Title

Stable oxygen isotope analysis confirms natural recruitment of Lake Michigan-origin lake trout (*Salvelinus namaycush*) to the adult life stage

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Abstract

Following Lake Michigan's lake trout (*Salvelinus namaycush*) population collapse in the mid-1900s, fisheries managers began stocking marked juveniles in 1965 in an effort to restore the population. However, there has been little evidence of naturally produced juveniles recruiting to the adult life stage, but recent collections of unmarked adults in Illinois waters of Lake Michigan suggest otherwise. Here, we provide stable isotopic evidence that confirms recruitment to the adult life stage is occurring in southwestern Lake Michigan. Our results indicate substantial differences in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values between hatchery-reared juvenile lake trout and the putative wild adults, effectively eliminating the possibility that unmarked adults were erroneously miss-clipped as hatchery-reared juveniles. We also found significant differences in $\delta^{18}\text{O}$ signatures between lakes Michigan and Huron and successfully classified over 90% of putative wild adult lake trout as Lake Michigan-origin. Trace element analyses were less effective in distinguishing fish from lakes Huron and Michigan than otolith $\delta^{18}\text{O}$. These findings indicate that Lake Michigan's lake trout restoration program is at least partially effective. Further investigation is required to determine what factors may be facilitating natural reproduction and adult recruitment in southwestern Lake Michigan.

Keywords: stable isotope analysis, lake trout, natural reproduction, otoliths, Great Lakes, native species

1. Introduction

Lake trout (*Salvelinus namaycush*) were once the focus of a thriving commercial fishery within Lake Michigan during the 1800s as well as a central component of the lake's fish community (Wells and McLain, 1973; Brown et al., 1981; Holey et al., 1995). Dramatic declines in catch rates began in 1944 (Eshenroder and Amatangelo, 2002) following the invasion of sea lampreys (*Petromyzon marinus*) in 1936 (Smith and Tibbles, 1980). Catch rates were reduced by 95% in 1949, leading to a population collapse occurring in 1955 (Eschmeyer, 1957).

Efforts to re-establish Lake Michigan's lake trout population began in 1965 when the U.S. Fish and Wildlife Service, with state fishery management support, commenced large-scale stocking of yearling lake trout with the ultimate goal of restoring self-sustaining stocks (Holey et al., 1995). However, the ecological complexity inherent to Lake Michigan necessitated a multi-faceted restoration program that included creating no-harvest refuges protecting what was identified as the best spawning habitat available, experimental stockings of different genetic strains, and differential stocking rates depending on location (Holey et al., 1995; Bronte et al., 2003; Bronte et al., 2008). Despite limited collections of viable eggs (Marsden, 1994; Marsden and Janssen, 1997; Jonas et al., 2005; DeKoning et al., 2006) and emergent fry (Jude et al., 1981; Wagner, 1981) as well as intermittent reports of unmarked age-1 and older fish collected by agency sampling (Rybicki, 1991) within Lake Michigan, low-levels or intermittent natural recruitment of lake trout to the adult life stage has not been documented and has been thought not to occur in Lake Michigan (Holey et al., 1995; Bronte et al., 2007). Based on these findings, it is believed that little recruitment to the adult life stage is occurring within the lake (Holey et al., 1995; Bronte et al., 2007).

73 All lake trout stocked in the Great Lakes are marked by clipping one or more fins and any
74 lake trout without a fin clip is assumed to be of wild origin (Bronte et al., 2008). From 1999-
75 2009, the percentage of unmarked adult lake trout sampled in southwestern Lake Michigan
76 ranged from 1.2-7.5% (Patterson et al., in review). Estimates from 2005-2010, however, indicate
77 that approximately 2-3% of stocked fish were unmarked by manually clipping fins, although
78 error rates for fish stocked into particular areas could potentially be as high as 10% (D. Hanson,
79 unpublished data).

80 Recent data collected by the Illinois Department of Natural Resources (IDNR) show a
81 dramatic increase in both the number and proportion of unmarked adult lake trout captured in fall
82 spawning assessments. From 2009 to 2014, the mean percentage of unmarked adult lake trout
83 was 35.8%, and has increased each year since 2009 (Patterson et al., in review). Further, Hanson
84 et al. (2013) reported wild (i.e., unmarked) juvenile lake trout were caught as bycatch in bloater
85 (*Coregonus hoyi*) assessments in Lake Michigan from 2010-2012.

86 There are three potential sources of putative wild lake trout in Lake Michigan. First,
87 clipping errors at the hatchery, prior to stocking, may have resulted in unmarked fish that were
88 introduced to the system. Second, the documented natural reproduction and successful
89 recruitment to the adult stage in Lake Huron over the last 10-15 years (Riley et al., 2007; He et
90 al., 2012) has resulted in migration of adults produced in Lake Huron to southern Lake
91 Michigan. However, relatively few instances of lake trout marked with a fin clip and coded-wire
92 tag that were stocked into Lake Huron and recovered in Lake Michigan have been noted,
93 suggesting immigration of lake trout may be minimal (Bronte et al., 2007; S. Robillard,
94 unpublished data). Third, the collection of an increasing number of unmarked, presumably wild-
95 origin lake trout by IDNR personnel may indicate that natural recruitment to the adult stage is

increasing. Thus, confirming the origin of putative wild lake trout is critical to evaluating management strategies and determining progress towards rehabilitation goals for lake trout in Lake Michigan.

One strategy to determine natal origin in fishes is through the use of otolith chemistry analytical techniques. In the Great Lakes, otolith chemistry has most often been used to study the movements of adfluvial Great Lakes fishes (e.g., see Brazner et al., 2004; Whitley, 2009; Zeigler and Whitley, 2011), but relatively few studies have used the technique to determine natal origin. Yet, stock identification is a common application of otolith chemistry (e.g., Campana et al., 1999; Campana et al., 2000; Pangle et al., 2010; Schaffler et al., 2015), and is particularly robust when known-origin individuals are available with which to compare signatures (Barnett-Johnson et al., 2008). Recently, researchers have applied otolith chemistry techniques to differentiate between hatchery-reared and wild Chinook salmon (*Oncorhynchus tshawytscha*) in Lake Huron (Marklevitz et al., 2011) as well as lake trout in Lake Ontario (Schaner et al., 2007). Their findings demonstrated distinct chemical signatures between hatchery and lake environments. Together, these studies provide support for the application of otolith chemistry as a means of identifying the origins of unmarked lake trout in Lake Michigan.

The application of otolith chemistry analytical techniques to determine natal origin is based on properties of the otoliths themselves. Elements contained within the water column are principally incorporated into otoliths during respiration (Campana, 1999; Walther and Thorrold, 2006) with a secondary source incorporated through diet (Walther and Thorrold, 2006). Otoliths are metabolically inert and no material is resorbed during growth, unlike structures such as bones and scales where calcium may be resorbed during periods of starvation (see Campana and Thorrold, 2001). Therefore, otoliths retain a permanent record of the chemical signatures of the

environments a fish has occupied. Furthermore, the elemental compositions and stable isotopic ratios at a particular location within an otolith reflect local geological and biological processes unique to specific areas and thus act as a natural marker (Campana et al., 2000; Veinott and Porter, 2005; Pangle et al., 2010); the otolith core will reflect the chemical signature of a fish's natal environment and the outer edge of the otolith will reflect the latest environment it has occupied.

The overall goal of this study was to make a definitive determination as to the origin of putative wild lake trout captured in southwestern Lake Michigan. To accomplish this, our first objective was to characterize the otolith stable isotope and trace element signatures of lake trout from all possible hatcheries that provide lake trout for stocking into Lake Michigan. We also wanted to quantify signatures for adult lake trout captured at multiple sites in lakes Huron and Michigan to establish lake-specific signatures. Our second objective was to determine the origin of unmarked lake trout adults captured on Julian's and Waukegan Reefs in the fall of 2012 and 2013 by comparing their otolith signatures to those from hatchery-reared juveniles as well as the adults captured from Lake Huron and Lake Michigan.

2. Materials and methods

2.1. Study design. Sagittal otoliths were collected from adult lake trout caught in fall spawning assessments in lakes Michigan and Huron. The unmarked fish used in the analysis were sampled by the IDNR at Julian's and Waukegan reefs in southwestern Lake Michigan (Fig. 1) in 2012 (n = 71) and 2013 (n = 24). Fifty-eight of the 60 fish used from the 2012 collection were males, while the 24 fish from the 2013 collection demonstrated a more balanced sex ratio (Table 1). Wisconsin Department of Natural Resource and United States Geological Survey staff

collected marked adult lake trout from the Mid-Lake Refuge (n = 40) and Northern Refuge (n = 32), respectively (Fig. 1). Biologists from the United States Fish and Wildlife Service and the Ontario Ministry of Natural Resources captured adult lake trout from Lake Huron in northwestern areas (n = 20), the North Channel (n = 10), and southern areas (n = 9) (Fig. 1). Otolith edge material from adult fish was analyzed to establish stable isotope and trace element signatures by pooling data for each lake to determine if between-lake differences occurred. The presence of fin clips from the adult fish was not relevant to analyses of otolith edge material because lake trout are stocked as fingerlings where water from the hatcheries would only affect otolith cores. Otolith core material of unmarked adults collected in southern Lake Michigan was analyzed to establish early-life signatures for comparison to potential sources (i.e., hatchery or open lake origin).

Stocking records were accessed through the Great Lakes Fishery Commission's "Great Lakes Stocking Database" (www.glfc.org/fishstocking/) and used to determine all possible hatchery sources of lake trout stocked into Lake Michigan. Hatchery-reared yearling lake trout were randomly sampled in spring 2014 from the Jordan River (n = 24), Iron River (n = 25), and Pendills Creek (n = 24) National Fish Hatcheries as well as from the Marquette State Fish Hatchery (n = 24) in Michigan (Figure 1). Otolith core material from hatchery yearlings was analyzed to establish stable isotope and trace element signatures for each hatchery. Otoliths were removed from all fish using non-metallic forceps and stored dry in small, labeled Eppendorf tubes to avoid contamination (Procter and Thresher, 1998; Campana, 1999).

2.2. Stable isotope and trace element analysis. Southern Illinois University's Mass Spectrometry Facility carried out otolith stable isotope analysis to determine isotopic signatures

for ^{18}O and ^{13}C . Otoliths from individual hatchery-reared juveniles were first pulverized whole using a mortar and pestle and then a subsample of the resulting material was used for analysis. To obtain lake-specific signatures as well as examine core signatures of unmarked fish, otoliths from adults were micro-drilled at the edge and core, respectively, to obtain approximately 200 μg of material. The samples were analyzed for stable oxygen and carbon isotopes using a ThermoFinnigan Delta plus XP isotope ratio mass spectrometer fitted with a Gas Bench II carbonate analyzer. Stable oxygen and carbon isotope data is expressed in standard δ notation (units per mille ‰) as $\delta^{18}\text{O}$ or $\delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$ where R represents $^{18}\text{O}/^{16}\text{O}$ or $^{13}\text{C}/^{12}\text{C}$. Standards consist of Pee Dee Belemnite for both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (Whitledge, 2009).

Trace element analysis was performed at the University of Windsor's Great Lakes Institute for Environmental Research. Otoliths from adult fish ($N_{\text{total}} = 158$) were embedded in epoxy, ground, thin-sectioned using a low-speed ISOMET saw, and polished using aluminum-oxide 3M lapping films. The mounted otoliths were then rinsed in Milli-Q (Millipore) purified water and sonicated for five minutes in Milli-Q water to remove any surface contamination. The otoliths were then left to dry for at least 24 hrs and stored in clean Petri dishes before being analyzed.

Elements analyzed included Ca, Sr, and Ba using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS), which is the preferred method for analyzing otoliths for the presence of trace elements due to its precision and accuracy (Campana et al., 1997; Campana, 1999; Ludsin et al., 2006). A standard (NIST 610) was analyzed every 10-15 samples to account for potential effects of instrument drift (Campana, 1999). Trace element data were reported as element:calcium ratios (mmol/mol). To maximize our sample sizes, we did not exclude from analysis otoliths that contained vaterite (one of the crystalline forms of CaCO_3), but instead

attempted to target locations within the otolith core or edge that contained aragonite (another crystalline form of CaCO_3). This was done to maximize sample sizes and because these sections could not be confirmed through visual inspection alone. If vateritic sections were unintentionally analyzed, those could be identified by the observed expression of Mg, Mn, Ba, and Sr as aragonite and vaterite display opposite patterns (Melancon et al., 2005). Furthermore, trace element analysis, which was conducted following stable isotope analysis, was only used for lake trout captured from southwestern Lake Michigan in 2013 (putative wild group, $n = 21$), the Mid-Lake ($n = 11$) and Northern Refuges ($n = 14$), and Lake Huron ($n = 24$). Hatchery fish were excluded because $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ provided clear evidence of separation among the other groups of fish; further analysis would have been redundant and costly. We also subsampled the available otoliths from adult lake trout due to cost constraints.

2.3. Statistical analysis. Eleven unmarked, putatively wild lake trout obtained from southwestern Lake Michigan in 2012 were damaged and could not be used in our analysis of otolith core stable isotope data. Open-lake stable isotope signatures were attained from a subsample of 39 adult lake trout collected in the study. We used separate one-way ANOVAs to detect differences in mean $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ ratios using whole otolith material from hatchery yearlings and otolith edge material from adult lake trout, thus differentiating stable isotope signatures from hatcheries, lakes Michigan and Lake Huron fish. Following this procedure another one-way ANOVA was performed to determine whether stable isotope signatures could be differentiated among hatcheries and then compared to the putative wild adult lake trout. To further hone our ability to identify differences in otolith microchemistry between lakes, stable isotope and trace element data from otolith edges were combined for fish captured in both lakes

and separate, independent two-sample t-tests were used to assess differences in mean $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ as well as mean Sr:Ca and Ba:Ca ratios between lakes. Significantly different $\delta^{18}\text{O}$ signatures between the lakes prompted application of linear discriminant function analysis (LDFA) with a leave-one-out jackknife procedure to assign a lake of origin to the unmarked lake trout. Significance levels for all statistical tests were set at $\alpha = 0.05$.

Inferring natal environment of an individual fish using otolith core $\delta^{18}\text{O}$ is complicated because the relationship between water and otolith $\delta^{18}\text{O}$ is temperature-dependent (Devereux, 1967). Although Lake Michigan and Lake Huron (including Georgian Bay and the North Channel) have distinct water $\delta^{18}\text{O}$ values (Lake Michigan mean: $-5.9\text{‰} \pm 0.22$ SD referenced to Vienna Standard Mean Ocean Water (VSMOW); Lake Huron mean: $-7.4\text{‰} \text{VSMOW} \pm 0.44$ SD) (Dufour et al., 2005; Longstaffe et al., 2011), it was necessary to assess the potential confounding effect of temperature on lake of origin assignments based on otolith core $\delta^{18}\text{O}$ for putative wild lake trout. Therefore, we used water $\delta^{18}\text{O}$ data from Lake Michigan and Lake Huron and otolith core $\delta^{18}\text{O}$ to estimate natal environment temperature for putative wild lake trout and determined whether water temperatures calculated using water $\delta^{18}\text{O}$ values representative of each lake were reasonable given knowledge of water temperatures at depths occupied by age-0 lake trout. In particular, water temperature calculations using otolith core $\delta^{18}\text{O}$ of lake trout classified as being of Lake Michigan origin (based on LDFA) in combination with water $\delta^{18}\text{O}$ characteristic of Lake Huron enabled evaluation of whether it was feasible that the calculated temperatures could have been experienced during age-0 by these fish. A temperature-dependent oxygen isotope fractionation equation for *Salvelinus* spp. (Storm-Suke et al., 2007) was used to estimate water temperatures occupied by lake trout:

$$T\text{ }^{\circ}\text{C} = ((\delta^{18}\text{O}_{\text{Otolith(PDB)}} - \delta^{18}\text{O}_{\text{Water(PDB)}}) - 32.90) / -0.23$$

Although several temperature-dependent relationships between water and otolith $\delta^{18}\text{O}$ have been developed for fishes, we chose to use this equation because fractionation equations can differ substantially among species that are not closely related, resulting in significant error in calculated temperatures when applied to taxa other than the genus for which they were developed (Storm-Suke et al. 2007). However, we do acknowledge that species level differences may exist (Godiksen et al., 2010), but in the absence of an equation for *S. namaycush* this equation is the best suited of those available. Water temperature calculations were performed using mean, minimum, and maximum otolith core $\delta^{18}\text{O}$ values for putative wild lake trout classified as being of Lake Michigan origin based on LDFA and mean water $\delta^{18}\text{O}$ for each lake $\pm$ SD to estimate the range of natal environment temperatures potentially experienced by these fish. Prior to water temperature calculations, water $\delta^{18}\text{O}$ data were converted from values referenced to VSMOW to values referenced to PDB (Hoefs, 2004; Storm-Suke et al., 2007):

$$\delta^{18}\text{O}_{\text{Water(PDB)}} = (0.97002 \cdot \delta^{18}\text{O}_{\text{Water(VSMOW)}}) - 29.98$$

We also estimated the temperatures experienced by adult lake trout in Lake Michigan and in Lake Huron using mean, minimum, and maximum otolith edge $\delta^{18}\text{O}$ values for fish sampled from each lake and corresponding mean lake water $\delta^{18}\text{O} \pm$ SD to further assess whether temperature estimates generated from the fractionation equation for *Salvelinus* spp. were reasonable for Great Lakes lake trout. Otolith edge subsamples from adult lake trout integrated CaCO_3 accreted during multiple seasons and thus likely approximate average annual temperatures experienced by adult fish.

3. Results

We found distinct separation between stable isotope signatures from fish of hatchery origin and open-lake environments for both $\delta^{18}\text{O}$ ($F = 1835$; $df = 2, 132$; $P < 0.0001$; Fig. 2) and

$\delta^{13}\text{C}$ ($F = 2033$; $df = 2, 132$; $P < 0.0001$; Fig. 2). There were also significant differences between hatchery-origin juvenile lake trout and the putative wild fish $\delta^{13}\text{C}$ values ($F = 1369$; $df = 5, 174$; $P < 0.0001$; Fig. 3a) and in $\delta^{18}\text{O}$ values ($F = 6299$; $df = 5, 174$; $P < 0.0001$; Fig. 3b). A Tukey's HSD post-hoc test indicated otolith cores from putative wild lake trout collected in southwestern Lake Michigan were significantly more enriched in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ than whole otoliths from any of the hatchery yearlings ($P < 0.05$; Fig. 3). The large difference in $\delta^{18}\text{O}$ values between whole otoliths from hatchery juveniles and otolith core material from putative wild lake trout indicate that all of the unmarked lake trout in our sample were not of hatchery origin. We found differences in $\delta^{18}\text{O}$ signatures among hatcheries, but they were small compared to the differences between lake and hatchery signatures (Fig. 3). Pendills Creek and Jordan River Hatchery $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures showed no significant difference (Tukey's HSD, $P > 0.05$; Fig. 3), though were significantly different from the Iron River and Marquette Hatcheries (Tukey's HSD, $P < 0.05$; Fig. 3). There was no difference between otolith core $\delta^{13}\text{C}$ signatures of adult lake trout collected between 2012 and 2013 (Fig. 3a), although $\delta^{18}\text{O}$ values were significantly more enriched for adult lake trout collected from Julian's and Waukegan Reefs in Illinois waters in 2013 than in 2012 (Tukey's HSD, $P < 0.05$; Fig. 3b).

Comparisons of stable isotope signatures of otolith core material from the putative wild lake trout to otolith edge material from other fish collected in lakes Michigan and Huron showed significant differences for both $\delta^{13}\text{C}$ ($F = 1853$; $df = 3, 215$; $P < 0.0001$) and $\delta^{18}\text{O}$ ($F = 3738$; $df = 3, 215$; $P < 0.0001$; Fig. 2). Post-hoc tests revealed that putative wild lake trout $\delta^{13}\text{C}$ values were significantly different from both open-lake environments (Tukey's HSD, P 's < 0.05). $\delta^{18}\text{O}$ values for putative wild lake trout were significantly different from Lake Huron values (Tukey's HSD, $P < 0.05$), but were similar to Lake Michigan values (Tukey's HSD, $P > 0.05$). Differences

between lakes were only evident for $\delta^{18}\text{O}$ values, with Lake Michigan being significantly more enriched in ^{18}O isotopes (Tukey's HSD, $P < 0.05$; Fig. 2; Table 2).

Trace element analysis of Sr:Ca and Ba:Ca ratios revealed a significant difference between lakes for Ba:Ca (independent two-sample t-test; Ba:Ca, $t = -3.04$, $df = 65.754$, $P = 0.003$) with Lake Michigan having higher mean Ba levels than Lake Huron (Table 3). In addition, observed ranges for both otolith Ba:Ca and Sr:Ca showed some overlap between lakes (Table 3). Our results also indicated that of the 158 fish used in the trace element analysis, 26 (15.9%) had vateritic sections within the otoliths, which additionally confounded the analyses.

Given the differences in mean otolith $\delta^{18}\text{O}$ and Ba:Ca between lakes, we performed linear discriminant function analyses with a leave-one-out jackknife procedure to determine the accuracy with which adult lake trout could be assigned to the lake in which they were collected based on otolith edge $\delta^{18}\text{O}$ or Ba:Ca. Individual unmarked, putative wild fish collected from southwest Lake Michigan were assigned to Lake Michigan with >90% accuracy using otolith edge $\delta^{18}\text{O}$ (only 3 of 39 subsampled fish were mis-classified), whereas accuracy in assigning adult fish to the lake in which they were captured ranged from 58-87% based on otolith edge Ba:Ca. Thus, we applied the LDFA developed using otolith edge $\delta^{18}\text{O}$ data to identify the lake of origin for putative wild adult lake trout. Fish were classified as Lake Michigan origin if their otolith core $\delta^{18}\text{O}$ was greater than -4.30‰, Lake Huron origin if their otolith core $\delta^{18}\text{O}$ fell below -4.59‰, and origin was uncertain if otolith core $\delta^{18}\text{O}$ was between -4.59 and -4.30‰. Over 90% (76 of 84) of the putative wild lake trout were classified as Lake Michigan-origin; the remaining eight fell within the range of uncertainty.

Estimates of water temperatures experienced by adult lake trout using the range of observed otolith edge $\delta^{18}\text{O}$ values for fish sampled from each lake and corresponding mean lake

water $\delta^{18}\text{O}$ values ($\pm$ SD) ranged from 0.7-6.8 °C for Lake Michigan fish (predicted temperature 4.0 °C using mean otolith edge and mean Lake Michigan water $\delta^{18}\text{O}$) and 2.0-7.8 °C for Lake Huron fish (predicted temperature 3.2 °C using mean otolith edge and mean Lake Huron water $\delta^{18}\text{O}$). Estimates of natal environment temperature using otolith core $\delta^{18}\text{O}$ for individual fish classified as being of Lake Michigan origin and mean Lake Michigan water $\delta^{18}\text{O} \pm$ SD ranged from 1.9-7.3 °C (predicted temperature 4.9 °C using mean otolith and water $\delta^{18}\text{O}$ values). In contrast, natal environment temperatures calculated using otolith core $\delta^{18}\text{O}$ values for putative Lake Michigan-origin individuals and mean Lake Huron water $\delta^{18}\text{O}$ ranged from -3.5 to 0.1 °C (mean -1.4 °C). Likewise, using mean Lake Huron water $\delta^{18}\text{O} - 1$ SD (-6.96‰ VSMOW) in temperature calculations yielded some estimates of natal environment temperature for putative wild lake trout of Lake Michigan origin that were < 0.0 °C (range -1.6 to 1.9 °C; mean 0.4° C).

4. Discussion

This study provides the first confirmation in over 50 years of population restoration efforts that lake trout are successfully recruiting to the adult life stage in Lake Michigan. Although it is assumed that unmarked lake trout are of wild origin (Bronte et al., 2008), until now this assumption has not been tested in Lake Michigan. Our results show clear separation between unmarked adult lake trout and hatchery-reared yearlings. By comparing stable isotope signatures of putative wild adult lake trout to each hatchery that currently produces or has historically produced lake trout for stocking purposes, we can confidently assert that unmarked lake trout collected by IDNR staff are indeed of natural origin based on two lines of evidence. First, large differences in otolith core $\delta^{13}\text{C}$ values were observed between the putative wild and hatchery fish. The differences likely were not a result of metabolic rate effects because the analysis targeted similar regions of the otolith (i.e., the core) when both groups of fish were

juveniles. Second, large differences in $\delta^{18}\text{O}$ values between hatchery-reared fish and our putative wild group reflect very different geographic locations. For instance, Jasechko et al. (2014) calculated considerable differences in $\delta^{18}\text{O}$ values among tributaries of the Great Lakes and the lakes themselves. Their findings support our results that average $\delta^{18}\text{O}$ values are nearly 2.5-fold higher in open-lake signatures compared to hatcheries, whose facilities would be fed, at least in part, by tributary water. Even the least ^{18}O enriched Great Lake, Lake Superior, showed considerably different $\delta^{18}\text{O}$ signatures from its tributaries (Jasechko et al., 2014). We similarly found large differences between the $\delta^{18}\text{O}$ signatures of hatchery-reared fish and putative wild adult lake trout, which are consistent with findings by Schaner et al. (2007). These differences are likely a function of thermal regimes of hatcheries and lake environments, where warmer water temperatures of hatcheries may be associated with ^{18}O depletion (i.e., more negative $\delta^{18}\text{O}$ values) in otoliths (e.g., see Kalish, 1991a). Indeed, both Kalish (1991b) and Tomida et al. (2014) found lower $\delta^{18}\text{O}$ values for hatchery-reared Australian salmon (*Arripis trutta*) and pink salmon (*Oncorhynchus gorbuscha*) fry, respectively, compared to wild individuals. Taken together, the results of our study further corroborate the utility of ^{18}O isotopes for distinguishing between hatchery-reared and wild fishes.

Implicit within our data is the assumption that differences between lake and hatchery signatures are consistent over time. Data presented in Jasechko et al. (2014) indicate stability in $\delta^{18}\text{O}$ signatures of lake water between seasons and across depths. In addition, the relatively long hydraulic residency time of Lake Michigan water (i.e., 62 years; Quinn, 1992) suggests that temporal variation in $\delta^{18}\text{O}$ signatures may be unlikely. Indeed, Dufour et al. (2005) and Longstaffe et al. (2011) both demonstrate very little temporal variation in $\delta^{18}\text{O}$ values for the Great Lakes. Data from Patterson et al. (1993) show temporal stability of $\delta^{18}\text{O}$ signatures as

evidenced by large differences observed between hatchery-reared lake trout relative to lake-caught individuals. Additionally, because the difference in $\delta^{18}\text{O}$ signatures between hatcheries and both Lake Michigan as well as Lake Huron are so large, temporal variability would be unlikely to yield overlap between hatchery-reared and putative wild fish for any year class.

Similar methodologies have been used only one other time with Great Lakes lake trout. Schaner et al. (2007) sought to ascribe a natal origin to unmarked juvenile and adult lake trout collected from Lake Ontario using $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signatures. However, the authors were hesitant to assign a definitive origin to the unmarked fish for two reasons. First, their results showed overlap in $\delta^{13}\text{C}$ signatures between hatcheries and unknown origin fish, which was attributed to differences in diet. Second, they did not sample juveniles from all possible hatcheries that have provided lake trout for stocking into Lake Ontario. In contrast, the findings of this study provide greater evidence that putative wild lake trout are indeed wild. Hatcheries providing lake trout specifically for stocking into Lake Michigan had $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values significantly different from the putative wild fish collected in both 2012 and 2013. We share similar opinions with Schaner et al. (2007) that oxygen isotopes may be most appropriate for identifying the geographic origin of an individual. This is because there is a well-established relationship among $\delta^{18}\text{O}$ and latitude, temperature, and precipitation amounts (Dansgaard, 1964; Siegenthaler and Oeschger, 1980; Dutton et al., 2005), while $^{13}\text{C}/^{12}\text{C}$ ratios are known to change with diet, temperature, metabolic rate, and carbon fixation pathway (e.g., DeNiro and Epstein, 1978; Kalish, 1991a; Rubenstein and Hobson, 2004; Zeebe and Wolf-Gladrow, 2001).

Stable oxygen isotopes have been successfully used in a number of studies to identify the spatial origins of different fish species. Even in large aquatic environments such as oceans, the thermal changes across latitudes have permitted researchers to differentiate stocks of fishes such

as tunas (*Thunnus* spp.; Rooker et al., 2008; Shiao et al., 2010; Wells et al., 2012), Patagonian toothfish (*Dissostichus eleginoides*; Ashford and Jones, 2007), grey mackerel (*Scomberomorus semifasciatus*; Newman et al., 2010), and haddock (*Melanogrammus aeglefinus*; Begg and Weidman, 2001) using $\delta^{18}\text{O}$ values. In freshwater environments, biologists have successfully used $\delta^{18}\text{O}$ values to identify the natal origins of species such as sockeye salmon (*Oncorhynchus nerka*; Gao et al., 2007) and American shad (*Alosa sapidissima*; Walther et al., 2008; Walther and Thorrold, 2008). Only the present study, Schaner et al. (2007), and Dufour et al. (2005) have used otolith stable oxygen isotopes to identify the natal origin of Great Lakes fishes. The results of our study indicate that stable oxygen isotopes may prove more useful than stable carbon isotopes for differentiating between lakes Michigan and Huron; Lake Michigan $\delta^{18}\text{O}$ signatures showed significantly greater enrichment of the heavy ^{18}O isotope than those of Lake Huron. Based on the range of $\delta^{18}\text{O}$ values for each lake, over 90% of unmarked adult lake trout sampled in southwestern Lake Michigan were classified as Lake Michigan-origin. Our research represents the first to distinguish between two of the Great Lakes (Michigan and Huron) using stable isotopes.

The results of our trace element analysis also enabled us to distinguish between lakes Michigan and Huron. However, sufficient geologic differences must exist between or among locations if the goal is to use trace element data to study stock or natal origins of different fishes. For instance, while Bronte et al. (1996) showed some ability to identify different stocks of Lake Superior lake herring (*Coregonus artedii*), considerable overlap existed among several locations. Their study indicated that using trace element data alone was not sufficient for distinguishing among spawning locations within the lake. In contrast, Marklevitz et al. (2011) successfully applied trace element analysis to differentiate among tributaries of multiple geologic regions

surrounding Lake Huron when studying natal origins of Chinook salmon (*Oncorhynchus tshawytscha*). Most other applications of trace element data to study fishes in the Great Lakes have been applied to adfluvial species (e.g., Brazner et al., 1996; Dufour et al., 2005; Whitley, 2009; Pangle et al., 2010). The use of trace element data to distinguish different locations within Lake Michigan, such as potential spawning sites of lake trout, requires further investigation.

Our trace element data also showed evidence of vateritic otoliths that only came from stocked fish. The CaCO_3 crystalline structure of fish otoliths most commonly takes the form of aragonite, but under certain conditions fish may switch to a vaterite crystalline structure. This is thought to occur during periods of acute or chronic stress and often occurs in hatchery-reared fish (Melancon et al., 2005; see Oxman et al., 2007). We did not have complete biological data (e.g., fin clips) for each of the 26 fish (15.9% of total fish used in the otolith trace element analysis) with vateritic otoliths, but at least 20 of them were confirmed to be hatchery-reared.

Calculations of natal environment temperature for putative wild lake trout collected in southwestern Lake Michigan provided evidence that our conclusions regarding lake-of-origin for these fish were not confounded by the effect of temperature on otolith $\delta^{18}\text{O}$. Estimates of water temperatures experienced by adult lake trout using the range of observed otolith edge $\delta^{18}\text{O}$ values for fish sampled from lakes Michigan and Huron and representative lake water $\delta^{18}\text{O}$ values were within the range of temperatures occupied by adult lake trout in the Great Lakes recorded in archival temperature tagging studies (Bergstedt et al., 2003, 2012). These findings indicate that the temperature-dependent fractionation equation for *Salvelinus* spp. (Storm-Suke et al., 2007) may yield reasonable temperature predictions for Great Lakes lake trout. However, Godiksen et al. (2010) advocates the need for species-specific equations because significant

differences may exist between species within the same genus. Future work should focus on developing a species-specific equation for lake trout.

Natal environment temperatures calculated using otolith core $\delta^{18}\text{O}$ values from putative wild lake trout classified as Lake Michigan-origin fish based on our LDFA and mean Lake Huron water $\delta^{18}\text{O}$ substantiated our conclusion that most of these fish did not originate in Lake Huron, as calculated natal environment temperatures consistently $< 0.0\text{ }^{\circ}\text{C}$ are nonsensical. In contrast, when water $\delta^{18}\text{O}$ values representative of Lake Michigan were used in calculations of natal environment temperature for these fish, water temperature estimates were consistent with observed temperatures at spawning locations and depths occupied by age-0 lake trout (Bronte et al., 1995). Thus, natal environment temperature calculations based on otolith and water $\delta^{18}\text{O}$ support our conclusion that Lake Michigan is the principal source of unmarked lake trout sampled from southwestern Lake Michigan. Unmarked lake trout with otolith core $\delta^{18}\text{O}$ signatures that did not enable definitive assignment of lake-of-origin potentially may have immigrated to Lake Michigan from Lake Huron or may have inhabited slightly warmer waters in Lake Michigan (up to $8.7\text{ }^{\circ}\text{C}$ based on the *Salvelinus* spp. fractionation equation) during early life. However, the fact that a low percentage of unmarked fish are found in northern Lake Michigan relative to the southern portion of the lake (M. Kornis, pers. comm.) is evidence that the putative wild fish in this study likely did not emigrate from Lake Huron.

Although we were able to assign lake of origin to most of the unmarked lake trout sampled in southwestern Lake Michigan, the question of within-lake origin, which is fundamental to management and rehabilitation of Lake Trout, was beyond the scope of this study. This study represents a first step in identification of the source of natural recruitment in Lake Michigan and allows researchers to now focus on questions related to the mechanisms

facilitating successful natural reproduction in southwestern Lake Michigan. Researchers should also seek to identify and characterize the habitats in which lake trout natural reproduction may be occurring.

5. Conclusion

Our findings provide evidence of progress toward re-establishing a self-sustaining population of lake trout in Lake Michigan waters. Although stocking efforts have managed to create abundant spawning populations of lake trout, the central goal of Lake Michigan fisheries managers to have self-sustaining lake trout populations is still elusive (e.g., Eshenroder et al., 1995; Dexter et al., 2010). Nevertheless, the results presented in this study support the assumption that wild lake trout can be identified by a lack of fin clips. The current study also further underscores the utility of stable isotope analysis, particularly that of ^{18}O -isotopes, at elucidating natal origins in wild fish species. Yet, perhaps most importantly, this study suggests that lake trout rehabilitation efforts are working and that managers are moving closer toward achieving their goal of creating a self-sustaining lake trout population within Lake Michigan.

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Table 1. Summary biological data for unmarked adult lake trout captured on Julian’s and Waukegan Reefs in southwestern Lake Michigan.

Year	Sex	Total length (mm) mean ± SD (range)	Number
2012	F	752.5 ± 3.5 (750-755)	2
	M	660.3 ± 45.0 (550-755)	69
2013	F	730.9 ± 43.3 (650-805)	11
	M	671.5 ± 46.5 (615-765)	13

Table 2

Table 2. Otolith edge $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values (‰) for adult lake trout collected from Lake Michigan and Lake Huron.

Group	Location	$\delta^{13}\text{C}$ mean $\pm$ SD (range)	$\delta^{18}\text{O}$ mean $\pm$ SD (range)
Lake Michigan	Julian's and Waukegan Reefs (2012)	-4.58 ± 0.68 (-5.35 – -3.78)	-3.86 ± 0.16 (-4.03 – -3.63)
	Julian's and Waukegan Reefs (2013)	-4.97 ± 0.46 (-5.56 – -4.38)	-3.44 ± 0.23 (-3.82 – -3.19)
	Northern Refuge	-5.11 ± 0.45 (-5.85 – -4.65)	-4.28 ± 0.28 (-4.59 – -3.99)
	Mid-Lake Refuge	-4.62 ± 0.67 (-5.56 – -3.77)	-3.33 ± 0.34 (-3.62 – -2.74)
Lake Huron	North Channel	-4.90 ± 0.45 (-5.90 – -4.46)	-4.74 ± 0.42 (-5.53 – -4.30)
	Various Locations	-5.06 ± 0.68 (-6.16 – -4.19)	-5.21 ± 0.34 (-5.64 – -4.77)
Combined	Lake Michigan	-4.82 ± 0.58 (-5.85 – -3.77)	-3.73 ± 0.45 (-4.59 – -2.74)
	Lake Huron	-4.99 ± 0.58 (-6.16 – -4.19)	-4.99 ± 0.44 (-5.64 – -4.30)

Table 3

Table 3. Sr:Ca and Ba:Ca (mmol/mol) for putative wild lake trout (core) and signatures for Lakes Michigan and Huron (edge).

Element	Lake	Otolith Site	N	Mean $\pm$ Standard Error	Minimum	Maximum
Sr	Huron	Edge	24	441.39 $\pm$ 12.88	347.86	599.09
	Michigan	Edge	46	424.41 $\pm$ 8.22	368.32	802.08
	Michigan	Core	88	512.48 $\pm$ 7.06	325.12	599.92
Ba	Huron	Edge	24	0.65 $\pm$ 0.045	0.35	1.23
	Michigan	Edge	46	0.86 $\pm$ 0.052	0.35	2.16
	Michigan	Core	87	2.88 $\pm$ 0.26	1.02	16.60

Fig. 1. Map indicating the locations of Julian's Reef and Waukegan Reef (denoted by +) where the putative wild group of adult lake trout was sampled. Also shown are the hatcheries yearling lake trout were collected from as well as the boundaries of the Mid-Lake Refuge and Northern Refuge (outlined in solid, bold lines) where additional adults were sampled to establish Lake Michigan stable isotope and trace element signatures. Adults from Lake Huron were also captured in the North Channel and southern areas to quantify signatures for this lake.

Fig. 2. Dual stable isotope ratios of unmarked adult lake trout captured in southwestern Lake Michigan (cross) compared to signatures from hatchery yearlings (triangles), Lake Michigan (solid diamond), and Lake Huron (open circle).

Fig. 3. Otolith core $\delta^{13}\text{C}$ (a) and $\delta^{18}\text{O}$ (b) values for unmarked adult lake trout (cross-hatched) and yearling lake trout from four hatcheries (solid). Letters indicate statistically similar groupings based on Tukey's HSD post-hoc test. SW LM denotes the southwest Lake Michigan collection site.

Figure 1

Fig. 1. Map indicating the locations (cross symbols) of Julian’s Reef (JR) and Waukegan Reef (WR) in Illinois (IL) waters where the putative wild group of adult lake trout was sampled. Also shown are the hatcheries yearling lake trout were collected from as well as the boundaries (thick black lines) of the Mid-Lake Refuge (MLR) and Northern Refuge (NR) where additional adults were sampled to establish Lake Michigan stable isotope and trace element signatures. Adults from Lake Huron were also captured in northwestern areas of the lake including the North Channel and southern areas to quantify signatures for this lake.

Figure 2

Fig. 2. Dual stable isotope signatures of putative wild adult lake trout captured in southwestern Lake Michigan (crosses) compared to signatures from hatchery yearlings (triangles) as well as the pooled signatures for Lake Michigan (solid diamonds) and Lake Huron (open circles).

Figure 3

Fig. 3. Otolith core $\delta^{13}\text{C}$ (a) and $\delta^{18}\text{O}$ (b) values for unmarked, putative wild adult lake trout (grey) and yearling lake trout from four hatcheries (black). Letters indicate statistically similar groupings based on Tukey's HSD post-hoc test. SW LM denotes the southwest Lake Michigan collection site.