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Research Article

Using fin ray elemental signatures and growth zone width to estimate onset of sexual maturity in lake sturgeon (*Acipenser fulvescens*)

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Abstract

Characterizing inter- and intra-population levels of variability in age at sexual maturation for long-lived fishes provides insight into year-class strength and recruitment dynamics, allowing for more effective management practices. Here we analyzed the ontogenetic chronology of pectoral fin ray annuli trace elemental concentration profiles as well as changes in annuli growth in lake sturgeon (*Acipenser fulvescens*) to determine onset of sexual maturity (OSM). Elemental concentrations and growth-zone width were used to build a random forest classification model to discriminate year-specific signatures to before or after OSM from 98 individual fin rays from both sexes across multiple populations and watersheds. The model demonstrated an overall accuracy of 98.8%. Ba was the most important variable related to OSM discrimination success followed by zone width, Pb, Mn, Mg, Zn, Cu, and Sr. Fin ray elemental concentrations began to increase at approximately age 24 (±4.7 years) in females and 15 (±3.1 years) in males while zone width decreased. This study implies that new applications for fin ray microchemistry are possible and could benefit future fisheries management strategies specifically by adding nonlethal or less invasive sampling techniques for evaluating OSM in sturgeon.

Key words: lake sturgeon, microchemistry, sexual maturation, elemental signatures, random forest, fisheries management

Introduction

Calcified structures have been used to characterize past life history traits in fish, with most studies focusing on elemental signatures characterizing natal or current resident environments resolved by interrogation of growth annuli of calcified structures including otoliths, fin rays, or scales (Tzadik et al. 2017; Hüssy et al. 2020). Typically, elemental signatures are defined as a pattern in the element concentration across a hard structure (Loeppky et al. 2020). Applications known as biomineral microchemistry studies were built on the assumption that element incorporation is strongly correlated to environmental availability at the time of uptake (Sturrock et al. 2015; Reis-Santos et al. 2022). Specifically, that elemental concentrations remain relatively constant throughout the pathway from uptake to deposition in tissues, ultimately providing a historical record of past or recent environmental exposure if episodes of exposure (e.g., annuli delineating annual periods of deposition) can be ascertained. Because of this constraint, most applications examine habitat-specific elemental signatures to characterize diadromous migration (Allen et al. 2009*a*; Smith and Kwak 2014), pollutant exposure (Nelson et al. 2015; Daros et al. 2022), diet (Ferenbaugh et al. 2009), natal origin, (Loeppky et al. 2020; Stewart et al. 2021; Mikheev et al. 2022), or habitat use (Arai et al. 2003; Feitosa et al. 2020). However, the physiological condition of the animal may also contribute to depositional elemental signatures (Sturrock et al. 2015; Hüssy et al. 2020). Indeed, the chemical composition of calcified structures has been observed to differ between species, populations, sex, and life stages, indicating that physiological processes may have an overall effect that can outweigh signals of environmental elemental availability (Sturrock et al. 2014; Charapata et al. 2022).

The incorporation of elements into calcified structures is the result of elemental uptake from the environment or diet through specific cellular pathways, across the gills or gastrointestinal tract (Campana 1999). Once in the blood, elements are actively transported or diffuse into the extracellular fluid surrounding a hard structure and may be incorporated in place of calcium (Ca) within the organic crystal of

Table 1. Sample information for lake sturgeon ($n = 98$) used in this study.

Dataset	Sample years	Sex	Age range	Total length (cm)	Mass (kg)	*n*
1	2021–2022 (April–May)	♂	(16–32)	146.4 ± 10.9 (119–165)	23 ± 9 (10–58)	30
1	2021–2022 (April–May)	♀	(20–62)	169.7 ± 12.6 (137–187)	42 ± 10 (18–60)	49
2	1999–2007 (April–October)	n/a	(34–58)	144.3 ± 11.2 (121–160)	24 ± 8 (15–48)	20

Note: Values of total length and mass are given as mean ± standard deviation with the range of values in parentheses below. Male = ♂, female = ♀. Pectoral fin rays were collected from a geographically closed population (Dataset 1: Black Lake Michigan) and seven other geographically separated populations (Dataset 2: Churchill River, Nelson River, and Kiskittogisu Lake, Manitoba; Whitedog River, Norman Dam, and Sturgeon Falls, Ontario: The Lake Winnebago System, Wisconsin.). Total length and mass were measured at time of pectoral fin ray removal.

that structure (Tillett et al. 2011; Kerr and Campana 2014). Internal factors such as metabolic rate, reproductive status, or ontogenic stage may influence elemental deposition due to changes in protein binding along the pathway from uptake to tissue incorporation (Hüssy et al. 2020). Specifically, energy demands related to sexual maturation and spawning may lead to differential deposition of elements in calcified structures after the onset of sexual maturity (OSM) in individuals that invest heavily in gamete production (Sturrock et al. 2015). Reproduction is energetically demanding and has been linked to changes in otolith elemental signatures coinciding with spawning in bearded rock cod (*Pseudophycis barbatus*) (Kalish 1991), grass goby (*Zosterisessor ophiocephalus*) (Granzotto et al. 2003), atlantic salmon (*Salmo salar*) (Clarke and Friedland 2004), and european plaice (*Pleuronectes platessa*) (Sturrock et al. 2015). Specifically, strontium (Sr) and zinc (Zn) concentrations in european plaice otoliths have been found to be significantly correlated to the gonadal somatic index in sexually mature females (Sturrock et al. 2015). Other elements such as Ca, magnesium (Mg), manganese (Mn), copper (Cu), selenium (Se), and lead (Pb) are also assumed to be under physiological control related to development and maturation (Sturrock et al. 2014; Hüssy et al. 2020). Se and Cu are also potentially related to reproductive effort (Martinho et al. 2020; Hüssy et al. 2021).

The aims of this study were to examine the influence of physiology on pectoral fin ray elemental signatures in lake sturgeon. To examine whether physiological influences related to reproductive effort are reflected in elemental isotopic signatures in calcified structures, it is crucial to choose a study population that does not experience a dramatic change in ambient water chemistry during spawning events, such as, a population that does not migrate large distances but still invests a significant amount of energy into gamete development. For this reason, this study focused on a closed population of lake sturgeon (*Acipenser fulvescens*) that has been closely monitored since 2001 with the use of radio-frequency identification and passive integrated transponder (PIT) tags. Lake sturgeon are one of the largest freshwater fish species in North America, known to significantly invest in gamete development due to the amount of eggs/sperm required during broadcast spawning. An adult female may even mature as many as 500 000–1000 000 eggs in preparation for spawning (Scott and Crossman 1998; Peterson et al. 2007), which requires significant investment in advance of the spawn. Males also require a substantial investment within a spawning season (Larson et al. 2020), and males spawn more frequently than females (every 1–2 years for males and every 2–7 years for females; Forsythe et al. 2012) and require large amounts of sperm due to the broadcast spawning method of fertilization (Marshall and Bolton 2007). High investment in gamete development relative to somatic tissue, particularly when the individual first matures, may lead to differential deposition of elements in calcified structures related to sexual maturity status.

In this study, we examined elemental concentrations in pectoral fin rays (hereafter, ray or rays) of lake sturgeon from a closed population and compared concentrations to rays collected from populations that are geographically distinct. Our overall goal was to quantify elemental signatures before and after presumed OSM, and to build a classification model using these data to predict sexual maturity status in lake sturgeon. Specific objectives were to (1) compare elemental signatures and growth zone width before and after OSM; (2) develop a model to discriminate year-specific signatures and zone width to before or after OSM in a closed population; (3) determine the model's ability to correctly predict maturity status in rays from the same population; and (4) determine the model's ability to correctly predict maturity status in rays across geographically distinct populations. We hypothesized that reproductive effort related to OSM would result in a detectable difference in the elemental composition and/or morphology across rays.

Methods

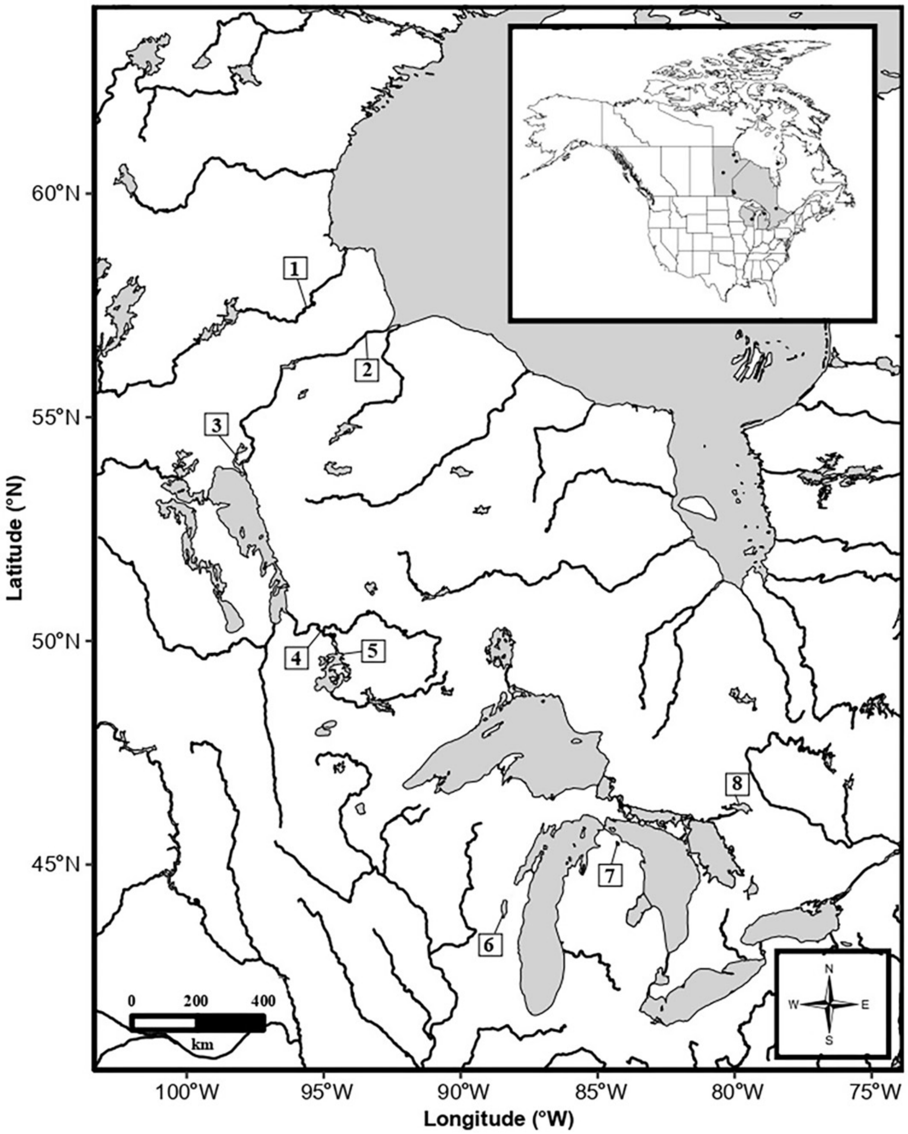
Data sets

Pectoral fin rays used in this study were divided into two data sets (Table 1).

Dataset 1: Black Lake population

Dataset 1 consisted of rays collected from the black lake sturgeon population in the northern lower peninsula of Michigan, USA (Fig. 1). Lake sturgeon were captured during the annual mid-April to late-May spring spawning survey of 2021 and 2022. All rays were collected from known sexually mature individuals of known sex, verified by either gamete expression upon capture and/or genetic sexing using DNA extracted from dorsal fin clips (Kanefsky et al. 2022). The black lake sturgeon population was chosen for this study because individuals are confined to a small geographic range (i.e., Black Lake; approx. 4000 ha) with dams present both upstream and downstream of the lake they inhabit year-round (see supporting information Table S1 for water chemistry data). Since 2001, adults have been captured annually during

Fig. 1. Map of all study areas. Site 1 = Churchill River, Manitoba ($n = 1$), site 2 = Nelson River, Manitoba ($n = 9$), site 3 = Kiskittogisu Lake, Manitoba ($n = 2$), site 4 = Whitedog River, Ontario ($n = 1$), site 5 = Norman Dam, Ontario ($n = 1$), site 6 = the Lake Winnebago System, Wisconsin ($n = 3$), site 7 = Black Lake, Michigan ($n = 78$), and site 8 = Sturgeon Falls, Ontario ($n = 3$). Dataset 1 = site 7. Dataset 2 = sites 1–6 and 8. Base map data source: https://www.naturalearthdata.com. Map projection is NAD 83.



the spring spawning survey. The use of PIT tags was implemented in 2001 and >95% of the adult spawning population of approximately 1200 adults (Pledger et al. 2013) has been tagged. PIT tags allow captured individuals to be scanned and identified, allowing confirmation of sex, reproductive frequency, and sexual maturity status based on past survey documentation (see Larson et al. 2020 for description). In total, this dataset consisted of 79 rays (30 males and 49 females).

Dataset 2: seven other geographically separated populations

Dataset 2 consisted of ray samples available from a reference collection at the University of Manitoba. Individuals greater than 33 years old (estimated age) were assumed to be sexually mature and therefore were selected for this study (COSEWIC 2006). This dataset consisted of 20 rays from seven distinct populations that were geographically separated from the Black Lake population. These rays were sampled from 1999 to 2007 in Churchill River Manitoba ($n = 1$), Kiskittogisu Lake, Manitoba ($n = 2$), Nelson River, Manitoba ($n = 9$), Norman Dam, Ontario ($n = 1$), Sturgeon Falls, Ontario ($n = 3$), Whitedog River, Ontario ($n = 1$), and the Lake Winnebago System, Wisconsin ($n = 3$) (Fig. 1). Background information for these samples including sex and known age was not available.

Fin ray preparation and elemental analysis

Rays were prepared for laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS) following procedures outlined in Smith and Whitledge (2010) and Loeppky et al. (2020). Rays were laid out in rows, set in epoxy resin, and cured for at least 24 h under a laminar flow hood. Using a Buehler IsoMet^{TM} low-speed saw (0.3 mm blade thickness), rays were sectioned to 1 mm at the articulating process and polished by hand using dampened silicon carbide paper (30, 9, and 0.3 μm). After surface polishing, rays were set in 2.54 cm rings ($n = 1$–2 rays/ring) and covered in epoxy to cure overnight. The surface of the rings was polished (Buehler Meta SERV 250 grinder-polisher) to expose rays. All rings were rinsed with distilled water and ethanol and stored in Kimwipes ™ until analysis.

Rays were scanned (LA ICP-MS, *Perkin-elmer DRC II*) in a similar fashion to Loeppky et al. (2020) and Loeppky (2021). Elements with potential to be under physiological regulation related to reproduction (Sturrock et al. 2014, 2015; Izzo et al. 2018) were chosen to be quantified. Epoxy rings were placed separately in the ablation chamber and line transects for each ray were created to quantify elemental concentrations along the longest axis of the structure from the first growth zone to the marginal edge. All line transects were pre-ablated (30 μm beam size, 100 μm/s scan speed, 10 Hz repetition rate, and 60% energy) to remove surface contamination before final quantification. Transect lines were then ablated (Dataset 1: 30 μm beam size, 5 μm/s scan speed, 20 Hz repetition rate, and 80% energy; Dataset 2: 30 μm beam size, 2 μm/s scan speed, 20 Hz repetition rate, and 100% energy) outward from the core with the following abundant isotopes of trace elements selected to be quantified: ^{137}Ba, ^{43}Ca, ^{63}Cu, ^{57}Fe, ^{7}Li, ^{25}Mg, ^{55}Mn, ^{208} Pb, ^{66} Zn, ^{33}S, ^{77}Se, and ^{88}Sr. Thirty second gas blanks were run before each ablation to clear residue between samples and calculate limits of detection (LOD). To account for possible instrument drift, glass standards (NIST 610, National Institute of Standards and Technology, Gaithersburg, MD, USA) were ablated (110 s scan time, 30 m beam size, 5 Hz repetition rate, and 65% energy) before and after each ring. After ablation, rays were imaged using a polarizing microscope (Nikon Eclipse 50i) (2–20x magnification) to ensure the entire transect line and corresponding growth zones were distinguishable for future age assessments.

Using Igor Pro graphing software (Iolite version 7), data reduction was performed to correct for changes in ablation yield during analysis (Paton et al. 2011). An internal standard was determined using Ca (^{43}Ca) in counts per second. For each ray, baseline concentrations measured from gas blanks were used to calculate LOD for each trace element. Any value falling below LOD was not significant and was therefore removed. Elements with >25% of their measures below LOD and that did not show any transient increase across the scan line were removed (Croghan and Egeghy 2003). Therefore, the final data set consisted of 7 elements (Mg, Mn, Cu, Zn, Sr, Ba, and Pb).

Data preparation

Element concentrations can often vary between individuals and populations due to ambient environment availability and individual-specific physiological influences (Sturrock et al. 2014 and references within). Due to issues regarding differences in elemental concentrations between individuals and locations, relative elemental concentrations were calculated for each ray by dividing each value by the average over the entire profile (Hüssy et al. 2015). To examine if OSM has an influence on elemental concentrations present in rays, the scan-line portions (μm) for each individual growth zone were determined to attain year-specific elemental signatures (outlined below). Two readers worked together simultaneously to identify age based on each growth zone (a white opaque band followed by a dark translucent band) and determine the corresponding scan line portion for each year. Elemental profiles were laid over ray images to help aid in growth zone determination since seasonal oscillations of certain elements may provide more accurate age estimates in Lake Sturgeon (Taylor et al. 2024).

To obtain year-specific signatures before and after presumed OSM mean elemental concentrations and growth zone width were calculated for each year before and after age 15 in males and 20 in females. Though the exact year the fish became sexually mature is unknown, a standard age was chosen based on current documented age ranges for OSM in lake sturgeon populations (COSEWIC 2017). For rays collected from individuals without confirmed sex (Dataset 2; $n = 20$) presumed OSM was set at 20, the same as females.

Statistical analysis

All statistical analyses were conducted in R statistical software 4.2.2 (R Core Team 2022). Following log transformation, elemental data did not meet the assumptions of normality and homogeneity of variance to warrant parametric analysis, therefore non-parametric methods were used. Utilizing the stats and rcompanion packages (R Core Team 2022; Function: Wilcox.test | Mangiafico 2023: Function: WilcoxonZ), paired sample Wilcoxon's signed-rank tests were used to test for differences in each individual variable before and after OSM. Using the vegan and ggplot2 packages (Oksanen et al. 2022; Function: metaMDS | Wickham 2016; Function: ggplot), a nonmetric multidimensional scaling (NMDS) ordination plot was created to visually evaluate similarities (or dissimilarities) between before and after OSM for both datasets. NMDS clustering significance was evaluated using a multiple response permutation procedure (Oksanen et al. 2022; Function: mrpp). Mean concentrations for all elements and growth zone width were calculated before and after OSM to summarize variability for both categories.

In total 78 rays were collected and successfully analyzed from the Black Lake population (Dataset 1), and these were randomly divided (70:30) to create training ($n = 55$) and test sample sets ($n = 24$). To build a classification model to discriminate year-specific elemental signatures to before or after the OSM, random forest was implemented on the training sample ($n = 55$) using the randomForest package (Liaw and Wiener 2002; Function: randomForest). This function had a built-in cross-validation method that uses random sampling with replacement to calculate an overall out-of-bag classification error rate (Breiman 2001). The eight predictor variables in the final data set (Ba, Cu, Pb, Mn, Mg, Sr, Zn, and growth zone width) were used to analyze a total of 255 potential formula combinations. The model with the best classification success (lowest out-of-bag classification error rate) was then chosen to be used on the test samples from Dataset 1 ($n = 24$) and Dataset 2 ($n = 20$). This process was done both with and without the variable growth zone width to determine the model's capacity to predict before versus after OSM using elemental signatures alone. All packages allowing for mixed effect random forest (merf: Hajjem et al. 2012; MixRF: Wang and Chen 2016; and MEml: Ngufor et al. 2019) currently cannot account for a categorical response variable and therefore were not able to be used in this study.

Because the exact age at OSM was not known for samples in the test dataset, the chosen model was run on signatures for the last growth zone as well as year five to determine the model's ability to correctly discriminate year-specific signatures to before or after OSM. Therefore, each ray from the test sample had two year-specific signatures tested; one to represent before OSM and one to represent after OSM. All rays used in the test dataset were from known sexually mature individuals or those that had an estimated age of ≥33 and therefore the last growth band was believed to represent a signature after OSM (COSEWIC 2006). Likewise, there has been no documentation of lake sturgeon either male or female being sexually mature at age five and the variables calculated for this year were believed to represent a signature before OSM. The "mean decrease in accuracy", which measures classification accuracy deprivation when a variable is excluded from the analysis (averaged across all trees), was used to quantify variable importance for the model (Breiman 2001; Wright et al. 2018). Utilizing the ULT package (Maugoust 2022; Function: multkw) a multivariate extension of the nonparametric Kruskal–Wallis test (He et al. 2017) was used to quantify dissimilarity in mean elemental concentrations and zone width among all possible combinations before and after OSM to further determine potential variable importance. For all statistical tests, significance was accepted at $\alpha = 0.05$.

Results

Variable differences before and after OSM

Elemental trends in fin rays were seen across individuals for both datasets. When assigning scan-line portions to corresponding growth zones, readers identified visual elemental increases occurring across certain profiles (Figs. 2 and 3). Elemental levels were relatively constant throughout the core and early years but began to visually increase towards the middle to end of rays. This elemental increase was seen to occur on mean (±SD) at age 24 years (±4.7) in females (range 14–34) and 15 years (±3.1) in males (range 9–21). Readers determined the age at increase by first agreeing when it occurred for each individual and matching it to the individual's corresponding age. The elements that increased varied by individual but usually occurred in Ba, Mg, Mn, Pb, and Zn.

Likewise, for both datasets, values of Ba, Mg, Mn, and Pb were significantly higher after OSM while growth zone width was significantly lower (Fig. 4; Table 2). Sr values did not show clear patterns across datasets with values significantly higher after OSM for Dataset 1 but statistically similar before and after OSM in Dataset 2. Zn values also did not show clear patterns across datasets with values significantly higher after OSM for Dataset 1 (Wilcoxon's signed rank: $Z = -6.78$, $P < 0.001$) but only slightly higher after OSM for Dataset 2 (Wilcoxon's signed rank: $Z = -2.05$, $P = 0.04$). Cu values were similar before and after OSM across datasets showing no differences before and after OSM. The ordination plot showed full dissociation of ray signatures and growth zone width before and after OSM with clear overlap between both datasets indicating that variables for each category were similar across geographic locations (Fig. 5).

Random forest OSM classification success

Classification accuracy for the training samples was highest when all variables were included in the model with an overall out-of-bag error rate of 12.87% (number of trees 500). The final model (using all 8 predictor variables) demonstrated an overall classification accuracy of 98.8% when run on the test samples, correctly classifying 100% of ray signatures from the same population as training samples (Dataset 1) and 97.5% of ray signatures from geographically separated populations (Dataset 2). There was only one observation that was misassigned being classified as after OSM rather than before OSM (Table 3).

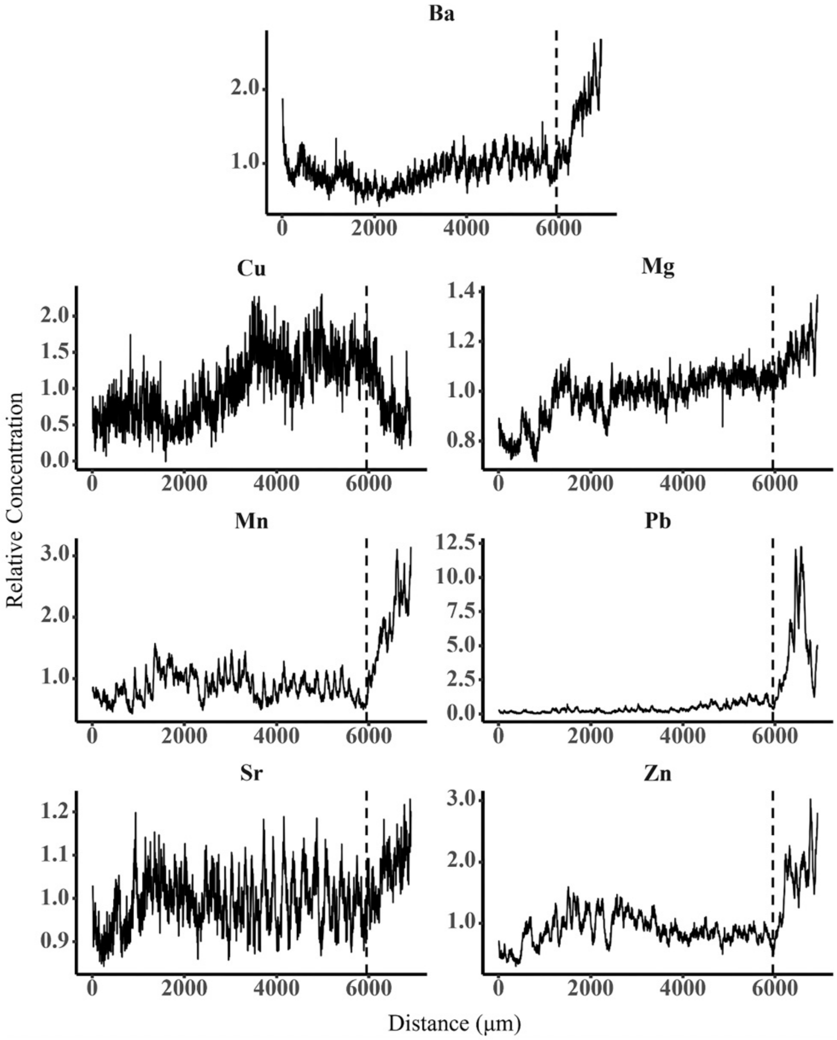
According to the multivariate analysis performed on all variables simultaneously, ray elemental signatures and growth zone width were significantly different in both datasets before and after OSM (multivariate Kruskal–Wallis: $H = 170.9$, df = 8, $P < 0.001$), indicating the usefulness of all variables for model discrimination. Ba, zone width, and Pb were found to be the three most important model discriminators since they had the lowest P-value when the analysis was performed on all three variable combinations (multivariate Kruskal–Wallis: $H = 167.7$, df = 3, $P < 0.001$). Results of "mean decrease in accuracy" measurements further validated this finding indicating that Ba was the most important variable related to classification success (70.6%), followed by zone width (60.7%), Pb (40.6%), Mn (39%), Mg (25.3%), Zn (22.7%), Cu (19.7%), and Sr (11.5%). Even so, classification accuracy for the training samples was still relatively high when growth zone width was excluded from the analysis, with an overall out-of-bag error rate of 13.45%. Accuracy results for the test data were the exact same with or without growth zone width (98.8% classification success), indicating that zone width was not needed to successfully discriminate OSM signatures.

Discussion

Discrimination success

Models based upon ray elemental concentration signatures and fin ray annuli growth zone width successfully discriminated year-specific signatures to before or after OSM in lake sturgeon from eight geographically distinct populations with an overall classification accuracy of 98.8%. Populations were associated with water bodies in regions characterized by different superficial geology and elemental profiles. Discrimination success was highest when all variables were included in the model; however, the three most discriminatory variables were consistently Ba, Pb, and growth zone width. Based on these variables, classification success was at 96.3%. Even

Fig. 2. Elemental profiles from core to outer pectoral fin ray edge of a 37-year-old, 1710 mm TL, female lake sturgeon captured in the Upper Black River, Michigan in 2021. Vertical lines represent the point of elemental increase identified by reader consensus (age 26).



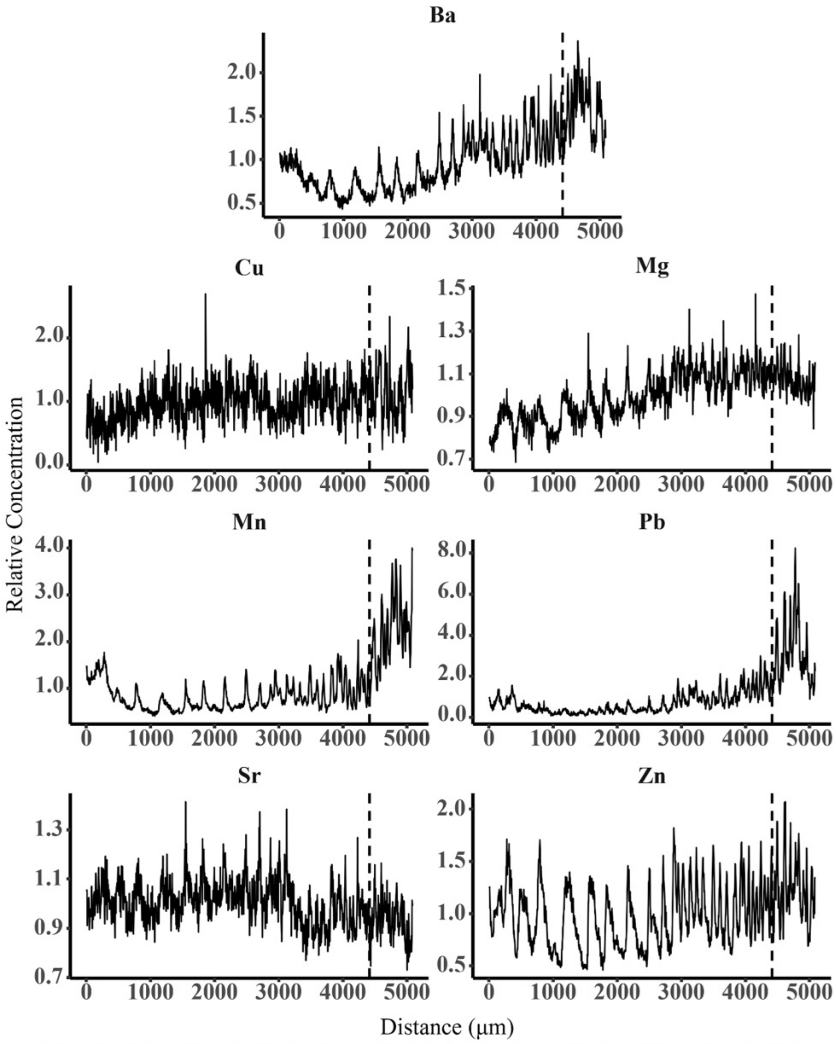
though zone width was significant it was not required to correctly discriminate OSM signatures, as shown by the accuracy results for the test data, which were the same with or without it (98.8% classification success). The results of the classification models were further supported by visual inspection of the ordination plot, which revealed clear dissociation of ray signatures and growth zone width before and after OSM with an overlap between the two datasets indicating that changes to signatures were comparable across geographic locations, confirming that sexual maturity influences elemental and morphological signatures across lake sturgeon populations.

Variable differences before and after OSM

Reproduction leads to a proliferation in gamete production which typically reduces somatic growth, which is then reflected in the annual deposition width on rays (Bakhshalizadeh et al. 2017). Growth zone width was one of the most important variables for discrimination success (Fig. 6), as it was found to significantly decrease after OSM which is consistent with previous research as numerous studies have reported a decrease in otolith or ray growth zone formation after OSM for a variety of fish species (Pulliainen and Korhonen 1994; Rijnsdorp and Storbeck 1995; Francis and Horn 1997; Reglero and Mosegaard 2006; Denechaud et al. 2021). This phenomenon has also been documented in sturgeon with studies using growth zone width to help identify OSM in female persian sturgeon (*Acipenser persicus*), starry sturgeon (*Acipenser stellatus*) (Bakhshalizadeh et al. 2017), and lake sturgeon (Roussow 1957).

Certain elements may be under greater physiological control around the time of sexual maturity. In our study Ba, Mg, Mn, Pb, and Zn were higher after OSM, and increased towards the end of the fin ray, proportionate with current documented age ranges of OSM in lake sturgeon. Age at sexual maturity in lake sturgeon is sex-specific and can vary by population but usually occurs later in females (~15–30) than in males (~12–20) (COSEWIC 2017). Elemental increases identified across structures were found to occur on average at age 24 in females (range 14–34) and 15 in males (range 9–21) suggesting that elements may begin to increase coincident with

Fig. 3. Elemental profiles from core to outer pectoral fin ray edge of a 36-year-old, 1409 mm TL, lake sturgeon (sex unknown) captured in the Nelson River, Manitoba in 1999. Vertical lines represent the point of elemental increase identified by reader consensus (age 23).

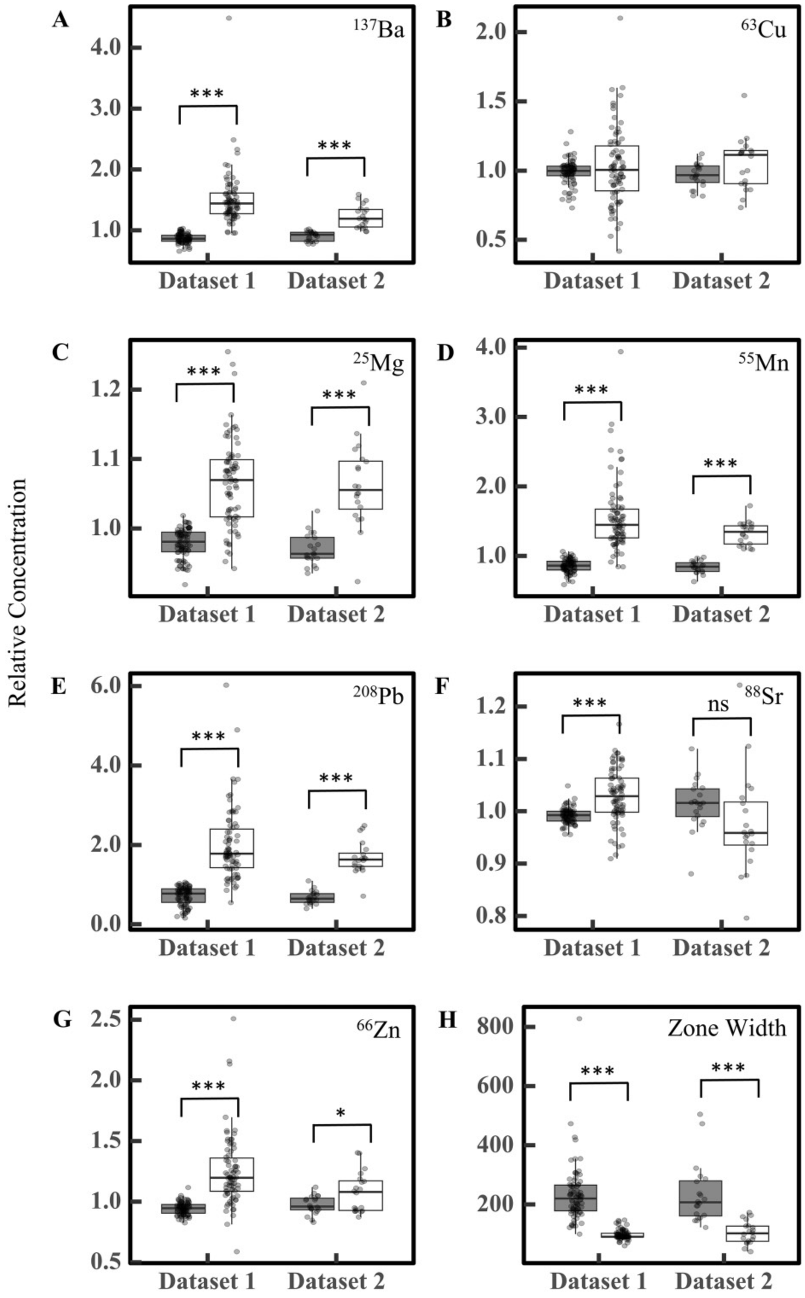


OSM (Roussow 1957, males = 12–19, females 14–23; Magnin 1966, males = 18–20, females 20–23; Harkness and Dymond 1961, males = 12–22, females 14–33). In contrast, Cu levels remained the same across life stages and, thus, may not be affected by sexual maturity status.

Factors that could cause element deposition in hard structures to change after OSM are currently unknown. Sturgeons have not yet been studied for the effects of maturation on ray elemental concentrations, and reports regarding otolith mineralization in other species have been inconsistent or uninformative (Hüssy et al. 2020). According to available research, the direct mechanistic basis for changes in otolith concentration of Ba, Mg, Mn, or Cu following sexual maturation is unknown (Hüssy et al. 2020). However, when examining element concentrations in otoliths from female european plaice, Sturrock et al. (2015) found a relationship between sexual maturation and otolith element concentrations where increases in otolith Sr and Zn were significantly correlated with gonadal somatic index. Furthermore, evidence for the effects of growth rate on otolith elemental concentrations has been well documented as otolith Mg, Zn, and Cu concentrations are reported to be positively correlated with fish growth. Whereas Ba, Mn, and Sr show a conflicting relationship to growth since positive, negative, and non-significant relationships have been reported (Martin and Thorrold 2005; Walther et al. 2010; Limburg et al. 2011; Hüssy et al. 2020).

The effect of OSM on elemental concentrations in sturgeon might be attributed to Ca requirements throughout the reproductive cycle. Ca is an essential element required for a variety of physiological processes related to reproduction in fish, such as the formation of vitellogenin (a yolk precursor protein that facilitates oocyte development) in females as well as for the regulation of sperm motility in males (Allen et al. 2009*b*; Alavi et al. 2011). Furthermore, total plasma Ca was reported to increase significantly during reproduction in sexually mature females and to increase marginally in males (Allens et al. 2009*b*). This investment, which causes the rerouting of Ca for progenitive use, may result in a decrease of avail-

Fig. 4. Box plots presenting comparisons of pectoral fin ray mean elemental concentrations and growth zone width before (gray) and after (white) onset of sexual maturity in lake sturgeon ($n = 98$) from the geographically closed Black Lake population (Dataset 1) and seven other geographically separated populations (Dataset 2) (map in Fig. 1). Median values are indicated by a horizontal bar within each box. Vertical lines above and below each box represent 5 and 95 percentiles. Comparisons were performed by Wilcoxon's signed rank test. Symbols: $* = P \leq 0.05$, $** = P \leq 0.01$, and $*** = P \leq 0.001$. Note: Relative elemental concentrations were calculated for each pectoral fin ray by dividing each measurement by the average over the entire profile.



able Ca during the calcification of hard structures and/or an increase of other divalent cations entering the fish via calcium channels. Scute/scale quality (i.e., size and toughness) has been seen to diminish in older slow-growing members of the Acipenser family (Allen et al. 2009*b* and references therein). Divalent cations (e.g., Mg^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Cu^{2+}, and Pb^{2+}), such as those seen to increase after OSM in this study, replace Ca on the hydroxyapatite matrix within the crystalizing structure (Doubleday et al. 2013; Kerr and Campana 2014; Izzo et al. 2016). As a consequence, a decrease in ray percent Ca could result in higher replacement by other similar divalent cations which would be reflected in higher elemental concentrations.

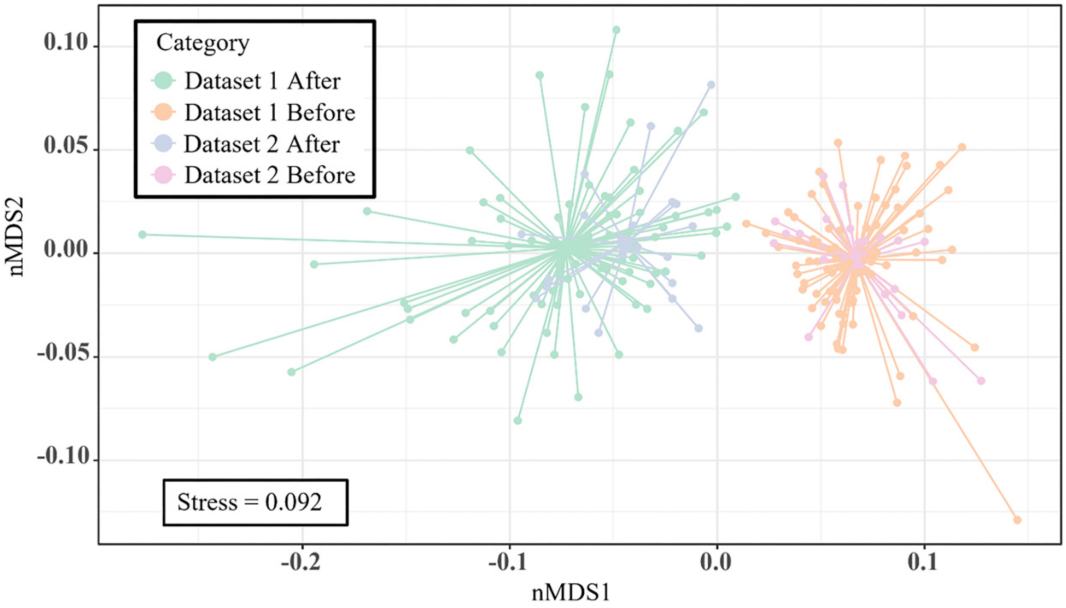
Interestingly, Sr and Cu were the least important discriminators in the model. Sr levels for this study varied by geographic location indicating its importance as an environmental tracer not as strongly influenced by OSM. Indeed, Sr is the most common and often only element examined in adult

Table 2. Results of statistical analysis (Wilcoxon's signed rank test) comparisons of pectoral fin ray mean elemental concentrations and growth zone width before and after onset of sexual maturity (OSM) in lake sturgeon ($n = 98$) from the geographically closed Black Lake population (Dataset 1; $n = 78$) and seven other geographically separated populations (Dataset 2; $n = 20$).

	Dataset 1				Dataset 2			
Variable	Before OSM	After OSM	Z	*P*	Before OSM	After OSM	Z	*P*
Ba	0.87 ± 0.08	1.51 ± 0.45	−7.62	<0.001	0.91 ± 0.08	1.23 ± 0.19	−3.81	<0.001
Cu	0.99 ± 0.09	1.03 ± 0.28	−0.451	0.654	0.97 ± 0.09	1.05 ± 0.19	−1.46	0.154
Mg	0.98 ± 0.02	1.07 ± 0.06	−6.79	<0.001	0.97 ± 0.02	1.06 ± 0.06	−3.47	<0.001
Mn	0.86 ± 0.10	1.56 ± 0.50	−7.57	<0.001	0.84 ± 0.09	1.33 ± 0.17	−3.92	<0.001
Sr	0.99 ± 0.02	1.03 ± 0.05	−4.59	<0.001	1.01 ± 0.05	0.98 ± 0.09	1.61	0.114
Zn	0.94 ± 0.05	1.25 ± 0.29	−6.78	<0.001	0.97 ± 0.08	1.08 ± 0.16	−2.05	0.039
Pb	0.72 ± 0.24	2.00 ± 0.90	−7.5	<0.001	0.68 ± 0.17	1.68 ± 0.42	−3.88	<0.001
Zone width	234.3 ± 98.4	97.9 ± 16.35	7. 67	<0.001	235.5 ± 102.9	104.7 ± 37.6	3.81	<0.001

Note: Relative elemental concentrations were calculated for each pectoral fin ray by dividing each individual element measurement by the average of the corresponding element's profile. Mean ± standard deviation measurements are shown. Ba, barium; Cu, copper; Mg, magnesium; Mn, manganese; Sr, strontium; Zn, zinc; and Pb, lead.

Fig. 5. Nonmetric multidimensional scaling (NMDS) ordination plot showing elemental and morphometric variation among lake sturgeon pectoral fin rays ($n = 98$) before and after presumed onset of sexual maturity (OSM) from the geographically closed Black Lake population (Dataset 1) and seven other geographically separated populations (Dataset 2) (map in Fig. 1). Each dot represents an individual's year-specific signature and the relative distance between two dots represents the similarity (or dissimilarity) quantified across measured variables using the Bray–Curtis approach. Clustering was found to be significant ($P = 0.001$) using a multiple response permutation procedure.



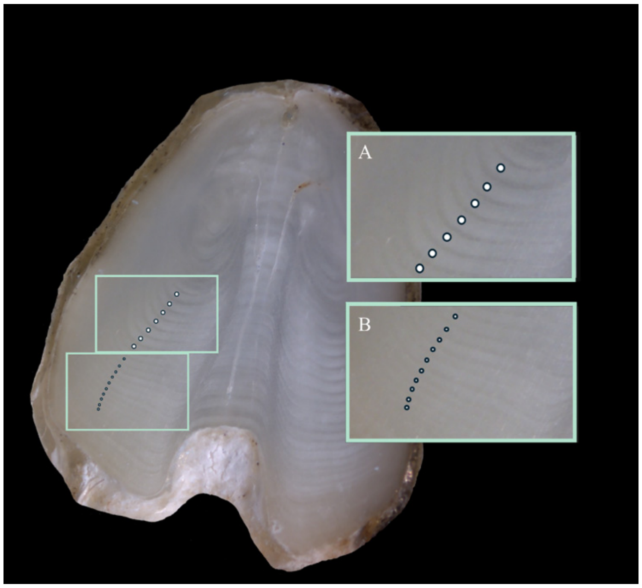
sturgeon microchemistry studies since it is considered an environmental indicator allowing both large and small-scale habitat differentiation (Sellheim et al. 2017; Allen et al. 2018; Gunn et al. 2019; Neary et al. 2024). Surprisingly, levels of Cu, a divalent cation, were found to remain the same across life stages which was not expected based on the results of other similar elements. As a cofactor for several enzymes, Cu is one of the most important essential elements in fish (Lall and Kaushik 2021). Highly regulated in the body, fish require Cu for a variety of intercellular processes related to brain and red blood cell development, cholesterol and glucose metabolism, and the development of bone tissue (Lall and Kaushik 2021; Wang et al. 2024). Ambient water Cu concentrations were extremely low compared to other essential elements indicating that Cu may be more reflective of dietary sources rather than environmental, but this assumption has not been validated (see supporting information S1). Interestingly, Cu concentrations were also extremely low compared to other essential elements (Mn, Mg, and Zn) incorporated in the rays (Table 4). Reasons why this may occur could be related to environmental availability or to just how vital this element is to the body, which may result in it being more highly regulated both before and after OSM (Chandrapalan and Kwong 2021). Similarly, Pb concentrations were also relatively low, but Pb is not regulated as strictly as Cu because it is considered a nonessential element and therefore it is possible that a decrease

Table 3. Random forest classification success of year-specific signatures to before or after onset of sexual maturity derived from lake sturgeon pectoral fin rays from a geographically closed population (Dataset 1, $n = 24$) and seven other geographically separated populations (Dataset 2, $n = 20$).

	Dataset 1				Dataset 2		
	Predicted		Correctly		Predicted		Correctly
Actual	Before	After	Classified	Actual	Before	After	Classified
Before	24	0	100%	Before	19	1	95%
After	0	24	100%	After	0	20	100%
Overall correct class. rate			100%	Overall correct class. rate			97.5%

Note: Two observations for every pectoral fin ray (year 5 and last growth zone) were tested in the model to classify both a before and after onset of sexual maturity signature for each fish. Signatures for the last growth zone as well as year 5 were tested to determine the model's ability to correctly discriminate year-specific signatures to before or after onset of sexual maturity.

Fig. 6. Pectoral fin ray cross section of an adult female lake sturgeon captured in Black Lake, Michigan. The top box (A) depicts growth zone width earlier in life while box (B) depicts growth zone width later in life. Note the decrease in overall growth zone width with increased age.



in Ca may lead to higher replacement (Hüssy et al. 2020). In fact, Ba and Pb, the only two non-essential elements measured (excluding Sr), were the two most important elemental discriminators in the model, indicating that non-essential elements may experience the most change after OSM. Since non-essential elements are not required for biological function, they are less regulated and therefore have the potential to be more readily available during calcification compared to essential elements (Wood et al. 2011; Izzo et al. 2016).

Due to the species declining abundance and distribution, several biomineral microchemistry studies have been performed on sturgeon populations to assess important population dynamics and implement policies to improve stock structure. The majority of studies that have analyzed sturgeon rays through microchemistry have concentrated on Sr variation in an attempt to distinguish habitat use, with other elements often omitted from published analysis (Allen et al. 2009*a*; Phelps et al. 2012, 2016; Sellheim et al. 2017; Ziegeweid et al. 2021). Studies that have reported on other elements examined specific time points and did not report differences between early and late life stages (Jarić et al. 2010; Balazik et al. 2012; Gunn et al. 2019; Bakhshalizadeh et al. 2021; Loeppky 2021). Thus, to our knowledge this is the first study to document variations between Ba, Cu, Mg, Mn, and Zn between adult and juvenile sturgeon.

Conclusion and future directions

This study is the first description of elemental variation in rays coinciding with OSM for any species. Results indicated that sexual maturity may influence element deposition and growth zone width in calcified structures. Findings across populations indicated some small dissimilarities (specifically in Sr), but overall trends were similar with most divalent cations (specifically Ba, Mg, Mn, Pb, and Zn) found to be significantly higher after OSM and growth zone width found to be significantly lower. Additionally, elements were found to visually increase at documented ages of OSM in lake sturgeon, suggesting that the change in signatures may be distinguishable using visual cues or a quantitative method (i.e., multivariate multiple change point analysis: Matteson and James 2014; Function: e.divisive) (see https://github.com/AlainaTaylor/Lake_Sturgeon_Microchemistry_R). This new discovery suggests that fin ray chemistry could be used to estimate an age of when each fish reaches sexual maturity. Furthermore, since the age at OSM in sturgeon is sex-specific (males 12–20: females 15–30; COSEWIC 2017) this increase may also prove useful in identifying sex for individuals on the marginal ends of their range. Although, discrimination success was high, development of a new model using the point of elemental increase to discriminate year-specific maturity signatures may be another area of future research. A model using this increase will most likely result in high discrimination success in signatures closer to the actual point of OSM.

Currently, an ultrasound, surgical biopsy of the internal gonads, or blood removal for plasma sex steroid analysis is required to determine maturity status in sturgeon (Wildhaber et al. 2007; Chapman and Van Eenennaam 2008; Webb et al.

Table 4. Pectoral fin ray mean elemental concentrations (±standard deviation) for lake sturgeon ($n = 98$) from a geographically closed population (Black Lake-Dataset 1: Location 1) and seven other geographically separated populations (Dataset 2: locations 2–8).

Location	Region	n	^{137}Ba	^{63}Cu	^{25}Mg	^{55}Mn	^{88}Sr	^{66}Zn	^{208}Pb
1	Black Lake	78	19.4 ± 10.7	1.28 ± 1.03	4401.4 ± 613.8	27.1 ± 16.4	113.4 ± 11.2	158.6 ± 61.0	2.14 ± 3.03
2	Churchill River	1	51.1 ± 11.9	0.83 ± 0.60	3031.2 ± 291.7	24.9 ± 10.3	190.0 ± 33.5	69.4 ± 20.1	0.43 ± 0.25
3	Kiskittogisu Lake	2	52.8 ± 12.7	0.74 ± 0.42	3036.5 ± 295.7	34.4 ± 20.0	198.6 ± 24.3	106.8 ± 33.0	0.81 ± 0.54
4	Nelson River	9	50.4 ± 19.4	0.91 ± 0.52	3268.5 ± 478.8	22.9 ± 13.5	184.6 ± 25.9	87.9 ± 31.3	0.64 ± 0.66
5	Norman Dam	1	23.0 ± 6.77	0.50 ± 0.29	3473.9 ± 550.9	21.2 ± 8.75	100.9 ± 13.6	122.1 ± 31.4	2.78 ± 3.13
6	Sturgeon Falls	3	21.8 ± 8.41	0.60 ± 0.28	3186.6 ± 384.5	37.3 ± 16.1	96.5 ± 9.24	133.7 ± 53.1	1.51 ± 1.76
7	Whitedog River	1	17.7 ± 3.07	1.13 ± 0.48	3437.9 ± 285.0	27.5 ± 13.1	108.0 ± 11.8	111.5 ± 37.7	0.49 ± 0.48
8	Lake Winnebago System	3	17.0 ± 6.61	3.96 ± 1.31	4519.8 ± 551.5	17.6 ± 7.18	115.3 ± 21.1	225.1 ± 65.6	1.89 ± 1.38

Note: Elemental concentrations are expressed in ppm = μg/g. Ba, barium; Cu, cpper; Mg, magnesium; Mn, manganese; Sr, strontium; and Zn, zinc.

2017). Although this process has become less invasive over time, using the pectoral fin ray to identify OSM could result in less stress during handling of the fish, especially if the ray is already being removed for age and growth analysis. Further, the current methods described only identify whether or not an individual is mature; they cannot ascertain an individual's relative age at OSM. In addition, if year-specific signatures are used to identify maturity status, it would be possible to expand this method to use the second fin ray in place of the fin spine, for less invasive removal (Baremore and Rosati 2014; Izzo et al. 2021). This structure could result in not only a less invasive procedure but also less time and money spent on ray preparation and elemental analysis.

It is important to note that sample sizes for certain sites were limited in this study (particularly for sites 1, 3–6, 8; Fig. 1). Because of this, it is impossible to ascertain whether trends in elemental concentrations before versus after OSM reflect the properties of the entire population for those sites. Results for these areas should be taken with caution and future work with larger sample sizes is required to further validate the influence of OSM on elemental signatures for rays collected for these populations.

Importantly, when using the microchemistry of calcified structures to interpret important population metrics, one should consider the effects of physiological influences, specifically in species that are known to invest dramatically throughout the reproductive cycle. It is possible for elemental data to be misinterpreted if physiological factors influence elemental signals, ultimately resulting in mismanagement of the fishery. Although the reasons for changing elemental concentrations remain unclear, this study suggests that year-specific signatures in rays can be used as a potential tool for identifying OSM in long-lived species such as lake sturgeon. Future work is required to determine if this technique is transferrable to other sturgeon species particularly those that are anadromous (i.e., white sturgeon (*Acipenser transmontanus*); atlantic sturgeon (*Acipenser oxyrinchus*)). Even so, this research highlights new applications for fin ray microchemistry, which could greatly improve current fisheries management practices that strictly use the chemistry of structures to examine environmental influences.

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Data availability

Data from this study can be obtained by contacting the corresponding author.

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Competing interests

The authors declare there are no competing interests.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2023-0279.

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