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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 $\delta^{18}O$ and $\delta^{13}C$ 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 $\delta^{18}O$ 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 $\delta^{18}O$. 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.

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).

All lake trout stocked in the Great Lakes are marked by clipping one or more fins and any lake trout without a fin clip is assumed to be of wild origin (Bronte et al., 2008). From 1999–2009, the percentage of unmarked adult lake trout sampled in southwestern Lake

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Michigan ranged from 1.2 to 7.5% (Patterson et al., in review). Estimates from 2005 to 2010, however, indicate that approximately 2–3% of stocked fish were unmarked by manually clipping fins, although error rates for fish stocked into particular areas could potentially be as high as 10% (D. Hanson, unpublished data).

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

There are three potential sources of putative wild lake trout in Lake Michigan. First, clipping errors at the hatchery, prior to stocking, may have resulted in unmarked fish that were introduced to the system. Second, the documented natural reproduction and successful recruitment to the adult stage in Lake Huron over the last 10–15 years (Riley et al., 2007; He et al., 2012) has resulted in migration of adults produced in Lake Huron to southern Lake Michigan. However, relatively few instances of lake trout marked with a fin clip and coded-wire tag that were stocked into Lake Huron and recovered in Lake Michigan have been noted, suggesting immigration of lake trout may be minimal (Bronte et al., 2007; S. Robillard, unpublished data). Third, the collection of an increasing number of unmarked, presumably wild-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; Whitledge, 2009; Zeigler and Whitledge, 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; Schaffer et al., 2015), and is particularly robust when known-origin individuals are available with to 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 (Fig. 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

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