QUANTIFYING DIFFERENCES IN OTOLITH CHEMISTRY OF CHINOOK SALMON IN LAKE MICHIGAN TO DETERMINE NATAL ORIGINS

By

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ABSTRACT

QUANTIFYING DIFFERENCES IN OTOLITH CHEMISTRY OF CHINOOK SALMON IN LAKE MICHIGAN TO DETERMINE NATAL ORIGINS

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Alexander C Maguffee

Previous research has indicated that a substantial amount of hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*) migrate from Lake Huron to Lake Michigan, likely due to greater foraging opportunities in Lake Michigan, indicating the potential for wild Chinook salmon to exhibit similar movement patterns. Thus, an increased priority has been placed on quantifying the movement of wild Chinook salmon from Lake Huron to Lake Michigan. The goal of this research was to determine the feasibility of quantifying inter-basin movement of wild Chinook salmon using otolith microchemistry techniques. Chinook salmon otolith pairs were extracted from juvenile and adult fish collected in 2015 and 2016 from tributaries in six predefined regions. Otoliths were analyzed using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA ICP MS) to determine trace metal concentrations, and various multivariate classification algorithms were evaluated for accuracy of classification. Juvenile data reclassified to their natal regions with classification success at a basin level comparable to previous Great Lakes otolith studies. Applying the juvenile-fit models to the adult data resulted in moderate success at a basin level. MANOVAs indicated significant differences in otolith microchemistry between juvenile year classes, and these differences negatively affected classification accuracy. These findings suggest that otolith microchemistry can be used to estimate wild Chinook salmon inter-basin movement, and that classification accuracy will be much higher if the model is developed from the same year class as the assessment sample.

This thesis is dedicated to my parents, who have always encouraged me to pursue my passions and instilled me with the drive to do so.

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INTRODUCTION

Chinook salmon (*Oncorhynchus tshawytscha*) were introduced to the Laurentian Great Lakes in the late 1960s with intentions of establishing a sport fishery and controlling invasive alewife (*Alosa pseudoharengus*) (Kocik & Jones 1999). Previous attempts to introduce Pacific salmonids to the region in the late 1800s failed to produce a sustainable recreational fishery (Tody & Tanner 1966, Emery 1985, Parsons 1973). Since these attempts, the Great Lakes have experienced several major ecosystem changes, driven primarily by introductions of non-native species (Mills et al. 1993), which allowed for the eventual successful introduction of Chinook salmon. Until the mid-1900s, the native lake trout (*Salvelinus namaycush*) was the dominant predator species in the Great Lakes (Smith 1968, Hansen and Holey 2002). Due to predation by invasive sea lamprey (*Petromyzon marinus*), in combination with substantial fishing pressure from the commercial fishery, lake trout populations collapsed in the mid-1900s (Smith 1968, Hansen and Holey 2002). In the absence of the lakes' top predator, populations of invasive alewife drastically increased (Smith 1970, O'Gorman and Stewart 1999). Alewife dominated the fish biomass of the Great Lakes in the 1960s, and experienced large spring die-offs that caused the fouling of beaches (Mills et al. 1993, Brown 1968, Brown 1972). Recognizing the need for a return of predatory species, and the potential for successful non-native salmonid introduction, Great Lakes managers stocked Chinook salmon into Lake Michigan in 1967 and Lake Huron in 1968 (Kocik and Jones 1999, Tody and Tanner 1966, Emery 1985). These introductions were highly successful due to the large biomass of alewife, and helped to develop a world-class recreational fishery in the Great Lakes (Tanner and Tody 2002, Emery 1985, Mills et al. 1994).

Chinook salmon are now a critical component of the multi-billion dollar fishery in the Great Lakes, which is enjoyed by over 9 million anglers each year (Hansen and Holey 2002, Tanner and Tody 2002, U.S. Department of the Interior 2006).

Chinook salmon were successfully introduced in both Lake Michigan and Lake Huron, although the lakes have experienced very different histories since these introductions. In Lake Michigan, stocking rates gradually increased through the 1970s and 1980s to a maximum of 7.86 million smolts in 1989 (Lake Michigan Technical Committee, Salmonid Working Group (SWG), unpublished data). Harvest rates remained high until the late 1980s, when a bacterial kidney disease (BKD) epizootic resulted in sharp declines in survival (Holey et al. 1998, Madenjian et al. 2002). Stocking rates have since declined to 3.2 million smolts in 2012, while harvest increased following the BKD epizootic in the 1980s and 1990s (Lake Michigan Technical Committee, Salmonid Working Group (SWG), unpublished data). While Lake Huron has experienced similar patterns in stocking rate reductions (Grischke 2011), harvest has notably declined in large part due to the 2004 Lake Huron alewife collapse (Roseman and Riley 2007, Riley et al. 2008). This collapse was documented by Riley et al. (2008), who found that the abundance of many deepwater demersal fish had declined significantly since the 1980s and 1990s, perhaps due in part to substantial predation from offshore fish predators such as Chinook salmon or lake trout (Johnson et al. 2005, Dobiesz et al. 2005, He et al. 2015), causing a destabilization of the lakes' predator-prey balance (Stewart et al. 1981, Tsehaye et al. 2014a, Tsehaye et al. 2014b). The decline of alewife in Lake Huron led to a decrease in Chinook salmon catch rates, growth rates (Dobiesz et al. 2005, Roseman and Riley 2007), and survival (Brenden et al. 2012); the statistical catch-at-age assessment conducted by Brenden et al. (2012)

indicated that the most recent declines in predator abundance were associated with a decrease in the abundance of alewives.

Chinook salmon populations were initially sustained via stocking, but naturally produced smolts have contributed to the lake wide populations since the early 1970s (Claramunt et al. 2012). Since their introduction, the natural reproduction of Chinook salmon has steadily increased. Wild smolt production in Lake Michigan tributaries has been estimated from various analyses, including recaptures of adult Chinook salmon marked with oxytetracycline (OTC) and stream electrofishing surveys (Jonas et al. 2008, Carl 1982, Keller et al. 1990, Hesse 1994, ESR and DFC, unpublished data, RMC and J. Johnson, unpublished data). These analyses have shown an increase in the number of wild smolts contributing to the population in Lake Michigan (Jonas et al. 2008). Due to increasing natural reproduction, in combination with stocking cuts, the proportion of wild fish in both lakes has increased. Williams (2012) used OTC analyses to determine the proportion of wild fish harvested in the Lake Michigan recreational fishery. He found that the fishery harvest consisted of approximately 53% to 70% wild fish among the four year classes between 2006 and 2009 (Williams 2012, Jonas et al. 2008). Johnson et al. (2010) also used OTC techniques to assess the contribution of wild fish to the Lake Huron fishery, and found that wild fish contributed greater than 80% to the harvest between 2000 and 2003. This variation in the contribution of wild fish to fishery harvest highlights yet another difference in Chinook salmon stocks between the Lake Michigan and Lake Huron basins.

The production rates of natural recruits varies substantially within each basin as well, particularly among Lake Michigan tributaries (Carl 1982, Creque et al. 2005, Ed Rutherford, NOAA, personal communication). Electrofishing surveys conducted in Lake Michigan by state

agencies have revealed that the majority of wild Lake Michigan Chinook salmon originate from tributaries along the eastern shore of Lake Michigan. For example, it was estimated by Carl (1982) that natural recruits produced in eastern shore tributaries such as the Manistee, Muskegon, Pere Marquette, White, Platte, Jordan, and Boyne Rivers represented 23% of the total recruitment into the fishery, including hatchery plants, in the late 1970s, when hatchery plants contributed more to the total population than naturally produced smolts. It is to be expected that tributaries in the state of Michigan contribute a greater amount of smolts to the total population in Lake Michigan, as these streams exhibit a number of qualities that make them ideal spawning and rearing habitat for Chinook salmon, including cold temperatures, high river discharge rates, and the presence of coarse gravel substrate (Groot and Margolis 1991, Raleigh et al. 1986), whereas some of these qualities are lacking in other Lake Michigan streams. More recently, Rutherford developed a Geographic Information System (GIS) model to estimate the theoretical contribution of individual streams to the Chinook salmon fishery based on the stream habitat classification system of Seelbach et al. (1997) (Creque et al. 2005, Ed Rutherford, NOAA, personal communication). This GIS model translated several stream attributes into fish production categories to estimate wild smolt production for each tributary in Lake Michigan and Lake Huron (Creque et al. 2005). While these techniques can estimate the potential for wild smolt production among several Great Lake tributaries, the actual production of natural recruits from individual streams is unknown.

Smolts produced in the tributaries of Lake Michigan represent a substantial contribution to the fishery in Lake Michigan, but another potential source of recruits might be wild fish migrating from Lake Huron. Movement of hatchery-reared fish from Lake Huron to Lake

Michigan has been documented (Clark and Bence 2012, Johnson et al. 2005). Johnson et al. (2005) examined the movement of adult Chinook salmon between Lake Michigan and Lake Huron by comparing the recapture rates of adults that were tagged as juveniles with coded wire tags (CWT), and found that an increasing proportion of Lake Huron stocked fish captured in Lake Michigan waters in the 1990s and early 2000s. Clark and Bence (2012) hypothesized that Chinook salmon from Lake Huron may be migrating to Lake Michigan due to potentially greater foraging opportunities in Lake Michigan, particularly following the 2004 Lake Huron alewife collapse (Riley et al. 2008). The potential for movement was tested by comparing the recapture rates of tagged hatchery fish released at a Lake Michigan site (Medusa Creek) and a Lake Huron site (Swan River), both located within 50 miles of the Mackinaw Bridge. Based on the absolute number of recaptures, recapture rates of Lake Huron stocked fish in Lake Michigan increased from about 5% in 1994 to a maximum of approximately 80% in 2003. Since 2000, on average more than 50% of the recaptures of Lake Huron stocked fish were in Lake Michigan, while 2% or less of the recaptures of Lake Michigan stocked fish were in Lake Huron. The results suggest that a large number of stocked fish move from Lake Huron to Lake Michigan, while few move in the other direction (Clark and Bence 2012).

Given this movement of hatchery fish to Lake Michigan from Lake Huron, it is likely that wild fish exhibit similar movement patterns. Indirect evidence indicates the potential for the inter-basin movement of wild Chinook salmon. Williams (2012) found that the percent wild fish in a cohort increased with age in Lake Michigan. For all cohorts, age-1 Chinook salmon averaged 55% wild, and age-2 fish averaged 64% wild. Williams (2012) concluded that the most likely explanation for the increase in percent wild fish from age-1 to age-2 was wild Lake Huron fish

migrating to Lake Michigan after their first year. In addition, it has been observed that the Lake Huron Chinook salmon fishery has shifted from one that is year round to one in which fish are harvested mostly in the early spring and fall (Clark et al. 2016, Dave Gonder, OMNR, personal communication). These fish are apparently feeding elsewhere during the summer, some likely in Lake Michigan. In recent years, low estimated prey fish biomass in both lakes has caused the Lake Huron and Lake Michigan Technical Committees to place elevated priority on quantifying this movement.

While the GIS model developed by Rutherford can be used to estimate smolt production of Great Lakes streams to the recruitment of the fishery (Creque et al. 2005; Seelbach et al. 1997; Ed Rutherford, NOAA, personal communication), this approach does not take into account the potential differences in survival and movement once smolts reach the lake. A more definitive approach would be to apply marks to juveniles in each tributary and assess their relative contributions to the fishery by collecting them as adults. However, this would likely be costly and difficult. A potentially useful tool for differentiating juvenile stream sources of Chinook salmon is analysis of otolith microchemistry. Otolith microchemistry provides an alternative, natural tag that avoids these issues. Natural tags, or markers that occur naturally, provide benefits over traditional mark-recapture studies involving artificial tags, such as avoiding the need to physically tag fish and allowing each fish to count as marked (Thorrold et al. 2002). Genetic markers are a commonly used natural tag, and have been used to distinguish among source populations of salmon and various other species (Thorrold et al. 2002, Davies et al. 1999, Kordos and Burton 1993). Parasites provide another natural tag which can be used to determine if a fish has visited a particular environment by determining whether or not a fish is

infected by a particular species of parasite (MacKenzie and Abaunza 1998). Finally, environmental markers, such as developmental differences in size and growth, can be used to discriminate fish populations (Thorrold et al. 2002, Swearer et al. 1999).

More recently, environmental markers such as geochemical signatures in otoliths have been used to discriminate source populations (Thorrold et al. 1998). Otoliths incorporate trace elements from the aquatic environment relative to their proportion in the water column, which have been found to vary considerably among individual streams and geographic regions. Trace element sources, and the resulting variation in otolith chemical signatures, are influenced by factors such as bedrock and surficial geology, atmospheric deposition, and anthropogenic impacts (Campana 1999, Campana and Thorrold 2001, Elsdon et al. 2008). This method makes use of the differences in otolith chemical signatures between sites at various scales, which are permanently and continually incorporated into the calcium carbonate structure (Campana 1999, Campana and Thorrold 2001).

While otolith microchemistry has historically been used to distinguish fish population sources, more recent work has indicated the potential for its use to quantify movement. For example, Hoover (2012) used otolith microchemistry to determine the inshore-offshore movement patterns of Atlantic croaker (*Micropogonias undulatis*) and black sea bass (*Centropristis striata*). While this study was mostly focused on the timing of movement, it also examined spatial differences in otolith microchemistry as a proxy for time, indicating that spatial differences in otolith microchemistry may be a means by which movement can be quantified. Multiple studies have concluded that otolith microchemistry has the potential to

quantify movement, particularly in the Great Lakes region (Pangle et al. 2010, Brazner et al. 2004).

Several studies have applied otolith microchemistry techniques in the Great Lakes on various species. For example, Pangle et al. (2010) used otolith microchemistry to determine differences in geographically distinct source populations of larval yellow perch (*Perca flavescens*) in Lake Erie. Other studies have used otolith microchemistry to examine chemical signatures unique to yellow perch from various spawning habitats in Lake Superior (Brazner et al. 2004), determine the stock structure of Lake Superior lake herring (Bronte et al. 1996), and identify the natal sources of invasive sea lamprey in the Great Lakes (using statoliths; Hand et al. 2008).

Recent studies have also applied otolith microchemistry to salmonids in the Great Lakes. Watson (2016) used otolith microchemistry to differentiate wild sources of Lake Michigan steelhead, as well as to determine differences in chemical signatures between wild and hatchery fish. In Lake Huron, Marklevitz et al. (2011) found that otolith microchemistry could be used to discriminate juvenile Chinook salmon between rearing environments, collection sites, and geological regions. Following this work, Marklevitz et al. (2016) then used otolith microchemistry to test the assumption of a mixed stock fishery, concluding that stocks in Lake Huron were more heterogeneously mixed than previously thought. Given the success of this method on Lake Michigan steelhead and Lake Huron Chinook salmon, it is likely that otolith microchemistry can be used to successfully distinguish Chinook salmon harvested in Lake Michigan as well.

While these recent studies examined differences in otolith microchemistry among spatially distinct regions (Watson 2016, Marklevitz et al. 2011, Marklevitz et al. 2016), they did not examine the potential for the use of these microchemistry differences to quantify movement. Mark-recapture studies of hatchery fish have supplied us with movement estimates for stocked fish (Adlerstein et al. 2007, Adlerstein et al. 2008, Johnson et al. 2010), but the movement rates of wild Chinook salmon have yet to be quantified. A better understanding of the contribution of wild Lake Huron smolts to the Lake Michigan fishery could lead to more informed management decisions such as stocking rates and fishing regulations.

Juvenile natal river sources can be classified at a regional level (groups of streams) with up to 80-90% success (Watson 2016, Marklevitz et al. 2011, Marklevitz et al. 2016, Pangle et al. 2010, Brazner et al. 2004). Given this success, it is likely that adult Chinook salmon can be correctly classified to their natal origins as well. However, there is the potential for otolith microchemistry to exhibit variation among years (Tanner et al. 2012), thereby complicating spatial classification. For example, Pangle et al. (2010) found that larval yellow perch could not successfully classify to their natal origins when using data from different years, indicating significant inter-annual variability in otolith microchemistry. It is important to account for annual variation if a goal is to identify the source composition of adults from the open lake. For example, if inter-annual variability is found to exist, it may be necessary to match adults to the signatures of juveniles from the same cohort.

This research aims to quantify the movement of wild Lake Huron Chinook salmon to Lake Michigan. Our primary goal was to develop and test a model that can be used to

determine if there is movement of wild fish between Lake Michigan and Lake Huron. Assuming this movement is observed, future studies may quantify the magnitude of this movement.

Data were gathered over multiple years and study sites, allowing us to answer multiple questions related to our goals. Juveniles were collected in their natal streams to assess the differences in otolith microchemistry over multiple scales, and multiple years of data were collected to assess the potential for variability in otolith microchemistry between year classes. Adults were collected in the streams. Given that most Chinook salmon adults captured in a stream are returning to their natal stream (Quinn and Fresh 1984), this allowed us to examine the success of assigning known origin adult fish using juvenile data. Given the scope of our data, we used otolith microchemistry to examine three specific research objectives related to our primary research goal:

(1) Develop a model to discriminate natal source by geographic regions for juvenile Chinook salmon within and between Lake Michigan and Lake Huron, as well as between wild and hatchery fish;

(2) Determine the model's capacity for quantifying movement by classifying adult fish of assumed known origin;

(3) Determine the effect of year-class on discrimination by comparing the classification success of juvenile models between years, and by building single-cohort and multiple-cohort models.

Based on previous literature, we hypothesized that (1) otolith microchemistry can be used to discriminate juvenile natal sources at multiple scales with a classification success comparable to previous otolith studies (upwards of 80%), (2) stream collected adults can be

classified to their natal sources based on juvenile data with moderate success, and (3) otoliths from juvenile fish of different year classes will have different chemical signatures, which will negatively affect classification accuracy when using one juvenile year class to classify the other.

METHODS

Sample Collection

Wild juvenile Chinook salmon were collected between the months of April and August in 2015 (n=223) and 2016 (n=143) (Table 1). Fish were collected with a model 12-B Smith-Root backpack electrofisher by Michigan State University, Central Michigan University, and Michigan Department of Natural Resources (MDNR) personnel. Wild adult Chinook salmon were collected in the tributaries during the months of October and November in 2015 and 2016. The majority (n=49 in 2015, n=42 in 2016) of adults were obtained as carcasses by Michigan State University personnel; their sagittal otoliths were extracted on site. Two samples were collected via angler volunteers in 2015. The remaining wild adult otoliths (n=16 in 2015, n=17 in 2016) were collected at the Swan River, Medusa Creek, Little Manistee River, and Boardman weirs by MDNR personnel and the Strawberry Creek weir by Wisconsin Department of Natural Resources (WDNR) personnel. The presence or absence of an adipose fin clip was recorded for each adult sample and used to distinguish between wild and hatchery-reared fish. The sagittal

Table 1.—Summary of juvenile and adult fish collected and successfully analyzed from each region in 2015 and 2016.

Region Number	Region Code	Region Description	2015 Juveniles	2015 Adults	2016 Juveniles	2016 Adults
1	UPP	Upper Peninsula (MI)	10	2	17	0
2	NLP	Northern Lower Peninsula (MI)	63	32	65	34
3	SLP	Southern Lower Peninsula (MI)	81	7	44	8
4	WIS	Wisconsin	48	12	0	10
5	NLH	Northern Lake Huron (ONT)	0	0	13	0
6	SGB	Southern Georgian Bay (ONT)	21	14	4	7
		Total	223	67	143	59

otoliths of all juvenile fish and the angler-collected adult fish were extracted at Michigan State University. All otoliths were cleaned of adhered tissue and left to dry in microcentrifuge vials (juveniles) or sample envelopes (adults).

Sampling occurred within the streams of 6 pre-selected regions in the Lake Michigan and Lake Huron basins, which were selected based on surficial and bedrock geology and evidence of previous spawning (Marklevitz et al. 2011). Within each region, 3-12 collection sites were sampled to help obtain a sufficient sample size for analysis (Table 2, Figure 1). The regions were: Upper Peninsula (UPP), Northern Lower Peninsula (NLP), Southern Lower Peninsula (SLP), Wisconsin (WIS), Northern Lake Huron (NLH), and Southern Georgian Bay (SGB).

Table 2.—Summary of streams where fish were collected in 2015 and 2016 within each region of collection. All numbers correspond to the locations in Figure 1. Note: Un-Named Creek is a tributary in the St. Joseph watershed that did not have a name.

Region of Collection	Stream	
Lake Michigan		
UPP	(1) Days River, MI	(2) Eighteen Mile Creek, MI
NLP	(3) Boyne River, MI	(4) Kids Creek, MI
	(5) Little Betsie Creek, MI	(6) Little Manistee River, MI
	(7) Little South Branch- Pere Marquette, MI	(8) Middle Branch- Pere Marquette, MI
	(9) Pine Creek, MI	(10) Platte River, MI
	(11) Weldon Creek, MI	
SLP	(12) Bigelow Creek, MI	(13) Muskegon River, MI
	(14) Prairie Creek, MI	(15) Silver Creek, MI
	(16) Un-Named Creek, MI*	
WIS	(17) Casco Creek, WIS	(18) Hibbards Creek, WIS
	(19) Kewaunee River, WIS	(20) Sauk Creek, WIS
Lake Huron		
NLH	(1) Manitou River, ON	
SGB	(2) Bighead River, ON	(3) Silver Creek, ON

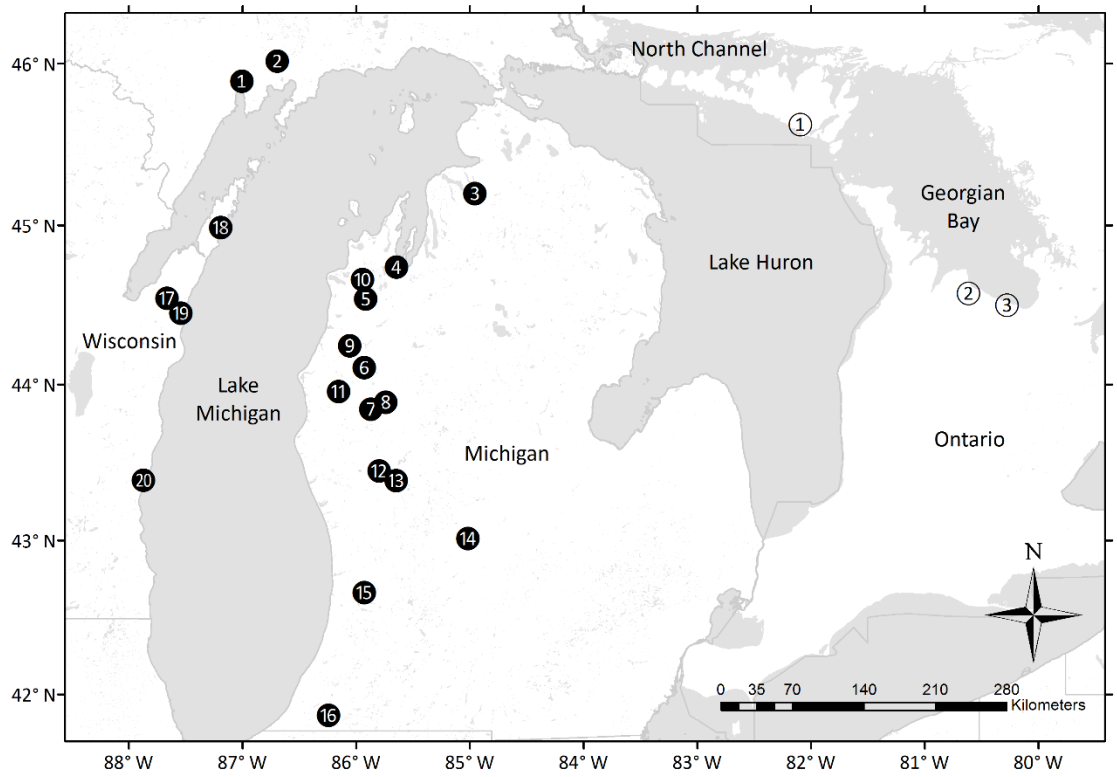

Figure 1.—Summary of streams in which fish were collected in 2015 and 2016. Numbers correspond to the names of streams in Lake Michigan (solid circles with transparent text) and Lake Huron (open circles with black text) in Table 2.

Otolith Analysis

Otoliths were sectioned and polished at Michigan State University. For each fish, an otolith was randomly sampled (left or right) for analysis. Otoliths that were cracked, broken, or contained vaterite deposits were excluded from analysis; it has been observed that vaterite calcium carbonate structures incorporate elements differently than the aragonite structures typical of otoliths (Melancon et al. 2005, Melancon et al. 2008). Otoliths were embedded in hard-setting EpoFix epoxy resin. The embedded otoliths were sectioned using an Allied isomet

saw by cutting 1mm sections along the transverse plane where the dorsal-ventral width was widest. Otoliths were ground to the core to expose the primordia using 3M lapping film with 5 µm and 3 µm grit sizes. Further fine polishing was accomplished using 1 µm and 0.3 µm alumina slurries.

Otoliths were analyzed for their microchemistry using a laser-ablation inductively-coupled plasma mass spectrometer (LA ICP MS) at the Center for Elemental and Isotopic Analysis (CELISA) at Central Michigan University (Mt. Pleasant, MI). A Photon Analyte 193 nm Excimer laser was used to ablate each otolith along the posterior-anterior section across the core; this was done to ensure that transects were standardized and variability was minimized (Campana 1992). Surface contaminants were removed prior to LA ICP MS analysis using an 80 µm square spot with a repetition rate of 2 Hz. Otoliths were analyzed using a 40 µm diameter spot size, a repetition rate of 10 Hz, and speeds at 2 µm s^{-1} for juvenile samples and 2-4 µm s^{-1} for adult samples. Ablated material was carried via argon gas to a Thermo-Finnigan Element 2 ICP MS.

Nine elements were measured: magnesium (^{25}Mg), calcium (^{43}Ca), manganese (^{55}Mn), copper (^{65}Cu), zinc (^{66}Zn), rubidium (^{85}Rb), strontium (^{88}Sr), barium (^{137}Ba), and lead (^{208}Pb). Background microchemistry signatures were accounted for by analyzing a 40s gas blank prior to laser ablation. Limits of detection were set using NIST 612 and NIST 610 glass standards and a MACS 3 pressed pellet standard. NIST 612 standards were also analyzed between every 3-8 otoliths to account for instrument drift. Due to their values being below the limits of detection, both copper and lead were removed from the data set. Raw counts-per-second data were post-processed at Central Michigan University to obtain elemental concentrations in parts per

million (ppm). Variation in the volume of ablated material was accounted for by standardizing microchemical concentrations to measured Ca in the otolith (Watson 2017, Marklevitz et al. 2016, Marklevitz et al. 2011, Pangle et al. 2010). The data were then further processed to convert ppm values to molar ratios of Ca within the otolith using the following formula:

$$C_{MR} = C_{PPM} * (\frac{1}{MW_X} \div \frac{0.4}{MW_{Ca}})$$

where C_{MR} is the concentration as a molar ratio to Ca, C_{PPM} is the concentration in parts per million, MW_X is the molar weight of a particular element, and MW_{Ca} is the molar weight of calcium. Ca concentrations within the otolith were assumed to be fixed to account for variation in the amount of ablated material (Marklevitz et al. 2016).

A transect was run from the posterior edge to the anterior edge across the core for the juvenile otoliths. Peaks in manganese concentrations were observed at the primordia during LA ICP MS analysis, and were used to identify the primordia when examining the post-processed data. Microchemical signatures within 100 µm of the primordia were excluded; this was the distance for each otolith at which the switch to exogenous feeding was assumed to have occurred (Zhang et al. 1995). Signatures 20 µm from the transect edge were excluded to account for contamination issues arising during sectioning and polishing. A mean of the remaining signatures was taken from the posterior side of the otolith and used for all further analyses.

For the adult otoliths, a transect was run from the posterior side to the anterior side across the core starting and ending at the first annulus. Following ablation, transects of varying distances set 100 µm from the primordia on the posterior side of the otolith (in 20 µm increments) were tested to see which transect length resulted in the highest classification

accuracy (see “Model Selection”) when applying the 2015 juvenile model to the 2015 adult data (see “Model Application”). A 400 µm transect was selected for all further analyses, and was used for all model applications to standardize comparisons.

Model Selection

Several methods (hereby referred to as “classification models”) have been proposed for the assignment of fish to their natal origin using otolith microchemistry. As described by Mercier et al. (2011), these classification models are: linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), artificial neural networks (ANN), and random forests (RF). LDA and QDA have been frequently used for otolith microchemistry classification in prior studies (Pangle et al. 2010, Gillanders & Kingsford 2000). More recently, machine learning algorithms such as ANN and RF have also been successfully applied (Marklevitz et al. 2016, Marklevitz et al. 2011, Mercier et al. 2011).

Classification accuracy (i.e., the percentage of observations correctly classified to their natal region) was evaluated for each proposed classification model and each combination of elements using each year of juvenile data and combined data set consisting of all samples from both years; a total of 63 possible element combinations were evaluated using the six elements in the final data set: magnesium (^{25}Mg), manganese (^{55}Mn), zinc (^{66}Zn), rubidium (^{85}Rb), strontium (^{88}Sr), and barium (^{137}Ba). MANOVAs using the Wilk’s lambda test statistic were used to confirm differences in otolith microchemical signatures among all elements for each year of

juvenile data at $\alpha = 0.05$. Principal components analysis (PCA) was also used to illustrate patterns in the data for both the 2015 and 2016 juveniles using all elements.

Due to difficulties in comparing the performance of classic statistical classification models together with machine learning algorithms, a method described by Mercier et al. (2011) was used to evaluate classification accuracy, in which the juvenile data sets were randomly sampled to obtain training and testing data sets. The training data sets comprised 75% of the data, and the remaining 25% formed the testing data set. This cross-validation procedure was used to avoid issues associated with using the same data to fit and test the classification models (Mercier et al. 2011, Kohavi 1995). Fifty replicates were run for each model and each combination of elements, resampling the training and testing data set each time to avoid a sampling effect (Mercier et al. 2011). Mean classification accuracies and their associated 95% confidence intervals were obtained for each model, and the best combination of variables for each classification model was evaluated based on the maximum classification accuracy. Classification accuracy was evaluated on the regional scale previously mentioned, as well as on a scale (referred to as “basin-wide”) in which fish were divided into two groups (Lake Michigan and Lake Huron). While broad, this alternative scale was considered because the scope of this project focuses on basin-wide implications. For each juvenile data set (2015 samples, 2016 samples, and all samples), due to similarities among models in performance on a basin-wide scale, the models with the greatest classification accuracy on a regional scale were selected for further analysis.

Adult Classification Success

The three selected models were used to evaluate the regional and basin-wide classification accuracy for each year of adult data and a combined data set consisting of all adult samples from both years (a total of 9 model applications). Because of discrepancies in the regions in which fish were collected, classification accuracy was only evaluated for the regions in which samples were present in both the juvenile data used to fit to the model being tested and the adult data used to evaluate the model. To account for stochasticity associated with machine learning classification algorithms, fifty replicates were run for each application of the models. Classification tables were retained for each application to evaluate the sources of misclassification. Median values across replicates for each classification scenario were obtained.

Annual Otolith Microchemistry Variation

Inter-annual otolith microchemical variation was evaluated by performing MANOVAs to test for the differences in mean otolith microchemical signatures between 2015 and 2016 juveniles for each region in which fish were present in both years (UPP, NLP, SLP, SGB). Then, for each region in which the MANOVAs indicated significant inter-annual differences in otolith microchemistry, the effect of inter-annual otolith microchemistry variation on classification success was evaluated by using models fit to each year of juvenile data to classify fish from the other year. This was then compared to the within-year classification accuracy, which was

determined within the random forest algorithm. Error estimates were generated for each tree within the forest by assigning samples excluded from the model fit. The individual error estimates were then averaged across all trees to obtain an overall error estimate, and this value was subtracted from 100 to determine the within-year classification accuracy. Fifty random forest algorithms were run and evaluated for both the 2015 and 2016 juveniles, and mean classification accuracy and 95% confidence intervals were obtained. Independent two-sample t-tests assuming unequal variances were performed to test the null hypothesis of no difference in classification accuracy within and among year classes at $\alpha = 0.05$.

RESULTS

Model Selection

MANOVA results indicated significant differences in otolith chemical signatures of juvenile Chinook salmon among regions for both the 2015 (Wilk's lambda = 0.597, $p < 0.001$) and 2016 (Wilk's lambda = 0.782, $p < 0.001$) data sets. PCA illustrates patterns in the variables, with the first two principal components explaining greater than 91% of the variation in 2015 (Figure 2) and 94% of variation in 2016 (Figure 3). Principal component loadings for each PCA analysis are depicted in Table 3; for both analyses, the first principal component was highly positively correlated with Mg, and the second principal component was highly positively correlated with Sr.

Table 3.—Component loadings for the 2015 and 2016 PCA analyses.

	2015 PCA		2016 PCA	
Element	**PCA 1**	**PCA 2**	**PCA 1**	**PCA 2**
Mg	0.972	0.225	0.999	-0.003
Mn	0.113	0.027	0.116	0.023
Zn	0.340	0.201	-0.255	0.158
Rb	-0.088	0.211	0.099	-0.026
Sr	-0.483	0.875	0.052	0.994
Ba	-0.041	0.514	0.020	0.160

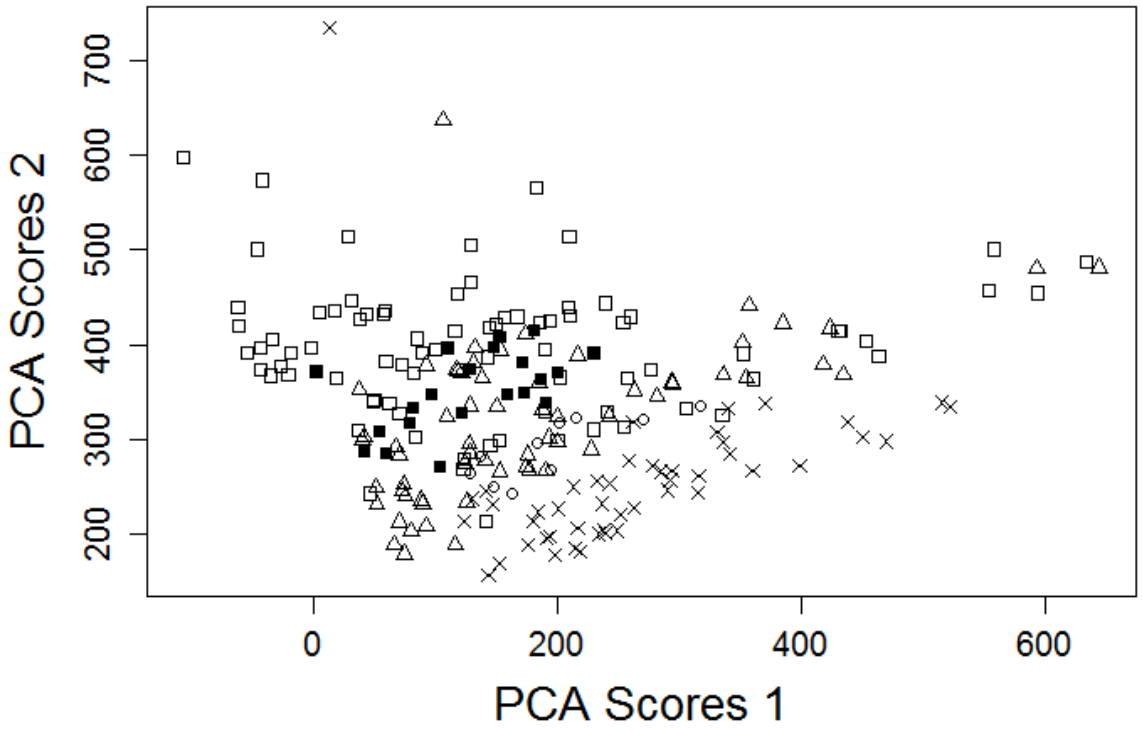

Figure 2.—Results of a principal components analysis (PCA) conducted on the 2015 juvenile data set, showing the first two principal components. Regions represented are UPP (open cirlces), NLP (open triangles), SLP (open squares), WIS (crosses), and SGB (closed squares).

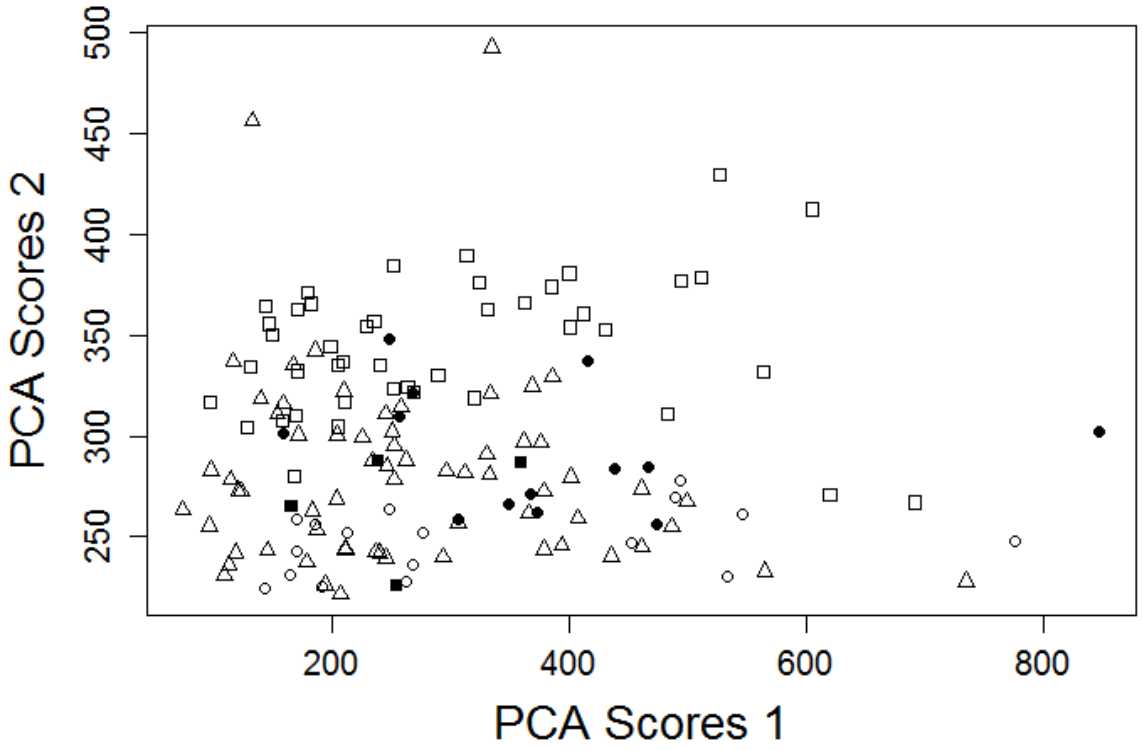

Figure 3.—Results of a principal components analysis (PCA) conducted on the 2016 juvenile data set, showing the first two principal components. Regions represented are UPP (open circles), NLP (open triangles), SLP (open squares), NLH (closed circles), and SGB (closed squares).

Classification accuracy varied depending on the combination of elements, classification model, and the years of data that were used to fit the model (Figure 4, Table 4). For 2015 juveniles, maximum classification accuracies on a regional scale ranged from 58.6% to 81.4% for all classification models. Regional accuracies were maximized with a combination of 3 or 5 elements depending on the classification model. On a basin-wide scale, maximum classification accuracies ranged from 92.1% to 98.3% for all classification models. Basin-wide accuracies were maximized with a combination of 2 or 4 elements. For the 2016 juvenile data, maximum classification accuracies

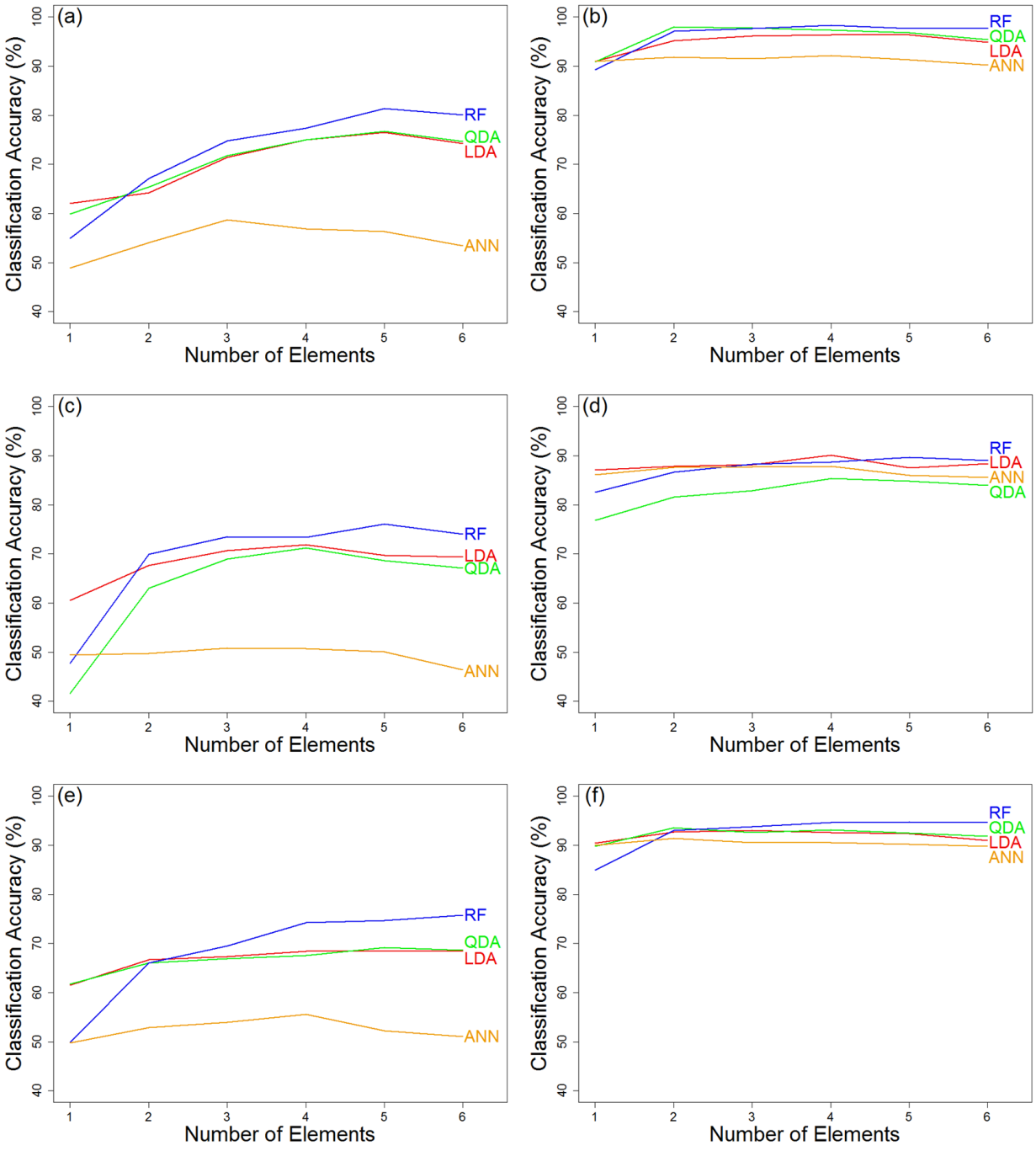

Figure 4.—Maximum classification accuracy for classification models including 1 to 6 elements, based on 2015 data at regional (a) and basin-wide (b) scales, 2016 juvenile data at regional (c) and basin-wide (d) scales, and both years of juvenile data at regional (e) and basin-wide (f) scales. The classification models used were linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), artificial neural networks (ANN), and random forests (RF).

on a regional scale ranged from 50.8% to 76.1% for all classification models. Classification accuracies on a regional scale maximized with a combination of 3 to 5 elements. Maximum classification accuracies on a basin-wide scale ranged from 85.3% to 90.1%, and accuracies were maximized with a combination of 4 or 5 elements. For the combined juvenile data set, maximum classification accuracies on a regional scale ranged from 55.6% to 75.8% for all classification models. Classification accuracies on a regional scale maximized with a combination of 4 to 6 elements. Maximum classification accuracies on a basin-wide scale

Table 4.—Maximum classification accuracy and 95% confidence intervals, and the number and combination of elements resulting in the most accurate model for each classification method, scale, and selection of juvenile data.

Data Fit	Scale	Method	Maximal Accuracy (%)	Number of Elements	Element Combination
2015	Regional	LDA	76.5 ± 1.5	5	Mg, Mn, Rb, Sr, Ba
		QDA	76.8 ± 1.7	5	Mg, Mn, Rb, Sr, Ba
		ANN	58.6 ± 1.9	3	Mn, Rb, Ba
		RF	81.4 ± 1.3	5	Mg, Mn, Rb, Sr, Ba
	Basin-Wide	LDA	96.3 ± 0.7	4	Mg, Mn, Sr, Ba
		QDA	98.0 ± 0.5	2	Sr, Ba
		ANN	92.1 ± 1.1	4	Mn, Rb, Sr, Ba
		RF	98.3 ± 0.5	4	Mn, Zn, Sr, Ba
2016	Regional	LDA	71.9 ± 1.8	4	Mn, Zn, Sr, Ba
		QDA	71.2 ± 2.3	4	Mn, Zn, Sr, Ba
		ANN	50.8 ± 2.1	3	Mn, Rb, Ba
		RF	76.1 ± 2.1	5	Mn, Zn, Rb, Sr, Ba
	Basin-Wide	LDA	90.1 ± 1.5	4	Mg, Rb, Sr, Ba
		QDA	85.3 ± 1.7	4	Mn, Zn, Sr, Ba
		ANN	87.8 ± 1.1	4	Mn, Zn, Rb, Sr
		RF	89.7 ± 1.2	5	Mn, Zn, Rb, Sr, Ba
2015 + 2016	Regional	LDA	68.4 ± 1.2	6	Mg, Mn, Zn, Rb, Sr, Ba
		QDA	69.2 ± 1.4	5	Mg, Mn, Zn, Sr, Ba
		ANN	55.6 ± 3.1	4	Zn, Rb, Sr, Ba
		RF	75.8 ± 1.2	6	Mg, Mn, Zn, Rb, Sr, Ba
	Basin-Wide	LDA	93.0 ± 0.6	3	Rb, Sr, Ba
		QDA	93.5 ± 0.6	2	Sr, Ba
		ANN	91.4 ± 0.8	2	Sr, Ba
		RF	94.7 ± 0.6	5	Mg, Mn, Rb, Sr, Ba

ranged from 91.4% to 91.7%, and accuracies were maximized with a combination of 2 to 5 elements. (Figure 4, Table 4).

For 2015 juvenile data, classification accuracies were maximized through the use of RF on a regional scale. Maximum classification accuracies for a combination of 2 or more elements were achieved using RF. On a basin-wide scale, all methods performed with a classification accuracy greater than 90%, with QDA and RF performing the most optimally. Using the 2016 juvenile data, classification accuracies were maximized through the use of LDA or RF on a regional scale. Maximum classification accuracies for a combination of 3 or more elements were obtained using RF. The use of LDA resulted in the best classification accuracy on a basin-wide scale using a combination of 4 elements; the use of RF at this scale resulted in greater maximum classification accuracies using combinations of 5 or 6 elements (Figure 4, Table 4). For the combined juvenile data set, classification accuracies were maximized by RF on a regional scale. The use of RF also resulted in the best classification accuracy on a basin-wide scale using a combination of 5 elements.

The element combinations that achieved maximum accuracy for each model varied depending on the classification model, scale, and the years of data fit to the model (Table 4). For 2015 juveniles, the optimal element combination on a regional scale consisted of either a combination of Mn, Rb, and Ba (ANN) or the entire suite of elements with the exclusion of Zn (LDA, QDA, RF). On a basin-wide scale, the optimal element combinations varied substantially among all classification methods. Ba was the only element present in all optimal element combinations using the 2015 juvenile data. Using the 2016 data, the optimal element combinations on a regional scale consisted of Mg, Zn, Sr, and Ba (LDA, QDA), Mn, Rb, and Ba

(ANN), or all elements with the exclusion of Mg (RF). On a basin-wide scale, the optimal element combinations varied substantially. No elements were consistent in any of the optimal element combinations using the 2016 juvenile data (Table 4). For the combined juvenile data set, the optimal element combination consisted of the entire suite of elements with the exclusion of Mg and Mn (ANN), the entire suite of elements with the exclusion of Rb (QDA), or the entire suite of elements (LDA, RF) on a regional scale. On a basin-wide, the optimal element combinations consisted of Sr and Ba (QDA, ANN), Rb, Sr, and Ba (LDA), or the entire suit of elements with the exclusion of Zn (RF). The juvenile models selected for further analysis were a RF using a combination of all elements with the exclusion of Zn fit to the 2015 juvenile data (hereby referred to as the "2015 model"), a RF using a combination of all elements with the exclusion of Mg fit to the 2016 juvenile data (hereby referred to as the "2016 model"), and a RF using a combination of all elements fit to the combined juvenile data set (hereby referred to as the "Combined model").

Adult Classification Success

Application of the juvenile models to adult data resulted in classification accuracies that varied depending on the juvenile model used and adult data that were classified (Table 5). Applying the three selected models to each selection of adult data resulted in regional classification accuracies ranging from 31.9% to 51.0% and basin-wide classification accuracies ranging from 74.2% to 87.8%. The maximum regional classification accuracy resulted from the

application of the 2015 model to 2015 adult data, and the maximum basin-wide accuracy was obtained by applying the 2016 model to 2016 adult data (Table 5).

Examination of the sources of misclassification from application of juvenile models to adult data reveal several patterns (Table 6). On a regional scale, the most common source of misclassification occurs due to adults from NLP being classified into the SLP region; this accounts for between 13% and 54% of the total misclassification across all scenarios. On a basin-wide scale, the most common source of misclassification occurs due to Lake Huron fish being classified into the Lake Michigan group; this accounts for between 6% and 17% of the total misclassification across all scenarios.

Table 5.—Classification accuracies and 95% confidence intervals for each application of the juvenile models to the adult data on regional and basin-wide scales.

Model	Adults Classified	Scale	Classification accuracy (%)
2015	2015	Regional	51.0 ± 0.4
		Basin-Wide	80.5 ± 0.2
	2016	Regional	39.2 ± 0.6
		Basin-Wide	74.2 ± 0.3
	2015 + 2016	Regional	42.1 ± 0.6
		Basin-Wide	77.8 ± 0.1
2016	2015	Regional	40.5 ± 0.4
		Basin-Wide	82.4 ± 0.4
	2016	Regional	31.9 ± 0.3
		Basin-Wide	87.8 ± <0.1
	2015 + 2016	Regional	35.6 ± 0.3
		Basin-Wide	83.8 ± 0.2
Combined	2015	Regional	39.8 ± 0.3
		Basin-Wide	82.2 ± 0.1
	2016	Regional	34.2 ± 0.3
		Basin-Wide	78.0 ± 0.1
	2015 + 2016	Regional	36.5 ± 0.2
		Basin-Wide	80.7 ± 0.1

Table 6.—Classification tables showing each application of the 2015 and 2016 models to the 2015 and 2016 adult data. Rows represent actual group membership, while columns indicate predicted group membership; values along the diagonal (bold) indicate correct classifications. Values for each classification scenario represent the median value over 50 replicates (the number of adults classified is presented for each table). Regions in which fish were not present in either data set were excluded from analyses.

Regional

2015 Juveniles -> 2015 Adults
Adults Classified = 67

	UPP	NLP	SLP	WIS	SGB
UPP	**0**	1	1	0	0
NLP	1	**20**	9	0	2
SLP	0	0	**7**	0	0
WIS	0	2	5	**0**	5
SGB	0	0	6	0	**8**

2015 Juveniles -> 2016 Adults
Adults Classified = 59

	NLP	SLP	WIS	SGB
NLP	**14**	18	0	2
SLP	1	**7**	0	0
WIS	1	0	**0**	9
SGB	1	3	0	**3**

2015 Juveniles -> All Adults
Adults Classified = 126

	UPP	NLP	SLP	WIS	SGB
UPP	**0**	1	1	0	0
NLP	4	**29**	29	0	4
SLP	0	1	**14**	0	0
WIS	0	2	5	**0**	14
SGB	0	1	9	0	**11**

2016 Juveniles -> 2015 Adults
Adults Classified = 55

	UPP	NLP	SLP	SGB
UPP	**0**	0	2	0
NLP	0	**11**	21	0
SLP	0	0	**7**	0
SGB	0	5	5	**5**

Basin-Wide

	LM	LH
LM	**46**	7
LH	6	**8**

	LM	LH
LM	**41**	11
LH	4	**3**

	LM	LH
LM	**87**	18
LH	10	**11**

	LM	LH
LM	**41**	0
LH	9	**5**

Table 6 (cont'd)

2016 Juveniles -> 2016 Adults
Adults Classified = 49

	UPP	NLP	SLP
NLP	**8**	26	0
SLP	1	**7**	0
SGB	3	3	**1**

	LM	LH
LM	**42**	0
LH	6	**1**

2016 Juveniles -> All Adults
Adults Classified = 104

	UPP	NLP	SLP	WIS
UPP	**0**	0	2	0
NLP	0	**19**	47	0
SLP	0	1	**14**	0
SGB	1	8	8	**4**

	LM	LH
LM	**83**	0
LH	17	**4**

All Juveniles -> 2015 Adults
Adults Classified = 67

	UPP	NLP	SLP	WIS	SGB
UPP	**0**	1	1	0	0
NLP	1	**12**	17	0	2
SLP	0	0	**7**	0	0
WIS	0	1	7	**0**	4
SGB	0	0	6	0	**8**

	LM	LH
LM	**47**	6
LH	6	**8**

All Juveniles -> 2016 Adults
Adults Classified = 59

	NLP	SLP	WIS	SGB
NLP	**9**	25	0	0
SLP	0	**8**	0	0
WIS	1	0	**0**	9
SGB	1	3	0	**3**

	LM	LH
LM	**43**	9
LH	4	**3**

All Juveniles -> All Adults
Adults Classified = 126

	UPP	NLP	SLP	WIS	SGB
UPP	**0**	1	1	0	0
NLP	2	**19**	43	0	2
SLP	0	0	**15**	0	0
WIS	0	2	7	**0**	13
SGB	0	0	9	0	**12**

	LM	LH
LM	**90**	15
LH	9	**12**

Annual Otolith Microchemistry Variation

MANOVAs indicated significant differences in otolith microchemical signatures between juvenile year classes in the UPP (Wilk's lambda = 0.067, $p < 0.001$), NLP (Wilk's lambda = 0.852, $p < 0.01$), and SLP (Wilk's lambda = 0.873, $p < 0.05$) regions, and no significant differences in otolith chemical signatures in the SGB region (Wilk's lambda = 0.626, $p = 0.158$). The inclusion of regions in which significant differences in otolith microchemistry were observed (UPP, NLP, SLP) to determine the effect of inter-annual variation on classification success resulted in higher classification accuracy when applying the models to data from the same year rather than data from the other year (Table 6). Because the regions included in this analysis were all in the Lake Michigan basin, classification accuracy could only be evaluated on a regional scale. Applying the 2015 model resulted in a higher classification rate for 2015 juveniles (79.6%) than for 2016 juveniles (56.3%). Similarly, the application of the 2016 model resulted in a higher classification rate for 2016 juveniles (84.3%) than for 2015 juveniles (61.1%). All results were significant at $\alpha = 0.05$ (Figure 5).

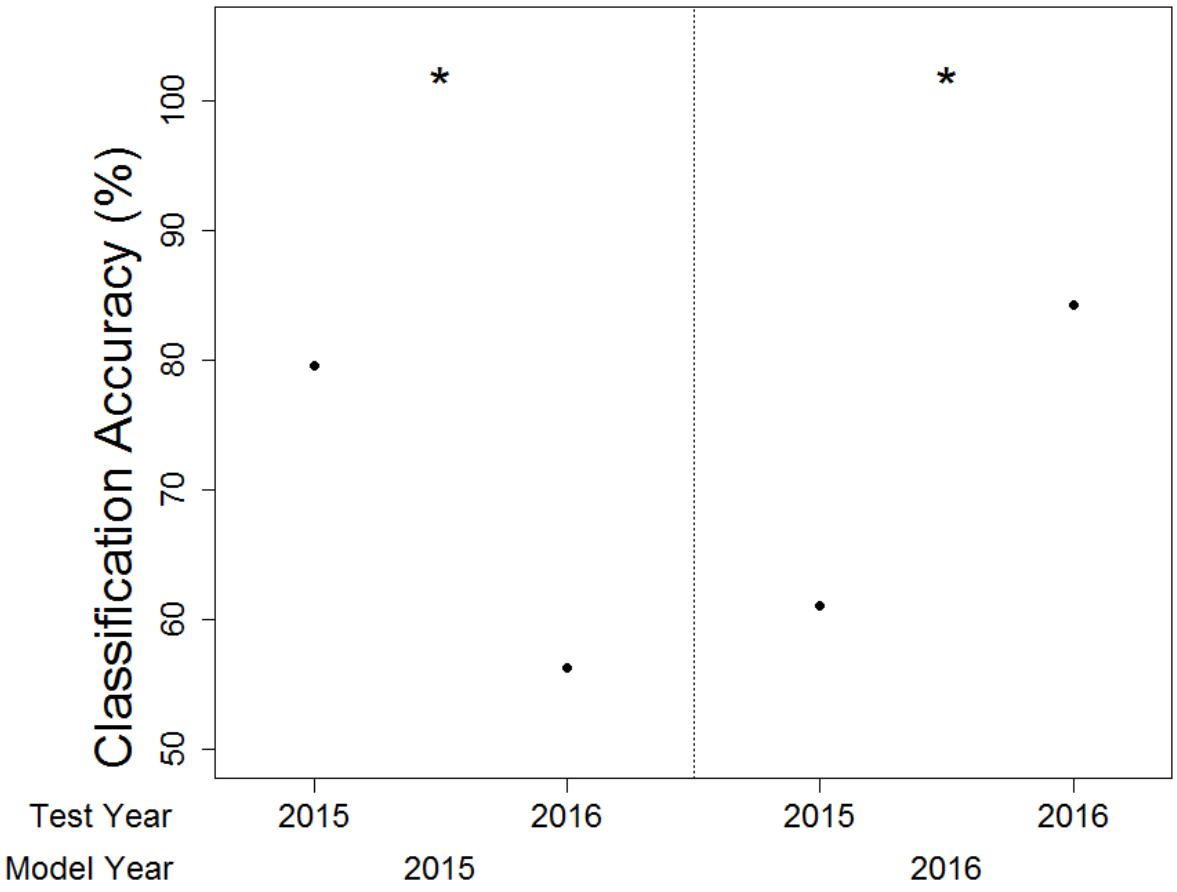

Figure 5.—Summary of the analyses of juvenile data to determine the effect of inter-annual otolith microchemistry variation on classification success. Mean classification accuracies are denoted by solid dots; due to the narrowness of the confidence intervals, error bars are not shown. Labels along the x-axis represent the model used to classify juveniles (2015 or 2016), and the year of juveniles that was tested (2015 or 2016). Significance between test years at $\alpha=0.05$ is indicated by a star (*) at the top of the chart. Results are depicted on a regional scale, as only regions in the Lake Michigan basin were included in this analysis.

DISCUSSION

Model Selection

Our analyses revealed that juvenile Chinook salmon otolith microchemistry varied significantly on regional and basin-wide scales. MANOVA results showed significant differences in otolith microchemistry among regions using all elements. In addition, the first two principal components of our PCAs explained over 90% of the variation in both 2015 and 2016, with Mg highly positively correlated with the first principal component and Sr highly positively correlated with the second principal component for both juvenile year classes. Regional classification accuracies using the top models were comparable to previous Great Lakes otolith studies (Marklevitz et al. 2011, Marklevitz et al. 2016, Pangle et al. 2010, Watson 2016). Classification accuracy was noticeably higher at a basin-wide scale; for the 2015, 2016, and combined data sets, classification accuracies were above 90%.

The top models from 2015, 2016, and the combined juvenile data sets all contained four common elements: Mn, Rb, Sr, and Ba, indicating that these four elements are likely the most important overall discriminators among regions. In particular, we expected that Sr and Ba were the key discriminators, as many of these elements were included in our best models, regardless of the classification model used. In addition, many previous otolith studies in the Great Lakes have indicated the importance of Sr and Ba in discriminating fish among regions and rearing environments (Marklevitz et al. 2011, Pangle et al. 2010, Watson 2016).

While the three top models were the ones that resulted in the highest regional classification success of the test data, a number of other models performed similarly. On a regional scale, 13 models for 2015 juveniles, 13 models for 2016 juveniles, and 9 models for the combined juveniles had a classification accuracy within 5% of the top models. On a basin-wide scale, over 50 models tested for each juvenile data set exhibited a classification accuracy within 5% of the selected models. We chose the models with the highest classification accuracy for further analysis because they resulted in the highest observed separation among groups. We assumed that the application of these models would also result in the highest classification accuracy for adult data. While this may not necessarily be the case, the similar performance of many of the tested models, particularly on a basin-wide scale, indicated that other models would have similar performance to the top models when applied to the adult data.

The maximum classification accuracy for the 2016 model was lower than the maximum classification accuracy for the 2015 model on both regional and basin-wide scales. There are a few possible reasons for the lower classification accuracy in 2016. First, classification accuracy may be lower due to differences in the numbers and locations of juvenile fish we collected in both years. We collected and successfully analyzed fewer fish in 2016 (143) than 2015 (223); fitting the model to fewer observations may have caused the classification accuracy to be lower for the 2016 data. In addition, we only analyzed four fish from the SGB region in 2016, which may have affected our results; when extracting the training and testing data sets, we fit the model to only one or two data points for some of the replicates, which likely resulted in a decline in classification accuracy. While we collected fish from streams within the same regions

in both years, the number of fish we collected at each site varied from year to year. This also has the potential to affect our results, and may have led to a lower classification success.

The maximum classification accuracy for the combined model was lower than the maximum classification accuracy of both the 2015 and 2016 models on a regional scale, whereas the maximum basin-wide classification accuracy was higher than that of the 2016 model but lower than that of the 2015 model. The effect of combining years on classification success is difficult to predict because of the trade-off between increasing overall sample size, which we would expect to improve classification accuracy, and adding data from years in which the signatures may be different, which we would expect to have a negative effect on classification accuracy.

Adult Classification Success

The application of the top juvenile models to the adult data resulted in lower classification accuracy than the accuracy in classifying the juvenile data. Classification on a regional scale was substantially lower for the adult data, with classification accuracies between 31.9% and 51.0% across all applications. Classification accuracies on a basin-wide scale were also adversely affected, but remained above 74% for all applications of the juvenile models. Because this project focuses on movement at a basin level, we were able to conclude that the success of applying the top juvenile models to the adult data on a basin-wide scale indicates that these models have the potential to quantify Chinook salmon movement between Lake Michigan and Lake Huron.

While misclassification patterns differed depending on the model used, common sources of misclassification emerged when examining the classification tables for each application of the juvenile models. The most common source of misclassification was NLP being misclassified as SLP fish. This is likely due to the close spatial proximity of the two regions; streams in these regions may have contained similar stream chemical signatures, and thus similar otolith chemical signatures.

There are several possible reasons for the reduced classification success of the adult otoliths. First, annual otolith microchemistry variation may have caused the classification accuracy to be lower. Adults that we classified did not match the year class of the juveniles, so it is possible that annual variation may have caused a decline in adult classification success. Second, a small number of adult Chinook salmon may spawn in a tributary different from the one in which they were reared, which is contrary to our assumption of 100% homing. While it is widely accepted that most Chinook salmon home to their natal streams to spawn, a small amount may stray to different rivers (Quinn and Fresh 1984). If a significant number of fish stray, this has the potential to negatively affect classification accuracy. Finally, the data that we selected for analysis from our adult otoliths may have affected classification accuracy. A 400 µm transect was selected for analysis from all adult otoliths, which we assumed represented the time each fish was exposed to stream chemistry in their first year of life. Transects of a fixed length were selected because of the difficulty we had in identifying differences between stream and lake chemical signatures. While a shift in Sr and Ba chemical signatures is often observed when examining species moving between marine and freshwater environments (Hoover 2012), we did not observe this shift in our freshwater system. Due to our use of a fixed distance

transect, we were unable to account for variation in daily otolith growth rates among individual fish. A possible solution may be to standardize adult otolith transect lengths to the average juvenile transect lengths in each region, or to limit the variation in juvenile transect lengths by removing some of the longest or shortest samples.

Annual Otolith Microchemistry Variation

Testing for differences in otolith microchemistry between juvenile year classes resulted in significant differences in three regions, indicating substantial inter-annual variation in otolith microchemistry. Using these regions to test the effects of inter-annual variation on classification success resulted in a significant drop in regional classification accuracy when classifying between juvenile year classes. These results indicated that inter-annual variation has a substantial negative effect on classification success, therefore potentially requiring the matching of juvenile and adult year classes.

Significant differences in otolith microchemical signatures were found for the UPP, NLP, and SLP regions, and significant differences were not found for the SGB region. This is most likely due to the small sample sizes in the SGB region for both years, particularly in 2016, when only 4 individuals were successfully analyzed. This likely affected the within year variation for the SGB region, and may have influenced the MANOVA results.

Significant differences were found when comparing classification accuracy by classifying within and between juvenile year classes at a regional scale. Classification accuracy was only compared at a regional scale due to only Lake Michigan regions being included in the analysis. It

is likely that inter-annual variation has a negative effect on classification accuracy at a basin-wide scale as well, although we could not determine the magnitude of this effect. The results indicated that, even within the Lake Michigan basin, significant differences in otolith microchemical signatures negatively affects classification accuracy when classifying between year classes.

Conclusions and Future Research

Otolith microchemistry appears to vary enough among regions to quantify the movement of wild Chinook salmon from Lake Huron to Lake Michigan. Juveniles discriminate with a high degree of success among regions and basins, showing that there is sufficient discrimination between regions for use on adult otoliths. The use of these models on adult data appears to be limited at a regional scale, at least when different year classes are involved, but adults classify with moderate success on a basin-wide scale. Lower classification accuracy of adults at regional and basin-wide scales may be due to annual variation in otolith chemical signatures, indicating the potential need to match juvenile and adult year classes.

Confidence in our classification models may have been affected by the limitations of our data. We did not collect as many samples as we had intended, particularly from the NLH region in Lake Huron. Based on the reduced amount of collections in these regions, it appears that wild Chinook salmon appear to be relatively uncommon in Lake Huron outside of the Southern Georgian Bay. Should Chinook salmon otolith studies continue in the future, regions in which few samples were collected should be targeted for additional samples.

Future research involving the use of otolith microchemistry to examine Chinook salmon inter-basin movement should focus on examining and refining the models that were developed during this research. We recommend the examination of fishery caught wild Chinook salmon to determine the spatial and seasonal differences in the contribution of wild Lake Huron Chinook salmon to the Lake Michigan fishery. Due to our observation of annual otolith microchemistry variation, we also recommend the validation of a year class effect by analyzing the otoliths of adults collected in the streams in 2018 and 2019. By collecting and classifying adults that match the year classes of our juvenile data, we expect to see an improvement in classification accuracy.

We expect these models will be used to calculate the movement of open-lake fish by classifying the otoliths of fish landed in the Lake Michigan recreational fishery. Further refinements of our models will likely lead to improved accuracy in the quantification of wild Chinook salmon movement from Lake Huron to Lake Michigan, and will result in more precise estimates of predatory demand due to this inter-basin movement. Ultimately, these models will allow us to assess the risks associated with a variety of Chinook salmon stocking alternatives. This research also indicates the potential for the use of otolith microchemistry to examine the movement of migratory fish species in freshwater environments.

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