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Abstract

We tested the hypothesis that otolith trace elemental signatures of mottled sculpin *Cottus bairdi*, slimy sculpin *C. cognatus*, and juvenile coho salmon *Oncorhynchus kisutch* were predictive of those of juvenile steelhead *O. mykiss* across many streams within the Lake Michigan basin. Laser ablation inductively coupled plasma mass spectrometry was used to generate otolith trace elemental signatures for each individual fish. For each species pair, statistical correlations of mean otolith concentrations of Mg, Mn, Cu, Zn, Sr, Ba, and Pb for each site were estimated. Linear equations describing these relationships were used to transform juvenile steelhead otolith chemistry data to those of each of the other species. Transformed otolith chemistry data were subjected to random forest classifications developed for mottled sculpin, slimy sculpin, and juvenile coho salmon to assess interspecific natal source assignment accuracies. Steelhead otolith concentrations of Sr were significantly correlated with those of each of the other species, whereas otolith concentrations Ba and Mn were significantly correlated among some species pairs, but not others. Natal source assignment accuracies of juvenile steelhead to stream and watershed generally decreased when otolith trace elemental data were transformed to those of mottled sculpin, slimy sculpin, and coho salmon. Miss-assigned fish often classified into nearby watersheds within larger management units, leading to higher assignment accuracies at coarser geographical resolutions (75-97% correct assignment to management unit for each species). These findings suggest that applications of otolith chemistry data may extend beyond the species from which it is collected.

Executive summary

Exploited fish populations in the Great Lakes are generally comprised of different stocks that originate from distinct natal locations. Effective management of such populations requires an explicit consideration for how stocks (i.e., locations) differentially contribute individuals. Salmon and trout, which are comprised of mixed-stock populations including stocked and naturally produced fish, are economically and ecologically important, to the Lake Michigan community and its watershed, representing millions of dollars in value to the region.

Conventional techniques for assessing survival of fish to adulthood, such as mass marking programs, are impractical to implement for naturalized salmonines, because it is not feasible to collect and mark a large number of wild juveniles in each tributary. The microchemical signature of otoliths offers a more informative diagnostic for the identification of fish origin. This chemical mark on the otolith is naturally imprinted on all fish inhabiting a given water body, thus overcoming the impracticalities associated with artificial marking techniques. Findings from a previous GLFT-funded project on juvenile steelhead in the Lake Michigan basin support the utility of otolith chemistry. This project, led by Pangle and Jonas, has analyzed the otolith chemistry of steelhead collected in 2013 and 2014 from 28 different watersheds throughout the basin and has found unique chemical signatures within a watershed, between watersheds, and between broad regional classifications. One important, but open question is how transferrable are these chemical signatures to other fish species.

Herein, we tested the hypothesis that otolith trace elemental signatures of mottled sculpin *Cottus bairdi*, slimy sculpin *C. cognatus*, and juvenile coho salmon *Oncorhynchus kisutch* were predictive of those of juvenile steelhead *O. mykiss* across many streams within the Lake Michigan basin. Laser ablation inductively coupled plasma mass spectrometry was used to generate otolith trace elemental signatures for each individual fish. For each species pair, statistical correlations of mean otolith concentrations of Mg, Mn, Cu, Zn, Sr, Ba, and Pb for each site were estimated. Linear equations describing these relationships were used to transform juvenile steelhead otolith chemistry data to those of each of the other species. Transformed otolith chemistry data were subjected to random forest classifications developed for mottled sculpin, slimy sculpin, and juvenile coho salmon to assess interspecific natal source assignment accuracies.

Steelhead otolith concentrations of Sr were significantly correlated with those of each of the other species, whereas otolith concentrations Ba and Mn were significantly correlated among some species pairs, but not others. Natal source assignment accuracies of juvenile steelhead to stream and watershed generally decreased when otolith trace elemental data were transformed to those of mottled sculpin, slimy sculpin, and coho salmon. Miss-assigned fish often classified into nearby watersheds within larger management units, leading to higher assignment accuracies at coarser geographical resolutions (75-97% correct assignment to management unit for each species). These findings suggest that applications of otolith chemistry data may extend beyond the species from which it is collected.

SECTION 1: INTRODUCTION

Otolith microchemical signature analysis is becoming an increasingly important tool used to reconstruct environmental histories of fish (Campana 1999; Campana and Thorrold 2001; Pracheil et al. 2014). Otoliths are paired, calcified structures located near the brain of teleost fish that are important for fish hearing and orientation. Otoliths of fish residing in different environments have been shown to exhibit differentiable trace elemental signatures, due in large part to intrinsic, regional differences in ambient water properties among fish habitats (Campana and Thorrold 2001; Elsdon et al. 2008; Sturrock et al. 2014). Because otoliths grow continuously and do not undergo chemical resorption, these trace elemental signatures serve as a permanent chronological record of the environment(s) in which a fish has resided during its lifetime (Thresher 1999; Campana and Thorrold 2001). A primary application of otolith microchemical signature analysis has been to develop classification models using measurements obtained from fish of known environmental histories (e.g., stock, natal source, migration patterns, etc.) and apply these to fish whose environmental history is unknown and of interest (Mercier et al. 2011; Tanner et al. 2016). Such research and applications have generally been intraspecific in scope. However, otolith microchemical signatures are natural tags imparted on all bony fishes occupying a particular location, and the extent to which interspecific relationships exist between the otolith chemistries of the different species of fish that share aquatic habitats remains largely unexplored. If significant interspecific relationships exist for trace elements that exhibit variation among natal sources, then otolith trace elemental signatures obtained for one species may be used to predict those of co-occurring species for which natal source otolith chemistry information is unavailable (Hamer and Jenkins 2007). In

this study, we test the hypothesis that predictive relationships exist between the otolith trace elemental chemistries of four co-occurring fish species across multiple sites over a large geographic range.

Our study was focused on the North American Great Lakes, where a multispecies assemblage of introduced migratory Pacific salmonines (genus *Oncorhynchus*) supports highly valued and intensively managed recreational fisheries (Tanner and Tody 2002; Thayer and Loftus 2013; Tsehaye et al. 2014; Clark et al. 2016). Chinook salmon *Oncorhynchus tshawytscha*, coho salmon *O. kisutch*, and steelhead *O. mykiss* are stocked annually throughout much of the Great Lakes basin (Crawford 2001; Claramunt et al. 2013), and substantial natural reproduction in tributaries supports diverse mixed-stock fisheries of each species (Claramunt et al. 2013; Claramunt and Clapp 2014). To promote ecosystem stability and resilience, there is a strong desire among Great Lakes management agencies to undertake strategies that promote self-sustaining fish stocks (Eshenroder 1990; Jonas et al. 2008; Great Lakes Fishery Commission 2011). However, the extent to which natural reproduction in any particular Great Lakes tributary contributes individual fish to the intermixed fisheries and populations is unknown. Accurate information on the natal sources of adult fish caught in each fishery is needed to quantify wild salmonine recruitment dynamics, and in turn, identify the factors underlying these dynamics at appropriate spatial scales.

Thus far, stock delineation of naturally-reproduced Pacific salmonines in the Great Lakes has been hampered by the logistical and economic impracticalities of collecting and uniquely marking (e.g., tag, fin-clip) a large number of wild juveniles from enough natal sources and year-classes to ensure sufficient samples sizes of marked adult fish are later recovered. Low genetic

differentiation among stream populations of introduced salmonines in the Great Lakes would likely preclude identification of natal source streams using genetic markers (Bartron and Scribner 2004; Weeder et al. 2005). Promisingly, Marklevitz et al. (2011) classified wild juvenile Chinook salmon with 87% accuracy among tributaries to Lake Huron using otolith trace elemental analysis, and Marklevitz et al. (2016) applied similar methods on unknown-origin adult Chinook salmon captured in the recreational fishery to assess temporal and spatial variability in stock admixture. Additional studies have successfully applied otolith microchemistry to distinguish environmental histories for multiple species in the Great Lakes region (Pangle et al. 2010; Reichert et al. 2010; Boehler et al. 2012; Schoen et al. 2016), suggesting that differences in otolith trace elemental signatures may be conserved across species.

To test for similarities among species, we collected and statistically compared otolith chemistry data from multiple co-occurring fish species across a wide geographical range within the Lake Michigan basin. In a separate investigation, we had built an otolith microchemistry database for juvenile steelhead from >30 streams across the Lake Michigan basin, so age-0 steelhead chemistry data were already in-hand for sampling years 2014 and 2015 (N. Watson, Central Michigan University, unpublished data). To perform among-species comparisons, 16 sites were revisited in 2015 and 2016 and sampled for sculpin (*Cottus* spp.) and age-0 coho salmon (species that were most commonly observed co-occurring with age-0 steelhead), as well as additional age-0 steelhead. The species selected for investigation all exhibit behavior particularly conducive to stock delineation via otolith microchemistry analysis. For both steelhead and coho salmon, the juvenile in-stream phase is particularly important for

recruitment, and they typically reside in streams for more than one year. The longer river residence time ensures that stream signatures are present in a substantial portion of their otoliths (in contrast with Chinook salmon that smolt at age-0). Both sculpin species have typical home ranges in streams that are <50 m, with movement distances rarely exceeding 0.5 km (Keeler 2006; Breen et al. 2009). Thus, the otolith chemical signatures of these sculpin are expected to reflect the histories of the ambient environmental properties of the sites sampled over the lifespans of the sculpin analyzed (Brennan et al. 2015). Herein, we tested the hypothesis that the trace elemental signatures of mottled sculpin *Cottus bairdi*, slimy sculpin *C. cognatus*, and coho salmon were predictive of those of juvenile steelhead by assessing the significance of the interspecific correlations of the otolith concentrations of each trace element measured.

SECTION 2: METHODS

Sample and data collection.-Fish collected for this research are described in Table 1. Stream sites chosen (Fig. 1) were Lake Michigan tributaries previously shown to support successful anadromous salmonine natural reproduction (e.g., Avery 1974; Seelbach and Whelan 1988; Rutherford 1997; Hirethota and Burzynski 2015), and from which sculpin were observed to be abundant in sampling events prior to this study. Because steelhead were collected in 2014 prior to when sculpin started being collected in 2015, we focused analyses generally on sculpin age-2 and older (identified from length-frequency histograms) in order to encompass the age-0 duration of the steelhead collected in 2014. All samples were collected using backpack electrofishing and stored frozen in ice until lab processing.

Upon processing, fish were thawed and total lengths measured to the nearest mm. Sagittal otoliths were removed from each fish, cleaned of adhering tissue and endolymph using hydrogen peroxide, rinsed with reverse-osmosis water, and placed in sorting trays to dry. For each fish, a single otolith was mounted in Epofix cold-setting embedding resin in silicone molds and allowed to harden for at least 48 hours. A PICO155 Precision Cutter saw (Pace Technologies, Tucson, AZ) outfitted with four-inch diamond wafering blades was used to cut thin sections of the embedded otoliths. These thin sections were hand-polished to the plane of the core sequentially with 600 and 1200 grit silicon carbide paper followed by 1 μm Alumina polishing film. We checked that the polished sections were smooth and absent of any deep scratches using a microscope. Polished thin sections were then mounted onto etched petrographic slides using super glue (Loctite, Westlake, OH). The day prior to chemical analysis,

the mounted slides were sonicated in ultrapure water (18.2 MΩ·cm) for seven minutes and stored in a laminar flow hood to air dry.

Otolith sections were analyzed for trace elements using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) at Central Michigan University's Center for Elemental and Isotopic Analysis. Laser ablation was performed using a Photon Analyte 193 nm Excimer laser system in conjunction with a computer operated X-Y-Z sample chamber following methods similar to those used by Schoen et al. (2016). Ablated material was carried from the sample chamber to a Thermo-Finnigan Element 2 ICP-MS unit using helium carrier gas (1.6 ± 0.1 L/min) to which argon makeup gas (1.2 ± 0.15 L/min) was added. LA-ICP-MS operating parameters were tuned to achieve a thorium to uranium ratio ($^{232}\text{Th}:^{238}\text{U}$) of 1.0, and a thorium to oxygen ratio ($^{232}\text{Th}:^{16}\text{O}$) of <1.8% at the start of each session. Laser fluence was estimated to be within 3-6 J/cm³.

We measured the otolith concentrations of magnesium (^{25}Mg), manganese (^{55}Mn), copper (^{65}Cu), zinc (^{66}Zn), strontium (^{88}Sr), barium (^{137}Ba), and lead (^{208}Pb). Data were collected for a transect running from 200 μm opposite the primordium to the otolith edge (2-6 μm/sec velocity, 40 μm circular spot size, 70% laser output). In all cases, the path of the transect was perpendicular to the growth circuli. To remove surface contamination prior to analysis of each otolith section, a double-pulse of an 80 μm-wide raster was ablated along the length of the transect. To correct for instrument measurement drift, at the start and end of otolith analysis, as well as every 60-90 minutes during analysis, the international glass reference material NIST 612 was analyzed (4 x 140 μm transects). Three transects each of the NIST 610 and MACS3

(USGS carbonate standard) reference materials were also analyzed at the beginning and end of each daily session as internal check standards.

Trace element concentrations in the otoliths were determined using the Trace Element Internal Standardization Routine within the Lolite mass spectrometry software package (version 2.31; Paton et al. 2011). Calcium was used as an internal standard at 40% weight as in stoichiometric calcium carbonate relative to the NIST 612 glass concentration values reported in the GeoReM database (Jochum et al. 2005). For every sample and standard analysis, 30-50 seconds of carrier/makeup gas (He and Ar) background signal was measured prior to laser ablation to quantify trace element background signals that were subtracted from the raw isotope count rates measured during ablation. Background-corrected isotope count rates were further adjusted and converted to parts per million (ppm) based upon the known and measured isotope:Ca ratios in the NIST 612 standards bracketing each sample. Although we only measured one isotope of each element, we report total elemental concentrations based upon known, naturally-occurring isotopic compositions of each respective element.

Analysis.-For each sample, median concentrations of each trace element were calculated from the primordium to the otolith edge. The position of the primordium was visually identified by generating seven-panel plots of the trace element signatures for each fish and locating the position of the transect around which symmetry in trace element concentrations could be observed. We chose to use median trace elemental values in favor of means due to non-normality in the distributions of the data that was not resolvable among all samples with any particular data transformation. Preliminary analyses performed using mean trace elemental values showed very similar results.

For each site, we averaged the median trace elemental concentrations among all steelhead and linearly regressed these against those of coho salmon and both sculpin species. To assess which elements exhibited significant interspecific patterns, we calculated interspecific Pearson correlation coefficients for each element. Significance of the correlations is reported at an α level of 0.05.

We then assessed the degree of importance of each trace element for correctly assigning fish to their streams of origin. To do this, random forest (RF) classifications (Breiman 2001) were developed for each species and the variable importance of each element was determined. For straightforward descriptions of the RF classification method employed, we direct readers to Liaw and Wiener (2002, p. 18) and Mercier et al. (2011, pp. 1355-1356). In the RF classification for each species, the levels of the class target comprised the set of sites from which that species was sampled, and the interval input variables were the median concentrations of the seven trace elements measured for each fish. We implemented these classifications in R (R Core Team 2016) using the default settings of the `randomForest()` function (randomForest package; Liaw and Wiener 2002). For the RF classification of each species, variable importance (*I*) was determined for each element as the mean decrease in classification accuracy when that particular element was permuted across all trees, and all other elements were left unchanged (Liaw and Wiener 2002).

Lastly, we transformed the steelhead otolith trace elemental concentrations to those of each of the other three species using the equations determined from the aforementioned interspecific linear regressions. We then applied the coho salmon, mottled sculpin, and slimy sculpin RF classifications to the corresponding transformed steelhead otolith chemistry data to

assess how well each of these species could inform steelhead natal origin assignments. We present these results in terms of the proportion of steelhead correctly assigned to their natal origin at three spatial resolutions – stream, watershed, and management unit. Sites located in Michigan were assigned to management units according to the Michigan Department of Natural Resource’s Lake Michigan management unit boundaries. We assigned Fischer Creek, the only site located outside of Michigan, to what we deemed the Wisconsin management unit.

SECTION 3: Results

Of the trace elements measured, otolith concentrations of Sr, Ba, and Mn, were the most correlated between all species pairs examined (Table 2). There were significant relationships between steelhead and coho salmon for otolith Sr and Ba concentrations, between steelhead and mottled sculpin for otolith Sr and Mn concentrations, and between steelhead and slimy sculpin for otolith Sr, Mn, and Ba concentrations (Table 2). Otolith concentrations of Mg, Cu, Zn, or Pb were not significantly correlated among any of the species pairs. Relationships between species pairs of otolith elemental concentrations followed a generally linear pattern (Fig. 2). Examination of the regressions with respect to the 1:1 relationship line indicates that incorporation of Sr into the CaCO_3 otolith matrix tended to be lower for juvenile steelhead than the other three species (Fig. 2). Juvenile steelhead also incorporated Ba into their otoliths at lower concentrations than coho salmon, while otolith concentrations of Mn were higher for steelhead compared to both mottled sculpin and slimy sculpin (Fig. 2).

RF classifications revealed that Sr, Ba, and Mn were also the three most important elements for correctly classifying coho salmon, mottled sculpin, and slimy sculpin to their streams of origin (Table 3). For steelhead, Sr and Ba were the two most important elements, followed by Mg and then Mn (Table 3). Distributions of the individual otolith concentrations of Mn, Sr, and Ba showed varying degrees of overlap and variability by site and management unit for steelhead and coho salmon (Fig. 3) and the two sculpin species (Fig. 4).

Steelhead RF classifications based upon the steelhead otolith chemistry data supported classification accuracies of 69% to stream, 73% to watershed, and 89% to management unit for the streams analyzed (Table 4). When steelhead otolith chemistry data were transformed,

based upon the regression equations determined for the otolith chemical relationships between steelhead and mottled sculpin, and subjected to the mottled sculpin RF classification, assignment accuracy rates of steelhead were 55% to stream, 57% to watershed, and 75% to management unit (Table 4). When steelhead data were transformed according to the regression equations determined between steelhead and slimy sculpin, and subjected to the slimy sculpin RF classification, assignment accuracies for steelhead were 67% to stream, 72% to watershed, and 96% to management unit (Table 4). When steelhead data transformed according to the regression equations determined between steelhead and coho salmon, and subjected to the coho salmon RF classification, assignment accuracies for steelhead were 39% to stream, 45% to watershed, and 97% to management unit (Table 4). Note that individual fish could only assign to streams, watersheds, or management units for which there were data for each respective species pair. Which, in the cases of the slimy sculpin and coho salmon RF classifications, particularly limits classification options. To illustrate, otolith chemistry data of steelhead from Fischer Creek were not transformed and subjected to the slimy sculpin model, nor could individual steelhead be assigned to Fischer Creek based upon the slimy sculpin model, because the slimy sculpin model was not informed by slimy sculpin collected from Fischer Creek.

SECTION 4: DISCUSSION

We tested the hypothesis that for elements whose otolith incorporation is highly correlated with environmental concentrations, spatial variability in otolith microchemistries should show similar patterns among co-occurring species (Hamer and Jenkin2 2007). We found significant interspecific relationships between otolith concentrations of Sr among all species pairs assessed, as well as Ba in two of three species pairs examined (Table 2). These elements are primarily incorporated into otoliths via cation substitution, which is ideal because otolith concentrations of elements incorporated in this manner are most likely to reflect physicochemical water properties of the environment in a consistent, predictable way (Campana 1999; Doubleday et al. 2014). Thus, our findings support the hypothesis of Hamer and Jenkins (2007). Over a similar geographical extent, Schoen et al. (2016) found positive linear relationships between water and otolith Sr:Ca and Ba:Ca of yellow perch *Perca flavescens* from 12 wetland sites throughout lakes Huron and Michigan. Schoen et al. (2016) did not find significant relationships between water and otolith element:Ca ratios for Mg, Cu, or Zn, and did not draw conclusions about Mn and Pb because they were below detection limits in many samples. Our finding of significant interspecific relationships between the otolith Mn concentrations of juvenile steelhead and those of mottled and slimy sculpins (Table 2) is contrary to expectations for this physiologically essential element. Nonetheless, Mn, in addition to Sr and Ba, is often an important trace element for interpreting fish environmental histories (reviewed by Pracheil et al. 2014). Future research should examine the extent to which this finding may be driven by spatial differences in environmental Mn:Ca between sites.

When environmental variability in $(\text{element:Ca})_{\text{water}}$ exists among different fish habitats, researchers and managers seeking to describe patterns of fish habitat use can use otolith microchemistry analysis as a tool for discriminating fish environmental histories (Campana and Thorrold 2001; Pracheil et al. 2014; Tanner et al. 2016). Our findings suggest that such environmental variability exists across the Lake Michigan basin. Environmental concentrations of both Ca and inorganic trace elements play important roles in contributing to differentiable otolith microchemistries among freshwater fishes with varying environmental histories (Campana 1999; Wells et al. 2003; Olley et al. 2011). The water in which fish reside provides the majority of Ca which comprises the CaCO_3 makeup of bony structures such as otoliths. It is through the process of branchial uptake that inorganic trace elements are also primarily taken up into the blood plasma (Farrell and Campana 1996). Ratios of inorganic trace elements to Ca in water are positively correlated with concentrations of trace elements in the otolith, and lower Ca concentrations in the water result in a greater proportion of the trace elements being absorbed by the gills (Campana 1999).

Physiological barriers also influence the pathway of inorganic elements from water to otolith, affecting the degree to which variability in $(\text{element:Ca})_{\text{water}}$ is reflected in otolith chemistries among fish of varying environmental histories and among different species (Campana 1999; Hamer and Jenkins 2007; Sturrock et al. 2014). Physiologically non-essential trace elements Sr and Ba are predominantly (>98%) incorporated into the mineral components of otoliths through cation substitution for Ca (Izzo et al. 2016). In contrast, >27% of physiologically essential Mn, Cu, and Zn in otoliths is incorporated into the protein component (Doubleday et al. 2014; Izzo et al. 2016). These physiologically essential elements are restricted

in the degree to which they can exhibit environmental differences because their concentrations in the blood plasma are highly regulated and poorly correlated with ambient conditions (Campana 1999; Sturrock et al. 2014). Thus, physiological barriers likely contributed to the interspecific otolith chemistry differences that we found for Sr and Ba, and could have limited our ability to detect otolith chemical differences in Mn, Cu, and Zn between sites, even if ambient concentrations of these elements exhibited environmental variability.

Campana (1999) suggests that the wide range of otolith trace elemental concentrations observed across habitats, species, and studies may also be related to differences in the relative rates of protein synthesis and otolith crystallization. Such a phenomenon may be contributing to our finding of distinct relationships between otolith Sr concentrations of juvenile steelhead and each of the sculpin species (Fig. 2). This finding was somewhat unexpected for many reasons. First, mottled and slimy sculpin are closely-related sister species (Yokoyama and Goto 2005) that are capable of hybridization (Strauss 1986). Furthermore, the two species exhibited similar sizes at age and occupy presumably similar ecological niches, suggesting that the two species exhibit differences in the physiological pathway regulating trace elemental uptake. While we did not perform otolith morphometry analyses, visual differences between mottled and slimy sculpins otoliths shapes were readily observable and may be indicative of different otolith crystallization rates between species. Because some streams within our study area contained both mottled and slimy sculpins, future research could investigate the role of crystallization rates on interspecific differences in otolith trace elemental concentrations. We note that the sculpin samples included in our study were identified to species in the lab prior to otolith excision by counting the number of pelvic fin rays following Hubbs et al. (2004); sculpin

with three rays per fin were identified as slimy sculpin and those with four rays were identified as mottled sculpin. The high correlations and diverging relationships between each sculpin species and juvenile steelhead confirms our distinction of the two species based on pelvic fin ray counts, and underscores the importance of accurate species identification for otolith chemistry provenance studies.

Our investigation into the interspecific relationships between juvenile steelhead, coho salmon, and resident sculpin species was conducted supplementary to a larger, ongoing assessment of juvenile steelhead otolith chemistries among more than 30 streams within the Lake Michigan basin. Because the aim of that larger project is ultimately to build a database of juvenile steelhead otolith chemistries in order to classify unknown-origin adult steelhead, sample sites targeted regions of high juvenile steelhead densities. This led to an uneven spatial distribution of sites with respect to watersheds and management units. A more experimental sampling strategy in which multiple streams were sampled within a set number of watersheds for each management unit would have allowed us to better assess classification performance at these coarser resolutions. We were limited in our site selection for this study to those streams for which we had juvenile steelhead samples already in hand and at which the presence of sculpin and coho salmon had been previously recorded. Nonetheless, our sampling encompassed a broad geographic area within the Lake Michigan basin (Fig. 1), and the otolith trace elemental concentrations we measured encompassed similar ranges of values to those found among yellow perch from nearshore wetlands throughout lakes Huron and Michigan (Schoen et al. 2016) and Chinook salmon from Lake Huron tributaries (Marklevitz et al. 2011). As such, we feel that our sampling coverage was sufficient to investigate the hypothesis that

otolith trace elemental chemistries of juvenile coho salmon and river-resident sculpin species are predictive of those of juvenile steelhead sampled among Lake Michigan tributaries.

Overall, understanding variation in recruitment among individual stocks, and the effects of harvest and management approaches on these dynamics, is critical to effective and efficient use of management resources (Houde 2008; Molton et al. 2012; Ludsin et al. 2014). When the structure of mixed-stock fisheries and populations is unknown, researchers and managers seeking to understand controlling mechanisms may waste a considerable amount of effort in investigating environmental (e.g., temperature, physical processes, predators, and prey) or population demographic variables (e.g., spawning stock biomass, age structure, recruitment) at appropriate spatial resolutions (Myers et al. 1997; Houde 2008). In reality, recruitment to mixed-stock fisheries is more likely regulated by disparate, region-specific mechanisms, with parameters governing recruitment at the stock level potentially uncorrelated with dynamics of the whole mixed-stock fishery (Einum and Nislow 2005; DuFour et al. 2015). Our finding that the elements most correlated between species were also the most informative for classification suggests that increased understanding of interspecific otolith trace elemental relationships may prove useful for streamlining future collections and data acquisition to inform fisheries research and management. Assignment accuracies to stream and watershed decreased by 2-30% and 1-28%, respectively, when otolith trace elemental signatures of juvenile steelhead were transformed to those of mottled sculpin, slimy sculpin, and coho salmon; however, mis-assigned fish often classified into nearby watersheds within the larger management unit, leading to relatively high assignment accuracies (75-97%) at the coarsest geographical resolution (Table 4). This is especially promising because there likely exist many other large

systems for which otolith chemistry on multiple species has been collected, and for which the determination of interspecific chemical relationships could bolster origin assignments. For example, otolith chemistry data sets for multiple species across numerous natal sources now exist for Lake Erie and its tributaries: steelhead (Boehler et al. 2012), walleye *Sander vitreus* (Bigrigg 2008), yellow perch (Pangle et al. 2010; Reichert et al. 2010), and white bass *Morone chrysops* (Davis 2013). We believe there are increased opportunities, efficiencies, and cost savings to be realized by further exploring otolith chemistry relationships among species.

SECTION 5: LITERATURE CITED

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SECTION 6: FIGURES AND TABLES

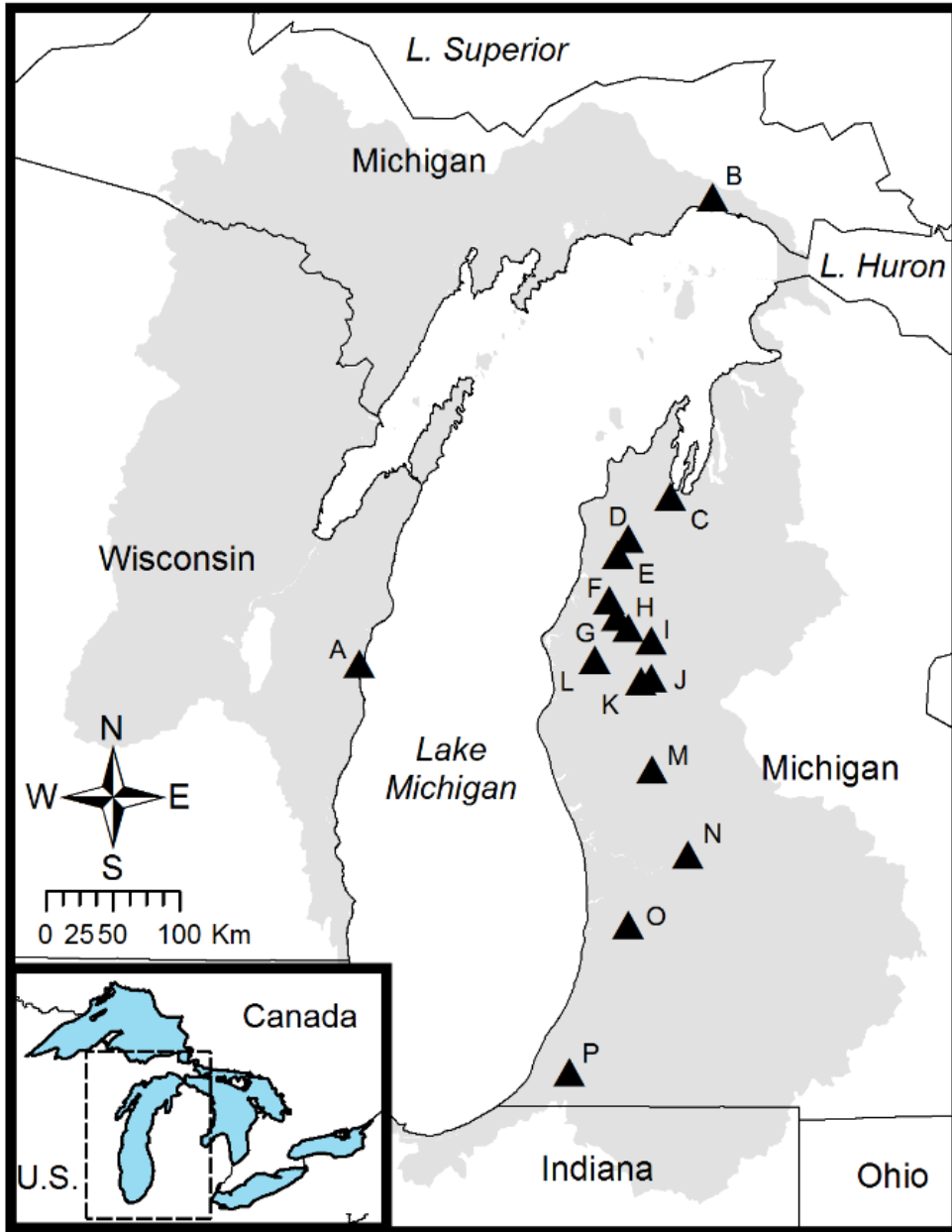


Fig. 1 Locations of the sites sampled are indicated by the triangles and the letters correspond to the site IDs in Table 1. The shaded gray region depicts the surface area within the Lake Michigan basin

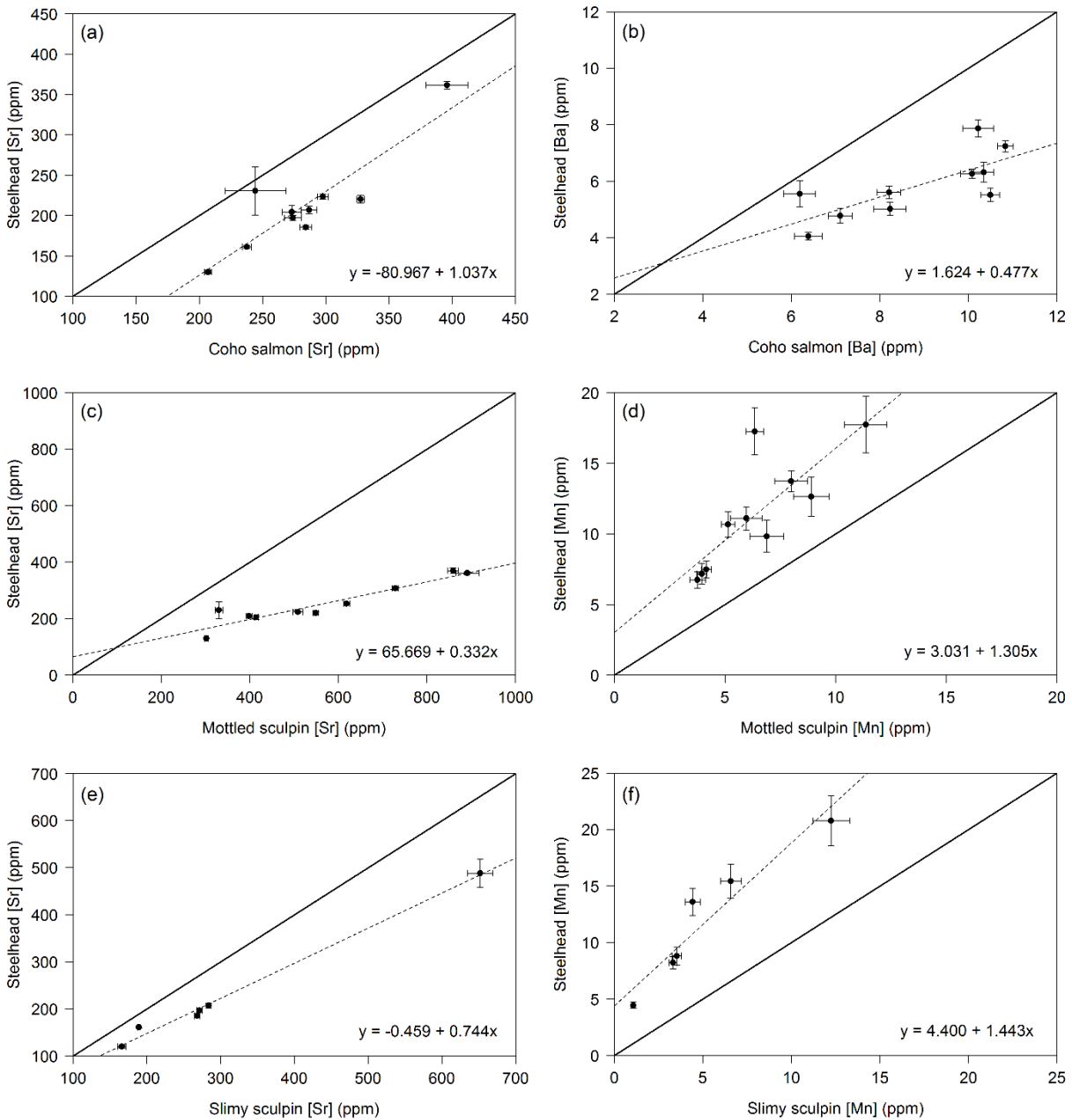


Fig. 2 Linear regressions of the interspecific otolith trace elemental signatures between species.

Points correspond to the average of the median individual elemental concentrations at each site for which both species were sampled. Error bars represent one standard error. The solid line represents a 1:1 relationship, and the dashed line depicts the least-squares regression

equation at the bottom right of each panel. Pearson correlation coefficients and p -values for these regressions are shown in Table 2

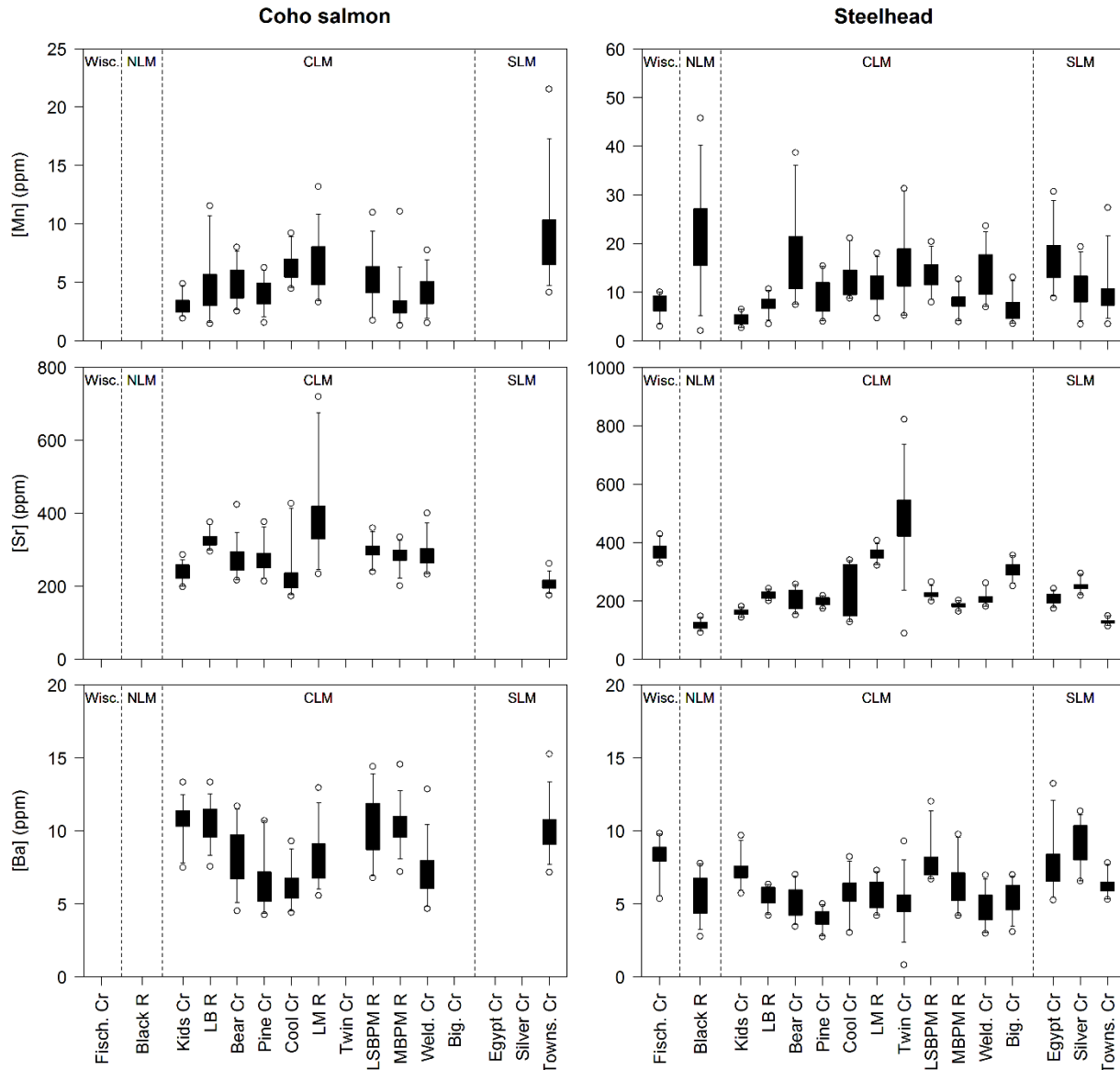


Fig. 3 Box and whisker plots describing the distributions of the median otolith Mn, Sr, and Ba concentrations among all individual age-0 coho salmon (left) and age-0 steelhead (right). Boxes encompass the 25 to 75 percentiles and whiskers extend to the 2.5 and 97.5 percentiles. The dashed vertical lines separate sites among the Wisconsin (Wisc.), northern Lake Michigan (NLM), central Lake Michigan (CLM), and southern Lake Michigan (SLM) management units.

Note that the y-axis scales differ between species

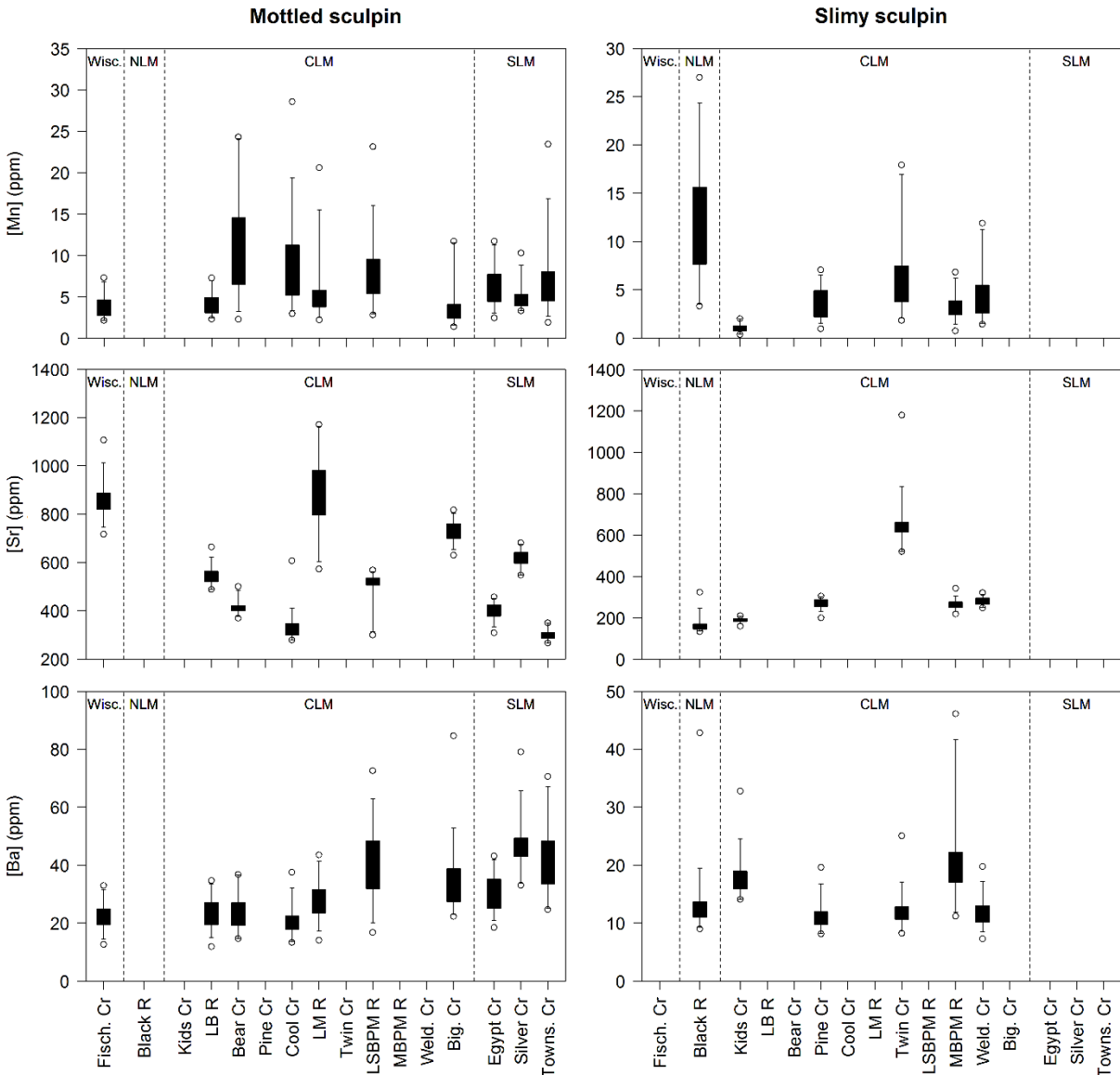


Fig. 4 Box and whisker plots describing the distributions of the median otolith Mn, Sr, and Ba concentrations among all individual mottled sculpin (left) and slimy sculpin (right). Boxes encompass the 25 to 75 percentiles and whiskers extend to the 2.5 and 97.5 percentiles. The dashed vertical lines separate sites among the Wisconsin (Wisc.), northern Lake Michigan (NLM), central Lake Michigan (CLM), and southern Lake Michigan (SLM) management units. Note that the y-axis scales differ between species

Table 1 The number of age-0 steelhead, age-0 coho salmon, mottled sculpin, and slimy sculpin from which otoliths were analyzed in this study, collected in years 2014, 2015, and 2016. Site IDs are used in Figure 1 to depict the location of the streams sampled

Site ID	Stream	Sample size (2014, 2015, 2016)			
		Steelhead	Coho salmon	Mottled sculpin	Slimy sculpin
A	Fischer Cr	0, 10, 0	0, 0, 0	0, 16, 20	0, 0, 0
B	Black R	10, 10, 0	0, 0, 0	0, 0, 0	0, 19, 18
C	Kids Cr	11, 10, 0	0, 20, 20	0, 0, 0	0, 20, 20
D	Little Betsie R	0, 10, 0	0, 20, 20	0, 14, 20	0, 0, 0
E	Bear Cr	10, 10, 0	0, 10, 20	0, 17, 20	0, 0, 0
F	Pine Cr	12, 10, 0	0, 13, 19	0, 0, 0	0, 20, 17
G	Cool Cr	0, 10, 0	0, 13, 0	0, 20, 19	0, 0, 0
H	Little Manistee R	10, 10, 0	0, 20, 20	0, 11, 17	0, 0, 0
I	Twin Cr	11, 10, 0	0, 0, 0	0, 0, 0	0, 20, 19
J	Little S Br Pere				
	Marquette R	10, 10, 0	0, 20, 19	0, 11, 19	0, 0, 0
K	Middle Br Pere				
	Marquette R	10, 10, 0	0, 20, 14	0, 0, 0	0, 20, 20
L	Weldon Cr	9, 10, 0	0, 20, 18	0, 0, 0	0, 20, 19
M	Bigelow Cr	10, 10, 0	0, 0, 0	0, 20, 19	0, 0, 0
N	Egypt Cr	0, 13, 0	0, 0, 0	0, 20, 19	0, 0, 0
O	Silver Cr	11, 10, 0	0, 0, 0	0, 17, 11	0, 0, 0
P	Townsend Cr	10, 10, 0	0, 20, 19	0, 9, 20	0, 0, 0

Table 2 Pearson correlation coefficients (R^2), degrees of freedom (df), and associated p -values describing the correlations between median individual steelhead otolith trace elemental concentrations and those of coho salmon, mottled sculpin, and slimy sculpin among all sites where each species pair was sampled. Significant relationships ($\alpha = 0.05$) are bolded

Statistic	Element						
	Mg	Mn	Cu	Zn	Sr	Ba	Pb
Steelhead ~ coho salmon							
R^2	-0.16	0.31	0.33	0.01	0.89	0.75	0.11
df	8	8	8	8	8	8	8
p	0.67	0.38	0.35	0.97	<0.01	0.01	0.34
Steelhead ~ mottled sculpin							
R^2	0.41	0.81	0.11	-0.12	0.94	0.54	-0.18
df	8	8	8	8	8	8	8
p	0.24	<0.01	0.77	0.75	<0.01	0.11	0.63
Steelhead ~ slimy sculpin							
R^2	-0.44	0.95	-0.52	-0.33	1.00	0.83	-0.05
df	4	4	4	4	4	4	4
p	0.38	<0.01	0.29	0.52	<0.01	0.04	0.93

Table 3 Variable importance (*I*) values describing the mean decrease in classification accuracy when a particular element was permuted across all trees of the random forest classifications built for each species. Higher values denote higher variable importance. Numbers in parentheses are *I* value ranks for each species

Element	Variable importance (<i>I</i>)			
	Coho salmon	Mottled sculpin	Slimy sculpin	Steelhead
Mg	28.1 (4)	18.1 (4)	8.4 (6)	37.0 (3)
Mn	43.8 (3)	30.2 (3)	37.1 (3)	31.8 (4)
Cu	8.0 (7)	9.4 (7)	12.4 (4)	10.8 (5)
Zn	12.9 (6)	14.1 (5)	8.0 (7)	1.2 (7)
Sr	68.6 (1)	75.3 (1)	59.1 (1)	63.9 (1)
Ba	55.5 (2)	55.1 (2)	41.3 (2)	46.4 (2)
Pb	15.2 (5)	10.1 (6)	8.5 (5)	4.4 (6)

Table 4 The proportions of steelhead natal origins assigned correctly at three spatial resolutions. Site IDs correspond to those in

Table 1.

Sample origin		Proportion steelhead assigned correctly											
		Steelhead model			Mottled sculpin model			Slimy sculpin model			Coho salmon model		
Site ID	Watershed	Stream	Watershed	Unit	Stream	Watershed	Unit	Stream	Watershed	Unit	Stream	Watershed	Unit
Wisconsin													
A	Fischer Cr	0.90	0.90	0.90	0.20	0.20	0.20						
Northern Lake Michigan (MI)													
B	Black R	0.95	0.95	0.95				0.80	0.80	0.80			
Central Lake Michigan (MI)													
C	Boardman R	0.95	0.95	1				0.71	0.71	1	0.95	0.95	1
D	Betsie R	0.50	0.50	1	0.60	0.60	0.90				0	0	1
E	Manistee R	0.60	0.65	0.95	0.20	0.20	0.95				0.20	0.20	1
F	Manistee R	0.86	0.86	1				0.41	0.41	1	0.09	0.09	1
G	Little Manistee R	0.10	0.40	0.80	0.30	0.60	0.80				0	0	0.90
H	Little Manistee R	0.70	0.80	1	0.80	0.80	0.85				0.60	0.60	1
I	Little Manistee R	0.90	0.95	1				0.95	0.95	0.95			
J	Pere Marquette R	0.60	0.60	0.60	0.75	0.75	0.80				0.35	0.35	1
K	Pere Marquette R	0.35	0.55	0.90				0.65	0.80	1	0	0.40	1
L	Pere Marquette R	0.42	0.47	1				0.47	0.68	1	0.58	0.74	1
M	Muskegon R	0.70	0.70	0.95	0.60	0.60	0.95						
Southern Lake Michigan (MI)													
N	Grand R	0.31	0.31	0.31	0.38	0.38	0.38						
O	Kalamazoo R	0.71	0.71	0.71	0.38	0.38	0.38						
P	St. Joseph R	0.95	0.95	0.95	1	1	1				0.75	0.75	0.75
Overall		0.69	0.73	0.89	0.55	0.57	0.75	0.67	0.72	0.96	0.39	0.45	0.97

