 0.727, SD = 0.0003) (Fig. 2A). The Swan Lake walleye Sr/Ca core values (n = 2, M = 1.3, SD = 0.18) were higher compared with the Sr/Ca edge values (n = 2, M = 0.56, SD = 0.11; Fig. 2B). The Swan Lake walleye $^{87}Sr/^{86}Sr$ core values (n = 2, M = 0.712, SD = 0.0002) were lower compared with the $^{87}Sr/^{86}Sr$ core values of the lake trout (n = 3, M = 0.733, SD = 0.0007; Fig. 2A). The Swan Lake walleye Sr/Ca core values (n = 2, M = 1.3, SD = 0.18) were higher compared with the Sr/Ca values of the lake trout (n = 3, M = 0.574, SD =0.06; Fig. 2B).

$^{87}Sr/^{86}Sr$ and Sr/Ca values showed discriminatory power based on intrawaterbody similarities and interwaterbody differences (Fig. 3). The mean within-waterbody standard deviation in $^{87}Sr/^{86}Sr$ core values was 0.00013, and the mean standard deviation in Sr/Ca values equaled 0.072. In contrast, we found interwaterbody range in $^{87}Sr/^{86}Sr$ values of 0.723–0.708 and a Sr/Ca range of 1.35–0.39 (Fig. 3). We used $^{87}Sr/^{86}Sr$ and Sr/Ca otolith core values from three walleye captured in popular walleye fisheries in the state of Montana (Table 1) and the Columbia River to train the DFA and predict the natal origin of the two illegally introduced Swan Lake walleye (Fig. 3). Individual geochemical data for walleye otoliths used in the DFA model can be found in the online Supplementary information[1] (Table S1). The DFA correctly classified 95% of individuals in the training data set based on jackknifed leave-one-out predictions, which assess the accuracy of the predictions by excluding one observation, formulating a DFA using the remaining data, and using the function to classify the excluded observation. The only misclassifications occurred in Fresno and Tongue River

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2018-0082.

Fig. 2. (A) Otolith $^{87}Sr/^{86}Sr$ core to edge profiles of fish captured in Swan Lake, including the two walleye and three lake trout. (B) Otolith Sr/Ca core to edge profiles of fish captured in Swan Lake, including the two walleye and three lake trout.



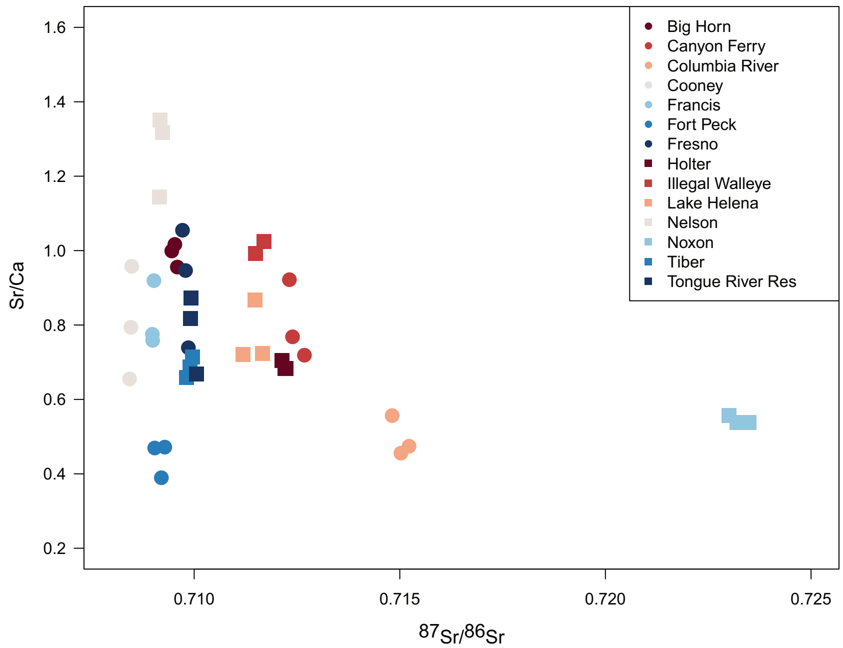
Reservoir (Fig. 3). Using the DFA to predict the origin of the two Swan Lake walleye, both individuals classified to Lake Helena with 0.99 and 0.98 posterior probabilities.

Discussion

Our analysis determined Swan Lake walleye were introduced to Swan Lake based on an abrupt shift in geochemical signatures from the otolith core to edge, representing a chemical inflection point (Fig. 2), which corresponds to a shift in water chemistry. The geochemical inflection point has been used to infer life history shifts in natural populations (Miller et al. 2011; Hegg et al. 2013), as well as in illegal introductions (Munro et al. 2005). The rapid geochemical change in the walleye core to edge transect was unlike the stable geochemical signature of lake trout from Swan Lake, which indicates the lake trout spent their entire lives in a waterbody with homogeneous geochemistry. Comparing the core to edge geochemical signatures of these two species helped confirm the walleye were illegally introduced and not progeny of a reproducing population in Swan Lake. Munro et al. (2005) found a 256% change in Sr/Ca signatures during otolith core to edge geochemical analysis of lake trout illegally introduced to Yellowstone Lake. We found a 2.2% mean change in $^{87}Sr/^{86}Sr$ and 49.44% change in Sr/Ca between core to edge geochemical signatures (Fig. 2). Similar to this study, Munro et al. 2005 found lake trout otolith samples with stable otolith core to edge geochemical signatures, which they concluded were naturalized in Yellowstone Lake.

An alternative explanation for the rapid geochemical change in the walleye core to edge transect is a migration within the inter-

Fig. 3. Bivariate scatterplot showing the distribution in strontium isoscapes from otoliths of fish sampled throughout Montana for the walleye geochemical database, including the two illegally introduced Swan Lake walleye. Each symbol represents an individual walleye otolith from its color-coded sample location.



connected Swan River system, in their recent life history. Although we did not collect data on Swan River geochemical signatures, such an explanation is very unlikely given the fact that 35 years of annual electrofishing in the Swan River and its tributaries, and extensive long-term gillnetting in Swan Lake, have not detected walleye.

We formed a geochemical database of Sr geochemical signatures (^{87}Sr/^{86}Sr, Sr/Ca) using three walleye otoliths from the 13 populations that had the greatest number of annual angler days in Montana. Although many studies have used ^{87}Sr/^{86}Sr and trace elements to predict the natal origin of fishes (Bacon et al. 2004; Walsworth et al. 2014; Walther and Nims 2015), to our knowledge this is the first study to predict the origin of illegally introduced fish over a large geographic area (state of Montana). The DFA model showed high classification accuracy (95% accurate with jackknife resampling) because of the unique and temporally stable geochemical signatures of the otoliths from the analyzed waterbodies (Fig. 3). The DFA suggests that of the waterbodies sampled, Lake Helena is the probable source of the Swan Lake walleye illegal introduction. When classifying the natal origins of invasive fish, a study in the Upper Colorado River Basin also found distinctive and temporally stable reservoir values of ^{87}Sr/^{86}Sr on a decadal scale (Wolff et al. 2012). Large waterbodies likely contain temporally stable ^{87}Sr/^{86}Sr geochemical signatures because their large drainage areas dampen effects of hydrology and climate fluctuation on water storage, thus mixing sufficiently to contain uniform water chemistry (Wolff et al. 2012). Although it did not affect our discriminatory power, we found minor variability in Sr/Ca geochemical signatures within otoliths because of natural physiological, biological, and temporal fluctuation in fish uptake rates (Walther and Thorrold 2009).

Recognizing that the walleye could have originated in numerous places over a large geographic area, waterbodies with walleye populations were sampled based on walleye angler days and proximity to Swan Lake. We hypothesized that these factors were important in determining probable sources of the illegal introduction and were included in the geochemical reference baseline (Munro et al. 2005; Wolff et al. 2012). However, given the technological advances in the aquarium equipment and boat live well tanks, it is possible to keep walleye alive for many days after capture. Therefore, we cannot eliminate the possibility that the illegally introduced walleye could have originated in a waterbody that was not sampled in this study and possesses a geochemical signature similar to Lake Helena. Additionally, given the similar geochemical values of Canyon Ferry and Holter reservoirs to Lake Helena, as well as their close proximity and fluvial connectivity, it is possible that the walleye originated from these waterbodies within this complex of Missouri River impoundments.

The threat of invasive non-native species to global biodiversity and ecosystem heath is a complex ecological, social, and economical problem (Jenkins 2003; Simberloff et al. 2013). The rapid increase in non-native freshwater fish invasions (Leprieur et al. 2009; Cucherousset and Olden 2011) is particularly alarming given the mechanism has shifted from agency-authorized introductions of game and forage fishes in the 20th century to unauthorized introductions of game fish in the 21st century (Rahel 2004). Here we highlight the value in collecting suspected illegally introduced fishes' otoliths, not only to investigate the natal source, but also to build a geochemical database of waterbodies that can be used to identify sources of future illegal fish introductions. MFWP is constructing a database for all geochemical samples collected in lotic and lentic waterbodies that would allow quick response to the detection of future illegal fish introductions. Expansion and inclusion of samples from waterbodies across jurisdictional boundaries would expand this Sr isoscape database and improve the usefulness of this tool for fisheries managers addressing the problem of illegal fish introductions.

This case study provides a tool to fisheries professionals that may improve the problem of illegal fish introductions by providing law enforcement information about where the crime was initiated. Such awareness can aid investigators by determining a place to start looking for additional evidence such as a personal witness, webcams, boat registrations, or fishing license sales. Additionally, the analysis showed the walleye were not naturalized in Swan Lake, which provides managers information pertaining to the population status of the introduced species, therefore determining if or when actions such as suppression are imperative. Previous research has described the human dimensions and motivations behind the crime of illegal fish transport and introduction, concluding that education and outreach are key strategies in addressing the issue (Rahel 2004; Johnson et al. 2009). In this instance, a US$30 000 reward for information leading to the conviction of the person that introduced the walleye to Swan Lake was initiated. Large fines and loss of fishing privileges through a conviction and subsequent sentencing could help deter further illegal introductions. Ultimately, confronting the problem of illegal fish introductions will require a large resource investment that incorporates a public outreach program to protect the value of the fisheries they threaten.

Acknowledgements

We are grateful to the MFWP biologists that collected walleye otoliths throughout the state, including Leo Rosenthal, Ryan Kreiner, Heath Headley, Eric Roberts, Dave Yerk, Mike Ruggles, Cody Nagel, and Ben Bailer. The geochemical analysis was conducted at the Woods Hole Oceanographic Institution by Jerzy S. Blusztajn. Mark Deleray, Matt Boyer, and two anonymous reviewers provided helpful comments that improved this manuscript. SLB was supported by Bonneville Power Administration grant No. 199101903 to MFWP while preparing this manuscript.

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