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## Otolith-Chemistry-Based Discrimination of Wild- and Hatchery-Origin Steelhead across the Lake Michigan Basin

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### Abstract

Effective conservation and management of mixed-stock fisheries depends upon accurate stock identification of individual fish. We evaluated the utility of otolith chemical analysis as an approach to classify juvenile, premigratory steelhead *Oncorhynchus mykiss* collected in tributaries of Lake Michigan as either wild or hatchery origin. Two primary hypotheses proposed were that otolith chemistry can be used (1) to classify juvenile, hatchery-origin steelhead to the correct hatchery of origin and (2) to correctly classify stream-collected juvenile steelhead as wild or hatchery origin. Using laser-ablation inductively coupled plasma mass spectrometry, we analyzed the concentrations of seven elements (using  $^{25}\text{Mg}$ ,  $^{55}\text{Mn}$ ,  $^{65}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{88}\text{Sr}$ ,  $^{137}\text{Ba}$ ,  $^{208}\text{Pb}$ ) in the otoliths of juvenile steelhead collected from five hatcheries and 25 streams in the Lake Michigan basin. When discriminating among hatcheries, only 4.41% of fish misclassified to an alternate hatchery when subjected to the best random forest classification algorithm that included all elements as predictor variables. Distinct chemical signatures between fish of wild and hatchery origin supported 100% classification accuracies of known-wild, age-0 steelhead as wild origin for 19 of the 25 streams sampled. Misclassification of wild, age-0, and hatchery-origin fish, which tended to occur for streams that were located in close proximity to the hatchery, never exceeded 3.4% for a given stream. Our findings demonstrate highly successful discrimination of hatchery-origin and wild juvenile steelhead across a broad geographic range. Applying the classification algorithms developed herein to unknown-origin steelhead provides the ability to infer survival of year-classes from specific hatcheries. Further, the ability to differentiate hatchery- and wild-origin fish will assist in stock assessment efforts allowing for increased effectiveness of conservation and management of the species.

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Discrimination of stocked and wild conspecifics is a common fisheries management concern (e.g., Johnson et al. 2010; Schröder and Garcia de Leaniz 2011; Hinrichsen et al. 2016). Management agencies differentiate wild and stocked fish in many ways, including fin-clipping, internal and external tagging, and chemical marking (e.g., oxytetracycline marking) (McFarlane et al. 1990; Pine et al. 2012); however, accurate quantification of the proportion of a fishery derived from stocked fish requires marking all stocked fish each year, and such an undertaking may not be feasible for various reasons. For example, fin-clipping and tagging may not be viable for early life stages or small fish sizes, and the cost and labor required to mark all fish may be prohibitive (Hammer and Blankenship 2001).

Alternatively, fish origin may be determined through analysis of characteristics imparted differentially upon fish occupying either hatcheries or natural environments. For example, growth pattern analyses have been developed for scales (Seelbach and Whelan 1988) and fin spines (Siegwarth 1994) to differentiate between wild- and hatchery-origin fish. More recently, analysis of fish otolith microchemical signatures has become a primary tool for reconstructing fish environmental histories, including fish origin (Campana 1999; Campana and Thorrold 2001; Pracheil et al. 2014), and in distinguishing hatchery- and wild-origin fish (Coghlan et al. 2007; Marklevitz et al. 2011). Briefly, the incorporation of various trace elements into the calcium carbonate matrix of otoliths is related to both the availability of these elements in the environment and physicochemical properties of the water in which fish reside (Campana and Thorrold 2001; Elsdon et al. 2008; Sturrock et al. 2014). Thus, diagnostic differences in otolith trace elemental concentrations may arise given sufficient differences in the properties governing trace element uptake among sites (Wells et al. 2003; Pangle et al. 2010; Schoen et al. 2016). Importantly for distinguishing between wild and stocked fish, otolith microchemical analysis may overcome the common limitations of fish-marking programs because this methodology has successfully been applied to fish as young as the larval life stage (Pangle et al. 2010; Reichert et al. 2010), all fish naturally receive this “mark,” and costs are only expended on the fish analyzed.

Both wild- and hatchery-origin steelhead *Oncorhynchus mykiss*, the anadromous and potamodromous forms of Rainbow Trout, are important components of a world-class, multispecies, recreational salmonine fishery throughout Lake Michigan and its tributaries—representing millions of dollars of value to the region (Tanner and Tody 2002; Tsehaye et al. 2014; Clark et al. 2016). The contemporary Lake Michigan steelhead fishery is supplemented with annual plantings of more than one million juvenile steelhead raised at five hatcheries among the states of

Illinois, Indiana, Michigan, and Wisconsin. Nonetheless, wild, naturally reproduced steelhead have long comprised a considerable proportion of the total steelhead population (Tody and Tanner 1966; Seelbach and Whelan 1988; Barron et al. 2004). Most of the steelhead stocked within the Lake Michigan basin are not marked by any means; thus, otolith chemical analysis may constitute a cost-effective approach of determining the origins of unmarked steelhead in this population. Such information may provide valuable insights as to the effectiveness of the multistate juvenile steelhead stocking program relative to fishery management objectives.

To assess the ability of otolith microchemical analysis to discriminate wild- and hatchery-origin Lake Michigan steelhead, we propose two primary hypotheses:

1. Among the five hatcheries that contribute juvenile steelhead to the Lake Michigan basin, otolith chemistry can be used to classify juvenile, hatchery-origin steelhead to the correct hatchery of origin.
2. Within stream systems into which juvenile steelhead are stocked, otolith chemistry can be used to correctly classify juvenile steelhead as wild or hatchery origin.

These hypotheses were based on several lines of evidence specific to the Great Lakes basin, including the following: (1) a preliminary evaluation of the water chemistry of the hatcheries (K. Pangle, unpublished data) indicating that the hatcheries had distinct chemical signatures, likely due to their use of well water, (2) the geological heterogeneity of the Great Lakes basin, which gives rise to strong spatial differences in water chemistries (Alexander et al. 1996; Pangle et al. 2010), (3) a previous otolith chemistry study that differentiated the origin of Lake Erie steelhead among different hatcheries (Boehler et al. 2012), and (4) previous otolith chemistry studies that successfully differentiated between hatchery and wild origin for other fish species in different Great Lakes (Schaner et al. 2007; Marklevitz et al. 2011; Landsman et al. 2017).

To test hypothesis 1, we assessed the ability of a classification model built using otolith microchemistry data to assign fish to the correct hatchery of origin. Otolith microchemistry data for this analysis were obtained from juvenile steelhead sampled at each of the hatcheries that raise steelhead to be stocked within the Lake Michigan basin. To test hypothesis 2, we assessed the extents to which stream-specific classification models could correctly discriminate between known-wild-origin juvenile steelhead sampled from 25 Lake Michigan tributaries and hatchery-origin juvenile steelhead from the hatcheries that source the stockings into those streams.

While many published otolith chemistry provenance studies have assessed the performance of classification models on known-origin samples, relatively few studies

have subsequently applied these predictive models to fish of unknown origin (e.g., Vasconcelos et al. 2008; Engstedt et al. 2010). To demonstrate how such predictive models can be used to determine the natal sources of unknown-origin fish, we applied our stream-specific classification models on unknown-origin, age-1 steelhead sampled from these same streams to predict their origin (i.e., hatchery versus wild). Natal source predictions of unknown-origin samples are presented in the context of uncertainty measures derived from each stream-specific model's performance on known-origin samples.

## METHODS

**Sample collection.**— Juvenile steelhead collected for this research are described in Tables 1 and 2. Age-0 stream-collected and hatchery fish were considered to be of known origin. Age-1 stream-collected fish were considered unknown origin. Hatchery sites constituted all of the hatcheries at which steelhead stocked into the Lake Michigan basin between 2014 and 2016 were raised. Stream sites comprised tributaries previously shown to support potamodromous salmonid natural reproduction (e.g., Avery 1974; Seelbach and Whelan 1988; Rutherford 1997; Hir-ethota and Burzynski 2015) and were also selected to maximize spatial (Figure 1) and geologic representations of the Lake Michigan basin. Hatchery fish were collected by hatchery personnel, and stream-caught fish were sampled using backpack electrofishing. All samples were stored frozen in water until lab processing.

**Otolith chemical analysis.**— Fish were thawed immediately before processing. The total lengths of each fish were measured to the nearest millimeter. Scale samples were taken from the dorsal side of each fish, midway between the dorsal and anal fins, and stored in paper-lined scale envelopes. Scale aging was used to verify known, wild-origin juvenile steelhead as age 0. Sagittal otoliths were removed from each fish, cleaned of adhering tissues using hydrogen peroxide, rinsed with reverse osmosis water, and placed in sorting trays to dry. Aberrant-shaped otoliths,

or otoliths with clear and glassy deposits, were identified as vateritic otoliths. Because vaterite uptakes trace elements differently than aragonite, the predominant crystal form of calcium carbonate comprising steelhead sagittal otoliths, we excluded vateritic otoliths (24 out of 984 otoliths, total) from our analyses (Melancon et al. 2005). For each fish, the right sagittal otolith was mounted in Epofix cold-setting embedding resin using silicone molds and allowed to harden in a desiccation chamber for 24–48 h. A PICO 155 Precision Saw was used to cut a 300–400- $\mu\text{m}$  section of each embedded otolith along the transverse plane. Otolith sections were polished to the plane of the core sequentially with 600- and 1,200-grit silicon carbide paper followed by 1- $\mu\text{m}$  Alumina polishing film. We checked that the polished sections were smooth and absent of any deep scratches using a microscope. Sections were then mounted onto an etched petrographic slide (30–40/ slide) using brush-on Crazy Glue, sonicated in ultrapure water (18.2 M $\Omega$ -cm) for 7 min, and stored in a laminar flow hood until chemical analysis.

Otolith sections were analyzed for trace elements using laser-ablation inductively coupled plasma mass spectrometry (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 ( $1.6 \pm 0.1$  L/min) to which argon makeup gas ( $1.2 \pm 0.15$  L/min) was added. Laser and ICP-MS operating parameters were tuned to achieve a thorium : uranium ( $^{232}\text{Th}:$  $^{238}\text{U}$ ) ratio of 1.0 and a thorium : thorium oxide ( $^{232}\text{Th}:$  $^{232}\text{Th}^{16}\text{O}$ ) ratio of <1.8% at the start of each session. Laser fluence was estimated to be within 3–6 J/cm<sup>3</sup>.

We measured the otolith trace element concentrations based on the isotopes magnesium ( $^{25}\text{Mg}$ ), calcium ( $^{43}\text{Ca}$ ), manganese ( $^{55}\text{Mn}$ ), copper ( $^{65}\text{Cu}$ ), zinc ( $^{66}\text{Zn}$ ), strontium ( $^{88}\text{Sr}$ ), barium ( $^{137}\text{Ba}$ ), and lead ( $^{208}\text{Pb}$ ). Data were

TABLE 1. Sampling information for hatchery-origin juvenile steelhead.

Hatchery description	Abbreviation	Sample date	Year-class	<i>n</i>	Mean TL $\pm$ SE (mm)
Bodine State Fish Hatchery, Mishawaka, Indiana	BSFH	Apr 10, 2014	2013	8	190 $\pm$ 9.2
		Nov 17, 2015	2015	10	157 $\pm$ 3.9
Jake Wolf Memorial Fish Hatchery, Topeka, Illinois	ILFH	Oct 20, 2015	2015	10	175 $\pm$ 6.0
Kettle Moraine Springs State Fish Hatchery, Adell, Wisconsin	KMFH	Nov 1, 2015	2015	10	171 $\pm$ 6.1
Thompson State Fish Hatchery, Manistique, Michigan	TSFH	Apr 25, 2014	2013	10	205 $\pm$ 4.7
		Apr 13, 2015	2014	10	190 $\pm$ 5.8
Wolf Lake State Fish Hatchery, Mattawan, Michigan	WLFH	Aug 28, 2015	2015	10	182 $\pm$ 4.2

TABLE 2. Sampling information for wild-origin, age-0 juvenile steelhead and wild-captured, age-1 juvenile steelhead of unknown origin.

Site ID	Site	Known-origin age-0 fish		Unknown-origin age-1 fish	
		<i>n</i>	Mean TL ± SE (mm)	<i>n</i>	Mean TL ± SE (mm)
<b>Michigan streams</b>					
UP1	Days River	10	76.1 ± 2.3	0	
UP2	Haymeadow Creek	10	73.4 ± 1.6	14	163.9 ± 6.5
UP3	Eighteen Mile Creek	9	70.0 ± 2.1	7	128.8 ± 5.9
NLP1	Horton Creek	10	79.5 ± 2.8	20	149.0 ± 3.9
NLP2	Jordan River	10	62.0 ± 3.8	18	154.1 ± 5.7
NLP3	Kids Creek	21	55.5 ± 1.9	20	114.7 ± 4.0
NLP4	Platte River	10	52.9 ± 2.3	10	148.0 ± 13.3
NLP5	Lemon Creek	10	77.1 ± 3.2	4	157.3 ± 9.9
NLP6	Bear Creek	20	77.3 ± 3.1	20	142.8 ± 4.1
NLP7	Pine Creek	22	68.4 ± 3.1	15	114.8 ± 9.0
NLP8	Weldon Creek	19	74.5 ± 2.8	20	133.6 ± 4.0
NLP9	Sanborn Creek	15	69.5 ± 1.4	17	124.7 ± 5.5
NLP10	Middle Branch Pere Marquette River	20	73.7 ± 2.5	10	107.5 ± 4.7
NLP11	Little South Branch Pere Marquette River	20	76.9 ± 2.9	20	148.6 ± 7.1
NLP12	Bigelow Creek	20	73.4 ± 1.8	19	138.3 ± 8.0
NLP13	Muskegon River tributary	10	57.3 ± 1.5	10	72.3 ± 1.7
SLP1	Prairie Creek	20	87.1 ± 2.5	14	178.9 ± 8.3
SLP2	Egypt Creek	13	114.9 ± 3.5	7	184.1 ± 4.9
SLP3	Honey Creek	10	91.2 ± 2.2	10	168.4 ± 2.7
SLP4	Silver Creek	21	57.4 ± 2.1	20	116.6 ± 4.5
SLP5	Townsend Creek	20	60.9 ± 2.8	20	136.3 ± 3.8
SLP6	Dowagiac River tributary	10	63.8 ± 3.4	10	112.2 ± 4.6
<b>Wisconsin streams</b>					
W1	Sauk Creek	20	87.5 ± 3.8	10	167.6 ± 12.2
W2	Fischer Creek	13	75.2 ± 1.8	19	132.5 ± 5.1
W3	Hibbard Creek	19	68.1 ± 2.4	20	145.4 ± 5.1

collected for a transect running from 200 µm opposite the primordium to the otolith edge (12-Hz ablation rate, 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, an 80-µm-wide raster was ablated using single laser pulses 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 min during analysis, the international glass reference material NIST 612 was analyzed (4-µm × 140-µm transects). Three transects each of the NIST 610 and MACS3 (U.S. Geological Survey 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 Iolite mass spectrometry software package (version 2.31; Paton et al. 2011). Calcium

(measured as <sup>43</sup>Ca during each analysis) 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 s of carrier or 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 : <sup>43</sup>Ca ratios in the NIST 612 standards bracketing the samples. 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.

*Statistical analyses.*—Our statistical analyses employ a machine learning classification method called random forests (R package “randomForest”), hereafter abbreviated

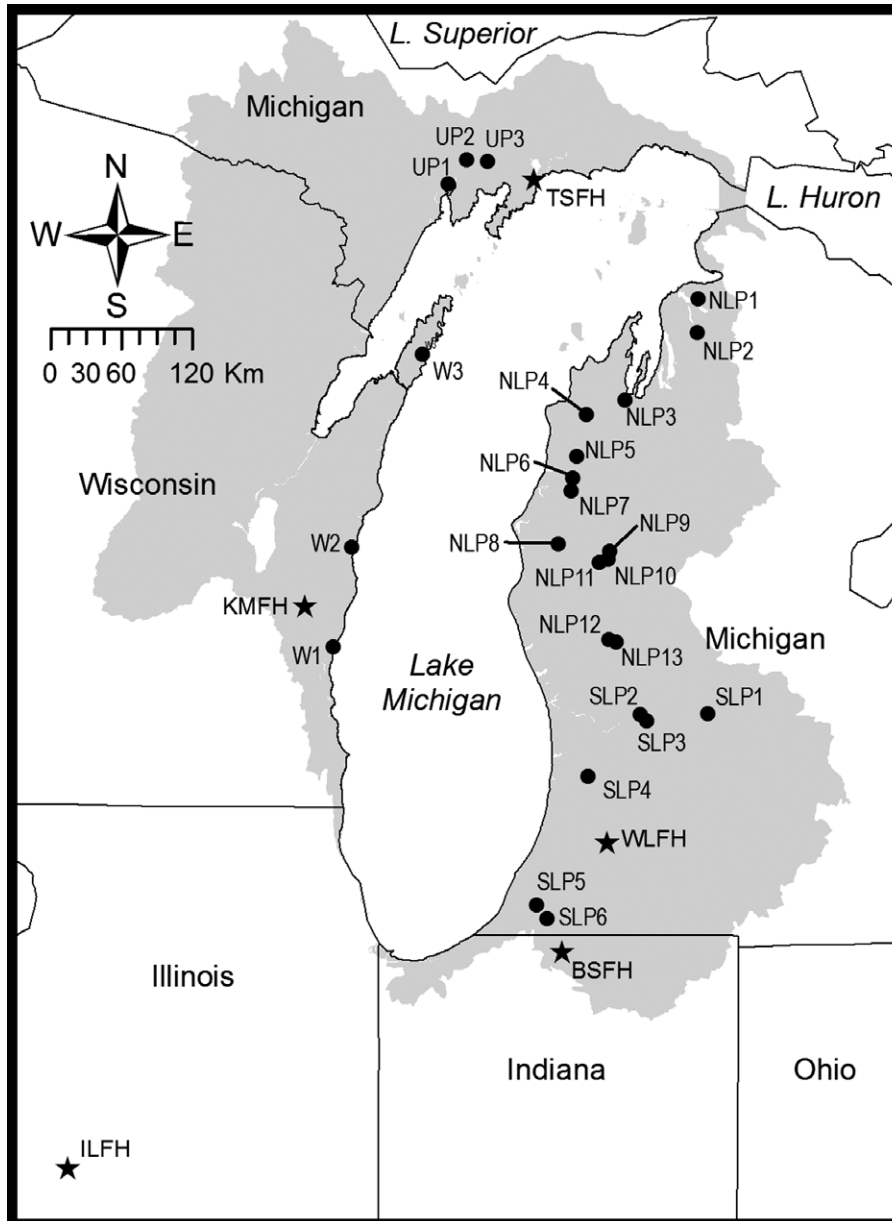


FIGURE 1. Stream sampling locations (circles) and hatchery sites (stars) included in this study. Hatchery site abbreviations correspond to those in Table 1, and stream site IDs are those described in Table 2. The shaded gray region indicates the Lake Michigan drainage basin.

RF (Breiman 2001). We chose to use the RF algorithm because it performed well, was fairly easily to decipher, produced a predictive model, and was not constrained by statistical assumptions. Linear discriminant analysis, a classification technique commonly applied to otolith microchemistry datasets, is dependent upon predictor variables following normal distributions among sampling units, whereas the RF algorithm is not (Breiman 2001; Mercier et al. 2011). Unlike analysis of variance methods, RF models can also be used to predict the assignments of unknown-origin samples. When compared with other

classification techniques, RF models performed better than linear or quadratic discriminant analysis and were more interpretable than similarly performing artificial neural networks (Mercier et al. 2011).

An RF model can be described as the aggregated results of an ensemble or “forest” of individual classification and regression trees (commonly referred to as CART models) that are each built using a random bootstrap of the data. Each tree is constructed as a hierarchy of bifurcating data partitions, called decisions, which minimize misclassification error (Sarma 2013). Each decision is based on the value of

one predictor variable (e.g., trace element concentration), and in RF models each decision is determined as the best split among a random subset of the predictors—thus adding a second element of randomness to model development (Breiman 2001; Liaw and Wiener 2002).

Once the entire forest of trees is built, samples are partitioned according to all of the trees, and the class of the response variable that a sample classifies to most often (natal origin location, in this case) is called the assignment. For each sample, the proportion of classifications to each of the classes of the response variable can be interpreted as the predicted probability for that assignment (Liaw and Wiener 2002). Cross validation is embedded into the model development because each tree is built using a bootstrap of the original data, so the aggregated assignments of the “out-of-bag” data (i.e., data not in the bootstrap) can be used to accurately estimate model performance (Breiman 2001; Liaw and Wiener 2002). The two layers of randomness built into the RF algorithm help make it robust to overfitting that might arise from, for example, building an inordinate amount of trees or including a high number of predictor variables (Breiman 2001).

Temporal variability in otolith trace element concentrations among similarly aged fish of a given species at a particular site may be linked to temporal variability in site-specific conditions, such as fluctuations in the abundances of the measured elements, or dynamic environmental parameters, including temperature and water chemistry (Gillanders 2002; Elsdon and Gillanders 2006; Elsdon et al. 2008). Thus, the “shape” of the relationship between otolith transect position and the concentrations of the elements measured may be informative for distinguishing fish from different origins. To make use of such potential temporal variability, we divided transect distances into deciles and calculated mean elemental concentrations for each of these 10 equidistant intervals to be used as model predictor variables.

In order to appropriately compare time-varying otolith chemistries among fish of different natal origins, data

should comprise similar temporal extents within the lives of the fish analyzed. For our classification model development and natal origin predictions, we truncated transects and associated otolith chemistry data relative to the youngest fish (i.e., earliest sampling date) used to develop each model (see calculation of  $t_{2adj}$  in Figure 2). To do this, we assumed an otolith formation date of April 1, as this represents a midpoint spawn date for this species over our sampling area, and assumed a linear relationship between otolith age and transect length. Additionally, because otolith core chemistries of juvenile potamodromous salmonids vary with the spawning run timing of the mother (e.g., Miller and Kent 2009), data included in our analyses began 50  $\mu\text{m}$  outside of the core.

To test hypothesis 1, the RF algorithm was used to build a classification model to predict the hatchery of origin of juvenile steelhead sampled from the five hatcheries used for Lake Michigan plantings. Fish used to construct the model are those described in Table 1. We assessed the performance of a full model that incorporated all of the predictor variables corresponding to the 10 decile mean concentrations of the seven measured elements ( $p = 70$ ). We compared the performance of the full model against a subset of reduced models that contained fewer elements as predictor variables. The performance of each model was assessed at three values of  $m_{\text{try}}$ , the number of randomly subsetted predictor variables ( $p$ ) considered at each decision within the RF algorithm. The default  $m_{\text{try}}$  value implemented by the randomForest package is  $\sqrt{p}$ , and following Liaw and Wiener (2002), we assessed the performance of the full model at 0.5, 1, and 2 times the default value to examine the influence of the number of variables on model performance. Upon examination of the variable importance values (essentially the decrease in model performance absent a particular predictor variable; Liaw and Wiener 2002) produced by building the full model, we chose to examine only a subset of the potential reduced models most likely to perform as well or better than the

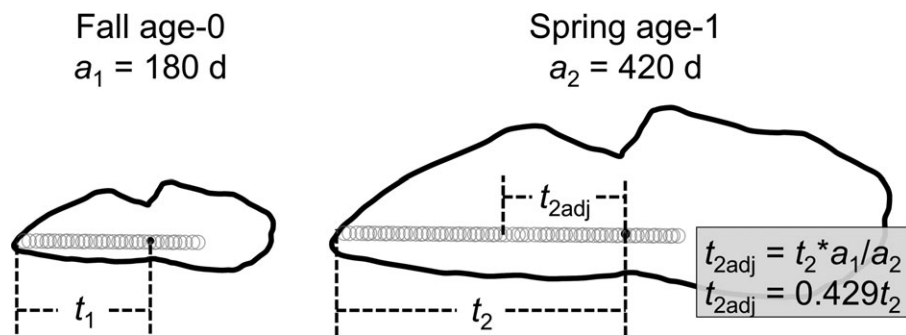


FIGURE 2. Graphical depiction of how transects and associated otolith chemistry data were truncated relative to the youngest fish used to develop each model. In this example, age-0 data extends from the core to the otolith edge and age-1 data extends from the core for a distance of  $0.429t_2$  (corresponding to  $t_1$  and  $t_{2adj}$ , respectively).

full model. All RF models were built using 200,000 trees. We report all results in terms of misclassification rates of the assignments of “out-of-bag” data.

To test hypothesis 2, the RF algorithm was used to build 25 site-specific classification models. Individual fish included in each site-specific model consisted of the wild-origin, age-0 steelhead sampled from that site (Table 2) and hatchery-origin steelhead from the hatcheries that sourced steelhead stocked proximal to that site from 2014 to 2016 (Table 1). Fish in this analysis are considered known-origin because the hatchery-origin juvenile steelhead were obtained directly from the hatcheries and wild-origin juvenile steelhead were obtained as age-0 fish sampled in the fall. We can be confident that age-0 juvenile steelhead sampled in the fall were truly wild origin as Indiana, Michigan, and Wisconsin Departments of Natural Resources only stocked juvenile steelhead either as yearlings in the spring or as fall fingerlings after our sampling was completed for streams included in our analyses. Each model thus consisted of a dependent variable whose classes were the potential origins of the fish (i.e., “wild” and any of the hatcheries included in each site-specific model), and the predictor variables were those from the best-performing model built to test hypothesis 1. For practical purposes, we report misclassification rates in terms of wild- or hatchery-origin assignment.

As additional measures of model performance, we present the following: (1) the minimum predicted probability among all fish for models with 100% classification accuracy and (2) the maximum predicted probability among misclassified fish for models in which there were misclassifications.

Lastly, we applied these stream-specific classification models to the unknown-origin, age-1 steelhead described in Table 2. To account for uncertainty in the RF model predictions of unknown-origin juvenile steelhead, we deemed predictions corresponding to predicted probabilities less than those of all misclassified, known-origin juvenile steelhead for a particular site-specific model as insufficient for natal-origin assignment (i.e., “unknown”).

## RESULTS

### Classification of Known-Origin Juvenile Steelhead

*Discrimination among hatcheries.*—The RF classification algorithm achieved the lowest misclassification rate (4.41%) when concentrations of all seven elements were included in the analysis and the number of predictor variables considered at each decisional node was  $m_{\text{try}} = 0.5p^{0.5}$  (Table 3). Of the reduced models explored, models using only Sr and Ba as inputs, or Mn, Sr, and Ba as predictors, achieved misclassification rates of 5.88% at  $m_{\text{try}}$  values of  $0.5p^{0.5}$  and  $p^{0.5}$ , respectively. Elemental

concentrations of Sr and Ba showed the greatest contrast among hatcheries across the otolith transects, and this was driven to a large degree by Sr concentrations in excess of 1,000 ppm at TSFH. All fish from BSFH and TSFH classified to the correct hatchery of origin (Table 4). One fish from each of ILFH and KMFH misclassified into the other, and one WLFH fish misclassified as having come from BSFH (Table 4).

*Site-specific hatchery versus wild classification.*—Overall, 19 of the 25 site-specific classification models exhibited 0.0% misclassification (Table 5). Of the sites stocked by only a single hatchery, only the model for Bigelow Creek (NLP12) had fish that misclassified (10.0%). All of the sites sourced by three hatcheries (i.e., BSFH, TSFH, and WLFH) had fish that misclassified, but improper discrimination between wild- and hatchery-origin never exceeded 3.4% (Table 5). In terms of misclassification rate and the predicted probabilities of assignments, the model for Eighteen Mile Creek performed the best with all predicted probabilities of assignment  $\geq 0.843$  (Table 5). Figure 3 shows how the elements most important for discriminating wild- and hatchery-origin juvenile steelhead differed between the Eighteen Mile Creek model (the best-performing model) and the Bigelow Creek model (the poorest-performing model).

### Natal Origin Predictions of Unknown-Origin Juvenile Steelhead

We predicted the natal origins of 353 age-1 steelhead of unknown origin (Table 6). The majority (89.0%) were predicted to be of wild origin. Based upon their otolith microchemistries, we detected fish from each of the hatcheries that sourced steelhead stockings into the tributaries

TABLE 3. Misclassification rates (%) of random forest classification models developed using otolith microchemistry data. Fish included in the models are the hatchery-origin juvenile steelhead described in Table 1. The parameter  $m_{\text{try}}$  describes the proportion of predictor variables ( $p$ ) considered at each tree split within the random forest algorithm.

Model	$m_{\text{try}} = 0.5p^{0.5}$	$m_{\text{try}} = p^{0.5}$	$m_{\text{try}} = 2p^{0.5}$
Hatchery ~ Mg, Mn, Cu, Zn, Sr, Ba, Pb	4.41	7.35	10.29
Hatchery ~ Mg, Mn, Zn, Sr, Ba	7.35	7.35	10.29
Hatchery ~ Mn, Sr, Ba	5.88	8.82	10.29
Hatchery ~ Zn, Sr, Ba	8.82	10.29	10.29
Hatchery ~ Sr, Ba	7.35	5.88	10.29
Hatchery ~ Sr	13.24	14.71	17.65
Hatchery ~ Ba	22.06	22.06	25.00

TABLE 4. Site-specific assignments of hatchery-origin juvenile steelhead based on random forest classification using otolith microchemistry data. Known origin is representative of the location fish were collected from; predicted origin represents the location to which the fish were classified to based on random forest analysis. The fish analyzed and the origin abbreviations are described in Table 1.

Known origin	Predicted origin					Error (%)
	BSFH	ILFH	KMFH	TSFH	WLFH	
BSFH	18	0	0	0	0	0.0
ILFH	0	9	1	0	0	10.0
KMFH	0	1	9	0	0	10.0
TSFH	0	0	0	20	0	0.0
WLFH	1	0	0	0	9	10.0

TABLE 5. Wild-origin (W) and hatchery-origin (H) assignments of known-origin juvenile steelhead based upon random forest classification models developed with otolith microchemistry data. The minimum predicted probabilities are presented for models with 100% classification accuracy, and the maximum predicted probabilities among misclassified fish are presented for models with classification accuracy < 100%.

Site ID	W-W <sup>a</sup>	W-H	H-H	H-W	Misclassified (%)	Minimum correct predicted probability	Maximum incorrect predicted probability
<b>KMFH-sourced streams</b>							
W1	20	0	10	0	0.0	0.722	
W2	13	0	10	0	0.0	0.681	
W3	19	0	10	0	0.0	0.712	
<b>TSFH-sourced streams</b>							
UP1	10	0	20	0	0.0	0.814	
UP2	10	0	20	0	0.0	0.838	
UP3	9	0	20	0	0.0	0.843	
NLP1	10	0	20	0	0.0	0.733	
NLP2	10	0	20	0	0.0	0.681	
<b>WLFH-sourced streams</b>							
NLP8	19	0	10	0	0.0	0.747	
NLP9	15	0	10	0	0.0	0.748	
NLP10	20	0	10	0	0.0	0.723	
NLP11	20	0	10	0	0.0	0.667	
NLP12	19	1	8	2	10.0		0.688
NLP13	10	0	10	0	0.0	0.750	
SLP1	20	0	10	0	0.0	0.705	
SLP2	13	0	10	0	0.0	0.768	
SLP3	10	0	10	0	0.0	0.591	
SLP4	21	0	10	0	0.0	0.685	
<b>TSFH- and WLFH-sourced streams</b>							
NLP3	21	0	30	0	0.0	0.683	
NLP4	10	0	30	0	0.0	0.517	
<b>BSFH-, TSFH-, and WLFH-sourced streams</b>							
NLP5	9	1	47	1	3.4		0.524
NLP6	20	0	47	1	1.5		0.512
NLP7	22	0	48	0	0.0		0.456 <sup>b</sup>
SLP5	20	0	46	2	2.9		0.511
SLP6	8	2	48	0	3.4		0.521

<sup>a</sup>Column headers (e.g., W-W) indicate the actual origin (first letter) and the predicted origin (second letter).

<sup>b</sup>There was 0.0% misclassification for the hatchery versus wild assignment, but not all hatchery fish assigned to the correct hatchery of origin.



that we sampled. Representative otolith chemistry plots of sites within the stocking domains of each possible hatchery that exhibited hatchery-origin assignments are shown in Figure 4.

## DISCUSSION

Random forest models based upon otolith chemistry discriminated among different hatchery sources and between wild- and hatchery-produced fish in the Lake Michigan basin. The distinctions were based largely on differences in strontium and barium, two elements that are readily incorporated into the crystalline matrix of the otolith at concentrations proportional to their abundance in the environment (Mugiya and Tanaka 1995; Farrell and Campana 1996; Bath et al. 2000) and that have been shown to be important in previous applications of otolith chemistry in the Great Lakes basin (Brazner et al. 2004; Pangle et al. 2010; Boehler et al. 2012; Prichard et al. 2018) and in studies that differentiated hatchery and wild Rainbow Trout outside of the Great Lakes basin (Coghlan et al. 2007; Gibson-Reinemer et al. 2009; Zitek et al. 2010). Concentrations of these elements were most extreme and unique in the otoliths of some hatchery fish. For example, fish reared in the Thompson State Fish Hatchery (Michigan) had Sr concentrations an order of magnitude greater than most wild fish. Thompson State Fish Hatchery's primary water source is a deep well and analysis of its water chemistry shows similarly elevated levels of Sr (K. Pangle, unpublished data). Though

unintentional, such anthropogenic influences on otolith chemistry can be very helpful in imparting unique chemical signatures in hatchery fish (Boehler et al. 2012).

Our study builds on previous applications of otolith chemistry that differentiated hatchery and wild fish (e.g., Coghlan et al. 2007; Gibson-Reinemer et al. 2009; Zitek et al. 2010) by evaluating these differences over a broad spatial scale. Results reveal the importance of spatial proximity in determining the degree of accuracy when assigning origin. For example, wild fish in streams located within 100 km of a hatchery tended to be the most difficult to discriminate from that hatchery. This result likely reflects region-specific characteristics of the underlying determinants of otolith chemistry, namely water chemistry, which in turn is strongly influenced by geology and hydrology. Previous studies have shown strong spatial heterogeneity of water chemistry in the Great Lakes basin and its effect on the otolith chemistry of different wild populations of Yellow Perch *Perca flavescens* (Pangle et al. 2010) and Chinook Salmon *Oncorhynchus tshawytscha* (Marklevitz et al. 2011). Our results here show that hatcheries, particularly those fed primarily by surface water, are essentially acting in the same fashion as natural streams.

Until now, when fish did not have obvious marks or a fin clip, the most common means of differentiating hatchery from wild steelhead in the Great Lakes has been through the identification of patterns in scale circuli that represent differing growth patterns (Seelbach and Whelan 1988). While this can be a beneficial tool, scale analysis may be limited in the details it provides. For example, scale analysis

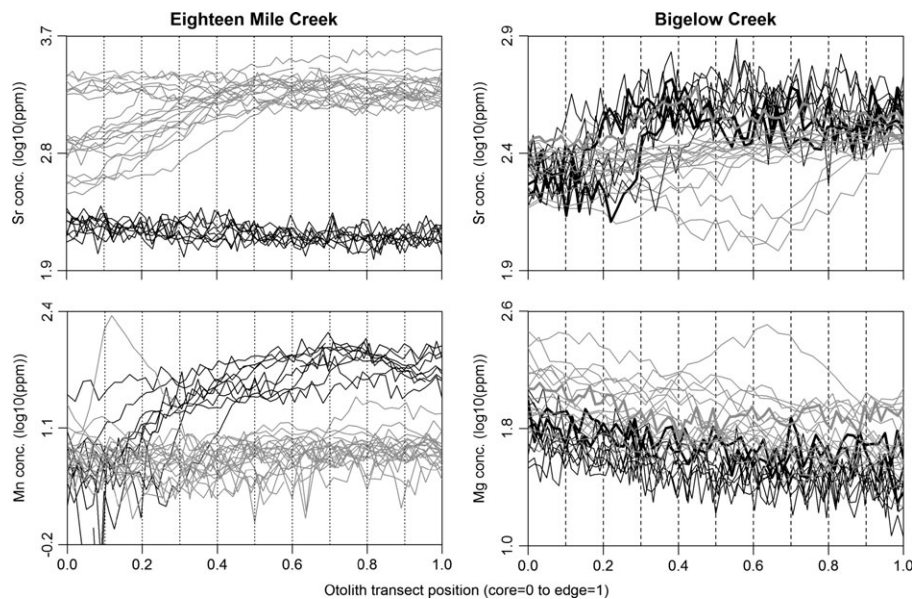


FIGURE 3. Raw elemental data of the known-origin wild (gray) and hatchery-origin (black) juvenile steelhead used to develop the site-specific classification models for Eighteen Mile (left panels) and Bigelow (right panels) creeks, the site-specific models that performed the best and poorest, respectively. Elements plotted are those that were most discriminatory for each respective site. Bold lines in the Bigelow Creek plots represent the fish that misclassified.

TABLE 6. Predicted natal origins of unknown-origin, age-1 steelhead based upon random forest classification models built using otolith microchemistry data from known-origin juvenile steelhead. Natal origin assignments were deemed “unknown” when predicted probabilities were less than those of all misclassified, known-origin juvenile steelhead for a particular site-specific model, hence the not applicable (NA) for models that exhibited 100% classification accuracy of known-origin juvenile steelhead (see Table 5).

Site	Assignment ( <i>n</i> )					
	Wild	BSFH	KMFH	TSFH	WLFH	Unknown
<b>KMFH-sourced streams</b>						
W1	7		3			NA
W2	15		4			NA
W3	15		5			NA
<b>TSFH-sourced streams</b>						
UP2	14			0		NA
UP3	14			0		NA
NLP1	20			0		NA
NLP2	17			1		NA
<b>WLFH-sourced streams</b>						
NLP8	20				0	NA
NLP9	17				0	NA
NLP10	10				0	NA
NLP11	19				1	NA
NLP12	15				1	2
NLP13	10				0	NA
SLP1	12				2	NA
SLP2	7				0	NA
SLP3	10				0	NA
SLP4	13				0	NA
<b>TSFH- and WLFH-sourced streams</b>						
NLP3	20			0	0	NA
NLP4	7			3	0	NA
<b>BSFH-, TSFH-, and WLFH-sourced streams</b>						
NLP5	0	0		0	0	4
NLP6	16	1		0	0	3
NLP7	15	0		0	0	0
SLP5	15	3		0	0	2
SLP6	8	2		0	0	0

does not provide reliable differentiation of premigratory juveniles or identify the specific hatchery of origin. Otolith microchemistry enables further discrimination of premigratory juvenile steelhead to specific hatcheries and streams of origin. Additionally, otolith chemical analysis can be applied to assess differences in survival and returns to creel of fish stocked from different hatchery facilities.

Although not a primary objective of our study, the specific assignments of the unknown-origin, age-1 steelhead was informative. Inclusion of unknown-origin, age-1 steelhead in our study allowed for the demonstration of successful classification of older fish using models developed from younger fish. Despite the large number of published otolith chemistry studies, such demonstrations are still relatively rare. Successful classification of age-1 steelhead increases our confidence in applying a similar approach to unknown-

origin adults. Fish assigned as “hatchery fish” most commonly occurred when collections were proximal to a stocking site and occurred shortly after spring stockings. The lack of hatchery classifications of fish collected during summer or fall supports the hypothesis that hatchery fish outmigrate soon after stocking and observations of age-1 steelhead are primarily confined to wild fish. Consequently, if there is an impact of hatchery fish on stream ecosystems, it will be localized and short lived.

Based on recent investigations, including this study, otolith microchemistry is likely to provide a useful tool for management of steelhead and other mixed-stock fisheries in areas inside the Great Lakes basin (Schaner et al. 2007; Marklevitz et al. 2011; Landsman et al. 2017) and outside of the basin (Mercier et al. 2011; Pracheil et al. 2014; Tanner et al. 2016). Our relatively high accuracy was on par

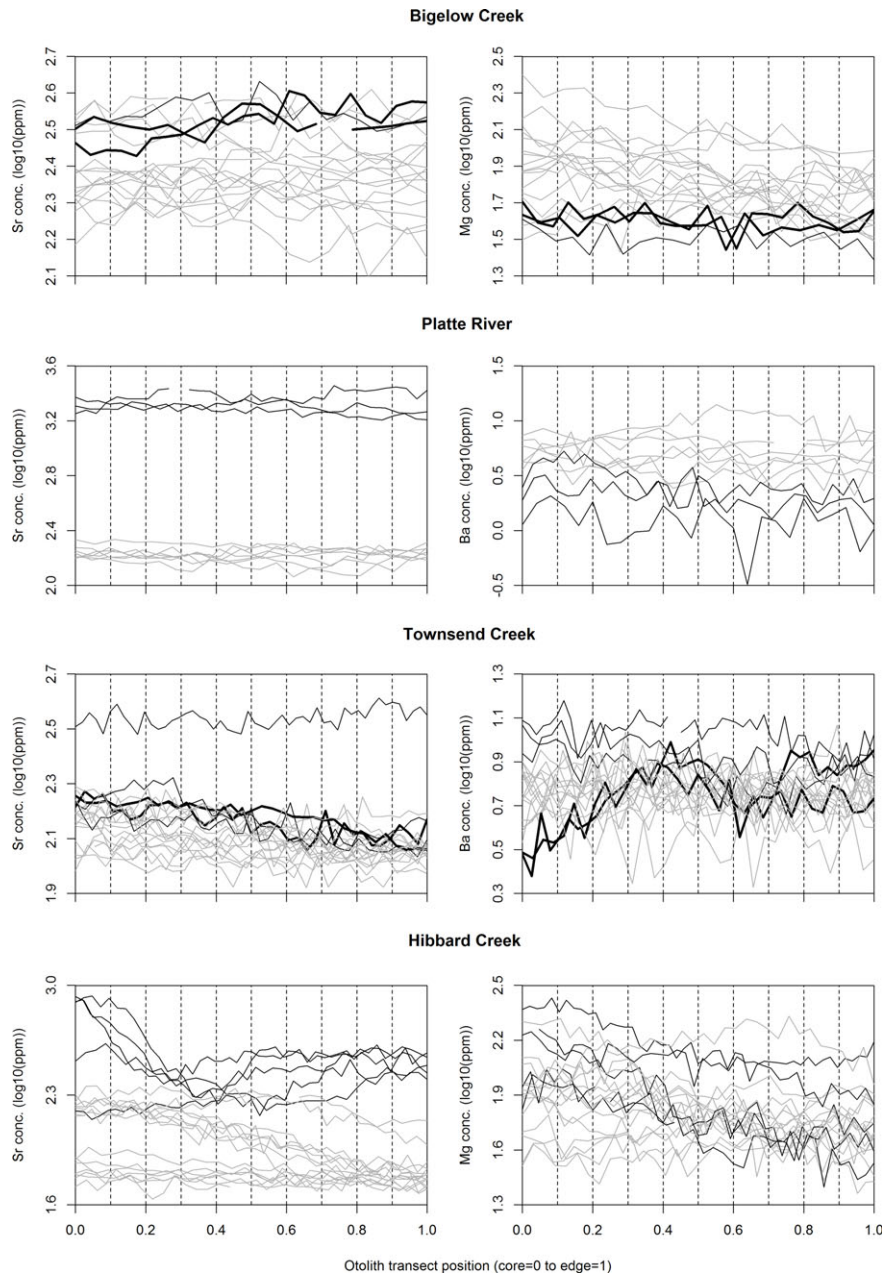


FIGURE 4. Raw elemental data of unknown-origin, age-1 juvenile steelhead (Table 1). Thin lines correspond to fish whose origin could be determined, and colors correspond to model-predicted origin assignments (black = hatchery origin, gray = wild origin; see Table 6). Bold lines in the plots of Bigelow and Townsend creeks represent fish whose natal origin could not be determined. Each site displayed had juvenile steelhead that assigned to a different hatchery. Elements plotted are those that were most discriminatory for each respective site.

with previous studies of Great Lakes fish species that differentiated hatchery and wild origins (Schaner et al. 2007; Landsman et al. 2017). Such reliable discrimination will provide a means to quantify relative contributions in the Great Lakes and tributary systems, as well as how contributions may vary among locations and from year to year. Better identification of stocked fish and the facilities at which they were reared could lead to a better understanding of

population dynamics and the influence of rearing and stocking strategies. In addition, our study identified two patterns that can be used to maximize differentiation between fish of hatchery and wild origins. First, chemical signatures of hatcheries can vary substantially in their relative degree of uniqueness. The use of hatcheries that impart very distinct chemistries, such as the Thompson State Fish Hatchery, will allow for clearer discrimination of wild fish.

Second, discriminatory power improves as distance between hatchery and stocking locations increases. This trend highlights the potential value in not stocking in close proximity to the hatchery if desiring distinct separation from wild fish. Our study demonstrates the ability to classify fish origin based on otolith chemical signatures of age-0 wild and age-1 hatchery fish and to apply these signatures to unknown-origin, age-1 fish. This process has the potential to determine origins of adult steelhead when focusing on the natal (age-0) region of the otolith, thus providing insight to the overall population structure and allowing for increased effectiveness of conservation and management of steelhead in the Great Lakes region.

Such applications of otolith chemistry also have limitations and alternatives that must be considered. For example, otolith chemistry analysis may be considerably more expensive than scale analysis, and the latter may be effective when discriminating hatchery versus wild adult steelhead (Seelbach and Whelan 1988). In addition, while we focused on the absolute concentrations of specific isotopes in our analysis, other studies have shown that isotopic ratios may also be useful in discriminating hatchery versus wild origin (Landsman et al. 2017), and chemical signatures based on isotopic ratios can be more temporally stable than absolute concentrations (Walther and Thorrold 2009). Finally, otolith chemistry analysis requires lethal sampling, which may be prohibitive, particularly when dealing with imperiled fish species.

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